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TOXICOLOGICAL REVIEW

OF

Phenol

(CAS No. 108-95-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2002

U.S. Environmental Protection Agency
Washington D.C.

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response assessment in IRIS pertaining to chronic exposure to phenol. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of phenol.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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This document and summary information on IRIS have received peer review by both EPA scientists and independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment underwent an Agency-wide review process whereby the IRIS Program Manager achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response (OSWER) of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are presented in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), an inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of milligrams per kilogram per day (mg/kg-day). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but it provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for the respiratory system (portal of entry) and effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of milligrams per cubic meter (mg/m^3).

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration that provide cancer risks of 1 in 10,000 1 in 100,000 or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for phenol has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment*

(U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a); *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The literature search was conducted in June 1999; selected key articles published after that date are also included.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Phenol is a monosubstituted aromatic hydrocarbon. In its pure state, it exists as a colorless or white solid. This pure compound is mixed with water and commercially sold as a liquid product. Phenol gives off a sweet, acrid smell detectable to most people at 40 ppb in air and at about 1–8 ppm in water (ATSDR, 1998). It evaporates more slowly than water and is moderately soluble in water. Phenol is also combustible.

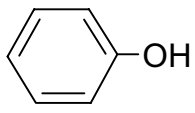
Phenol is produced through both natural and anthropogenic processes. It is naturally occurring in some foods, in human and animal wastes, and in decomposing organic material, and it is produced endogenously in the gut from the metabolism of aromatic amino acids. Phenol has been isolated from coal tar, but it is now synthetically manufactured. Currently, the largest use of phenol is as an intermediate in the production of phenolic resins, which are used in the plywood, adhesive,

construction, automotive, and appliance industries. Phenol is also used in the production of synthetic fibers such as nylon and for epoxy resin precursors such as bisphenol-A. Phenol is toxic to bacteria and fungi, and it is used as a slimicide and disinfectant. Because of its anesthetic effects, phenol is used in medicines such as ointments, ear and nose drops, cold sore lotions, throat lozenges and sprays (such as those sold under the Cepastat® and Chloraseptic® labels), and antiseptic lotions.

The greatest potential source of exposure to phenol is in the occupational setting, where phenol is used in manufacturing processes. People are also exposed via consumer products, such as medicines and lotions, and some foods and tobacco smoke. Phenol has been found in drinking water.

The physical and chemical properties of phenol are shown in Table 1.

Table 1. Physical Properties and Chemical Identity of Phenol

CAS Registry Number	108-95-2	Lide, 1993
Synonym(s)	Benzenol, hydroxybenzene, monophenol, oxybenzene, phenyl alcohol, phenyl hydrate, phenyl hydroxide	ATSDR, 1998
Registered trade name(s)	Carbolic acid, phenic acid, phenic alcohol	ATSDR, 1998
Melting point, °C	43	Lide, 1993
Boiling point, °C	181.8	Lide, 1993
Vapor pressure, at 25 °C	0.3513	HDSB, 1996
Density, at 20 °C relative to the density of H_2O at 4 °C	1.0576	Lide, 1993
Flashpoint (open cup)	85 °C	ATSDR, 1998
Water solubility, g/L at 25 °C	87	Lide, 1993
Log K_{OW}	1.46	HDSB, 1996
Odor threshold	0.047 ppm (0.18 mg/m^3) - 100% response 0.006 ppm (0.02 mg/m^3) - sensitive	U.S. EPA, 1986d
Molecular weight	94.12	Calculated
Conversion factors	1 ppm (v/v) = mg/m^3 x 0.260 1 mg/m^3 = ppm (v/v) x 3.85	Calculated
Empirical formula	C_6H_6O	Lide, 1993
Chemical structure		

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Phenol is readily absorbed by the inhalation, oral, and dermal routes. The portal-of-entry metabolism for the inhalation and oral routes appears to be extensive and involves sulfate and glucuronide conjugation and, to a lesser extent, oxidation. The primary oxidative metabolites include hydroquinone and catechol, which are also substrates for conjugation. Secondary products of hydroquinone or catechol metabolism, including benzoquinone and trihydroxybenzene, can also be formed. Once absorbed, phenol is widely distributed in the body, although the levels in the lung, liver, and kidney are often reported as being higher than in other tissues (on a per-gram-tissue basis). Elimination from the body is rapid, primarily as sulfate and glucuronide conjugates in the urine, regardless of the route of administration. Phenol does not appear to accumulate significantly in the body.

3.1. ABSORPTION

Extensive absorption following inhalation exposure has been demonstrated in both human and laboratory animal studies. Piotrowski (1971) studied lung and skin absorption of phenol in volunteers (seven male and one female) exposed to air concentrations of 6 to 20 mg/m^3 for 8 hours. The study subjects were staff of the research institute in Poland, and all had undergone a previous medical examination. In the lung absorption studies, the subjects inhaled phenol through a face mask, eliminating the potential for most dermal absorption. These subjects retained 60–88% of the inhaled phenol, and the percent retained did not vary with exposure concentration. The absorption rate leveled off after approximately 3 hours of exposure, indicating that absorption had reached steady state. In the skin absorption studies, subjects wore underwear and denim coveralls or were unclothed for different trials of the experiment; in each case they were supplied with fresh air from outside the chamber for breathing. The absorption coefficient did not appear to vary greatly with exposure for 6 hours to concentrations in air ranging from about 5 to 25 mg/m^3, and clothing did not appear alter the absorption rate. The mean absorption coefficient was 0.35 m^3/hr, indicating that the amount of phenol present in 0.35 m^3 of air was absorbed through the skin per hour. These data show that dermal absorption can contribute significantly to the systemic dose of phenol following exposure to phenol in air. However, the quantitative data from the dermal exposure study are limited for the development of an RfC because of the short duration of the exposure and the absence of a direct determination of whether the absorption rate had reached steady state.

Other studies of workers exposed to phenol provide evidence for significant absorption via the inhalation route; however, the contribution of dermal absorption from direct contact with liquid phenol or from phenol in air was not assessed in these studies. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. The total and conjugated phenol levels tended to increase with increasing air concentration, but free phenol levels were not affected. This suggests that at the concentrations studied (up to 12.5 mg/m^3), phenol conjugation was not rate limiting. The investigators did not specifically evaluate the levels of oxidative phenol metabolites, so no conclusion can be made regarding whether Phase I metabolism was rate limiting. On the basis of mass balance analysis, the authors concluded that phenol is efficiently absorbed from the lung, because the phenol dose (air concentration * air volume/hr) was similar to the total mass excreted in the urine.

Other occupational studies provide qualitative evidence for lung absorption, reporting increasing urinary excretion of phenol metabolites with increasing workplace air concentrations. In many cases, the data are not adequate to estimate the rate and degree of absorption through this route, and potential contributions of dermal absorption are often inadequately described (Ogata et al., 1986; ACGIH, 1991).

Absorption through the lung has also been evaluated in laboratory animals report following inhalation exposure or intratracheal administration. In an unpublished study, Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in Fischer 344 (F344) rats following inhalation exposure to 25 ppm (96 mg/m^3) for 6 hours (nose only).[1] Radioactivity in the blood was at steady-state levels at the first measured time point (120 minutes after beginning the 6-hour exposure), indicating rapid absorption kinetics. Hughes and Hall (1995) evaluated the disposition of phenol following intratracheal and intravenous (i.v.) administration of 63.5 nmol of ^{14}C-phenol to female Fisher 344 rats. The recovery of radioactivity in tissues and excreta for both routes was approximately 90% of the administered dose within 72 hours. Because the amount of radioactivity recovered was nearly equal for the intratracheal and the i.v. dose routes (and fecal excretion is minimal), the authors concluded that absorption was near 100%.

Hogg et al. (1981) administered ^{14}C-phenol intratracheally in isolated perfused rat lungs from MRC hooded rats. At the end of the experiment (perfusions were approximately 85 minutes),

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[1]This study has not been peer-reviewed, but it was well-conducted according to EPA guidelines for a pharmacokinetics study (with minor deviations).

approximately 92% of the administered radioactivity was in the perfusate, 6% was recovered in the lung lavage, and approximately 3% was associated with lung tissue. The high recovery in the perfusate indicated that phenol is nearly completely absorbed across the airways.

Human evidence for oral absorption indicates rapid and complete absorption. In a study of three human volunteers, Capel et al. (1972) found that 85–98% of a 0.01 mg/kg oral dose of ^{14}C-phenol was excreted in the urine—primarily as phenylsulfate and phenylglucuronide—within 24 hours. In addition, case reports of oral poisoning provide qualitative evidence for gastrointestinal absorption of phenol, but the ingested and absorbed dose were not estimated in these reports, and in some cases both oral and dermal exposure were involved (Tanaka et al., 1998).

Numerous laboratory animal studies have found that orally administered phenol is readily absorbed. In most cases, absorption rates were not calculated, but the rapid recovery of high percentages of administered doses in the urine—with only minimal recovery in feces—demonstrated nearly complete absorption. In various studies in the rat (Kao et al., 1979; Edwards et al., 1986; Kenyon et al., 1995), the percentage of the administered dose recovered in urine ranged from 65 to 96.5% over a wide range of doses. Varying degrees of absorption have been observed in a variety of other species. In a study of 18 animal species orally administered single doses ranging from 20 to 50 mg/kg ^{14}C-phenol, Capel et al. (1972) found that the percentage of radiolabel recovered in the urine within 24 hours varied from 31% of the administered dose in squirrel monkeys to 95% in Wistar rats. It is not clear, however, if these differences represent differences in the degree of absorption or in the rate of elimination.

Hughes and Hall (1995) found that in female F344 rats administered 63.5 nmol of ^{14}C-phenol by oral gavage, total recovery of radioactivity (in tissues and excreta) was approximately 90% of the administered dose within 72 hours. Because fecal excretion is approximately 1–3%, and the recovered dose was nearly equal for the oral and the i.v. dose routes, the authors concluded that the absorption was near 100%. The difference between the 90% recovery and 100% total absorption was attributed to losses that were consistent across doses.

Rapid absorption of orally administered phenol has been observed in a number of studies. Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in F344 rats following oral dosing by gavage or in drinking water. Total recovery of the administered radioactivity in the urine, feces, tissues and carcass, and exhaled air was approximately 95%, regardless of the dosing protocol. In the high-dose gavage animals (150 mg/kg), peak levels of radioactivity in blood were detected 15

minutes post-administration, indicating rapid uptake kinetics. Humphrey et al. (1980) found that free phenol levels in the plasma of rats given an oral dose of 300 mg/kg radiolabeled phenol reached a maximum of 26 μg/mL at the first measured time, about 10 minutes after dosing, and declined rapidly to background by 60 minutes. They observed similar results in beagle dogs given a 40 mg/kg dose, with rapid peak levels of 7.8 μg/mL and complete removal of free phenol by 1 hour.

More quantitative kinetics data are available from in situ perfusion studies. Humphrey et al. (1980) administered ^{14}C- phenol (1 mg/mL) to the gut lumen of rats by means of a duodenal cannula. The remaining radioactivity was measured at 3-minute intervals over 30 minutes in perfusate collected by an ileal cannula. The results from the intestinal perfusion studies indicated that removal of ^{14}C-phenol obeys first-order kinetics, with a lumenal $T_{1/2}$ of 5.5 minutes and a rate constant for absorption of 0.127 min^{-1}. These authors also measured the plasma concentrations of phenol in the portal vein and posterior vena cava of dogs following intraduodenal dosing with either 40 or 160 mg/kg phenol. At either dose, the concentration was already maximal in the portal vein plasma within 3 minutes after dosing (the first measurement taken) and had decreased to nondetectable levels within 1 hour at the low dose and to 33% at the high dose. These data show that in both species phenol is rapidly absorbed from the gut.

Powell et al. (1974) added ^{14}C-phenol to the mucosal medium of isolated rat gut preparations and measured the level of radioactivity in the mucosal and serosal medium over 2 hours. They found that 78% of the administered radiolabel had been transferred to the serosal medium over this period. Kao et al. (1979) administered ^{14}C-phenol (12.5 or 25 mg/kg) to rats intraduodenally. Recovery of the radioactivity was rapid, with more than 70% recovered in the urine within 2 hours.

The dermal route of exposure is an important one. Both absorption of phenol liquid directly in contact with skin and dermal absorption from exposure to phenol vapor are of concern. Significant dermal absorption can result from phenol in air, so that phenol in air results in both dermal and inhalation exposure (Piotrowski, 1971). On the basis of an analysis of the Piotrowski (1971) data, ATSDR (1998) concluded that in air concentrations ranging from 5 to 25 mg/m^3, the amount of phenol absorbed through the skin will be about half of that absorbed through the lungs. The conclusion was reached by estimating the amount of phenol absorbed through the lung as the product of the human ventilation rate of 0.8 m^3/hour and the steady-state lung retention fraction of 0.7 reported by Piotrowski (1971). The resulting lung absorption coefficient of 0.6 m^3/hr is nearly twice the skin absorption coefficient of 0.35 m^3/hr. This analysis is limited, however, because it is

not clear that the exposure duration was long enough for steady state to be reached in the dermal absorption studies. Absorption via the dermal route may be lower at steady state due to the potential for a back-pressure from phenol levels in blood.

A number of case reports and in vitro studies have led to quantitative estimates of phenol absorption through the skin. Baranowska-Dutkiewicza (1981) applied a reservoir of 2.5, 5, or 10 g/L phenol solution on a small area of the forearm of 12 male volunteers. The absorption rate was dependent on the concentration and ranged from 0.08 $mg/cm^2/hr$ at the low concentration to 0.301 $mg/cm^2/hr$ at the high concentration. At the low concentration, the total amount of phenol absorbed—but not the absorption rate—increased with increased time; approximately 13% of the applied dose was absorbed over a 30-minute period. In an in vitro study, 20% of applied doses ranging from 1.3 to 2.7 $\mu g/cm^2$ were absorbed from unoccluded human skin patches within 72 hours; addition of a Teflon cap resulted in 47% absorption over this same period (Hotchkiss et al., 1992).

Bentur et al. (1998) reported an accidental dermal poisoning case in which a solution of 90% phenol was spilled on the left foot (3% of body surface). The exposure site remained occluded, and no attempt at decontamination was made until the onset of symptoms, which began within 4.5 hours. Following admission to the hospital shortly afterwards, peak serum phenol levels of 21.6 µg/mL were measured. This study is presented here for completeness, but quantitative exposure data from studies at lower phenol concentrations are more relevant to environmental exposures.

The ability of phenol to be absorbed through the skin has also been evaluated in laboratory animals. Hughes and Hall (1995) administered 63.5 nmol of labeled phenol to an occluded dermal patch (2.5 cm^2) of female F344 rats. Maximal recovery of the radioactivity was approximately 70%. The site of dermal application was washed 72 hours post-treatment and yielded 14% of the recovered dose; 1.6% of the recovered dose was present in the skin at this site. Thus, approximately 15% of the dose was not absorbed within 72 hours. In an in vitro study, Hotchkiss et al. (1992) found that phenol absorption by rat skin is similar to that of human skin: approximately 20–50% in 72 hours, depending on the conditions.

Taken together, the human and laboratory animal data demonstrate that phenol is readily absorbed following exposure by all dose routes. The recovery of greater than 90% of the administered phenol dose as urinary metabolites provides direct evidence that the administered dose was nearly completely absorbed. The route of administration appears to play a limited role, with skin absorption reported as less extensive than absorption from the lung or gut. In most studies,

absorption rate constants have not been calculated; however, the measurement of peak blood phenol concentrations within minutes of dosing indicates that absorption is rapid

3.2. DISTRIBUTION

Studies in humans and laboratory animals indicate that phenol is widely distributed throughout the body regardless of exposure route. Because phenol is rapidly excreted, studies on tissue distribution typically evaluate only a small fraction of the absorbed dose.

Several fatal poisoning case studies evaluated phenol concentrations in multiple tissues (Tanaka et al., 1998). Generally, phenol is widely distributed. Higher tissue concentrations relative to blood have been reported for some organs, particularly for the liver and kidneys, although this finding has not been reported consistently across all studies.

Morrison et al. (1991) reported on the kinetics of phenol injected intramuscularly in a motor point block procedure in pediatric patients. Administered doses ranged from 6.7 to 70 mg/kg, and the blood phenol concentration was measured at 5, 15, 30, 60, and 120 minutes after the last injection. Phenol reached peak levels 5 to 15 minutes after administration and rapidly declined to 3 to 34% of peak levels within 120 minutes. Peak phenol concentration (μg/mL) in blood as a function of administered dose (x, in mg/kg) was determined ($y = 0.483x - 3.244$; $r = 0.873$). Pretreatment levels of blood phenol ranged from 0.3 to 0.8 μg/mL and post-treatment levels ranged from 2.5 to 36 μg/mL.

The laboratory animal data provide additional evidence for elevated tissue concentrations in the lung, liver, and kidney, although the magnitude of the tissue differences varies from study to study. Liao and Oehme (1981) evaluated the tissue distribution of 207 mg/kg ^{14}C-phenol orally administered to male Sprague-Dawley rats. Total radioactivity in tissues declined rapidly from a maximum of 28.4% of the administered dose at 0.5 hours to 16.6% at 1 hour and 0.3% at 16 hours. Tissue concentrations of radioactivity measured at time points between 0.5 and 16 hours were significantly greater than in plasma for the liver, spleen, kidney, and adrenal gland; tissue concentrations in lungs and thyroid were also marginally elevated. The liver had the greatest amount of radioactivity, accounting for 29–56% of the total radioactivity recovered from tissues at the various time points. The study authors attributed the high levels in the liver to both an elevated tissue concentration and the large relative organ size. Because the study measured total radioactivity without further identification of the radiolabeled compounds, it is not known whether the observed radioactivity represented phenol or its metabolites.

Dow Chemical Co. (1994) conducted a study of ^{14}C-phenol administered to F344 rats by oral gavage at 1.5, 15, or 150 mg/kg in drinking water at 5000 ppm or via nose-only inhalation at 25 ppm for 6 hours. Tissue levels of radioactivity were measured in the kidneys, liver, lung, muscle, skin, spleen, testes, ovaries, and carcass 24 hours after exposure by the various routes. The only sites with a statistically significant increase in radioactivity levels were the kidney and liver (levels 5- to 10-fold higher than in other tissues). This finding was consistent across dosing regimens.

Hughes and Hall (1995) evaluated the disposition of radiolabeled phenol administered dermally, by oral gavage, intravenously, or intratracheally to female F344 rats. When the rats were sacrificed 72 hours after administration by any of these four routes, tissue concentrations represented only 1–5% of the recovered dose. No tissue appeared to have higher concentrations of radiolabel following oral dosing, but the lung concentrations were markedly higher following intratracheal administration. There was no substantive difference across tissues following dermal dosing, although untreated skin had a slightly higher level. Marginal elevations in the liver and kidneys were observed following i.v. dosing. The authors concluded that phenol is distributed widely in tissues, with some accumulation in the large organs (lung, liver, and kidney, based on within-route comparisons to the levels in blood).

Powell et al. (1974) treated juvenile rats (50 g) with less than 1 mg/kg ^{14}C-phenol orally or intraperitoneally. Whole-body radiograms indicated that the liver was not a site for accumulation of the phenol; rather, it was widely distributed. It is not clear whether the difference between the findings of this study and others is due to the differences in the sensitivity of the analysis or to differences in dose levels. Thus, the data from animals studies at doses ranging from 1.5 to 207 mg/kg, which included several doses higher and lower than the chronic No Observed Adverse Effect Level (NOAEL) of 60 mg/kg (NTP 1983a; Argus Research Laboratories, 1997) (see Chapter 5), showed that phenol is rapidly distributed to a wide range of tissues.

No direct studies of the placental transfer of phenol were identified. However, Ghantous and Danielsson (1986) evaluated the placental transfer of benzene, of which phenol is a primary metabolite. B6 mice were exposed for 10 minutes to benzene (at a target concentration of 2000 ppm) in air on gestation day (GD) 11, 14, or 17. The investigators conducted whole-body radiography analysis and determined of tissue concentrations. Radioactivity was distributed to the fetuses, but it was not specifically identified as phenol. The concentration of volatile and nonvolatile radioactivity in the fetuses was, however, lower than that in maternal tissues.

The human and laboratory animal data indicate that phenol is widely distributed in the body. Although the human data are inconclusive, the laboratory animal data consistently indicate that highly perfused organs such as the liver, kidney, and lung have higher tissue concentrations in comparison to the blood concentration.

3.3. METABOLISM

Metabolic pathways for phenol are shown in Figure 1. Phenol is directly conjugated with sulfate or glucuronic acid. Phenol that is not directly conjugated can also be a substrate for oxidation reactions. The cytochrome P450 2E1 isozyme (CYP2E1) catalyzes the addition of one oxygen atom to a variety of low-molecular-weight substrates such as benzene and chloroform, and it is thought to be the primary P450 isozyme for phenol oxidation, although a minor role by other cytochrome P450 enzymes cannot be discounted. The oxidation products of phenol generated by CYP2E1 activity appear to be primarily hydroquinone and catechols, which can themselves undergo further oxidation by CYP2E1 to trihydroxybenzene or by peroxidation to benzoquinone. Alternatively, the hydroquinone or catechol metabolites can undergo conjugation reactions. In addition to P450-mediated oxidation, some studies have suggested that peroxidative metabolism of phenol can also take place, producing biphenols and diphenoquinones.

Direct sulfate and glucuronic acid conjugations are detoxifying mechanisms that represent the bulk of phenol metabolism, as evidenced by the metabolic profiles observed in both humans

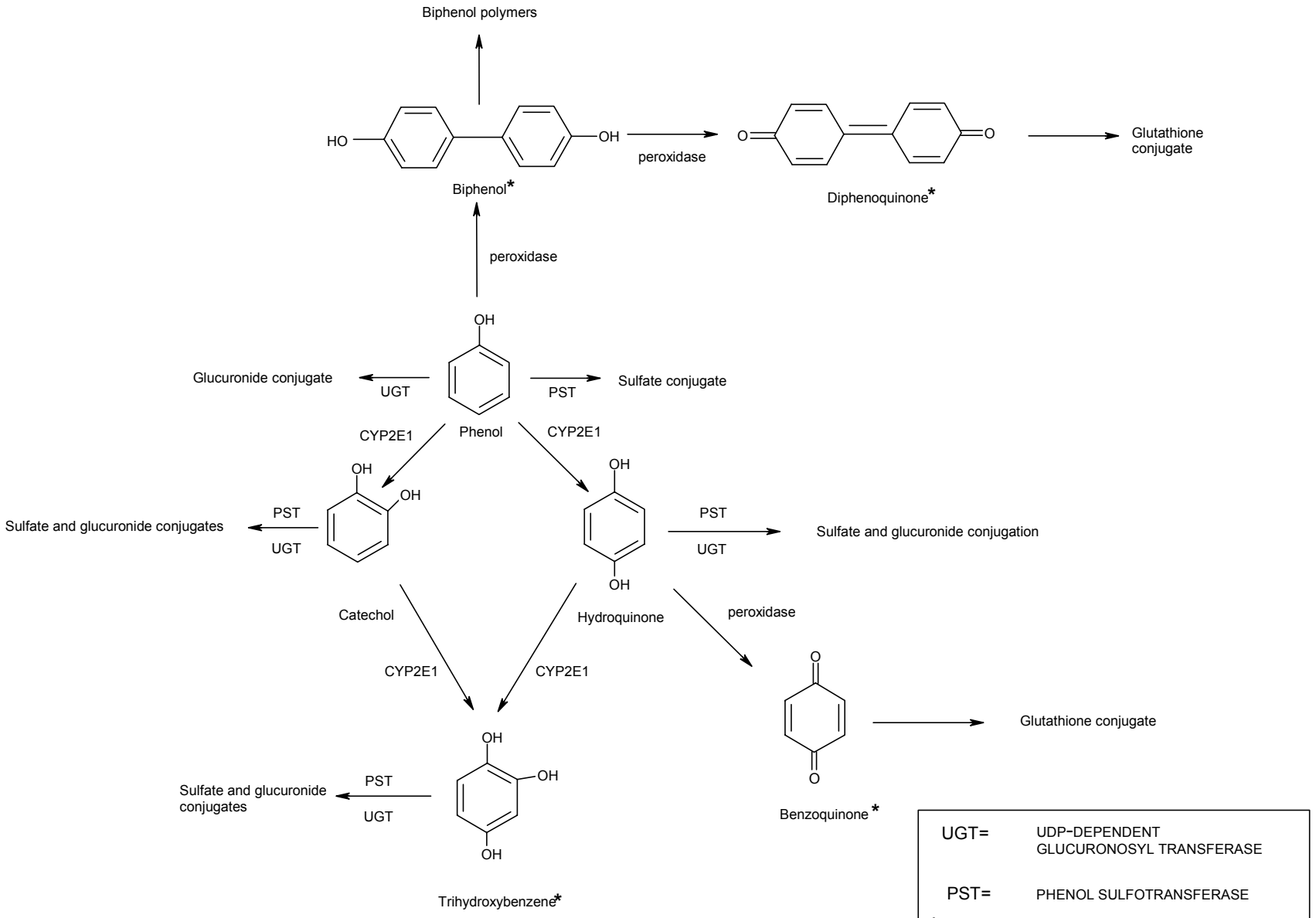

Figure 1. Metabolism of Phenol.

and laboratory animals. In humans and in most other species tested, sulfation predominates at the lower doses. Capel et al. (1972) studied the urinary metabolites following oral administration of 0.01 mg/kg ^{14}C-phenol to three male volunteers. In these men, 85–98% of the dose was excreted in 24 hours—69–90% as phenyl sulfate, 4–23% as phenyl glucuronide, and trace amounts as hydroquinone conjugates. This high degree of conjugation indicates that, at low doses, ingested phenol is nearly completely conjugated.

Laboratory animal studies have clearly shown that as the dose increases, the role of glucuronidation increases, until at sufficiently high doses it becomes the predominant reaction. The formation of oxidative metabolites (primarily by CYP2E1) also increases with increasing dose. These dose-dependent changes have been best characterized in rat studies, which show that at low doses sulfation predominates, with the glucuronidation beginning to predominate at approximately 133 µmol/kg (12.5 mg/kg) (Kao et al., 1979; Powell et al., 1974; Hogg et al., 1981; Koster et al., 1981; Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994), a dose that is below the chronic NOAEL of 60 mg/kg for phenol toxicity (NTP 1983a; Argus Research Laboratories, 1997), as discussed later in detail. There is considerable interspecies variation, however, in the contribution of the sulfation and glucuronidation pathways (Capel et al., 1972; Mehta et al., 1978).

The formation of oxidative metabolites increases at high doses. Dow Chemical Co. (1994) reported that hydroquinone conjugates represented 3, 7.8, and 17.0% of the eluted radioactivity in an analysis of urinary metabolites following single oral doses of 1.5, 15, and 150 mg/kg ^{14}C-phenol, respectively. Legathe et al. (1994) administered an intraperitoneal (i.p.) dose of 75 mg/kg to B6 mice and reported urinary metabolites as 34.5% phenyl sulfate, 28.5% phenyl glucuronide, and 32.4% hydroquinone glucuronide, indicating substantial contribution of oxidative metabolism at this high dose.

The formation of oxidative metabolites is thought to result primarily from reactions catalyzed by CYP2E1. Koop et al. (1989), using hepatic microsomes prepared from male New Zealand white rabbits, showed that CYP2E1 was the most active of six P450 isoforms tested. Treatment of the lysates with an antibody to CYP2E1 inhibited hydroquinone formation by 68% and 89% in acetone-induced and uninduced microsomes, respectively. Snyder et al. (1993) studied phenol metabolism in vitro in rat hepatic microsomal preparations. Addition of phenol to the CYP2E1 microsome preparation yielded hydroquinone and, to a lesser degree, catechol metabolites. Incubation of ^{14}C-phenol and ^{3}H-glutathione in the CYP2E1 microsome preparation yielded an additional metabolite that cochromatographed with the compound formed from the reaction of

benzoquinone with glutathione. The formation of the glutathione adduct was not dependent on addition of glutathione-S-transferase. Lunte and Kissinger (1983) also reported the formation of glutathione conjugates in microsomal (prepared from liver of male Swiss mice) metabolism of phenol to hydroquinone. In addition to benzoquinone, hydroquinone and catechol can also be oxidized to trihydroxybenzene (Sawahata and Neal, 1983).

Other in vitro studies using hepatic microsomes from rats treated with various P450 inducers or inhibitors have also provided evidence for the importance of CYP2E1 in phenol metabolism (Sawahata and Neal, 1983; Gilmour et al., 1986; Chapman et al., 1994; Kenyon et al., 1998). CYP2E1 appears to predominate phenol oxidation. Contributions by other P450 enzymes cannot be excluded, however, because only 68% of the induced hydroquinone formation was blocked by anti-CYP2E1 antibody, and several inducers of other P450 enzymes (such as phenobarbital and arochlor) enhanced phenol metabolism in these studies.

An alternative oxidative pathway involving peroxidation has been described for phenol. Several investigators have used in vitro cell preparations with high peroxidase activity, such as peritoneal macrophages or neutrophil preparations (Eastmond et al., 1986; Post et al., 1986; Eastmond et al., 1987; Kalf et al., 1990), purified peroxidase enzymes (Smart and Zannoni, 1984; Subrahmanyam and O'Brien, 1985), or cell lines that have high myeloperoxidase activity (Kolachana et al., 1993), to show that phenol can be metabolized in these reactions. Metabolites resulting from these reactions include 4,4'-biphenol and diphenoquinone. Although the peroxidation of phenol has been demonstrated in vitro, no direct in vivo evidence for these peroxidative reactions was identified.

The shift from sulfation to glucuronidation at increasing doses has been postulated to result from depletion of sulfate pools (Kim et al., 1995). Alternatively, it has been suggested that the difference between the K_m values for sulfate and glucuronide conjugation drives the conjugation shift (Weitering et al., 1979). The effects of differing metabolizing enzyme activity across the zones of the liver has also been suggested as an explanation for the metabolic profiles of phenol (Medinsky et al., 1995). The functional units of the liver include lobules with blood supply provided from the perimeter (periportal region) of the lobule though the portal vein and the hepatic artery. The blood flows from the periphery of the lobule toward the terminal hepatic vein (also called the central vein) at the center of the lobule through a series of differing metabolic regions or zones. Both sulfotransferases and glucuronosyltransferases are present in periportal zone 1, with the sulfotransferases predominating. Glucuronosyltransferases are present in zone 2, while both

glucuronosyltransferases and monooxygenases such as CYP2E1 are present in pericentral zone 3.

According to the model, phenol entering the hepatic circulation would be metabolized first in the periportal region, where sulfation predominates. Because the blood flows from the periportal region to the pericentral region and then to the central vein and general circulation, little unconjugated phenol is available for glucuronide conjugation or oxidation when it reaches the pericentral regions of the liver. This model is consistent with the shift from sulfation to glucuronidation at increasing doses. As the dose increases, more of the phenol reaches the pericentral region unconjugated, and thus is available for glucuronidation. The model also explains the increase in oxidative metabolites at high doses that exceed the conjugating capacity of zones 1 and 2 (Kenyon et al., 1995).

The model is also consistent with the observation that oral dosage with benzene results in greater production of hydroquinone than that seen after oral dosage with phenol, even though benzene is metabolized to hydroquinone via phenol. Benzene enters the liver in the periportal region, is oxidized to phenol and other metabolites in the pericentral region, and then leaves the liver via the hepatic vein. Because benzene must be oxidized before it is conjugated, more unconjugated phenol would be released into the blood following benzene exposure than following phenol exposure (Medinsky et al., 1995).

Direct evidence for this model was presented by Ballinger et al. (1995), who studied phenol and metabolite transport kinetics in isolated perfused liver from rats, and by Hoffmann et al. (1999), who conducted similar experiments in mice. The effects of enzyme distribution in the zones of the liver were studied by contrasting phenol metabolite profiles resulting from antero- and retrograde perfusions. It is noteworthy that the importance of the location of enzyme activities within the liver would only be significant at oral phenol doses that were not conjugated at the portal of entry, and thus were available for transport to the liver via the hepatic portal vein.

There is some evidence that the capacity for phenol conjugation varies with the portal of entry. Cassidy and Houston (1984) conducted an elegant series of experiments in which they injected rats intra-arterially, intravenously, or intraduodenally with phenol and then measured the systemic availability of phenol. This allowed them to evaluate the first-pass metabolism by different organ systems at doses ranging from 0.4 to 15 mg/kg. They were able to use this approach to determine metabolism by the liver and gut. However, results on metabolism by the respiratory tract from this study should be treated with caution, because environmental exposure results in exposure of the epithelial respiratory tract (i.e., the portion exposed to the outside), whereas this study

involved exposure of the endothelial respiratory tract (i.e., the portion exposed to the inside of the body). Thus, any differences between the metabolic capacity of the endothelial and epithelial cells would not be taken into account by the study design.

In this series of experiments, phenol that was systemically available had not been conjugated or metabolized. The doses at which phenol became systemically available thus reflected the doses at which the relevant metabolic enzyme systems became saturated. Metabolism became nearly saturated between 4.5 and 15 mg/kg for the endothelial lung, and between 0.4 and 1.5 mg/kg in the liver, and it was not saturated at the high dose for the gut. The affinity of metabolic pathways also varied among the organ systems. The liver and gut, which removed 88% and 86% (respectively) of phenol at the 0.4 mg/kg dose, demonstrated high affinity in comparism with the endothelial lung, which removed 58% of the phenol at this same dose. Taken together, these data indicate that the gut is a high-affinity and high-capacity site of metabolism, and the liver has high affinity but its capacity is readily exceeded.

The data also suggest that the lung provides substantial metabolizing capacity but has lower affinity than the gut and liver. Clear conclusions regarding the metabolic capacity of the lung following exposure by the inhalation route are not possible because of the potential for differences between the metabolic capacity of the epithelial and endothelial cells of the lung. However, one would expect the potential for metabolism of inhaled phenol to be similar to that seen in this study, because systemically absorbed phenol must pass through the endothelial cell layer.

The area under the blood concentration curve (AUC) for ^{14}C-phenol was route- and dose-dependent, reflecting the effects of portal-of-entry metabolism. In contrast, the AUC for phenol metabolites did not differ by dosing route, indicating that phenol is extensively metabolized and the effect of portal-of-entry metabolism is to reduce the amount of parent compound available for metabolism by other organ systems.

Studies using isolated perfused rat liver were also conducted and correlated well with the in vivo data. The percent of phenol removal from blood by first-pass metabolism declined from 73% at a blood concentration of 2.8 μg/mL to 26% at 26 μg/mL, indicating extensive saturation at the higher dose level.

Dow Chemical Co. (1994) studied the differential metabolism kinetics of phenol by differing exposure routes. This study evaluated the kinetics of ^{14}C-phenol in F344 rats following dosing regimens that included single or eight daily oral gavage doses of 1.5, 15, or 150 mg/kg 5000 ppm in

drinking water for 1 or 8 days or 25 ppm via inhalation for 6 hours (nose-only) for 1 or 8 days. The authors estimated the doses resulting from the drinking water and inhalation exposures. For drinking water administration (males only), doses were estimated by measurement of daily water consumption. The administered dose was 291 mg/kg for the single-day protocol and 405 mg/kg for the last day of the 8-day treatment, based on the water intake and the weight of each animal; thus, the drinking water doses were higher than the oral gavage doses.

The doses used in the drinking water study and the high dose in the gavage study were all higher than the chronic NOAEL of 60 mg/kg (NTP 1983a; Argus Research Laboratories, 1997), as discussed in detail in Section 5. In contrast, the inhalation doses were estimated as 11.5 and 17.8 mg/kg for males and females, respectively, following a single exposure period, and the dose was 21.4 mg/kg (males only) on the last day of the 8-day exposure protocol. Thus, the inhalation route more closely resembled the middle gavage dose level, and the absorbed dose via inhalation was lower than the chronic NOAEL (NTP 1983a; Argus Research Laboratories, 1997).

Metabolic profiles revealed ratios of 0.61 for glucuronide/sulfate conjugates in urine at the two lower gavage doses and were similar following inhalation (0.24–0.39). The ratio at the high gavage dose was 1.16, and it was similar following drinking water exposure (1.43 and 1.87 for the single and 8 day exposures). The observed formation of oxidative products, as shown by urine levels of hydroquinone glucuronide, was also dependent on total dose. The formation of oxidative metabolites following inhalation paralleled the low-dose gavage data, whereas the drinking water levels paralleled the high-dose gavage levels. The pattern of phenol metabolism correlated with the magnitude of the absorbed dose and did not appear to be dependent on the route of administration.

Metabolism of phenol appears extensive in the lung, liver, and gastrointestinal tract; however limited data are available for other organs. Metabolism appears to be extensive in the kidney (Tremaine et al., 1984). No data were identified that addressed portal-of-entry metabolism for the skin.

One consequence of the portal-of-entry metabolism of phenol is that phenol serum levels are not necessarily linear with dose or exposure levels. At low doses, almost all of the absorbed phenol is conjugated and excreted, without entering the bloodstream. At higher doses, free phenol and its metabolites appear in the blood and increase with dose. This nonlinearity of blood phenol levels with dose is illustrated by the data of Dow Chemical Co. (1994). Peak phenol blood concentrations in rats following an oral bolus dose of 150 mg/kg were 2320-fold higher than the peak blood concentrations following an oral bolus dose of 1.5 mg/kg.

The role of peak levels may be significant for induction of at least some aspects of systemic toxicity. Dow Chemical Co. (1994) sheds some light on the relationship between metabolism and toxicity. The high-dose gavage group in this study developed a cluster of behaviors that the authors termed "phenol twitching behavior (PTW)" that included tremors, sudden jerks, hyper-reactivity to stimuli, and excessive blinking. PTW began almost immediately after dosing and disappeared by 37 minutes post-dosing. Blood phenol levels also peaked almost immediately after dosing, and PTW was not apparent at blood phenol concentrations below approximately 3 μg/mL. PTW was not observed at the lower gavage doses or following inhalation exposure; peak blood phenol levels in these groups were well below 1 μg/mL.

Interestingly, PTW was also not observed in the drinking water exposure groups, even though the total dose in these groups was higher than the high gavage dose, and the drinking water doses had a similar metabolic profile to the high gavage dose. Unfortunately, blood phenol levels were not sampled in the drinking water groups, so the peak blood phenol level is not available. However, given the rapid clearance of phenol from the blood, it is likely that the peak blood level was much lower in the drinking water group than in the high-dose gavage group. This suggests that PTW is more closely related to peak phenol blood levels than to a measure of total dose, such as AUC. Because phenol metabolite levels paralleled those of phenol, these data cannot be used to distinguish between phenol and its metabolites being the toxic agent. These data do not identify the appropriate dose metric (e.g., peak concentration vs AUC) for other toxic endpoints.

One indication that the oxidative metabolites are important determinates of toxicity is based on experiments by Chapman et al. (1994). They studied the dysmorphogenic and embryotoxic effects of benzene and its metabolites to the whole rat conceptus in vitro. Phenol at 1.6 mM elicited only minor effects, but inclusion of S9 microsomal fractions greatly increased the potency of phenol, with significant effects observed at doses as low as 0.01 mM. Metabolite analysis indicated that hydroquinone and catechol were the primary metabolites. When evaluated singly, hydroquinone, catechol, and benzoquinone induced similar embryotoxicity, producing 100% lethality at 0.1 mM. The addition of phenol and hydroquinone together induced a more-than-additive embryotoxicity, which the authors suggested as evidence for a peroxidative mechanism for phenol bioactivation, based on the potential for electron cycling between phenol and hydroquinone.

Intraspecies variability has also been studied. Campbell et al. (1987) isolated human liver sulfotransferases, the enzymes responsible for the conjugation of phenol with sulfate, and analyzed their apparent activities toward *p*-nitrophenol (as a model compound for simple phenols).

The average phenol sulfotransferase (PST) activity measured in liver samples of 20 patients (13 male, 7 female) was 35.8±10.6 standard error of the mean (SEM) units/mg protein. No correlation between enzyme activity and patient age or gender was found, although the power to detect any such correlation was not noted. Seaton et al. (1995) studied the kinetics of phenol sulfation and hydroquinone conjugation, both of which varied over a range of approximately three-fold in a sample of liver fractions from 10 humans. Using lysates from a single human liver, saturation of phenol sulfation was apparent above 800 μM; the observed kinetics were consistent with two contributing enzymes, PST1 and PST2. The expression of two distinct PST enzymes has also been demonstrated in human nasal epithelium (Beckmann et al., 1995).

Kawamoto et al. (1996) studied the effect of various lifestyle factors and of genetic polymorphisms in five metabolizing enzymes, including aldehyde dehydrogenase (ALDH2), N-acetyl transferase (NAT2), cytochrome P450 1A1 (CYP1A1), CYP2E1, and glutathione-*S*-transferase mu (GSTM1) on urinary levels of phenol in a cohort of men who were not occupationally exposured. Step-wise multiple regression analysis was performed to identify important determinates of urinary phenol levels. On the basis of this analysis, there was no relationship between polymorphisms (including for CYP2E1) and background urinary phenol levels. In the total sample (n = 351), the geometric mean urinary phenol level was 7.64 mg/L and the geometric standard deviation was 2.9. No data are available, however, on how genetic polymorphisms affect the levels of metabolites produced from exogenously dosed phenol.

The changes in enzyme activity or expression of genes that encode enzymes important for phenol metabolism with age have been studied. The status of CYP2E1 in fetuses remains unclear, with conflicting results reported. Most of the existing studies indicate that this enzyme is expressed in human adults but not in human fetuses, even when measured using sensitive assays (reviewed in Hakkola et al., 1998). However, at least two studies (Carpenter et al., 1996; Vieira et al., 1996) indicate that CYP2E1 is expressed at least to some degree in fetal liver. Vieira et al. found that CYP2E1 protein could not be detected immunochemically in fetal human liver, and there was only minimal evidence of CYP2E1 mRNA or CYP2E1 activity in fetal liver microsomes. (The difference in assay results may be due to differences in sensitivity or to cross-reaction of CYP1A1 activity.) However, the authors found, that CYP2E1 protein levels rise rapidly in the first few hours after birth, with a slow increase in protein levels and in CYP2E1 mRNA levels during childhood.

Results of animal studies of developmental CYP2E1 regulation are consistent with the human data in providing uniform evidence of the rapid induction of this gene soon after birth (Song

et al., 1986; Umeno et al., 1988; Schenkman et al., 1989; Ueno and Gonzalez, 1990). Thus, overall, the data show that if CYP2E1 activity exists in human fetuses, levels are much lower than those in adults. Regardless of fetal CYP2E1 expression, the enzyme is rapidly induced upon birth. For this reason, children would be expected to be capable of phenol metabolism, although the amount of CYP2E1 may be less than that present in adults.

Age-dependent changes in phase II conjugation have also been evaluated. In an evaluation of how PST activity varies with age in rats, Iwasaki et al. (1993) studied ß-naphthol metabolism by PST in fetal rat liver, in the liver of 2-, 9-, 17-, and 25-day-old neonates, and in adult rats. Activity was analyzed in the livers of both sexes. The fetal liver had little conjugating ability, but this activity developed rapidly after birth. However, activity was substantially lower in neonates of all ages evaluated when compared with adult levels. Heaton and Renwick (1991) administered i.p. doses of 25 mg/kg ^{14}C-phenol to rats varying in age from 3 to 16 weeks and measured metabolites in urine collected in 24 hours. The percentage of the administered dose recovered in the urine in 24 hours ranged from 61–90% in males and 63–99% in females, with increasing recovery with age.

Importantly, the formation of hydroquinone conjugates was greater in the younger animals. In males, 38% of the administered dose was recovered as hydroquinone conjugates in the 3-week-old animals; 8.2% of the urinary metabolites was recovered in 16-week-old rats. In females, 17.8% of the administered dose was recovered as hydroquinone conjugates in 4-week-old rats and 10.5% was recovered in 15-week-old rats. Taken together, the evidence indicates that both sulfate conjugation and P450 metabolism are lower early in life and increase as adulthood is reached. However, even in the face of limited P450 activity, significant formation of oxidation products can occur because of limited sulfation capacity. The oxidative products become substrates for glucuronidation, and this does not appear to be limited in the young.

Phenol metabolism may also be gender dependent, although the data are less substantial than those for differences due to age. Iwasaki et al. (1986) reported that PST activity was similar in both sexes up to 3 weeks of age and was higher in males than in females in 7-week-old rats. Activity in 2-year-old rats of both sexes was similar and fell between the levels for males and females at 7 weeks to 1 year. Kenyon et al. (1995) administered ^{14}C-phenol to B6 mice of both sexes and observed that, males excreted a greater proportion of HQ-glucuronide than did females at all doses; the difference was roughly twofold at a dose of 40 μmol/kg. These results are consistent with the greater degree of hydroquinone conjugates excreted in the urine of male versus female rats reported by Heaton and Renwick (1991). Sex-based differences in metabolism have also been reported in

rats (Meerman et al., 1987), with slightly lower total recovered radioactivity in the urine of females versus males (i.e., more rapid metabolism in males). However, the magnitude of this difference (91.2% vs 87.3%) was limited.

Interspecies differences in phenol metabolism have also been evaluated. Seaton et al. (1995) found that the rates of both phenol sulfation and hydroquinone conjugation in mouse and rat liver were comparable to those of human liver preparations. Schlosser et al. (1993) reported that mouse liver microsomes metabolized approximately twice as much phenol as did rat liver microsomes, although the relative proportions of metabolites were roughly similar.

Phenol is formed endogenously in the gut by bacterial metabolism of aromatic amino acids in protein. The amount formed is related to the amount of protein ingested, but the amount in humans typically varies from 1 to 10 mg/day, corresponding to approximately 0.014 to 0.14 mg/kg-day (Bone et al., 1976; Lawrie and Renwick, 1987; Renwick et al., 1988).

A physiologically based pharmacokinetic (PBPK) model for the distribution of benzene and metabolites was developed by Bois et al. (1991). The model was developed to predict phenol and metabolite distributions to fat, well-perfused tissue, poorly-perfused tissue, bone marrow, liver, lung, and gut using Monte Carlo simulations of 64 parameters. The model was not validated using empirical data. The Bois et al. model consistently predicted that phenol administration would produce higher levels of phenol and hydroquinone in the blood than seen following benzene administration.

The first phase in the development of a model of the in vitro kinetics of phenol and benzene biotransformation by liver microsomes was described by Schlosser et al. (1993) and enhanced by Medinsky et al. (1995). The model described the following reaction sequences: benzene > phenol > catechol > trihydroxybenzene and phenol > hydroquinone > trihydroxybenzene. All reaction steps were assumed to be catalyzed by cytochrome P450 2E1, and benzene, phenol, catechol, and hydroquinone were all assumed to compete through reversible binding for the same reaction site on cytochrome P450. Parameters were identified that were successful at predicting the concentration with time of all five chemicals in incubations with rat or mouse liver microsomes (Schlosser et al., 1993). The observation of a lag time in the production of hydroquinone from benzene—in comparison to the rate of production of hydroquinone from phenol—supported the assumption that all of the substrates compete for the same enzyme reaction site.

Medinsky et al. (1995) extended the data into a conceptual model of the differences between phenol and benzene metabolism. The goals of the conceptual model included explaining the

observed differences between the carcinogenicity and genotoxicity of phenol and benzene and explaining why urinary hydroquinone levels are higher after benzene dosing than after phenol dosing. The latter observation would appear to be inconsistent with the prediction of the Bois et al. (1991) model that blood hydroquinone levels are higher following phenol dosing than following benzene dosing. As described earlier in this section, differences between benzene and phenol toxicity were attributed to zonal differences in the distribution of hepatic metabolic enzymes.

In summary, phenol is an endogenous metabolite that undergoes further metabolism efficiently. At low doses the bulk of the phenol appears to be conjugated with sulfate or glucuronide at the portal of entry. As the dose increases, the sulfation pathway becomes saturated, and the relative contribution of glucuronidation and oxidation reactions increases. Saturation of first-pass metabolism may be important for producing peak levels of phenol that correlate with acute systemic toxicity. In addition, saturation of conjugation, which leads to increases in oxidative metabolism, may also be an important determinate of toxicity. The data on intraspecies variability are limited, but they do not indicate great variation in metabolic capacity in humans. In rodents, males and younger animals appear to rely more heavily than females and adult animals on oxidative metabolism, respectively, but the differences are no more than twofold. The metabolism of phenol in humans and rodents appears to be similar, although some evidence suggests that mice metabolize phenol more rapidly than do humans or rats.

3.4. EXCRETION

The existing human and laboratory animal studies consistently report that phenol is rapidly excreted, with little tendency for accumulation. Elimination is primarily in the urine in both humans and laboratory animals, with only a minor contribution of elimination in the bile. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. Workers were exposed to phenol vapor by inhalation on a daily basis. The workers were also possibly exposed by the dermal route, but the contribution of this route to the total exposure was not directly measured. Analysis of urinary phenol levels at different times during the work shift and across work shifts indicated that in workers exposed to 7.8 to 9.6 mg/m^3, the urinary levels increased significantly from the beginning of the work shift to the end of the work shift, but they did not tend to accumulate across the work shifts. A slight increase in the morning sample on the sixth consecutive work day was observed, but after two days off, pre-shift samples were no longer elevated.

Rapid clearance from the blood in humans has also been observed. Bentur et al. (1998) presented a case report from a dermal poisoning in which a solution of 90% phenol was spilled on the left foot (3% of body surface). Even at this high exposure level, clearance from the blood was rapid, with blood levels decreasing from 21.6 to 2.8 μg/mL in the first 12 hours. The authors estimated that the half-life elimination was 13.86 hours, but they did not include the initial rapid decline in serum concentration that was apparent over the first 12 hours post-admission. Both absorption and clearance would be expected to be more rapid at lower exposure levels, as high exposure levels can lead to protein denaturation and saturation kinetics.

Laboratory animal studies have consistently found that phenol is rapidly excreted. Clearance of phenol from the blood is rapid. Rats given an oral dose of 300 mg/kg, a level comparable to the rodent median lethal dose (LD_{50})of 340 mg/kg (Deichmann and Witherup, 1944), had maximum blood concentrations of 26 μg/mL at the first measured time point (about 10 minutes), and blood levels declined rapidly to background by 60 minutes (Humphrey et al., 1980). Similar results were observed by the same authors in dogs given a 40 mg/kg dose, with rapid peak levels (7.8 μg/mL) and complete removal of free phenol by 1 hour. A half-life of 12 minutes in blood was reported for rats administered 150 mg/kg by gavage (Dow Chemical Co., 1994). Legathe et al. (1994) reported biphasic elimination kinetics from the blood, with a terminal half-life of 22 minutes. Similarly, Cassidy and Houston (1984) reported biphasic kinetics with a half-life of approximately 5 minutes following intra-arterial administration.

The elimination kinetics in multiple tissues was studied by Liao and Oehme (1981). Total radioactivity in tissues was maximal within 30 minutes of dosing, representing 28.4% of the administered dose. Tissue levels accounted for 16.6% of the administered dose at 2 hours and 0.3% at 16 hours. Although maximum levels varied considerably across tissues, the rate of elimination did not appear to differ with tissue type. Numerous laboratory animal studies indicate that urinary elimination of sulfate and glucuronide conjugates accounts for most of the excretion, ranging from 70 to 90% of the administered dose within 24 hours, whereas excretion in feces represents only a small fraction of the administered dose, approximately 1–3% (Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994; Hughes and Hall, 1995).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

The epidemiology data on phenol are limited. Epidemiology studies have typically included confounding exposures and have not adequately adjust for smoking. Kauppinen et al. (1986) reported a significant increase in respiratory cancer in phenol-exposed workers, but this observation appears to be due to confounding exposures, because there was no dose-response, and the effect decreased after accounting for latency. No effect on cancer mortality was observed in workers exposed to phenol in the rubber industry (Wilcosky et al., 1984) or in workers exposed to formaldehyde and phenol (Dosemeci et al., 1991). An occupational study (Shamy et al., 1994) and case studies (e.g., Merliss, 1972) have reported liver effects following exposure to phenol. Immune effects were also been reported in an occupational study of workers exposed to phenol as part of a mixture of solvents (Baj et al., 1994). Studies of populations whose drinking water was contaminated with phenol found elevated incidences of diarrhea, nausea, mouth sores, and dark urine (Jarvis et al., 1985; Baker et al., 1978).

4.1.1. Oral

Estimated lethal oral doses of phenol in adults vary widely, from 1 g (14 mg/kg, assuming an adult body weight of 70 kg) to as much as 65 g (930 mg/kg) (Deichmann and Klepinger, 1981). In another report (Bruce et al., 1987), the minimum lethal oral dose in adults was estimated as 140 mg/kg.

Jarvis et al. (1985) reported on illness associated with consuming water contaminated with phenol from a spill into the river that served as the drinking water source. A retrospective mail survey was sent to a total of 594 English households: 250 that were highly exposed, 94 that were exposed at low concentrations (from a reservoir that diluted the contaminated river water), and 250 that were unexposed (selected from a telephone book). On the basis of data from the water authority, the estimated phenol concentrations in drinking water in the low-exposure area (0.05 mol/L, equivalent to 4.7 μg/L) was roughly half that in the high-exposure area (0.11 μmol/L, equivalent to 10 μg/L) for the first 24 hours. The next day, the phenol concentration for both groups was 0.05 μmol/L, and the concentration was < 0.01 μmol/L (< 0.9 μg/L) by the third day after the contamination incident.

Chlorination of the water resulted in production of chlorophenols. The chlorophenol concentration followed a similar pattern, but the chlorophenol concentrations, which ranged from

0.43 to 0.2 μmol/L at the first measurement (84.7–39.4 μg/L, assuming all chlorophenols were in the form of trichlorophenols), were higher than those for phenol. There were no data on phenol concentrations in the unexposed area, but an unspecified local press report implicated a possibility of phenol contamination. Due to the similarity of the two exposed areas in the measured concentrations of phenol and chlorophenol, these two areas were combined in the data analysis.

The percentage of responding households was similar in all of the groups and ranged from 69 to 77%, resulting in 172 households (448 people) in the unexposed area and 254 households (754 people) in the exposed area being evaluated. The two groups had similar distributions in sex, age, and usual water usage. Compared with the unexposed individuals, those in the exposed area had significantly higher incidences of gastrointestinal illnesses, such as diarrhea, nausea, vomiting, and abdominal pain. Other symptoms, such as headache, rash, and malaise, were also observed at a significantly elevated incidence in the exposed group. The day of onset of symptoms corresponded with the period of elevated phenol concentrations in the contaminated drinking water. The associations were stronger among those who reported that they drank the water than among those in the exposed area who reported not drinking the water. (Others may have consumed the water in cooking.) In another analysis, gastrointestinal symptoms did not significantly correlate with whether the water tasted bad. However, because of the confounding exposure of chlorophenol in the water, the usefulness of the result for risk assessment is limited.

Baker et al. (1978) reported on phenol poisoning in humans due to an accidental contamination of their drinking water on July 16, 1974. A train derailment resulted in a spillage of 37,900 L of pure phenol onto the ground, and the spillage caused contamination of drinking water in wells of nearby houses. Two wells near the spill were tested initially on July 23 and were found to have phenol concentrations of 0.21 and 3.2 mg/L. Further testing in late July and August of the six wells nearest the spill found peak concentrations between 15 and 126 mg/L. Within approximately 2 months after the spill, "most families" began to obtain water from other sources (from neighbors or bottled water). Phenol concentrations in well water as high as 1130 mg/L were reported over the next 6 months, with the higher levels observed after flushing of the spill site (unspecified number of wells tested). The authors investigated the health effects in three groups of people. Group 1 (n = 39) consisted of all families living 120–310 m from the spill site and having at least one water test greater than 0.1 mg phenol/L (at least once between July and February). Group 2 (neighborhood control) (n = 61) consisted of all families adjacent to Group 1 (210–670 meters from the spill) whose wells had phenol concentrations of between 0.1 and 0.0001 mg/L. Group 3 (distant control)

(n = 58) lived at least 1.9 km from the spill and had no phenol in its wells.

Group 1 reported significantly more diarrhea, mouth sores, burning mouth, and dark urine than the combined control groups. About 44% of the individuals in Group 1 reported at least two of these responses in the 7 months following the accident and were considered "affected individuals"; only 8% and 3% of Group 2 or 3 subjects, respectively, had the same responses. Responses in Group 1 were primarily restricted to the first 2 months of exposure, before the use of bottled water began. Responses in the other two groups tended to occur throughout the 8-month period (July through February). Other than the four reported symptoms, no abnormal observations in physical examinations and serum biochemical evaluations were evident in Group 1 as compared with controls when monitoring was done in February.

On the basis of water testing data and water preference histories, the authors estimated that the daily oral dose of phenol for the 17 affected individuals in Group 1 was between 10 and 240 mg. However, this range may overestimate the amount of phenol ingested because phenol's unpleasant odor might have discouraged ingestion of water with concentrations above 0.1 mg/L. In contrast, this range does not include phenol that may have been absorbed during skin contact with contaminated water. It was also not clear whether the subjects continued to shower with the contaminated water after switching their drinking water source. Based on a default adult body weight of 70 kg, this daily oral dose corresponds to 0.14 to 3.4 mg phenol/kg-day. Thus, there is a considerable range in the estimated phenol dose associated with symptoms. In addition, because "most" (but apparently not all) families switched to other water sources within the first 2 months of exposure, the exposure duration for the affected individuals is not known. Therefore, it is difficult to use these data for quantitative analysis, although they might be useful for placing bounding estimates on the risk values for systemic effects estimated from laboratory animal studies.

4.1.2. Inhalation

Kauppinen et al. (1986) reported a case-control study on respiratory cancers and chemical exposures in the wood industry. A cohort of 3805 Finnish men who worked in the particle board, plywood, sawmill, or formaldehyde glue industries for at least 1 year between 1944 and 1965 was followed until 1981. From the cohort, 60 cases of respiratory malignant tumors were identified. The tissue locations of these tumors included tongue (1), pharynx (1), larynx or epiglottis (4), and lung or trachea (54). No cases with tumor in the mouth, nose, or sinuses were identified. Among the 60 cases, 2 were rejected due to a false preliminary diagnosis of cancer and 1 was rejected as

chronic lymphocytic leukemia. The final size of the group of cases was thus 57. The control group contained three subjects for each case, selected from the cohort and matched by birth year, for a total size of 171.

The job exposure was estimated from the industrial hygiene data of the plant, general hygiene data on exposures, and information on ventilation, work procedures, and other relevant factors at the plants. However, the authors, gave no information on direct phenol measurements; thus, the quality of the estimated exposure levels could not be evaluated. The work histories of the subjects were assessed primarily from plant registers and supplemented with personal interviews. Individual phenol exposures were determined qualitatively as "yes" or "no" and as a function of exposure time.

Smoking histories were determined by a mail survey that resulted in smoking information on 39 of 57 cases (68%) and on 130 of 171 controls (76%). Because there were few nonsmokers and information on the amount smoked was not as complete as on years of smoking, the subjects were compared only as light or heavy smokers, based on whether their years of smoking exceeded 35.

Phenol exposure resulted in a statistically significant odds ratio (OR) of 3.98 or 4.94 for respiratory tumors with or without the adjustment for smoking years, respectively. When the duration of phenol exposure was considered, both exposures $\leq$ 5 years and > 5 years resulted in a statistically significant OR $\leq$ of 5.86 or 4.03, respectively (i.e., no duration response). When a provision for a 10-year latency was introduced (excluding exposure during the 10 years immediately preceding the diagnosis of cases), phenol exposure resulted in a nonsignificant OR of 2.86 adjusted for smoking years but a significant or of 3.98 without smoking adjustment. Of the 39 cases for which smoking information was available, 12 had been exposed to phenol (9 to phenol in wood dust), and 7 had been exposed to phenol with a 10-year latency (4 to phenol in wood dust). Because the OR did not increase with duration of phenol exposure and the provision for the 10-year latency period resulted in lower values of ORs, a confounding factor may have been responsible for the observed statistically significant ORs.

One of the confounding factors could have been concurrent exposure to multiple pesticides, which was in the same study shown to increase the OR for respiratory tumors. An exclusion of workers exposed to both phenol and pesticides resulted in a change of the OR from a significant 4.9 to a nonsignificant 2.6. Thus, a confounding effect due to exposures to pesticides was very possible. Considering the location of the tumors, formaldehyde exposure was also a likely confounder.

Generally similar results were observed in this study for workers exposed to phenol in wood

dust. Exposure to phenol in wood dust resulted in a statistically significant OR with or without adjustment for smoking. For the workers exposed to phenol but not wood dust, provision for a latency period eliminated the observed statistically significant OR. Among the workers exposed to phenol in wood dust, however, the OR did increase with exposure duration and was statistically significant in those exposed > 5 years (OR of 4.77) but not in those exposed for ≤ 5 years (OR of 3.84). On the basis of these results, the phenol-exposed workers had an elevated risk of respiratory cancer, but phenol itself did not appear to be the causative agent; rather, it appears that there was a confounding exposure.

Wilcosky et al. (1984) reported a case-control study of cancer mortality and solvent exposures in the rubber industry. From a cohort of 6678 active and retired male rubber workers of a large plant in Ohio, 183 decedents from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, and respiratory cancer were selected as cases. As a control, 20% of an age-stratified random sample of the cohort (calculated as 1336 subjects) was selected. Including phenol, a total of 25 solvents were authorized to be used in the plant. The exposure to any particular solvent was determined from the records of annual authorization for use of these solvents in each work area. Only workers who had cumulative exposures of more than 1 year were considered exposed.

On the basis of the analysis of the age-adjusted exposure ORs, no association was seen between phenol exposure and mortality from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, or respiratory cancer. However, this study had several major limitations. One limitation was that the estimation of whether workers were exposed to a solvent was based solely on authorization and not on actual usage, which would tend to lead to an overestimation of exposure. In addition, the analysis was based solely on a qualitative evaluation of whether a given solvent was used; no estimates of exposure were made, and so no exposure-response assessment was conducted. Although smoking can confound evaluation of cancer risk, this factor was not investigated. Finally, it was common for workers to be simultaneously exposed to multiple solvents; therefore, solvents other than phenol may have affected the study outcome. In this study, phenol exposure was not associated with a risk of several cancers, but this lack of an association cannot be considered definitive because of the study limitations mentioned above.

In an occupational epidemiology study, Dosemeci et al. (1991) evaluated mortality among 14,861 white male workers in five companies that used formaldehyde and phenol. Unfortunately, the phenol exposure was confounded by co-exposure to other compounds, such as formaldehyde,

asbestos, urea, melamine, hexamethylenediamine, wood dust, plasticizers, carbon black, ammonia, and antioxidants. On the basis of phenol concentrations obtained from historical monitoring and industrial hygiene surveys, the investigators assigned each job/department/year combination to groups with no, low, medium, or high phenol exposure and then calculated cumulative exposure.

Compared with the entire U.S. population, the entire cohort, had no significant increases in standardized mortality ratios (SMRs) for all causes of death or any diseases. The phenol-exposed workers as a group had slightly elevated SMRs for cancers of the esophagus (1.6), rectum (1.4), kidney (1.3), and Hodgkin's disease (1.7); however, none of these increases were statistically significant when compared with those in general population. In addition, an analysis of mortality by level of cumulative exposure showed that none of these increases had dose-response relationships with exposure to phenol. The only significant observations were decreases of SMRs for infective and parasitic diseases and for accidents in the entire cohort and exposed workers. These observations were attributed to the healthy worker effect. This study provided no evidence of phenol-induced morbidity, mortality, or carcinogenicity.

Baj et al. (1994) reported an epidemiology study of 22 Polish office workers (18 females and 4 males) exposed to Ksylamit® vapor for 6 months and 27 age- and sex-matched healthy volunteers from the same town. The exact composition of the Ksylamit® vapor was not reported. The study authors stated that Ksylamit® consists of "a mixture of chlorinated benzenes, pentachlorophenol, -chloronaphthalene, chloroparaffin and kerosene." The only exposure information reported was that at the end of 6-month exposure period the concentrations of formaldehyde and phenol in the workplace atmosphere were 0.8 mg/m^3 and 1.3 mg/m^3, respectively. The study authors did not address how exposure to formaldehyde or phenol resulted from the reported product constituents. In addition, it cannot be determined from the presented information whether the analytical methods used would differentiate between phenol and pentachlorophenol (ATSDR, 1998).

The exposed workers reported chronic symptoms such as headache, cough and sore throats, burning eyes, and fatigue, but morbidity during the 6-month exposure period was comparable to that of the controls. Although all evaluated hematological parameters were normal in the exposed workers as a group, some statistically significant changes were observed in a subset of eight workers who had elevated urinary phenol levels 3 days after the last day of exposure (mean of 18.2 mg/L, compared with 12.1 mg/L in the exposed workers and 7.9 mg/L for the general population). Compared with the matched controls, there was a small, but statistically significant decrease among

in erythrocyte counts and a statistically significant increase in eosinophil and monocyte counts. Levels of CD3, CD4, and CD8 lymphocytes were also decreased in the exposed group, but there was no effect on the CD4/CD8 ratio, and the effect was not stronger in the apparently more highlyexposed subset. Decreases in lymphocyte proliferation induced by phytohemagglutinin and alloantigens were also observed in exposed workers, whereas reactivity to concanavalin A (Con A) was unchanged.

These results suggest that exposure to Ksylamit® could affect the immune and hematological systems. However, the poor characterization of the chemical exposure, including uncertainties regarding the source of the phenol as well as the marginal dose-response for phenol in urine, mean that conclusions regarding the contribution of phenol to the observed effects are limited.

Shamy et al. (1994) reported a cross-sectional investigation of phenol-induced biochemical changes in workers at an oil refining plant in Egypt. The study included 20 workers who were exposed to a time=weighted average concentration of 5.4 ppm phenol and 30 office workers who had no exposure to organic solvents. The phenol-exposed workers worked in the aromatic extraction of distillates; other potential exposures were not described. The mean concentration of phenol in spot urine samples was 68.6 and 11.5 mg/g creatinine in the exposed and control groups, respectively. The average duration of exposure was 13.15 years. At the end of the shift of the last working day of the week, blood samples were collected for hematological and serum biochemistry evaluations.

Small but statistically significant increases (approximately 55% and 80%, respectively) were observed in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). There were also small but statistically significant increases in hemoglobin, hematocrit, mean corpuscular hemoglobin, and mean corpuscular volume, although there was no effect on red blood cell count. This finding is in contrast with those of laboratory animal studies, in which decreases in erythrocytes and hematocrit have been reported. Other small, but statistically significant changes included increased basophils and neutrophils, decreased monocytes, and increased clotting time. A nonsignificant increase in prothrombin time and decrease in platelets was also observed.

Overall, these data suggest subclinical effects on the liver and hematopoietic system, based on the small changes in SGOT and SGPT; the observed increases in the hematology endpoints are not adverse. Although the authors described the workers as exposed to phenol alone and compared them with other workers exposed to mixed solvents, it appears that the phenol-exposed workers may

have also been exposed to other organic compounds that can cause hepatic or hematologic effects, and the observed effects cannot be clearly attributed to phenol exposure.

Merliss (1972) reported a case of phenol marasmus. A laboratory technician had been frequently exposed to phenol through vapor or skin contact for 13.5 years. He developed lessened appetite, weight loss, muscle pain in his legs and arms, and dark color in his urine. Serum biochemical evaluations indicated liver damage, with SGOT and SGPT at much higher than normal levels. The patient's symptoms improved after the exposure ended.

4.2. PRE-CHRONIC, CHRONIC STUDIES AND CANCER BIOASSAYS IN LABORATORY ANIMALS

4.2.1. Oral

Acute lethality of oral phenol has been evaluated in numerous animal studies. Oral LD_{50} values in rodents range from 300 mg/kg to 650 mg/kg (Deichmann and Witherup, 1944; Von Oettingen and Sharpless, 1946; Flickinger, 1976; Berman et al., 1995). The acute toxicity of phenol when administered by gavage appears to be at least partly dependent on phenol concentration or total administered volume (Deichmann and Witherup, 1944; NTP, 1983a), and it may be more severe in young animals (Deichmann and Witherup, 1944). In addition to lethality, acute oral dosing has been reported to induce adverse renal (tubular necrosis, protein casts, papillary hemorrhage), hematological (reduction in poly/normochromatic erythrocyte ratio), respiratory (dyspnea and rales), neurological (muscle twitching, decreased motor activity, ataxia, tremors, convulsions, coma), and reproductive and developmental effects (Berman et al., 1995; Narotsky and Kavlock, 1995; Liao and Oehme, 1981; Moser et. al. 1995; NTP, 1983a,b).

As shown in Table 2, there is an extensive database of oral studies relevant to the RfD. Chronic drinking water studies have been conducted in rats and mice, but the only noncancer endpoints evaluated were body weight and histopathology (NCI, 1980). Hematology and serum biochemical evaluations were included in a recent two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch, 1998).

These drinking water studies consistently found effects only at exposure levels where water consumption was also decreased, sometimes by as much as 80%. The decreased water consumption was presumably due to poor palatability of the drinking water. Effects seen in these studies included

tremors, decreased final body weight as compared with the controls (possibly as a result of dehydration), decreased motor activity, and kidney inflammation. Decreased pup survival was also observed in the two-generation study. The only drinking water study in which effects were seen in the absence of decreased water consumption was a 28-day study with mice by Hsieh et al. (1992). In that study, decreased hematocrit and decreased immune endpoints were observed at doses at least an order of magnitude lower than the NOAELs in the other drinking water studies. Although the Hsieh et al. study is useful for hazard identification, its findings need to be confirmed before they can be used in dose-response assessment. The differing findings of this study and the above-mentioned two-generation study in rats in which no immunological effects were observed suggest marked interspecies differences between rats and mice for this endpoint.

Toxicity in gavage studies with phenol is typically much higher than that in the drinking water studies. NOAELs for systemic effects were 5–10 - fold lower in gavage studies (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945) than those seen in the drinking water studies. Effects observed included tremor and liver and kidney histopathology. As described in greater detail in Section 4.5, this difference between gavage and drinking water exposure is

Table 2. Summary of Oral Toxicity Studies

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Systemic Toxicity								
F344 rat, 50/sex/ group	NCI, 1980	0, 2500, 5000 ppm; 0, 260, 585 (M), 0, 280, 630 (F) mg/kg-day	Drinking water	103 weeks	Kidney inflammation and decreased body weight (compared to controls) in both sexes, decreased water consumption at high dose	260	585	Study authors stated there were no noncancer effects, but independent evaluation for this assessment found significant increase in kidney inflammation.
B6C3F1 mouse, 50/sex/ group	NCI, 1980	0, 2500, 5000 ppm; 0, 450, or 660 mg/kg-day	Drinking water	103 weeks	Decreased body weight, decreased water consumption	450	660	Effect apparently secondary to decreased water consumption.
F344 rat, 10/sex/ group	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 16, 48, 160, 480, 800 (M), 0, 17, 51, 170, 510, 1140 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight, decreased water consumption.	480	800	Range-finding for bioassay; effect apparently secondary to decreased water consumption.
B6C3F1 mouse, 10/group	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 25, 75, 250, 450, 500 (M), 0, 26, 78, 260, 468, 520 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight, decreased water consumption.	450	500	Range-finding for bioassay; effect apparently secondary to decreased water consumption.

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Sprague-Dawley rat, 15/sex/ group	ClinTrials BioResearch, 1998	0, 200, 1000, 5000 ppm; 0, 18, 83.1, 308 (M), 0, 24.6, 107, 360 (F) mg/kg-day	Drinking water	13 weeks	Decreased motor activity in females, decreased body weight in males and females, decreased water consumption	107	360	Specialized neurotoxicity study; decreased body weight apparently secondary to decreased water consumption; unpublished GLP study BMDL = 219 mg/kg-day
CD-1 mouse, 5 M/group	Hsieh et al., 1992	0, 4.7, 19.5, 95.2 ppm; 0, 1.8, 6.2 or 33.6 mg/kg-day	Drinking water	28 days	Decreased immune endpoints (particularly in plaque-forming cell assay and ELISA), decreased red blood cells and hematocrit	6.2	33.6	Study limited by small sample size; confirmation in a study conducted according to modern methods would be useful.
Rat 10/group, strain & sex NS	Dow Chemical Co., 1945	0, 50, 100 duration adjusted: 0, 35.7, 71.4 mg/kg-day	Gavage/ volume NS	6 months, 5 days/wk	Liver and kidney histopathology; mortality in 4/10 at low and high doses	None	35.7	Unpublished study, small group size, incomplete reporting; authors raised questions about the mortality, although it is unclear what the issue was.
F344 rat, 8 F/group	Berman et al., 1995; Moser et al., 1995	0, 4, 12, 40, 120 mg/kg-day	Gavage/ 1 mL/kg	14 days	Tubular degeneration, tremor, increased rearing post-exposure	12	40	Small group size, small dosage volume
Reproductive and Developmental Toxicity								
Sprague-Dawley rat 30/sex/ group P1; 20/sex/ group F1	Ryan et al., 2001; IIT Research Institute, 1999	0, 200, 1000, 5000 ppm; 0, 14.7, 70.9, 301 (P1 M), 0, 20, 93, 320.5 (P1 F) mg/kg-day	Drinking water	2 genera-tions	Decreased parental and pup body weight, decreased pup survival, decreased water consumption	70.9	301	Study also included evaluation of hematology, serum biochemistry, and developmental landmarks. Effects may be secondary to decreased water consumption. Decreased uterine weight at all doses, but not considered adverse

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Sprague-Dawley Rat 25 F/group	Argus Research Laboratories, 1997	0, 60,120 or 360 mg/kg-day	Gavage 10 mL/kg	GD 6-15	Decreased maternal weight gain; Decreased fetal body weight and delayed ossification	60 (maternal) 120 (dev)	120 (maternal) 360 (dev)	Doses were divided into 3 administrations/day One dam died at 360 mg/kg-day Unpublished GLP study BMDL = 93 mg/kg-day
CD Rats 20-22 F/group	NTP, 1983a	0, 30, 60 or 120 mg/kg-day	Gavage 5 mL/kg	GD 6-15	Decreased fetal body weight	120 (maternal) 60 (dev)	None (maternal) 120 (dev)	None Developmental LOAEL is equivocal BMDL not calculated, because data on fetal weight by sex was not available
CD Rat 5-10 F/group	NTP, 1983a	0-250 mg/dg-day	Gavage 1-7.5 mL/kg	GD 6-15	Toxicity (tremors, liver and lung pathology, death) markedly higher in smaller dosing volume.	N/A	N/A	Range-finding studies.
CD-1 Mouse, 31-36 F/group	NTP, 1983b	0, 70, 140 or 280 mg/kg-day	Gavage 10 mL/kg	GD 6-15	4/36 dams died, tremors, reduced maternal body weight (10%); reduced fetal body weight	140 (maternal) 140 (dev)	280 (maternal FEL) 280 (dev)	None
F344 Rat 15-20 F/group	Narotsky and Kavlock, 1995	0, 40, 53.3 mg/kg-day	Gavage 1 mL/kg	GD 6-19	Maternal rales and dyspnea, marginal decreases in maternal body weight	N/A	N/A	Screening study

NS = Not Stated; dev = developmental; ELISA = enzyme-linked immunosorbent assay

consistent with toxicokinetic data suggesting that toxicity is correlated with peak blood concentrations rather than with total daily intake.

Several developmental toxicity studies are available (Argus Research Laboratories, 1997; NTP, 1983a; NTP, Narotsky and Kavlock, 1995). All of these studies were conducted via the gavage route, although the Argus Research Laboratories study used large dosing volumes and a divided-dosing protocol, apparently to reduce the effect of peak blood levels. The developmental toxicity studies found that the primary fetal effect is decreased body weight, which occurs at doses similar to those that cause decreased maternal body weight gain. The National Toxicology Program (NTP, 1983a) also found that toxicity increased markedly if the same phenol dose was administered in a lower dosing volume. Because the observed signs of toxicity included tremors and liver and kidney pathology and were not targeted to the portal of entry, the effect of dosing volume is not related to the concentration of a direct-contact toxicant.

The National Cancer Institute (NCI, 1980) conducted a carcinogenicity bioassay in which F344 rats (50/sex/group) received analytical-grade phenol (approximately 98.5% pure) in drinking water at concentrations of 0, 2500, or 5000 ppm for 103 weeks and were sacrificed 1–2 weeks later. Using the reference water intake of 0.13 and 0.14 L/kg-day for chronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 260, and 585 mg/kg-day for male rats and 0, 280, and 630 mg/kg-day for female rats. The doses shown here were adjusted to account for the reported water consumption of 80% and 90% of control at the low and high doses, respectively. The animals were observed daily for clinical signs and examined weekly for palpable masses. Body weights and food consumption were recorded every 2 weeks for the first 12 weeks and then monthly thereafter; water consumption was recorded weekly.

At the end of study, the animals were sacrificed, and complete gross and histopathological examinations were performed. Organs and tissues examined included the bone marrow, spleen, cervical and mesenteric lymph nodes, heart, liver, kidney, thyroid, reproductive organs, brain, and other major tissues. No evaluation of other noncancer endpoints, such as hematological effects or serum biochemistry, was conducted.

The survival rate at study termination was comparable among all three groups of males (approximately 50%) and females (approximately 75%). Dose-related decreases in body weight compared with the controls were observed in male and female rats, with a decrease of approximately 15% in high-dose males and approximately 10% in high-dose females. Water consumption was reduced by approximately 10% at the high dose. The authors stated that the non-neoplastic lesions were similar to those occurring naturally in aged F344 rats. However, an analysis conducted for this assessment found statistically significant increases (using a chi square test) in chronic kidney

inflammation in high-dose males and females; there were no significant changes at the low dose. No other differences in the incidence of non-neoplastic lesions between the controls and exposed rats were observed.

On the basis of increased kidney inflammation and decreased body weight as compared with controls at the high dose of 5000 ppm (585 mg/kg-day for males and 630 mg/kg-day for females), the NOAEL in this study can be considered to be the low dose, 260 mg/kg-day in males and 280 mg/kg-day for females, resulting in an overall study NOAEL of 260 mg/kg-day. These effects also indicate that the maximum tolerated dose (MTD) was reached.

In the NCI (1980) rat study, there were no dose-related trends in cancer incidence in male or female rats, but the authors reported several tumors for which statistically significant increases were seen in low-dose males only, based on pairwise comparisons. These increases were seen in the incidences of pheochromocytomas of the adrenal medulla (13/50, 22/50, and 9/50 in the control, low-, and high-dose groups, respectively) and "leukemias or lymphomas" (18/50, 31/50, and 25/50). The historical control incidences of pheochromocytomas in the bioassay program was 9% (data for the test laboratory were not reported), and the historical control incidence of leukemias or lymphomas in the test laboratory was 26%. The study authors stated that the leukemias were "of the type usually seen in untreated F344 rats." There were no significant increases in tumor incidence in any tissue in female rats. Because there was no clear dose-response in males and the tumors were not observed in female rats, an association between the tumors and phenol exposure cannot be established. NCI concluded that phenol was "not carcinogenic in male or female F344 rats." However, the report noted uncertainties regarding the possible increase in leukemia in male rats, and the NCI reviewers recommended that phenol be considered for a retest.

In a parallel study, NCI (1980) administered phenol at 0, 2500, or 5000 ppm in drinking water to B6C3F1 mice (50/sex/group) for 103 weeks and sacrificed the mice 1–2 weeks later. For B6C3F1 mice, the reference water intake was 0.24 L/kg-day for both sexes. The study found that water consumption was decreased to 75% and 50–60% of the control levels at the low and high doses, respectively. The resulting doses (adjusting for decreased water intake) were 0, 450, and 660 mg/kg-day for both sexes. Dose-related decreases in body weight as compared with the controls were attributed to the decrease in water consumption. No other clinical signs of toxicity were observed, and mortality rates (approximately 10% in males and 20% in females) were comparable between experimental and control groups.

Histopathological examination and statistical analyses revealed no phenol-related signs of toxicity or carcinogenicity; lesions in all systems observed in the dosed groups were comparable to those in the controls. NCI concluded that, under the conditions of the assay, phenol was not

carcinogenic in male or female B6C3F1 mice. On the basis of the decreased body weight as compared with controls observed at 5000 ppm, the low dose of 2500 ppm (450 mg/kg-day) can be considered the study NOAEL. The observed effect, however, is likely secondary to the decreased water consumption due to poor palatability.

In light of the marked decrease in water consumption, higher doses of phenol in drinking water probably could not have been tested. If the authors had attempted to overcome the palatability issue by administering the high dose by gavage rather than in drinking water, high toxicity would have been expected in light of the higher toxicity of phenol administered by gavage (see Section 4.5 and Table 2). These considerations suggest that an MTD was also reached in mice, although the conclusion is less clear than for rats.

In the range-finding test for the carcinogenicity bioassay (NCI, 1980), F344 rats and B6C3F1 mice (10/sex/group) were given drinking water containing 0, 100, 300, 1000, 3000, or 10,000 ppm phenol (at a purity of 98.47%) for 13 weeks. Using the reference water intake of 0.16 and 0.17 L/kg-day for subchronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 16, 48, 160, 480, and 800 mg/kg-day for male rats and 0, 17, 51, 170, 510, and 1140 mg/kg-day for female rats. The high doses shown here were adjusted to account for the decreased water consumption described below. For B6C3F1 mice, the reference water intake was 0.25 L/kg-day for males and 0.26 L/kg-day for females. The corresponding doses (adjusting for decreased water intake at the high dose) were 0, 25, 75, 250, 450, and 500 mg/kg-day for males and 0, 26, 78, 260, 468, and 520 mg/kg-day for female mice.

Body weights, appearance, behavior, and food and water consumption were recorded weekly. After 13 weeks, all animals were sacrificed and tissues were subjected to histopathological examinations. All of the rats and mice survived the phenol treatment. The only significant observation was the decreased final body weights (compared to controls) in rats of both sexes (11–14%) and in male mice (12%) that received 10,000 ppm. Because drinking water consumption in these groups was decreased to 50–70% (rats) and 20–60% (mice) of the control value, the decreased body weight was likely due to the low water consumption. No histopathological changes attributable to phenol treatment were observed.

This study suggests that the second-highest dose (480 mg/kg-day for male rats, 510 mg/kg-day for female rats, 450 mg/kg-day for male mice, and 470 mg/kg-day for female mice) was a NOAEL, based on the decreased final body weight (compared to controls) at 10,000 ppm, which was secondary to decreased water consumption due to poor palatability at the high dose.

In an unpublished 13-week neurotoxicity study conducted according to good laboratory practices (GLP) guidelines (ClinTrials BioResearch, 1998), groups of 15 male and 15 female Sprague-

Dawley rats received phenol via drinking water at concentrations of 0, 200, 1000, or 5000 ppm (at a purity of 100%) for 13 weeks, followed by a 4-week recovery period.[2] The authors calculated that the average doses were 0, 18.1, 83.1, and 308.2 mg/kg-day for males and 0, 24.6, 107.0, and 359.8 mg/kg-day for females. These concentrations were chosen on the basis of preliminary palatability studies conducted at a different laboratory (IITRI project No. L08657).[3]

During the exposure period, clinical signs and water intake were recorded daily, and body weight and food consumption were recorded weekly. In addition, a functional observational battery (FOB) and a motor activity test were conducted pre-study and once each during weeks 4, 8, 13, and 17. At the end of the exposure and the end of the recovery period, five rats/sex in the control and 5000 ppm groups underwent neuropathological evaluations (including a thorough evaluation of the brain and several nerves). The rest of the rats in the ClinTrials BioResearch (1988) study were sacrificed at the end of the 4-week recovery and were subjected to gross necropsy.

One high-dose female was euthanized on day 14 due to poor condition. Clinical signs observed in this female prior to sacrifice included dehydration, hunched posture, tremors, reduced activity, and cold to touch. Among the rest of the high-dose animals, the primary clinical sign was dehydration, which was accompanied by reduced activity and tremors in one female and by a thin appearance in additional animals. Dehydration was also observed in mid-dose rats (2/15 in each sex). Dehydration was assessed qualitatively and independently of drinking water consumption by grabbing the scruff on the back of the animal's neck; a delay in returning to the normal position was considered dehydration.

Dehydration was associated with marked decreases in water consumption at the high dose and smaller decreases at the mid dose. Decreases in water consumption were more pronounced in females than in males and were most evident during the first week of dosing. Water consumption was decreased to approximately 90% of the control level in mid-dose males and females, to approximately 60% of control levels in high-dose males, and to approximately 55% (40% during the first week) of control levels in high-dose females. Water consumption rebounded to levels higher than those of

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[2]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for a neurotoxicity screening battery, it is well documented, and it contributes useful information to the hazard identification and dose-response portions of the assessment. The study was designed to comply with the U.S. EPA Enforceable Consent Agreement for Phenol (Docket No. OPPTS-42150).

[3]Results of the palatability study were not provided in the IITRI study report (IIT Research Institute, 1999; Ryan et al., 2001), which reports the results of the two-generation reproduction study. The reproduction study was conducted during the same time period as the neurotoxicity study and reported similar problems of markedly decreased drinking water consumption at the high dose of 5000 ppm (see Section 4.3).

controls during the recovery period. The decreased water consumption was likely due to the poor palatability of phenol at high concentrations rather than being a manifestation of an overt toxicological effect. In addition, the high-dose group had decreased body weights when compared to controls (8% for males and 12% for females) and decreased food intake (approximately 10% for males and 10–20% for females).

The only toxicologically significant neurological effect was decreased motor activity in females. There was a statistically significant reduction in total group mean motor activity counts at week 4 in the 5000 ppm group. The authors reported that the rate of linear change of motor activity with time was also significantly decreased at weeks 8 and 13 in the 1000 ppm and 5000 ppm groups, although supporting data were not provided. Motor activity in females at week 4 exhibited a dose dependency at the first five (of six) analysis intervals, although the total counts for the low- and mid-dose groups were not significantly different from control. High-dose females also had markedly lower total activity counts than did controls and lower counts in the first four intervals, at week 4, although there was no statistically significant difference in mean total counts (Table 3). By contrast, the high-dose males had markedly lower group mean activity counts pre-study but activity comparable to or higher than the controls at weeks 4, 8, and 13.

The authors attributed the decreased activity to dehydration, noting that the control group mean total activity increased by >20% at week 4 as compared with pre-study levels, whereas activity of dehydrated females in the 5000 ppm group at week 4 was decreased by 17%, and activity of females in this group that were not dehydrated increased by 2%. To address whether the decreased activity could be attributed to dehydration, this assessment evaluated the data in greater detail. Table 4 presents the individual animal data for week 4 total motor activity counts and compares them with the individual animal dehydration data. If the individual clinical data reported an animal as dehydrated, the days of that notation are shown. With the exception of animal 4502 (which died) and animal 4507 (which had severe dehydration) dehydration was noted as slight or moderate. For clarity of presentation, the individual animal data are shown for the control and high-dose groups, but only the average data are shown for the low- and mid-dose groups.

Table 3. Total Activity Counts in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Dose Group	Prestudy (Mean ±SD)	Week 4 (Mean ±SD)	Week 8 (Mean ±SD)	Week 13 (Mean ±SD)
Females				

Control	384±116	468±118	436±75	309±77
200 ppm	386±89	451±149	440±99	338±66
1000 ppm	384±103	394±78	436±104¶	343±124¶
5000 ppm	372±142	337±127**	363±111¶¶	366±145¶¶
Males				
Control	354±109	339±89	320±90	260±68
200 ppm	340±107	346±132	323±88	256±78
1000 ppm	335±126	356±137	359±105	274±103
5000 ppm	277±59	321±95	352±91	275±116

**Significantly different from control, $p<0.01$ (T-test)

¶Linear constructed variable significantly different from control, $p<0.05$ (T-test)

¶¶Linear constructed variable significantly different from control, $p<0.01$ (T-test)

Table 4. Individual Data on Dehydration and Week 4 Motor Activity in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1501	383	No	2501	397	No	3501	322	No	4601	501	14, 21
1502	321	No	2502	529	No	3502	402	18, 21	4502	No data - sacrificed day 14 due to poor condition	
1502	621	No	2503	427	No	3603	270	No	4503	227	7, 14, 21, 28
1504	437	No	2504	558	No	3504	370	No	4504	258	14, 21, 28, 35, 42, 70
1505	630	No	2505	245	No	3505	572	21, 28, 35, 42, 49, 56, 70	4505	396	No
1506	365	No	2506	537	No	3506	452	No	4506	277	70
1507	591	No	2507	470	No	3507	461	No	4507	399	7-9, 11, 12, 13-15, 17, 20, 21, 70, 77
1508	318	No	2508	284	No	3508	342	No	4508	271	No

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1509	479	No	2509	527	No	3509	462	No	4509	387	No
1510	469	No	2510	823	No	3510	452	No	4510	450	No
1511	309	No	2511	561	No	3511	390	No	4511	439	7
1512	574	No	2512	424	No	3512	383	No	4512	130	No
1513	566	No	2513	302	No	3513	320	No	4513	242	49, 56
1514	381	No	2514	289	No	3514	403	No	4514	180	7, 14, 21, 28
1515	578	No	2515	386	No	3515	311	No	4515	556	No
Overall Average	468	--		451	--		394	--		337	--
Average - Dehydrated animals	N/A	--		N/A	--		487 (n=2)	--		315	--
Average - non-dehydrated animals	468	--		451	--		380	--		365	--

N/A = Not applicable

Only two animals in the mid-dose group were reported as dehydrated on any day, and neither of these animals had decreased motor activity. As shown, the average activity was lower in the dehydrated high-dose females than in those not reported as dehydrated, but an association of decreased activity with dehydration was not consistently supported on an individual-animal basis. (For the purpose of calculating averages, animals were considered dehydrated if they dehydrated at any point in the study. This is a limitation to the analysis, because some were reported as dehydrated only prior to week 4 and others were reported as dehydrated only after week 4. In addition, basing the analysis on the clinical sign of dehydration may not appropriately reflect whether the animals were dehydrated, because no objective measure of dehydration was used and because decreased water consumption in this group occurred throughout the study.)

As shown in Table 4, animal 4601 was reported as dehydrated on days 14 and 21, but it had one of the highest total activity counts. Conversely, animal 4512 had the lowest activity count, but it was never reported as being dehydrated. Furthermore, the mean activity of the dehydrated high-dose females was 67% of concurrent controls, compared to 78% of concurrent controls for the nondehydrated high-dose females. These data indicate that the difference between the control and high-dose animals was greater than the difference between the dehydrated and nondehydrated animals at the high dose.

Overall, the data indicate that there was not a tight linkage between dehydration and decreased motor activity in the high-dose females. The data for high-dose males also did not indicate a clear correlation between low activity and dehydration. The clinical signs for one high-dose male (4003) for week 2 included severe dehydration and decreased activity, but no effect (i.e., no dehydration or decreased activity) was seen when the animal underwent the objective activity analysis in week 4. The finding of dehydration in males without the accompanying decrease in activity further supports the conclusions that only severe (not mild or moderate) dehydration results in decreased motor activity levels and that the decrease observed in females was phenol related. Conversely, the absence of other findings in the FOB and the presence of a statistically significant effect on motor activity only at 4 weeks and not at later time points argue against a neurotoxic effect of phenol.

As an additional investigation of whether decreased motor activity was related to dehydration, the very limited literature on water deprivation and motor activity was reviewed. Campbell and Cicala (1962) evaluated the effects of terminal water and food deprivation (i.e., deprivation until death from dehydration or starvation) on motor activity of male and female Wistar rats. Motor activity was measured using a stabilimeter, which is similar to the figure-8 mazes used in

the ClinTrials BioResearch (1998) study in that ambulation (as opposed to simply movement) is measured. The study found that water deprivation alone did not result in decreased motor activity until approximately days 5–7 (depending on age), at which time activity continuously declined until death. By contrast, food deprivation resulted in an initial increase in activity followed by decreasing activity until death.

Only the pooled data for males and females were reported. These results are not directly comparable to the results of the ClinTrials study because the latter involved long-term, lower-level dehydration; however, they do support the conclusion that the decreased motor activity in high-dose females was due at least partially to phenol exposure. The most appropriate way to address this issue would be to conduct the neurotoxicity study with a water-restricted control group. Overall, based on the decreased motor activity, the study NOAEL in females was 1000 ppm phenol (107 mg/kg-day) and the Lowest Observed Adverse Effect Level(LOAEL) was 5000 ppm (360 mg/kg-day). No LOAEL was identified in males; the high dose of 308 mg/kg-day was a NOAEL. A 95% lower confidence limit on the benchmark dose (BMD) of 219 mg/kg-day was calculated for decreased motor activity in week 4 in this study (see Appendix B).

Hsieh et al. (1992) investigated the effects of phenol exposure on hematological, immune, and neurochemical endpoints in a study of 6-week-old male CD-1 mice administered actual concentrations of 0, 4.7, 19.5, or 95.2 ppm in drinking water for 28 days. On the basis of measured concentrations and water intake, the authors reported that the corresponding daily doses were 0, 1.8, 6.2, and 33.6 mg/kg-day.

The mice were housed in groups of five per cage. Drinking water was prepared and changed every 3 days. Drinking water was provided in glass water bottles with stainless sipper tubes containing ball bearings to minimize evaporation; the bottles were shaken frequently during treatment. Food and water consumption were monitored continuously, and the animals were weighed weekly. After 28 days, the mice were sacrificed by decapitation, gross pathological examinations were performed, and the liver, spleen, thymus, and kidney were weighed. Blood was taken at sacrifice for analysis. Splenocytes were prepared for analysis of mitogen-stimulated lymphocyte proliferation, mixed lymphocyte response, and cell-mediated cytolytic response.

Data were reported for five animals per group for each assay. During the 28-day exposure, no mortality and no overt clinical signs occurred in exposed mice. Phenol treatment had no effect on food or water consumption or on body weight gain. Exposed mice had no gross lesions in the liver, kidney, spleen, thymus, lung, heart, or brain, and there were no effects on organ weights for the liver, kidney, spleen, and thymus. As shown in Table 5, a decreased antibody response to sheep red

blood cells was observed, as indicated by both the plaque-forming cell (PFC) assay (expressed as PFC/million spleen cells and PFC/spleen) and the antibody titer using an enzyme-linked

Table 5. Effects of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters in Mice and Rats

Concentration (mg/L)	Dose (mg/kg-day)	PFC/10^6 splenic cells	PFC/total spleen	Antibody titer [b]
Hsieh et al. (1992) - 4-week study in CD-1 mice				
0	0	1,123 ± 99[a]	265,947 ± 53,099	0.446 ± 0.039
4.7	1.8	896 ± 60	214,678 ± 17,500	0.392 ± 0.068
19.5	6.3	795 ± 49[c]	207,659 ± 18,886	0.325 ± 0.019[c]
95.2	33.6	616 ± 83[c]	130,185 ± 18,202[c]	0.263 ± 0.037[c]
IIT Research Institute (1999); Ryan et al. (2001) - 2-generation study in Sprague-Dawley rats, effects in P1 generation				
0	0	1343±890[d]	$5.54x10^5$±$3.70x10^5$	Not assayed
200	15	1668±788	$6.42x10^5$±$3.40x10^5$	Not assayed
1000	71	1781±1151	$9.01x10^5$±$7.16x10^5$	Not assayed
5000	301	1880±865	$9.81x10^5$±$5.02x10^5$	Not assayed
Positive control (n=5)	cyclo-phosphamide	0±0	0±0	Not assayed

[a] Values are given as mean ± S.E. (n=5). [b]Arbitrary as change in absorbance at 490 nm, using 1:2000 diluted serum. [c] Significant ($P<0.05$) difference from the control value. [d] Values are given as mean ±SD (n=8-9)

immunosorbent assay (ELISA). Two of these measures were statistically significantly decreased at the mid dose, and PFC/spleen was significantly decreased only at the high dose. Decreases in the absolute splenocyte lymphoproliferative responses to mitogens and the mixed lymphocyte response (the proliferative ability of splenic lymphocytes in response to alloantigens) were also observed at the high dose; there was no effect on the cytolytic response to tumor cells at any dose.

Although these assays were conducted according to the methods of the day, the latter two assays do not conform to modern protocols, and there is little biological significance in the results of the mitogen response assay. In particular, the approach used cannot distinguish between an effect on the lymphocyte's ability to initiate a proliferative response and confounding due to contamination by nucleated red blood cell precursors.

A statistically significant, dose-related decrease in erythrocyte counts was observed at all doses (Table 6). The hematocrit was decreased only at the high dose. A decreased erythrocyte count in the absence of an effect on hematocrit may have been due to macrocytosis (enlarged erythrocytes), but insufficient data were provided to evaluate this possibility. In the absence of such data, the decreased erythrocyte counts are insufficient to form the basis for identification of a LOAEL. The erythrocyte counts in all dosed groups were markedly lower than the historical control values provided by the animal distributor (Charles River Laboratories, 1986), although the hematocrit concentration in all groups was above the historical control mean. There was no effect on total or differential leukocyte counts. Interestingly, total white blood cells for all groups, including the controls, were below the historical control data provided by the distributor.

Hsieh et al. (1992) also observed dose-related decreases in the concentration of several neurotransmitters and their metabolites in the brain, including levels of norepinephrine, indoleamine serotonin, and dopamine and their metabolites. In the absence of a clear correlation with clinical effects, the toxicological significance of these neurobiochemical findings is unclear.

Thus, this study found dose-related, statistically significant decreases in red blood cells at all doses, but the significance of this finding is uncertain, because decreased hematocrit was observed only at the high dose. Statistically significant decreases in antibody response were observed at the mid dose, and these decreases reached 40% (a value often used by immunotoxicologists as a rule of thumb for clinically relevant decreases) at the high dose. Identification of a NOAEL in this study is somewhat problematic, because immunotoxicity risk assessment guidelines have not been developed.

The determination of what degree of decrease is adverse is also problematic, because the clinical relevance of a decrement in immune function will depend on the magnitude and type of

Table 6. Effects of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters in Mice and Rats

Concentration (mg/L)	Dose (mg/kg-day)	Spleen cellularity (x 10^{-7})	WBC[a] (x 10^{-3})	RBC[a] (x 10^{-6})	Hematocrit %	Differential counts as % of WBCs		
						Lymphocyte	Neutrophil	Monocyte
Hsieh et al. (1992) - 4-week study in CD-1 mice								
0	0	8.59 ± 0.34[b]	6.06 ± 0.17	7.17 ± 0.56	48.00 ± 0.52	74.20 ± 1.83	17.00 ± 1.00	4.60 ± 0.51
4.7	1.8	7.94 ± 0.20	5.82 ± 0.60	4.90 ± 0.54[c]	49.10 ± 0.68	71.80 ± 2.06	19.40 ± 0.75	4.80 ± 1.02
19.5	6.3	7.31 ± 0.40	5.05 ± 0.53	4.64 ± 0.76[c]	48.20 ± 1.24	69.20 ± 3.25	21.80 ± 2.40	4.60 ± 0.81
95.2	33.6	7.26 ± 0.55	5.68 ± 0.69	3.23 ± 0.68[c]	44.10 ± 0.81[c]	73.60 ± 2.32	17.00 ± 1.55	6.20 ± 1.16
Historical control value[d]		Not available	9.0 (8.9-9.1)	7.6 (7.2-8.0)	42 (36-48)	70 (52-86)	25 (10-42)	4 (0-8)
IIT Research Institute (1999); Ryan et al. (2001) - 2-generation study in Sprague-Dawley rats, effects in P1 generation								
0	0	43.2±13.5[e]	13.1±2.01	9.22±0.37	46.5±1.44	N/A	N/A	N/A
200	15	38.3±8.78[f]	13.8±1.98	9.08±0.62	46.2±3.65	N/A	N/A	N/A
1000	71	48.1±11.0	14.5±2.42	9.03±0.34	46.4±1.56	N/A	N/A	N/A
5000	301	52.7±13.4	14.9±2.93	8.81±0.44	45.1±1.75	N/A	N/A	N/A

[a]Cells/mm^3 . [b]Values are given as mean ± S.E. (n=5); N/A = not assayed. [c]Significant ($P < 0.05$) difference from the control value. [d]Mean (Range ±2S.D) for mice 6-8 weeks of age, based on 20 studies, from Charles River Laboratories (1986). [e]Mean ±SD (n=9-10 for hematology endpoints and n=8-9 for spleen cellularity).
[f]Standard deviation reported in Ryan et al. (2001) has a typographical error; correct standard deviation obtained from IIT Research Institute (1999)

immune challenge, with a sufficiently large challenge resulting in illness even for unimpaired individuals. In a paper on the use of immunotoxicity data for risk assessment, Selgrade (1999) recommended that any statistically significant and consistent change be considered a risk for the purposes of hazard identification, but the degree of change considered adverse for the purposes of dose-response assessment was not addressed.

On the basis of magnitude of the decreases in antibody response observed in three related—assays, supported by decreased hematocrit and red blood cells—the high dose (33.6 mg/kg-day) can be considered the study LOAEL, and the mid dose (6.2 mg/kg-day) can be considered the study NOAEL. There is, however, considerable uncertainty regarding the reliability of these values because of issues of study interpretation and because the study used only five animals per group as opposed to the recommended eight per group (U.S. EPA, 1998c).

The results of BMD modeling conducted for this study are presented in Appendix B for completeness. However, it is unclear what the appropriate benchmark response (BMR) would be for an in vivo/in vitro immunotoxicity study, and so the modeling results are considered very preliminary. In particular, it is unclear whether the default of one standard deviation is appropriate as the BMR for this study design in light of the small sample size (n = 5) but the relatively tight data.

In contrast to the minimal effects observed in these drinking water studies, gavage dosing with phenol produces severe toxicity, including liver and kidney pathology, and death at doses that cause only minimal effects when delivered in drinking water.

Dow Chemical Co. (1945) administered 0, 50, or 100 mg/kg phenol by gavage 5 days/wk to 10 rats per group (sex and strain not reported) for 6 months (0, 35.7, or 71.4 mg/kg-day after adjusting for intermittent dosing). The dosing volume was not reported. Mortality occurred in 1/10, 4/10, and 4/10 rats in the control, low-, and high-dose groups. The authors raised questions about whether the mortality was treatment-related, but it is not clear whether they questioned whether the deaths were due to phenol or to gavage accidents. Other observed effects were slight cloudy swelling of the liver and of the tubular epithelium at the high dose and slight tubular degeneration at the low dose. This unpublished study[4] is limited by the incomplete reporting of methods and results, but the low dose of 35.7 mg/kg-day appears to be a LOAEL.

[4]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

In a series of toxicological screening tests, the systemic, neurological, and developmental effects of phenol in F344 rats following acute and short-term oral exposure were examined (Narotsky and Kavlock, 1995; Berman et al., 1995; and Moser et al., 1995; MacPhail et al., 1995). In these tests, systemic and neurological effects were examined on the same animals following exposure by gavage to a single dose of phenol or to 14 consecutive daily doses. Developmental toxicity was also examined in pregnant rats that received phenol by gavage on GDs 6–19. The dosing volume was 1 mL/kg (Moser et al., 1995; Narotsky and Kavlock, 1995).

In the acute toxicity study of this series, groups of eight female rats were given a single gavage dose (1 mL/kg volume) of phenol at 0, 12, 40, 120, or 224 mg/kg in water (Berman et al., 1995; Moser et al., 1995). An FOB evaluating autonomic and neuromuscular functions, activity, excitability, and sensorimotor and physiological measures was conducted prior to the exposure and at approximately 4 and 24 hours after exposure. Immediately after the 24-hour FOB, the animals were sacrificed, blood samples were collected for serum chemistry analyses, and the liver, kidneys, spleen, thymus, and adrenals were weighed and subjected to histopathological examinations. Two rats (25%) died within 4 hours of exposure to 224 mg/kg, and one rat died 24 hours after exposure to 120 mg/kg phenol. The only treatment-related effects observed were confined to these two dose groups, and they included tremor, decreased motor activity, and kidney pathology (necrosis, protein casts, and papillary hemorrhage). Hepatocyte necrosis was also observed at 40 and 120 mg/kg but not at 224 mg/kg. No other effects were reported at the lower doses, although the primary data were not provided.

In the short-term study, groups of eight female rats were given daily gavage doses of phenol in water at 0, 4, 12, 40, or 120 mg/kg-day for 14 consecutive days (Berman et al., 1995; Moser et al., 1995). As in the acute study, the FOB was conducted prior to exposure as well as on days 4 and 9 (before the daily dose) and approximately 24 hours after the last dose. After the last FOB, blood samples were collected for serum chemistry analyses, and internal organs were removed, weighed, and subjected to histopathological examinations. All rats administered the high dose died during the study, but deaths occurred over the entire dosing period. Tremor was also seen in the high-dose (120 mg/kg) group immediately after the first administration but not after subsequent treatment. Vacuolar degeneration of the liver, kidney necrosis and protein casts, and "necrosis or atrophy of spleen or thymus" were reported at 40 mg/kg-day. The increased incidences were not large enough to be statistically significant; the statistical power of the study was also low, with only 8 rats per group.

Additional information on this study is available from a preliminary abstract (Schlicht et al., 1992) and from a recent WHO (1994) review. According to these sources, the renal pathology

consisted of 3/8 rats with renal vascular stasis, 2/8 rats with tubular degeneration in the papillar region, and 1/8 rats with protein casts in the tubules. WHO (1994) states that, according to a personal communication from one of the study authors, the pathology report attributed the renal findings to decreased vascular perfusion.

The study also found slight but not statistically significant decreases in motor activity at 40 mg/kg-day. The only statistically significant effect in this group was increased rearing in the post-exposure measurement. The only effect at 12 mg/kg-day was "necrosis or atrophy in the spleen or thymus" in 1/8 rats. On the basis of the liver, kidney, and thymus/spleen pathology findings, which are rarely observed in control animals in 2-week studies, and the decreased motor activity, the second dose (40 mg/kg-day) was the study LOAEL, and the mid dose of 12 mg/kg-day was the study NOAEL.

4.2.2. Inhalation

The laboratory animal inhalation data for phenol are very limited, with only one 2-week toxicity study being conducted using modern methodology and documentation (Hoffman et al., 2001; additional details available in the unpublished version, Huntingdon, 1998). Although a subchronic study conducted with multiple laboratory animal species is available (Sandage, 1961), this latter unpublished study tested only one concentration and was insufficiently documented for definitive risk assessment purposes. Other short-term (Dalin and Kristoffersson, 1974) or subchronic (Deichmann et al., 1944) inhalation toxicity studies are limited by short duration, inadequate documentation, or lack of a modern exposure protocol. Nonetheless, the data are consistent that the respiratory tract, kidney, and nervous system are targets of inhalation exposures.

In conducting dosimetric conversions from animal studies to human exposure scenarios, U.S. EPA (1994b) classifies gases according to their water solubility and reactivity. Category 1 gases are highly water-soluble and/or rapidly reactive and do not penetrate the blood. Category 3 gases are water insoluble, and uptake from the lungs is limited by perfusion. Category 2 gases are intermediate between these two groups. They are moderately water-soluble and rapidly-reversibly reactive or moderately-to-slowly irreversibly metabolized in the respiratory tissue. On the basis of phenol's chemical/physical properties (see Table 1) of moderate water solubility and moderate reactivity (based on the evidence of irritation and corrosivity seen following direct contact), it can be considered a Category 2 gas. This conclusion is supported by the finding of both respiratory effects (from direct contact) and systemic (extrarespiratory) effects (from absorbed phenol) following inhalation exposure, as described below.

Because the equations for the regional gas dosimetry ratio (RGDR) for Category 2 gases are currently undergoing EPA reevaluation (eqs. 4-29 through 4-44, pages 4-52 through 4-57 of U.S. EPA, 1994b), dosimetric adjustments for extrarespiratory effects were made using the Category 3 equations (eq. 4-48, page 4-60 of U.S. EPA, 1994b), in which the RGDR is based on the blood:air partition coefficient for the chemical in the experimental animal species and in humans. No data on the blood:air partition coefficient for phenol in laboratory animals or in humans were located. Therefore, the default value of 1 for the ratio of the laboratory animal-to-human partition coefficient was used, and the human equivalent concentration (HEC) for systemic effects was the same as the duration-adjusted concentration.

HECs for respiratory tract effects were calculated using the equations of U.S. EPA (1994b) for a Category 1 gas (eqs. 4-17 through 4-28, pages 4-47 through 4-51). When the EPA reanalysis is complete, revised dosimetric conversions may be calculated. The inhalation toxicity data for phenol are summarized in Table 7.

The acute toxicity studies support the findings of the short-term and subchronic studies that the respiratory tract and nervous system are targets of inhaled phenol. For example, tremors were seen in rats and guinea pigs exposed to 187 or 540 ppm (720 or 2080 mg/m^3) phenol for 30 minutes in a whole-body inhalation chamber (UBTL, 1991). By contrast, no tremors were observed in rats exposed via nose-only inhalation at 25 ppm (96 mg/m^3) for 6 hours (Dow Chemical Co., 1994). Phenol also caused sensory irritation in mice, as evidenced by decreased respiratory rate (De Ceaurriz et al., 1981). The concentration associated with a 50% decrease in rate (RD_{50}) was estimated to be 166 ppm (639 mg/m^3). No acute lethality studies were identified for phenol following exposure by the inhalation route.

In a 2-week inhalation study conducted according to GLP guidelines (Hoffman et al., 2001; full study report available as Huntingdon, 1998), groups of 20 F344 rats per sex were exposed nose-only to actual concentrations of 0, 0.52, 4.9, or 25 ppm phenol (0, 2.0, 18.9, or 96.2 mg/m^3) 6 hrs/day, 5 days/wk for 2 weeks. The duration-adjusted concentrations were 0, 0.36, 3.4, and 17 mg/m^3, respectively. The animals were observed twice daily for mortality and abnormal clinical signs. Animal body weights and food consumption were recorded twice pre-test, weekly thereafter, and just prior to sacrifice. At the end of 2 weeks of exposure, 10 rats of each sex in each group were sacrificed. The rest of the rats were sacrificed after 2 weeks of recovery. Blood samples were collected just prior to sacrifice for hematological (including differential leukocyte count) and

Table 7. Summary of Inhalation Toxicity Studies

Strain Species, Sex	Reference	Exposure mg/m^3	Duration	Duration-Adjusted mg/m^3	Response	NOAEL/ LOAEL mg/m^3	NOAEL/ LOAEL (HEC) mg/m^3	Comments
F344 Rat (20/sex)	Huntingdon 1998	0, 2.0, 18.9, 96.2 Nose-only	6 hr/d 5 d/week 2 weeks	0, 0.36, 3.4, 17	Red nasal discharge, but no histopathology lesions.	17/None	2.5 or 17 /None	Well-conducted study, but authors did not note the clinical signs
Rat, 7 exposed, 11-12 controls (Strain & sex NS)	Dalin and Kristoffersson 1974	0, 100	15 days continuous	0, 100	Nervous system effects, increased serum liver enzymes	None/100	None/100	Exposure measurement not done according to modern methods, no histopathology exam
Guinea pig, 12 (Strain & sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 6 weeks	31 (based on midpoint of range)	FEL – 5/12 dead	None	None/ 31 is FEL	Minimal documentation, outdated exposure methods, no controls
Rabbit 6 exposed (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 13 weeks	31 (based on midpoint of range)	Pneumonia, heart inflammation, liver necrosis, kidney tubular degeneration	None	None/ 31	Minimal documentation, outdated exposure methods, no controls
Rat, 12 (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 74 days	31 (based on midpoint of range)	No effect, no evidence of histopathology	31/None	31/None	Minimal documentation, sensitivity of assay unclear, outdated exposure methods, no controls
Rhesus monkey 10 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study

Strain Species, Sex	Reference	Exposure mg/m^3	Duration	Duration-Adjusted mg/m^3	Response	NOAEL/ LOAEL mg/m^3	NOAEL/ LOAEL (HEC) mg/m^3	Comments
Sprague-Dawley rat 50 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study
Albino mouse 100 M/group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Lung pathology (Not further described)	None/18.2	Not determinable*	Pathology reported to be minimal, but limited by minimal description Unpublished study

NS = Not stated

* HEC cannot be determined because the region of the respiratory tract affected is not clear.

biochemical examinations. Gross pathological evaluations were conducted on all of the animals, and organ weights were determined. Histopathological examinations were conducted on the liver, kidney, and respiratory tract tissues (including three sections of the lungs with mainstem bronchi, the pharynx, and three sections of the nasal turbinates) of the control and high-exposure groups; the spleen of mid-concentration females was also analyzed.

During the exposure, one male rat in the low-concentration group accidentally died from trauma caused by turning itself within the nose-only restraint tube. All of the other rats survived until sacrifice at the end of the 2-week exposure or 2-week recovery periods. During exposure and recovery, there were no treatment-related changes in weekly physical examinations, body weight, weight gain, or food consumption. The authors reported no effect on clinical signs. However, there was a concentration- and duration-related increase in the incidence of a red nasal discharge in the males. The incidence was 0/20, 0/20, 3/20, and 4/20 at 0, 0.52, 4.9, and 25 ppm, respectively, in the first week and 0/20, 0/20, 7/20, and 10/20 in the second week of exposure. This detailed information on nasal discharge was presented only in the unpublished report (Huntingdon, 1998); the published version reported scattered observations of chromodacryorrhea and nasal discharge.

In an analysis done for this assessment, the incidence at the mid and high concentrations was statistically significant, using the Fisher exact test. In females, nasal discharge was seen in 1/20 at the low concentration and 3/20 at the mid concentration in the second week, but no discharge was reported in high-concentration females. Prior to exposure, a nasal discharge was observed in a single control male and a single high-concentration female. Considering the exposure chamber design, it does not appear that the discharge was an artifact of the rats' noses being in contact with phenol condensate on the chamber walls. Instead, in the absence of nasal histopathology, it is likely that the discharge reflected a nonspecific response to stress in the rats. A tear-like nasal discharge in rats can be a generalized response to stress from a variety of causes. Porphyrins in the discharge lead to a red color. In light of the dose-related response in males, it appears that the stress in this study was related to an effect of phenol, either as an irritant or a toxicant.

Hematological and biochemical examinations showed slight but statistically significant increases in prothrombin time at the low concentration only and in albumin concentration in high-exposure females; these changes were not considered to be biologically significant. No other significant changes in hematology or biochemistry were observed. The only statistically significant changes in organ weights were decreases in liver-to-body, spleen-to-body and spleen-to-brain weight ratios in mid-concentration (18.9 mg/m^3) females. Because the changes in organ weights did not occur at the highest phenol exposure concentration (96.2 mg/m^3), and the same responses occurred in

female rats but not in male rats, these responses were considered by the authors to be not toxicologically significant.

Gross pathological and microscopic examinations of these organs did not exhibit any differences from those of the controls. Although there were a number of histopathology findings in the respiratory tract (e.g., inflammatory cells in the nasolacrimal ducts, alveolar macrophages, and eosinophilic and basophilic material), these findings occurred at similar incidences in the control and exposed groups. The lesions reported were also those typically seen in control animals. The only lesion of concern was minimal to slight lung hemorrhage, which was reported in 4/10 control males and 6/10 high-exposure males at the terminal sacrifice. However, there was no clear concentration-related increase in incidence or severity, this lesion was not found in the females, and this lesion was not seen in exposed animals post-recovery or in control animals.

Thus, it appears that the only effect of concern in this study was the red nasal discharge, which was observed in males but not in females, and this effect was probably due to a nonspecific response to stress. In addition, no supporting histopathology was observed in a thorough examination. On the basis of these considerations, the highest concentration (96.2 mg/m^3) in this study was NOAEL. The HEC cannot be definitively determined in the absence of an affected endpoint. However, assuming that the respiratory tract would be affected first, as shown in other studies, a conservative NOAEL (HEC) based on a nasal effect would be 2.5 mg/m^3. Assuming that the nasal discharge reflects a nonadverse systemic stress response, the NOAEL(HEC) would be 17 mg/m^3. No LOAEL was identified.

Deichmann et al. (1944) conducted subchronic inhalation studies of phenol toxicity in rabbits, rats, and guinea pigs. Twelve guinea pigs, 6 rabbits, and 12 rats (strain and sex not reported) were exposed (whole body) in a single exposure chamber to phenol vapor at "a concentration ranging from 0.1 to 0.2 mg/L (100-200 mg/m^3)" for 7 hrs/day, 5 days/wk for 6 weeks, 13 weeks, or approximately 11 weeks, respectively. The actual exposure concentration apparently could not be controlled more precisely. Using the midpoint of 150 mg/m^3 as the exposure concentration, the duration-adjusted concentration was 31 mg/m^3.

Among the three tested species, the guinea pig was the most sensitive and the rat was the least sensitive to phenol exposure. Deaths were observed in 5/12 guinea pigs during the 6-week exposure period. Other signs of toxicity in the guinea pigs included decreased activity during the first week and respiratory difficulties and paralysis of hind quarters after 4 weeks of exposure. Histopathological evaluations revealed lesions of the lungs (pneumonia and bronchitis), heart (inflammation, fibrosis, and necrosis), liver (fatty changes and necrosis), and kidneys (tubular

degeneration and edema). At the end of exposure, the surviving guinea pigs had a concentration of 1.0 mg free phenol/100 mL blood and 0.4 mg conjugated phenol/100 mL blood. (Details on the analytical procedures used to measure phenol in blood were not reported.).

No deaths or clinical signs of toxicity were observed in the rabbits exposed for 13 weeks, but lobular pneumonia and fibrosis was observed in these animals. Histopathology lesions in the heart, kidney, and liver were similar to, but less severe than, those reported in the guinea pigs. After 37 days on study, the rabbits had a concentration of 0.5 mg free phenol/100 mL blood and 0.7 mg conjugated phenol/100 mL blood; similar concentrations were observed at the end of the exposure period.

The rats did not show any clinical signs of toxicity during the 74-day exposure period (approximately 11 weeks), and there was no histopathological evidence of any effect. Blood phenol levels were not reported for the rats, but an analysis of carcass homogenate found 0.2 mg free phenol and 0.35 mg conjugated phenol per 100 g carcass homogenate. These levels were reported to be within the normal range in unexposed rats. This study is limited by the use of only one exposure concentration, the absence of controls, the inadequate control of exposure levels, and the absence of reporting of the primary data. However, the results do appear to show that rats are much less sensitive than rabbits or guinea pigs to the inhalation effects of phenol. However, a comparison of blood levels suggests that the interspecies differences are due to both toxicokinetic and toxicodynamic differences.

In an unpublished 90-day study (Sandage, 1961), groups of 10 male rhesus monkeys, 50 male Sprague-Dawley rats, and 100 male albino mice were exposed to average phenol concentrations of 0 or 4.72 ppm (18.2 mg/m^3) continuously for 90 days.[5] Exposure was interrupted for 14 hours on day 39 and for 36 hours on days 68–69. The frequency of monitoring of the test atmosphere was not reported, but the phenol concentration was reported to remain in the desired range of 4.5–5.5 ppm "after the first three days." No further information on the concentrations during the first 3 days was reported.

During the exposure, no deaths were observed in the test animals. Body weight gain in mice was comparable to that in controls but was slightly higher in exposed rats and monkeys. A complete hematological examination showed no significant changes in the three test species following phenol

[5]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

exposure. Blood biochemistry (alkaline phosphatase, cholinesterase, amylase, lipase, and glutamic oxalacetic transaminase) was evaluated in monkeys only. Urinalysis was apparently conducted in all species, but kidney function tests (urine volume and specific gravity) were conducted only in monkeys and rats. The study authors reported that there were no effects on any of these endpoints but did not provide any supporting data.

At the end of the exposure period, "approximately half" of the animals underwent a stress test in which the animals swam in a smooth-walled tank until exhausted. These animals were sacrificed immediately after the test, and the other animals were held for a 2-week recovery period prior to sacrifice. Histopathological evaluations were conducted on only 5–8 organs (including the liver, kidney, and lung). It appears that all of the monkeys and about half of the rats and mice were evaluated, although it is not clear whether some of the rodents were evaluated after the recovery period.

The authors considered the histopathology findings "essentially negative" and did not provide any description of the observed lesions or the number of animals examined histopathologically. Liver and kidney pathology was observed in 30% and 20%, respectively, of the monkeys (compared with 0% of the controls). However, the authors did not consider these changes to be significant, and they noted that 6/7 reports of pathology in monkeys were considered "minimal or doubtful." Liver and kidney pathology was also reported in 20% of phenol-exposed rats (compared with 0% of the controls) and lung pathology was reported in 20% of the phenol-exposed mice (compared with 6% of the controls). The incidences of liver and kidney pathology in the rat and lung pathology in the mouse were statistically significant in a Fisher's exact test done for this assessment. Although the incidence of lung pathology was not affected in monkeys and rats, a relatively high incidence of lung pathology in the control animals (30% and 65%, respectively) decreased the sensitivity of the evaluation. No other significant pathological changes were reported in the test animals.

Although the authors concluded that there was no evidence that phenol exposure resulted in significant damage, there is some indication of liver, kidney, and lung pathology in this study, but the inadequate reporting precludes the determination of whether there was a treatment-related effect. For the purposes of this assessment, the single exposure level tested, 18.2 mg/m^3, should be considered a free-standing LOAEL, although it might be considered a minimal LOAEL if additional histopathology data were available. The LOAEL (HEC) for the kidney and liver lesions is also 18.2 mg/m^3. In the absence of additional information on the nature of the lung lesions, the LOAEL (HEC) for the lung cannot be determined. The study is also limited by the poor control of exposure levels and limited reporting of effects.

In a study published by Dalin and Kristoffersson (1974), seven white rats of an in-house strain were exposed to phenol at a concentration of 100 mg/m^3 continuously for 15 days. There is some uncertainty about this exposure measure, however, because the exposure chamber was not set up according to modern designs, and it does not appear that continuous monitoring of exposure levels was conducted. Unexposed rats (n = 11–12) were used as controls. Nervous system effects were observed from the first day after the start of exposure. These effects progressed from increased activity to imbalance, twitches, and disordered walking rhythm on days 3–4. These signs disappeared by day 5 and were replaced by sluggish behavior until the end of the exposure. A tilting-plane test was conducted before and after exposure in both groups, and a significant effect was observed on the exposed rats. There were no significant changes in food intake or water consumption during the exposure period. Although there was no significant difference in body weight of the exposed group compared with that of the controls, the average body weight of the exposed group decreased during exposure, whereas the controls gained weight.

The serum biochemical evaluations showed large, statistically significant increases in SGOT, SGPT, lactic dehydroganese (LDH), and glutamate dehydrogenase activities, indicating liver damage. Plasma potassium and magnesium levels were also increased. Although the significance of these changes is unknown, the authors suggested that the increased magnesium levels may have caused some of the nervous system effects. Hemoglobin and hematocrit were unaffected. No histopathology examination was conducted. On the basis of the observed nervous system effects as well as the serum enzyme changes indicating liver damage, the only exposure concentration was a free-standing LOAEL. The LOAEL (HEC) is 100 mg/m^3, but the actual exposure measurement is of low quality.

4.2.3. Dermal

Phenol is quite irritating, and dermal exposure to liquid phenol or to concentrated phenol vapor can result in inflammation and necrosis of the skin (Conning and Hayes, 1970; Patrick et al., 1985; Pullin et al., 1978). As discussed in Section 3.1, phenol is readily absorbed from dermal contact with phenol liquid or phenol vapor, so systemic effects can also result from dermal exposure. Several acute lethality assays have been reported. Conning and Hayes (1970) reported a dermal LD_{50} of 669.4 mg/kg for undiluted phenol applied for 24 hours to the skin of female Alderly Park rats. Acute dermal toxicity appears to be dependent on the concentration of phenol, with increased lethality observed with decreased concentration when the same total dose is applied (Deichmann and Witherup, 1944; Conning and Hayes, 1970). In addition to lethality, renal effects (severe

hemoglobinuria and hematin casts in the tubules), cardiovascular effects (cardiac arrhythmias and ventricular tachycardia), and neurological effects (severe muscle tremors, marked twitching, generalized convulsions, loss of consciousness, and prostration) were observed at 107.1 mg/kg in female Alderly Park rats following dermal exposure to undiluted phenol for 24 hours (Conning and Hayes, 1970). A similar array of effects have been reported in humans following accidental dermal exposures to large volumes of phenol (ATSDR 1998).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

No studies of the reproductive or developmental toxicity of phenol following inhalation exposure of laboratory animals were located. Several developmental toxicity studies in rats and mice conducted via the gavage route are available (Argus Research Laboratories, 1997; NTP, 1983a,b; Narotsky and Kavlock, 1995); the only developmental effect reported in these studies was decreased fetal body weight. In a two-generation drinking water study in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), decreased pup weight and decreased survival of pups pre-culling were observed, but these effects appeared to be secondary to decreased water consumption.

In the Ryan et al study, 30 Sprague-Dawley rats/sex/group were exposed to 0, 200, 1000 or 5000 ppm phenol in drinking water. Parental (P1) rats were given phenol for 10 weeks prior to mating, during a 2-week mating period, throughout gestation, lactation, and until sacrifice. The males were sacrificed after successful mating. All of the P1 females were allowed natural parturition and were sacrificed at F1 weaning. The authors calculated that the average daily phenol intake during week 10 was 0, 14.7, 70.9, and 301.0 mg/kg-day for P1 males and 0, 20.0, 93.0, and 320.5 mg/kg-day for P1 females.

For the F1 generation, the average phenol intake during week 10 was 0, 13.5, 69.8, and 319.1 mg/kg-day for males and 0, 20.9, 93.8, and 379.5 mg/kg-day for females. The F1 generation (20 rats/sex/group) was treated following a protocol similar to that used for the P1 generation, and F2 pups were sacrificed after weaning, on postnatal day (PND) 22. During treatment, rats were monitored for mortality, clinical signs, body weight, and food and water consumption. At sacrifice, the animals were necropsied, and reproductive organs from 20 animals per sex in the control and high-dose groups from the P1 and F1 generations were examined microscopically. In addition, the spleen, thymus, liver, and kidneys from 10 randomly selected P1 and F1 animals of each sex in the control and high-dose groups were examined.

Most of the treatment-related changes in P1 rats were observed in the high-dose groups. No treatment-related mortality was observed in P1 rats of either sex. Three high-dose F1 female pups died shortly after weaning. The deaths appeared to be associated with decreased water consumption associated with poor palatability, as these pups refused to drink the water containing the phenol. No other treatment-related mortality was reported in the F1 generation. The only significant observed clinical sign was redness around the nose fur, which occurred in the high-dose males and females of the F1 generation before mating and in P1 dams during lactation. As noted in the context of the Hoffman et al. (2001) inhalation study, this redness likely reflects a nonspecific stress response. A significant decrease in water consumption was observed throughout the study in P1 animals of both sexes (up to 23% for males and up to 39% for females) and was attributed to poor palatability. Decreased water consumption in the F1 rats at the high dose was of a similar or larger magnitude. The low water consumption at the high dose was accompanied by decreased body weights as compared with the controls (9% in P1 males and 16% in P1 females at sacrifice).

At birth, the high-dose F1 and F2 pups had decreased body weights as compared with the controls, and the differences were larger by PND 21. The authors noted that pups began drinking the water prior to weaning and that the decreased water consumption was also observed in the high-dose pups. Decreased absolute organ weights and increased relative organ weights were observed for a number of organs at the high dose in both the P1 and F1 generations. Most of these changes likely reflected the lower body weight and overall dehydration in these groups.

F1 females had a statistically significant, dose-related decrease in uterine weights at all doses, but P1 females were not affected. The authors suggested that some of this decrease may have been related to a lower incidence of uterine dilatation at the high dose. Because the stage in the estrus cycle can affect uterine weight, estrus cycle staging was also considered as an explanation, but the authors did not consider the small decreases in the number of rats in estrus (16/24, 15/24, 13/25, 9/22 in the control, low-, mid-, and high-dose groups) sufficient to account for the decreased uterine weight. Nonetheless, the decreased uterine weight was not considered adverse for several reasons. There was no evidence of a dose-response relationship for relative uterine weight across the three dose groups (Table 8). There was no effect on reproductive function and no histopathological changes in the uterus. Finally, the uterine weight was below the control range for only a few rats in each dose group, and the control group appeared to have greater variability (particularly at the high end) than each of the experimental groups.

Table 8. Selected Results of Two-Generation Drinking Water Study (Ryan et al., 2001; IIT Research Institute, 1999)

Endpoint	Control	200 ppm (14-21 mg/kg-day)	1000 ppm (70-94 mg/kg-day)	5000 ppm (301-380 mg/kg-day)
P1 body weight - males week 10 (g)	414±39.8	434±34.4	423±40.3	382±40.8*
P1 body weight - females week 10 (g)	259±24.5	260±21.8	258±19.3	234±21.2*
P1 water consumption - males week 10 (g/day)	30±4.6	32±4.6	30±3.3	23±3.1*
P1 water consumption - females week 10 (g/day)	24±3.7	26±5.5	24±4.1	15±2.1*
F1Absolute uterine weight (g) Uterine weight relative to body weight	0.81±0.33 0.27±0.11	0.62±0.13* 0.21±0.044*	0.60±0.17* 0.20±0.058*	0.53±0.17* 0.20±0.068*
F1 Absolute prostate weight (g) Prostate weight relative to body weight	0.90±0.20 0.18±0.039	0.77±0.17* 0.15±0.031	0.74±0.17* 0.15±0.027*	0.76±0.16* 0.18±0.036
F1 pnd0 litter weight (g)	6.73±0.42	6.85±0.51	6.63±0.51	6.38±0.27*
F2 pnd0 litter weight (g)	6.67±0.38	6.80±0.44	6.58±0.42	6.20±0.48*
Preputial separation (age, days) Body weight at onset (g)	44.0±2.16 229±23.0	45.4±2.05 236±23.4	44.4±2.02[2] 227±19.7	47.8±3.13* 195±23.6*
Vaginal patency (age, days) Body weight at onset (g)	34.6±1.85 126±13.7	34.5±1.83 127±13.5	34.1±2.25 121±12.6	38.3±2.21* 112±7.8*

[1]Mean ±Standard deviation

[2]Standard deviation reported in Ryan et al. (2001) has a typographical error; correct standard deviation obtained from IIT Research Institute (1999)

*Statistically significant, p 0.05

Absolute prostate weights were significantly reduced—by approximately 15%—in all dose groups of the F1 generation but not the P1 generation. Relative prostate weight was decreased in the low-dose (but not statistically significantly) and mid-dose F1 groups but not at the high dose. In the absence of a dose-response, the changes in prostate weight were not considered adverse. Dose-related statistically significant decreases in absolute adrenal and spleen weights were also observed in the mid- and high-dose F1 males. However, there was no effect on relative weights of these organs in these groups, there was no effect on these organ weights at any dose in the P1 males, and P1 females were only affected at the high dose (where decreased body weight would have played a role).

The pathological examinations showed no treatment-related lesions in the kidneys, spleen, liver, thymus, or reproductive organs. An immunotoxicity screen conducted with 10 male P1 rats per group found no significant effects on spleen weight, cellularity, or antibody-forming cells for any test group when compared with the control group; the expected results were found with a positive control group. The strength of this finding is reduced, however, by the relatively large standard variability in response (based on the standard error as a percent of the mean).

Complete hematological evaluations (including hematocrit, erythrocyte count, and differential white cell count) and serum biochemical evaluations were conducted on 8–10 P1 males/group prior to sacrifice. The only significant change in these evaluations was increased blood urea nitrogen (BUN) in the high-dose group. Because this change was not accompanied by increased creatinine and there was no associated kidney pathology, the BUN increase was not considered to be biologically significant. The authors also noted that all but one of the high-dose BUN values were within the control range.

There was no effect on fecundity or fertility in either generation. In addition, there was no effect on other indicators of reproductive toxicity, including the frequency of estrus, testicular sperm count, sperm motility, and sperm morphology. The survival of the high-dose F1 pups was significantly decreased on PND 4 (pre-culling), although there was no effect on overall F1 pup survival. In the F2 generation, high-dose pup survival was significantly decreased throughout the lactation period. This decreased survival of both generations of pups was likely secondary to the decreased maternal water intake and associated decreases in milk production. In the F1 generation, delayed vaginal patency and delayed preputial separation were observed at the high dose. The delay was considered secondary to decreased fetal growth at the high dose resulting from decreased water consumption due to poor palatability and associated decreased food consumption.

Thus, all of the adverse systemic and reproductive effects of phenol in this study occurred at the high dose, and they appear to be secondary to decreased water consumption due to poor

palatability rather than to a toxic effect of phenol. On the basis of the decreased parental and pup body weight (compared to the controls) and decreased pup survival, the high dose is a LOAEL. The study NOAEL is 70.9 mg/kg-day (based on the NOAEL corresponding to the lowest LOAEL in this study, in P1 males). BMD modeling was not conducted for this study because the observed effects appeared to be secondary to decreased water consumption and not reflective of phenol toxicity.

In an unpublished developmental toxicity study conducted according to GLP guidelines (Argus Research Laboratories, 1997), pregnant Crl:CDRBR VAF/Plus Sprague-Dawley rats (25 per group) received phenol by oral gavage on GDs 6 through 15.[6] Dosing was three times daily with 0, 20, 40, or 120 mg phenol/kg/dosage using a dosing volume of 10 mL/kg. The corresponding daily doses were 0, 60, 120, and 360 mg/kg-day. The authors noted that the test material was 90% phenol United States Pharmacopeia (USP); the authors adjusted the dosage calculations for test material purity.

The exposed dams were observed twice a day for viability and daily for clinical signs, abortions, and premature deliveries. In addition, the maternal body weights were recorded every day, and food consumption was also recorded periodically (every 1–2 days). The rats were sacrificed on GD 20, and gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. Each fetus was weighed, sexed, and examined for gross external alterations. One-half of the fetuses were examined for soft tissue alterations, and the rest were examined for skeletal alterations.

One high-dose dam died on GD 11. The study authors attributed this death to phenol treatment because it occurred only at the high dose, although there were no adverse clinical observations and no abnormal necropsy findings in this animal. Other high-dose animals exhibited excess salivation and tachypnea (rapid breathing). There were no other treatment-related clinical observations and no treatment-related necropsy findings. Dose-dependent decreases in body weight of the exposed animals as compared with the controls were observed. Statistically significant decreases in both maternal body weight (8%) and body weight gain (38% for GD 6–16) were observed at the high dose; although a statistically significant decrease in body weight gain (11%) was

[6]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for developmental toxicity studies, it is well documented, and it contributes useful information to the hazard identification and dose-response portions of the assessment. The study was designed to meet the U.S. EPA Pesticide Assessment Guidelines, Subdivision F, 83-3.

observed at the mid dose, the decrease at the mid dose (relative to controls) in absolute maternal weight at the end of dosing (3%) was not statistically significant. Dose-dependent decreases in food consumption were also observed during the dosing period (see Table 9).

Fetal body weights in the high-dose group were significantly lower than those of the controls, by 5–7%. The high-dose group had a statistically significant decrease in ossification sites on the hindlimb metatarsals, but it is unlikely that this small change is biologically significant. The incidence of litters with incompletely ossified or unossified sternal centra was 0/23, 0/25, 3/23, and 3/24; this increase was not statistically significant (Table 9). There were small, dose-related increases in the number of litters with fetuses with "any alteration" and with "any variation" at 120 mg/kg/day and higher. However, neither of these changes was statistically significant, and the response was not clearly dose-related. In addition, an increase in total variations is of questionable significance in the absence of any increase in individual variations. There were no other treatment-related effects on uterine contents, malformations, or variations.

The maternal NOAEL was 60 mg/kg-day, based on small decreases in maternal body weight gain at 120 mg/kg-day, and the developmental NOAEL was 120 mg/kg-day, based on decreased fetal body weight and delayed ossification at 360 mg/kg-day. Benchmark dose (BMD) modeling was also conducted for the decreased maternal weight. Defining the BMR as a one standard deviation decrease in maternal body weight gain, the BMDL was 93 mg/kg-day. Details on the BMD modeling are provided in Appendix B.

In a well-designed developmental toxicity study (NTP, 1983a), timed-mated CD rats were administered phenol by gavage at 0, 30, 60, or 120 mg/kg-day in 5 mL/kg distilled water on GDs 6 to 15 and sacrificed on GD 20. Females were weighed on GDs 0, 6–15 (prior to daily dosing), and 20 (immediately following sacrifice), and they were also observed during treatment for clinical signs of toxicity. A total of 20–22 females per group were confirmed to be pregnant at sacrifice on GD 20. The dams were evaluated at sacrifice for body weight, liver weight, gravid uterine weight, and status of uterine implantation sites. Live fetuses were weighed, sexed, and examined for gross morphological abnormalities and malformations in the viscera and skeleton.

The results of this study did not show any dose-related signs of maternal toxicity or any clinical symptoms of toxicity related to phenol treatment. The number of implantation sites was slightly higher in the dosed groups, but this change could not be treatment related because implantations in this strain

Table 9. Key Results in Argus Research Laboratories, (1997) Rat Developmental Toxicity Study

	Control	60 mg/kg-day	120 mg/kg/day	**360 mg/kg-day**
Maternal body weight GD 16 (g)	363.9±22.2	359.0±18.7	354.3±17.5	**334.2±20.1****
Maternal body weight gain (GD 6-16) (g)	64.0±10.7	58.0±9.4	56.8±10.8*	**39.8±9.5****
Maternal food consumption (GD 6-16) (g/day)	26.7±2.7	26.0±2.6	24.8±1.9**	**21.9±2.1****
Fetal body weight - all fetuses (g)	3.62±0.30	3.63±0.24	3.60±0.30	**3.41±0.35***
Fetal body weight - males (g)	3.71±0.30	3.73±0.25	3.71±0.31	**3.53±0.34**
Fetal body weight - females (g)	3.53±0.30	3.53±0.25	3.49±0.28	**3.29±0.35****
% Fetuses with any alteration/litter	1.0±2.6	2.4±4.6	3.7±4.4	**4.1±7.8**
% Fetuses with any variation/litter	1.0±2.6	2.4±4.6	3.4±4.4	**3.8±7.3**
Incompletely ossified or unossified sternal centra litter incidence	0	0	37337	**37338**
Ossification sites per fetus per litter, hindlimb metatarsals	4.00±0.00	4.00±0.00	4.00±0.00	**3.98±0.07***

[1]Mean ±Standard deviation

*Statistically significant, p 0.05

**Statistically significant, p 0.01

take place prior to GD 6 (prior to dosing). Significant increases in the litters with nonlive (dead plus resorbed) pups were observed in the low- and mid-dose groups but not in the high-dose group (Table 10). Because this response was not dose dependent, and the response in the high-dose group was comparable to that in the control group, this observation is not considered treatment-related. In addition, there was no effect on the more appropriate measure of nonlive pups per litter.

There was also no effect on live fetuses, sex ratio, malformations, or variations, but there was a clear dose-related downward trend in fetal body weight, although the changes at the two lower doses were small and the effect was statistically significant only at the high dose (Table 10). Fetal body weights in the high-dose group were 93% of the average of those in the control group; fetal body weights were not reported separately for males and females. Historical control data from the supplier report the average fetal body weight in this strain as being well below the weight in the high-dose group (Charles River Laboratories, 1988). (Concurrent control weight was 4.14 g, high-dose weight was 3.84 g, and historical control weight was 3.39 g.) The litter size in the high-dose group was also somewhat higher (but not statistically significantly) than in the controls, possibly contributing to the smaller fetal weight at the high dose.

As shown in Table 10, the total pup burden (total fetal weight) and the gravid uterine weight were highest in the low-dose group and then in the high-dose group; both values higher than those in the control group. In addition, the treatment-period maternal weight gain was very similar in the control and high-dose groups (but higher in the low-dose group), but the absolute maternal weight gain (i.e., adjusted for the gravid uterine weight) was much lower in the high-dose group than in the controls. The results from the low-dose group suggest that the dams could have borne a somewhat higher burden of the total in utero package. However, the results also suggest that the dams were near the limit of what they could carry, considering the lower absolute weight gain but unaffected treatment-period weight gain in the high-dose group. No dose-related signs of maternal toxicity and no clinical symptoms of toxicity related to phenol treatment were observed in this study.

From these considerations and the potential for the decreased fetal weight to reflect primarily the larger litter size, the decreased fetal weight in this study could be considered an equivocal LOAEL. Thus, on the basis of decreased fetal body weight, the mid dose in this study of 60 mg/kg-day was a NOAEL for developmental toxicity and the high dose of 120 mg/kg-day was an equivocal LOAEL. The high dose was a maternal NOAEL. BMD modeling could not be done for the decreased fetal weight because NTP did not have information on the fetal weight by sex,

Table 10. Key Results from Developmental Toxicity Study in Rats Administered Phenol by Gavage (NTP, 1983a)

	Control	30 mg/kg-day	60 mg/kg-day	120 mg/kg-day
Live fetuses/litter[1]	12.23±0.51	13.32±0.51	12.14±0.56	13.75±0.62
No. litters with nonlive (%)	0 (0%)	5 (22.7%)	7 (31.8%)	3 (15%)
Nonlive/liver	0	0.41±0.18	0.32±0.10	0.25±0.14
Historical control data for this strain on nonlive/litter (Implants/pregnant female minus live fetuses/pregnant female) (Charles River Laboratories, 2001): 0.6-0.8, based on 62 studies.				
Males/litter	6.23±0.38	7.36±0.52	5.14±0.56	7.35±0.57
Average fetal body weight per litter (g)	4.14±0.07	4.10±0.05	4.03±0.07	3.84±0.05**
Historical control fetal weight (from Charles River Laboratories, 1988)	Mean 3.39 g Range 3.04-3.52 g			
Total ave. fetal weight/dam (g) (calculated)	50.6	54.6	48.9	52.8
Gravid uterine weight (g)	76.9±3.0	82.9±3.4	75.4±3.2	81.7±3.7
Treatment period maternal weight gain (g)	41.0±1.1	47.2±1.7**	40.2±1.6	41.2±2.9
Absolute maternal weight gain (adjusted for gravid uterine weight) (g)	58.0±3.1	58.4±2.2	52.7±2.4	51.8±3.2

[1]Mean ±Standard error of the mean.

**Statistically significant, $p<0.01$

either in the report or in its archives.[7] Data on fetal weight by sex is needed for meaningful modeling because the average weight of males and females is different and the number of males per group varied.

The preliminary rat developmental toxicity studies (NTP, 1983a) found that phenol toxicity is increased by the use of small dosing volumes. For example, when phenol was administered by gavage on GDs 6–15 to pregnant CD rats at doses of 0, 125, 160, 200, or 250 mg/kg-day in a volume of 1 mL/kg, the mortality was 0% (0/9), 70% (7/10), 78% (7/9), 100% (9/9), and 100% (9/9), respectively. The deaths were preceded by dose-related signs of toxicity, including tremors, convulsion, and respiratory distress; mottled liver and congested lungs were found on necropsy. In contrast, when the same doses were administered in a volume of 5–7.5 mL/kg, the respective mortality was only 0% (0/24), 0% (0/5), 17% (1/6), 17% (1/6), and 71% (5/7), respectively. On the basis of these results, a volume of 5 mL/kg was used in the main developmental toxicity study.

In preliminary toxicity studies conducted with doses of 60–250 mg/kg-day in a volume of 5–7.5 mL/kg, decreased maternal body weight gain (or body weight loss) during dosing was observed at 160 mg/kg-day and up, doses at which mortality was also observed. In addition, tremors were observed sporadically in the phenol-dosed groups, without any clear dose-response. There were no treatment-related changes in prenatal viability, fetal sex ratio, or fetal structural development.

The study authors stated that when results of all of the preliminary studies were pooled, a statistically significant trend of decreasing fetal weight was observed, but there were no statistically significant differences from controls in pairwise analyses. The power of the pairwise tests was limited because only 4–6 litters were produced in the dose range 100–200 mg/kg-day.

In a standard mouse developmental toxicity study (NTP, 1983b), phenol was administered by gavage in water at 0, 70, 140, or 280 mg/kg-day on GDs 6 to 15 in a volume of 10 mL/kg. Groups of 31–36 plug-positive female CD-1 mice were used in each treatment group. The pregnancy rate in the controls was only 83%; the pregnancy rate in dosed animals ranged from approximately 83% in the low- and mid-dose groups to 71% at the high dose. In addition, 4/36 high-dose mice died; no deaths occurred in any other groups. The average maternal body weight gain during treatment was statistically significantly reduced at the high dose, as was the maternal body weight at terminal sacrifice on GD 17 (by 10%, compared with the control group). In addition, tremors were observed at the high dose throughout the dosing period. As in the rat study, there was a highly statistically significant decrease in fetal body weight per litter (18%) at the high dose. An increased incidence of cleft palate was also reported at the highest dose level, although the incidence was not significantly

[7]Michael Shelby, NTP, personal communication to Lynne Haber (TERA), March 13, 2002.

different from that of the other groups and there was no statistically significant increase in the incidence of litters with malformations. There was no other evidence of altered prenatal viability or structural development.

Thus, the high dose of 280 mg/kg-day was a maternal frank effect level based on the observed deaths; tremors and decreased body weight also occurred at this dose. The high dose was also a developmental LOAEL based on decreased fetal body weight (accompanied by a possible increase in the incidence of cleft palate) in the fetuses, an effect that was likely secondary to the severe toxicity in the dams. The study NOAEL for maternal and developmental toxicity was 140 mg/kg-day.

The series of oral screening studies mentioned above (Narotsky and Kavlock, 1995; Berman et al., 1995; Moser et al., 1995; MacPhail et al., 1995) also included a developmental toxicity screening study in which groups of pregnant F344 rats (15–20 animals/group) were given phenol at doses of 0, 40, or 53.3 mg/kg by gavage once daily on GDs 6–19 and then sacrificed on postnatal day (PND) 6 (Narotsky and Kavlock, 1995). The dosing volume was 1 mL/kg. Pups in each litter were examined and counted on PND 1, 3, and 6 and were weighed on PND 1 and 6. Uterine implantation sites were counted after the dams were sacrificed. Only minimal quantitative data were presented. No maternal deaths were observed. The authors reported that phenol treatment altered respiration (rales and dyspnea) at both dose levels, but no quantitative data were presented. Decreased (but not statistically significant) maternal body weight (compared with the controls) and decreased (statistically significant) maternal body weight gain were also reported at both doses, but there was no clear dose-response.

No statistically significant evidence of developmental toxicity due to phenol exposure was observed. The only evidence of developmental toxicity came from dams that exhibited severe respiratory signs. These signs of developmental toxicity included a dose-dependent increase in full-litter resorptions (one at the low dose and two at the high dose) and an excessive incidence of perinatal mortality and reduced pup weights on PND 1 in one litter at the high dose. However, these changes as a group were not significantly different from those in the controls. Nonetheless, the respiratory effects from oral dosing reported in this study are of interest, particularly as they were not reported in the related study of systemic toxicity (Moser et al., 1995). This difference may reflect differences in the completeness of the study reporting. Alternatively, it may suggest that pregnant females may be more sensitive than nonpregnant females to the toxic effects of phenol.

4.4. OTHER STUDIES

4.4.1 Initiation/PromotionStudies, Other Short-Term Tumorigenicity Assays, and Cancer Mechanism Studies

Several studies have tested the promotion potential of dermally administered phenol. These studies found that phenol promotes tumors initiated with dimethyl=benzanthracene (DMBA), but the phenol doses tested caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959) and thus were well above the MTD. One study (Wynder and Hoffman, 1961) found promotion of DMBA-initiated tumors by dermally administered phenol at a concentration that caused "no toxic reactions."

Salaman and Glendenning (1957) conducted an initiation/promotion study in which groups of 20 male "S" strain mice were initiated with a single dermal treatment with DMBA and promoted with dermal treatment with 0.5 mg/mouse phenol in acetone using two different treatment concentrations for the same applied dose. The phenol was applied beginning 3 weeks after the DMBA application for either 24 weeks in a volume of 0.025 mL as a 20% solution or for 32 weeks in a volume of 0.1 mL as a 5% solution (rotating the weekly applications among four application sites for both concentrations). The study did not report whether the application site(s) were covered or whether the animals were restrained from licking the site.

The high concentration produced local ulceration that healed just in time for the next treatment 4 weeks later, whereas the low concentration produced only transient light crusting that tended to decrease as the experiment progressed. It is unclear how severe the skin effects would have been if the low concentration had been repeatedly applied to the same site rather than being rotated among four sites. Tumors were observed in both treatment groups, with a shorter time to first tumor and a higher tumor burden in the group treated with the higher phenol concentration. A few histologically confirmed malignant tumors (primarily squamous epitheliomas) were observed in both groups. In mice that underwent the same phenol treatment but were not pretreated with DMBA, seven papillomas were observed at the high concentration. No tumors were observed at the low concentration, even though the weekly dose was the same and the total dose per mouse was higher (because the duration was longer).

This study had no control group on DMBA-only group, but the absence of tumors at the low concentration indicates that the observed tumors were phenol related. The authors noted that the observed tumors might have been related to the significant skin injury produced by phenol. This suggestion is supported by the strong effect of the concentration of applied phenol at the same total dose.

Boutwell and Bosch (1959) conducted a series of initiation/promotion studies with different strains of mice. The mice were pretreated with a single application of 75 μg DMBA in benzene followed by 5% or 10% phenol (1.25 or 2.5 mg per application) in benzene or with either dose of phenol alone twice weekly for 52 to 72 weeks. An additional group received DMBA alone, apparently followed by benzene vehicle, although there is some inconsistency between the text and the summary tables regarding whether the control group received the benzene vehicle. At the high phenol dose, dermal treatment with phenol resulted in decreased body weight (compared with the controls) and decreased survival. Skin wounds, hair loss, and reparative hyperplasia were also seen at the high dose, with the wounds predominantly seen in the first 6 weeks of treatment. By contrast, the authors stated that there was no evidence of ill effects of 5% phenol except for its promoting activity. This statement was based on external observation; no histopathology was conducted.

A dose-related increase in papillomas and in carcinomas was observed in the groups initiated with DMBA and promoted with phenol. Increased papillomas were also observed in one strain treated with the high dose of phenol alone. There was evidence of decreased activity when phenol was further purified, indicating that the activity was not due to a contaminant. Because the benzene vehicle is a defatting agent, it is unclear whether it would have contributed to the effect of phenol.

Wynder and Hoffmann (1961) also found that dermally applied phenol is a promoter. Female Swiss mice (28–30/group) were initiated with a single application of DMBA followed by treatment with 5% phenol three times weekly or 10% phenol two or three times weekly. The dilution vehicle was not reported. "No toxic reactions" were reported at 5% phenol, although the higher concentration was reported to be "rather toxic." Treatment was for 12 months, and the mice were observed for an additional 3 months; the percentage of animals with papillomas and with cancers was recorded monthly.

At 10 months, papillomas were seen in 33% of the low-dose group and > 80% of the high-dose group; cancer was seen in 3% of the low-dose animals and 30–60% of the high-dose groups. By contrast, there were no papillomas or cancers in female Swiss mice treated with phenol alone and only 10% papillomas (no cancer until week 12, and only 7% of the animals had cancer at study termination) in the mice treated with DMBA alone. Survival decreased markedly after week 10 in the high-dose groups but not the other groups. In another experiment, the onset of tumor formation was much earlier in mice treated with 0.005% benzo[a]pyrene three times weekly plus 5% phenol twice weekly than in mice treated with benzo[a]pyrene alone. Papillomas were observed by the second week in the groups receiving benzo[a]pyrene and phenol and were present in at least 33% of the animals by week 5, compared with 3% of the mice at week 5 in the benzo[a]pyrene-only group.

In a short-term assay, Stenius et al. (1989) found that phenol did not increase the production of gamma-glutamyl transpeptidase (GGT)-positive foci. Groups of 7–9 male Sprague-Dawley rats were partially hepatectomized and treated with diethylnitrosamine and then with 100 mg/kg phenol by gavage for 5 days (gavage volume not reported). Phenobarbital, the positive control, produced a marked increase in GGT-positive foci. This assay was based on the assumption that GGT-positive cells in enzyme-altered foci represent initiated cells and the observation that these cells are often resistant to toxic effects.

A decrease in tumor formation was seen in a co-carcinogenesis study of phenol and benzo[a]pyrene (Van Duuren and Goldschmidt, 1976; Van Duuren et al., 1971, 1973). Phenol was applied at a dose of 3 mg/application in acetone to the clipped skin of female ICR/Ha Swiss mice (50/group) three times a week for 1 year simultaneously with 5 μg of benzo[a]pyrene. The resulting number of tumors (both papillomas and squamous cell carcinomas) was markedly lower than in the animals receiving the benzo[a]pyrene alone. Phenol alone did not cause skin tumors. Neither the application volume nor the application surface area were reported, and no information was provided on any skin effects other than tumors.

In a test of a (TG •AC) transgenic mouse line carrying a v-Ha-ras gene fused to a ζ globin promoter, Spalding et al. (1993) found that phenol did not produce papillomas. This strain has genetically initiated skin and has been shown to be sensitive to the known promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Five male mice were dermally treated twice per week for 20 weeks with 3 mg phenol. Only one papilloma was observed; in contrast, strongly promoting agents produced five or more papillomas per mouse. This dose did, however, cause chronic irritation and hair loss.

A number of studies have investigated the reason why benzene is carcinojenic but orally administered phenol is not, in light of the fact that when phenol and many of its metabolites are significant products of benzene metabolism. Medinsky et al. (1995) noted that, on the basis of the urinary metabolite profile, higher levels of hydroquinone are produced after benzene exposure than after exposure to comparable doses of phenol. The potential production of other toxic metabolites, such as muconaldehyde, following benzene exposure but not phenol exposure was also noted. The authors explained the different metabolite profiles of phenol and benzene using the zonal distribution of metabolizing enzymes in the liver. As described in Section 3.3, phenol is conjugated in the gut and in zone 1 of the liver. This reduces the amount of phenol that reaches zone 3 of the liver, where oxidative activity is highest, and so decreases hydroquinone production. By contrast, conjugation of benzene in the gut and zone 1 is low, because benzene must be oxidized prior to conjugation. This

results in more free phenol reaching zone 3 of the liver following benzene exposure than after phenol exposure, and hence more production of hydroquinone.

Equivocal or negative results were obtained with phenol in a well-conducted and well-controlled interlaboratory study evaluating the usefulness of the Chinese hamster V79 cell metabolic cooperation assay for detecting tumor promoters (Bohrman et al., 1988). The study authors noted, however, that the assay was conducted in the absence of exogenous metabolic activation, and V79 cells have low intrinsic metabolic capacity.

Miyagawa et al. (1995) conducted a validation test in male B6C3F1 mice of the in vivo-in vitro replicative DNA synthesis test. The test was based on the hypothesis that nongenotoxic carcinogens are likely to increase cell proliferation. Phenol was negative in this assay, which was conducted at 0, 300, and 600 mg/kg administered via oral gavage.

4.4.2. Genotoxicity

The genotoxic potential of phenol appears to depend on the competing processes of activation to a genotoxic form and metabolic inactivation (e.g., via conjugation). Phenol tended to be negative in bacterial gene mutation assays (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983) but was positive or equivocal in mammalian cell gene mutation assays (McGregor et al., 1988a,b; Paschin and Bahitova, 1982; Tsutsui et al., 1997) (Table 11). Phenol tended to induce micronuclei in mice when administered intraperitoneally (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b) but was negative (or positive only at very high doses) when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in in vitro micronucleus tests with human lymphocytes (Yager et al., 1990) and Chinese hamster ovary (CHO) cells (Miller et al., 1995) and caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Results from DNA damage assays are inconsistent, but they tend to show that phenol can cause sister chromatid exchanges (Erexson et al., 1985; Ivett et al., 1989) or cell transformation (Tsutsui et al., 1997) if it is not metabolically inactivated.

Phenol was negative in a well-conducted assay *Salmonella typhimurium* reverse mutation assay performed with up to cytotoxic doses in the presence and absence of varying concentrations of S9 activation with strains TA1535, TA1537, TA1538, TA98, and TA100 (Pool and Lin, 1982). Phenol was tested in two independent laboratories as part of a large-scale test by NTP in salmonella strains TA1535, TA1537, TA98, and TA100 in the presence and absence of S9 activation (Haworth et al., 1983). Both laboratories found that phenol was negative. Rapson et al. (1980) also reported

that phenol was negative in a test in strain TA100, although no primary data were presented and it was unclear whether sufficiently high doses were tested. A weak positive response was reported with phenol in strain TA98 in the presence of S9 but not in the absence of S9 (Gocke et al., 1981). Other strains were also tested in that assay, but the results were unclear.

Positive or equivocal results have been reported in mammalian cell gene mutation assays. McGregor et al. (1988a, b) reported on a well-conducted mouse lymphoma L5178Y tk+/tk- assay of phenol performed as part of a test of 72 coded chemicals. In the absence of S9, the results were considered questionable or inconclusive in two independent assays because of the absence of a dose-related trend and increases occurring only in the presence of high cytotoxicity. In the presence of S9, the first test was questionable (no dose-related trend but statistically significant results at several doses), but a clear positive result was obtained in the confirmatory test. Overall, the study authors concluded that no definitive conclusion was possible.

Table 11. Summary of Genotoxicity Studies

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
In Vitro Studies				
Gene mutation-bacteria	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100	-/-	Tested to cytotoxic doses, varying S9 concentrations	Pool and Lin, 1982
	Salmonella typhimurium TA1535, TA1537, TA98, TA100	-/-	Part of NTP testing; tested in 2 laboratories	Haworth et al. 1983
	Salmonella typhimurium TA100	-1	Unclear if sufficiently high doses tested	Rapson et al. 1980
	Salmonella typhimurium TA98	-/w [2]	Other strains also tested, but results with them unclear	Gocke et al. 1981
Gene mutation - mammalian cell	Mouse lymphoma L5178Y cells	?/?[2]	Two independent assays conducted +/- S9	McGregor et al. 1988a, 1988b
	Chinese hamster V79 cells	NT[2]/+	S9 from phenobarbital-induced mice	Paschin and Bahitova 1982
	Syrian hamster embryo (SHE) cells	NT/+	None	Tsutsui et al. 1997
Clastogenicity	Micronuclei in human lymphocytes	+/NT	None	Yager et al. 1990
	Micronuclei in CHO cells	+/+	S9 from phenobarbital/beta-naphthoflavone induced rats	Miller et al. 1995
Chromosome aberration	CHO cells	-/+	Part of NTP testing	Ivett et al. 1989

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
DNA damage	Sister chromatid exchange, human lymphocytes	-/NT	Unclear if sufficiently high doses tested	Jansson et al. 1986
	Sister chromatid exchange, human lymphocytes	+/NT	Small increases	Morimoto et al. 1983; Morimoto and Wolff 1980
	Sister chromatid exchange, human lymphocytes	+/NT	None	Erexson et al. 1985
	Sister chromatid exchange, CHO cells	+/w	Part of NTP testing	Ivett et al. 1989
	Unscheduled DNA synthesis, SHE cells	+/NT	None	Tsutsui et al. 1997
	Single strand breaks mouse lymphoma cells	-	None	Pellack-Walker and Blumer, 1986
	Single strand breaks CHO cells	-	None	Sze et al. 1996
Cell transformation	Syrian hamster embryo (SHE) cells	+/NT	None	Tsutsui et al. 1997
In Vivo Studies				
Gene mutation	*Drosophila* sex-linked recessive lethal	-	None	Gocke et al. 1981
	Drosophila sex-linked recessive lethal	-	None	Sturtevant 1952
	Drosophila sex-linked recessive lethal	-	None	Woodruff et al. 1985
Clastogenicity	Mouse micronucleus i.p.	+	None	Marazzini et al. 1994

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
	Mouse micronucleus i.p.	+	Weak response	Chen and Eastmond 1995a
	Mouse micronucleus i.p.	-	No positive control, unclear if sufficiently high doses tested	Barale et al. 1990
	Mouse micronucleus i.p.	-	Small sample size	Gocke et al. 1981
	Mouse micronucleus oral	w	None	Ciranni et al. 1988b
	Mouse micronucleus i.p.	+	Clear effect at same dose as oral study	Ciranni et al. 1988b
	Mouse micronucleus oral	-	Number tested not reported	Gad el-Karim et al. 1985
	Mouse micronucleus oral	+	Pregnant females on GD13	Ciranni et al. 1988a
Chromosome aberration	Mouse, spermatogonia and spermatocytes	+	Inconsistencies in reporting	Bulsiewicz 1977
DNA damage	Single strand breaks, testicular cells, i.p.	-	None	Skare and Schrotel 1984

[1]Apparently in the absence of S9 - the presence of absence of S9 was not addressed.
[2]w = weak positive response; ? = questionable or inconclusive; NT = not tested

Other authors have also reported positive results in mammalian gene mutation assays. Paschin and Bahitova (1982) found that phenol was mutagenic in an in vitro assay for mutagenicity at the HGPRT locus of V79 Chinese hamster cells in the presence of S9 from the livers of phenobarbital-induced mice. Tsutsui et al. (1997) also reported that phenol induced a dose-related increase in mutation frequency in Syrian hamster embryo cells.

In contrast with these positive results in mammalian cells, a number of authors (Gocke et al., 1981; Sturtevant, 1952; Woodruff et al., 1985) found that phenol was negative in sex-linked recessive lethal assays conducted in drosophila using the feeding and injection routes.

The potential for phenol to induce micronuclei (a measure of clastogenicity) appears to be related to the route of dosing, with generally positive results when phenol is administered intraperitoneally but negative or equivocal results when it is administered orally. This route-related difference is likely due to the potential for first-pass detoxification of phenol when it is administered via the oral route but not when administered intraperitoneally. Several authors have suggested that the chromosomal effects of phenol result from phenol interactions with the spindle apparatus (Bulsiewicz, 1977; Yager et al., 1990). No in vitro studies of phenol clastogenesis were located.

Phenol was positive in the micronucleus test in male mice at an i.p. dose of 120 mg/kg (Marrazzini et al., 1994). Similarly, Chen and Eastmond (1995a) reported a weak increase in bone marrow micronuclei of male CD-1 mice treated with up to 160 mg/kg-day phenol intraperitoneally for 3 days. By contrast, Barale et al. (1990) found that phenol was negative when administered at i.p. doses of up to 160 mg/kg to male CD-1 mice. However, it is unclear whether sufficiently high doses were tested in that study, since no cytotoxicity and no clinical signs of toxicity were reported; also, the study did not include a positive control. Gocke et al. (1981) also found that phenol was negative in male and female NMRI mice treated with i.p. phenol at two daily doses of up to 188 mg/kg per dose, although the sample size of tested animals was small.

Ciranni et al. (1988b) found that an oral dose of 265 mg/kg phenol caused a slight increase in micronuclei and some myelotoxicity (decreased ratio of polychromatic to normochromatic erythrocytes, PCE/NCE ratio), but i.p. administration of the same dose caused clear increases in micronuclei and stronger myelotoxicity. Gad-el-karim et al. (1985) reported that a single oral dose of phenol (250 mg/kg) was negative for micronucleus formation in male CD mice, but they did not report the number of animals tested. This difference between the effects of i.p. and oral administration of phenol is also consistent with the metabolic effects of first-pass metabolism mentioned in Section 3.3.

Ciranni et al. (1988a) found that a single gavage dose of 265 mg/kg phenol caused a small but statistically significant increase in bone marrow micronuclei—accompanied by cytotoxicity—in pregnant female CD-1 mice treated on GD 13. There was no effect on fetal liver. Although no positive control was included, benzene did cause micronuclei in fetal liver, confirming the sensitivity of the assay.

Phenol was positive in an in vitro assay for the development of micronuclei in human lymphocytes in the absence of exogenous metabolic activation, although the dose-response was weak (Yager et al., 1990). Miller et al. (1995) also found that phenol was positive in an in vitro micronucleus test in CHO cells in the presence or absence of S9 from livers of phenobarbital/beta-naphthoflavone induced rats, although a stronger response was observed in the presence of S9.

Phenol was evaluated in the chromosome aberration assay in CHO cells as part of a series of tests by NTP to evaluate genotoxicity assays (Ivett et al., 1989). No significant increase was observed in the absence of S9 activation. In the presence of S9, significant increases in both simple and complex aberrations were observed. A delayed harvest time was used due to cell cycle delay.

In a five-generation study of chromosome aberrations in spermatogonia and spermatocytes in Porton strain inbred mice, Bulsiewicz (1977) observed dose-related increases in aberrations that tended to increase with successive generations. Polyploidy was also observed. Three dose groups and a control were treated by oral gavage. The dosing volume was reported as "2 mL of a solution of phenol" (sic) for the low-dose group; volumes were not reported for the other groups. Phenol was reported as being administered in a concentration of 0, 0.08, or 0.8 mg/L per day, or "8 g per liter" (sic).

A number of studies reported synergistic effects between phenol and hydroquinone in the micronucleus assay in mice (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a). At least part of this interaction is likely due to phenol enhancing the peroxidase-dependent metabolic activation of hydroquinone.

A number of studies have evaluated the potential for phenol to cause DNA damage. These studies tend to show that phenol can produce effects when it is metabolized to an active form, but that inactivation is likely to predominate over activation following oral dosing.

Jansson et al. (1986) found no effect on sister chromatid exchanges (SCEs) in an in vitro assay with human lymphocytes, although it was unclear whether sufficiently high doses were tested. Small but statistically significant increases in SCEs in cultured human lymphocytes were reported by Morimoto et al. (1983) and Morimoto and Wolff (1980). By contrast, Erexson et al. (1985) found a

dose-related increase in SCEs in human lymphocytes exposed to phenol in vitro. They attributed the difference between their results and negative results in other studies to differences in the procedure used. In particular, Erexson and colleagues used mitogenic stimulation of the lymphocytes 24 hours prior to the phenol exposure. This mean that the cells were blast-transformed and exposed in the G1-S phase (and so there was less opportunity for repair prior to DNA replication), and cytochrome P450 activity was elevated as a result of the blast transformation. Negative controls showed that the increases were not due to the mitogenic stimulation alone. As part of a series of tests by NTP to evaluate genotoxicity assays, phenol was tested for the induction of SCEs in CHO cells (Ivett et al., 1989). Phenol was positive in the absence of S9 and weakly positive in the presence of S9. Cell cycle delay was observed at all positive doses.

In an assay with Syrian hamster embryo cells, Tsutsui et al. (1997) reported that phenol induced a slight dose-related increase in transformed colonies as well as a dose-related increase in unscheduled DNA synthesis in the same cell line.

In an evaluation of effects on germ cells in vivo, Skare and Schrotel (1984) found no effect on single strand-breaks in testicular cells of Sprague-Dawley rats receiving a single i.p. injection of up to 79 mg/kg or five daily i.p. injections of up to 39.5 mg/kg-day. Phenol also did not induce single strand breaks in mouse lymphoma L5178YS cells (Pellack-Walker and Blumer, 1986) or in CHO cells in a test up to cytotoxic concentrations (Sze et al., 1996).

Reddy et al. (1990) reported that DNA adducts were produced in cultured rat Zymbal glands orally dosed with 750 μg/mL of either phenol or hydroquinone. The adducts were not chemically characterized and their intensities were not quantified, but no spots were observed autoradiographically in the untreated controls. By contrast, many different adducts were seen in the analyzed tissues (bone marrow, Zymbal gland, liver, spleen) from both untreated female Sprague-Dawley rats and from rats treated for 4 days by oral gavage with a dose of 75 mg/kg-day phenol or 150 mg/kg-day of a 1:1 mixture of phenol and hydroquinone. The adduct patterns and levels of adducts did not differ significantly between control and treated animals.

The authors noted that endogenous adducts would interfere with the determination of treatment-induced adducts that chromatograph similarly. To address this possibility, they compared the chromatograms resulting from in vitro and in vivo treatments. The absence of the major in vitro adducts of hydroquinone or benzoquinone in the in vivo samples suggested that these adducts were not formed in the whole animal. Conversely, the primary adduct of phenol formed in vitro was also observed in vivo, although the levels relative to controls were much higher under in vitro conditions.

The authors suggested that the higher level of adducts following in vitro treatment versus in vivo treatment could be attributed to detoxification of orally administered phenol, but they did not further address the possibility that there may be a significant basal load of adducts formed by endogenously produced phenol.

Using a fluorescence *in situ* hybridization approach, Chen and Eastmond (1995a) found that treatment with phenol alone resulted in micronuclei and breaks in euchromatin, whereas hydroquinone affected chromosome loss and chromosomal breakage, particularly in centromeric heterochromatin. They suggested that the different pattern of effects with phenol and hydroquinone indicates that the synergism between phenol and hydroquinone is not due solely to phenol-induced increases in hydroquinone metabolism. Instead, they suggested, phenol or its metabolites may also be inhibiting DNA repair. In a follow-up study, Chen and Eastmond (1995b) found that phenol alone did not affect the DNA repair enzymes topoisomerase I or topoisomerase II in vitro. However, mixing phenol with horseradish peroxidase to mimic the peroxidase metabolism of the bone marrow resulted in complete inhibition of topoisomerase II; no effect was seen when glutathione was added to the peroxidase mixture.

In an assay with NCTC 929 mouse fibroblast cells, Yang and Duerksen-Hughes (1998) found that phenol caused a dose-related increase in levels of the p53 protein. The authors noted that cells increased p53 levels in response to DNA damage.

4.4.3. Neurological Effects

As described above, tremors have been observed following relatively high exposures to phenol via the oral (Dow Chemical Co., 1994; Moser et al., 1995) or inhalation (Dalin and Kristoffersson, 1974) routes. Decreased motor activity and a statistically significant increase in rearing post-exposure were also reported in a screening study with rats (Moser et al., 1995), and altered balance was reported in rats exposed continuously via inhalation for 15 days (Dalin and Kristoffersson, 1974). However, in a 13-week drinking water neurotoxicity study that included extensive neurohistological analyses (ClinTrials BioResearch, 1998), the only observed effects were decreased motor activity and decreased body weight (compared with the controls), which were probably secondary to decreased water consumption as a result of poor palatability. On the basis of the results of a short-term screening study, neurotoxic effects do not occur at lower exposures than other systemic effects of phenol (Berman et al., 1995; Moser et al., 1995).

4.4.4. Immunotoxicity

As described in Section 4.2, Hsieh et al. (1992) reported immune effects in CD-1 mice administered phenol in drinking water for 28 days. The reported effects included decreased antibody response (based on the PFC assay and direct antibody quantification using ELISA), with some evidence of decreased lymphoproliferative response and decreased mixed lymphocyte response. The clearest response was seen in the PFC and ELISA, which are highly predictive of effects on host resistance (Luster et al., 1992, 1993). Confidence in the study results is decreased by the somewhat small sample size (five rather than eight per group). Berman et al. (1995) reported atrophy of the spleen or thymus of rats gavaged with phenol under conditions that greatly enhanced toxicity in comparison with drinking water exposure.

Although no effects on spleen weight, cellularity, or antibody-forming cells in the spleen were observed in a two-generation study of Sprague-Dawley rats exposed to phenol in drinking water at concentrations of up to 5000 ppm (approximately 300 mg/kg-day) (Ryan et al., 2001; reported in unpublished form as IIT Research Institute, 1999), qualitative and quantitative differences between rats and mice in the effects of chemicals on the immune response are not unusual (e.g., U.S. EPA, 2000c). There is also no consistent pattern between mice and rats regarding which species is more predictive of immunotoxic effects in humans. In light of Hsieh et al. (1992) who reported immunotoxic effects of phenol at unusually low doses, it would be useful to confirm the results of that study in mice using a protocol compliant with EPA immunotoxicity test guidelines (U.S. EPA, 1998c).

The National Institute of Environmental Health Sciences (NIEHS) and the NTP have elected to conduct a comprehensive series of tests to evaluate the potential of phenol to adversely affect the immune system (verbal communication from Dori Germolec, NIEHS to Monica Barron, EPA, 2002). Using test protocols designed to be consistent with EPA testing guidelines and GLP standards, the first assay considered the same dosing regimen used in the Hsieh et al. (1992) study. That is, inbred female B6C3F1 mice–rather than outbred CD-1 male mice–were exposed to drinking water concentrations of 0, 5, 20, and 100 mg/L phenol (approximately 0, 1.7, 6.7, and 33 mg/kg-day). The study also included a positive control. Preliminary results demonstrated immunosuppression (reduced antibody response) at all levels of exposure, confirming the Hsieh study outcome.

In order to characterize a wider range of response levels, a second assay was initiated using drinking water concentrations of 0, 1.25, 2.5, 5.0, 20, and 40 mg/L (approximately 0, 0.4, 0.8, 1.7, 6.7, and 13.3 mg/kg-day), overlapping the previous study's range of exposures. In addition, NIEHS/NTP has initiated host resistence studies. Depending on the final outcome of this series of

tests, including NIEHS/NTP peer review, EPA may reconsider and, if appropriate, reopen this assessment.

4.4.5. Other Studies

Eastmond et al. (1987) investigated the role of phenol in benzene-induced myelotoxicity. No suppression of bone marrow cellularity was observed in male B6C3F1 mice treated intraperitoneally with doses as high as 150 mg/kg twice daily for 12 days (daily doses up to 300 mg/kg). Only minimal suppression was observed in mice dosed with hydroquinone at up to 100 mg/kg twice per day. By contrast, marked statistically significant, dose-related suppression was seen in mice treated with 75 mg/kg phenol and 75 mg/kg hydroquinone under the same conditions. In further in vitro studies, the authors showed that phenol stimulates the horseradish peroxidase-mediated metabolism of hydroquinone, and they hypothesized that similar stimulation of local peroxidases occurs in the bone marrow. The observation of myelotoxicity following benzene treatment–but only minimally or not at all following phenol or hydroquinone treatment– was therefore explained by a more-than-additive interaction between phenol and hydroquinone.

Corti and Snyder (1998) evaluated gender- and age-specific differences in cytotoxicity of benzene metabolites in vitro. Bone marrow cells were harvested from adult unexposed male and female Swiss Webster mice as well as from pregnant females and from fetal males and females. Cultures of CFU-e (colony forming units-erythroid, an erythroid precursor cell particularly susceptible to benzene toxicity) were prepared and then exposed to different concentrations of the metabolites. Although most of the benzene metabolites caused marked cytotoxicity, only minimal toxicity (0–20% cytolethality) of phenol was observed up to the highest concentration tested (40 : M), compared with nearly 100% cytolethality at the same concentration of catechol or hydroquinone. The effects were strongest in cells isolated from fetal females or from virgin adult females, but the dose-response was inconsistent, and it appears that no statistical comparisons with the untreated control of the same life stage were done.

Zamponi et al. (1994) studied the mechanism of phenol-induced cardiac arrhythmia, including ventricular tachycardia. In an abbreviated report, the authors suggested that phenol caused cardiac arrhythmia by blocking batrachotoxin-activated cardiac sodium channels. Testing conditions, including doses tested, were not provided in the abbreviated report.

Bishop et al. (1997) investigated the effect of phenol exposure on total reproductive capacity in mice. Groups of 26 female hybrid (SEC x C57BL6) F1 mice were given a single i.p. injection of 0 (buffer solution) or 350 mg/kg of phenol, and the females were caged individually with an untreated male hybrid (C3H/R1 x C57BL10) F1 mouse following the day of injection for 347 days. The

animals were observed daily for producing newborn mice; the young mice were counted and discarded immediately after birth. Female reproductive performance was evaluated on the basis of the total number of offspring per female and the average number of litters per female. The numbers of offspring per female and litters per female in phenol-treated mice were comparable to those in the controls. Thus, phenol had no measurable detrimental effect on the parameters used for evaluating long-term reproductive effects in this study.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

Studies investigating the effects of orally administered phenol are summarized in Table 2. When phenol is administered in drinking water, the most common effect is decreased water consumption, presumably due to poor palatability. Effects seen concurrently with decreased water consumption, and probably secondary to dehydration, include body weights lower than those of the controls, decreased maternal body weight compared with that of developmental toxicity studies, and decreased pup survival prior to culling. Decreased motor activity was also seen in a drinking water neurotoxicity study, but it does not appear to be secondary to the decreased water consumption. The data also indicate that phenol causes immune effects in mice, but not in rats.

Oral exposure to phenol also affects the kidney and liver. Kidney inflammation was observed in the chronic drinking water study in rats (NCI, 1980). Liver and kidney pathology (tubular degeneration, kidney necrosis, and vacuolar degeneration) in rats also has been observed in short-term and subchronic toxicity studies using gavage dosing (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945). These results from experimental animal studies support the observations in case studies and epidemiology studies (Shamy et al., 1994; Merliss, 1972) that the liver is a target of phenol in humans.

A number of nervous system effects have been observed following phenol dosing. Tremors were observed in one animal that later died (apparently of dehydration) following dosing in drinking water (ClinTrials BioResearch, 1998). Tremors have also been observed in several gavage studies in rats and mice (NTP, 1983a; Dow Chemical Co., 1994; Moser et al., 1995). However, in a specialized 13-week neurotoxicity study in male and female rats that included an FOB and a detailed neurohistopathology evaluation (ClinTrials BioResearch, 1998), the only observed nervous system effects were tremors in one animal and decreased motor activity in females. A short-term gavage screening study (Moser et al., 1995) found that the only effect in an FOB was a marginal decrease in motor activity and increased rearing post-exposure.

Headaches and weakness were reported in workers exposed to a mixture of phenol and a number of other aromatic compounds (Baj et al., 1994). Due to the mixed nature of the exposure, the effects cannot be clearly attributed to phenol. Muscle pain in a laboratory technician who developed phenol marasmus after being frequently exposed to phenol vapor (Merliss, 1972) may have reflected neurological damage.

The data regarding the hematotoxic potential of phenol are conflicting. No hematological effects were observed in rats in a well-conducted two-generation study in Sprague-Dawley rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). By contrast, decreased hematocrit and erythrocyte counts were seen at much lower doses in a 28-day drinking water study in mice (Hsieh et al., 1992), although this study is limited by the use of only five males per dose. Data from these two studies are contrasted in Table 6. The differences between the two studies cannot be resolved by considering the results of the chronic drinking water studies conducted by NCI (1980) in mice and rats, because no hematological evaluation was conducted in those studies.

The negative finding in rats following oral exposure are supported by the absence of hematological effects in rhesus monkeys, male Sprague-Dawley rats, and male albino mice (strain not further identified) exposed to an average phenol concentration of 18.2 mg/m^3 continuously for 90 days (Sandage, 1961). Hemoglobin and hematocrit were also unaffected in a small study of rats exposed to phenol in air at 100 mg/m^3 continuously for 15 days (Dalin and Kristoffersson, 1974). Neither of these inhalation studies used modern exposure protocols, and both were limited by inadequate exposure monitoring. However, Dalin and Kristoffersson (1974) did both report systemic effects (nervous system and liver effects) in rats at exposure levels that did not cause hematological effects. Sandage (1961) found an indication of liver and kidney histopathology in the monkeys and rats, although not in the mice. Assuming that all of the inhaled phenol was absorbed (see Section 3.2), the systemic dose to mice in the Sandage (1961) study can be estimated at approximately 30 mg/kg-day, based on a body weight of 0.03 kg and an inhalation rate of 0.052 mg/m^3/day. This dose is comparable to the high dose in the Hsieh et al. (1992) study.

The negative results in the inhalation studies raise further questions about the reliability of the hematotoxicity effects seen by Hsieh et al. (1992) in mice–particularly in the light of the small sample size–as well as the relevance of those results to humans. Because portal-of-entry conjugation is more efficient following ingestion rather than following inhalation of phenol (see Section 3.3), it is not surprising that the systemic toxicity (i.e., liver and kidney effects) of a given absorbed dose may be higher for inhaled phenol. Human data on hematotoxic effects of phenol are limited. Baj et al. (1994) reported a small but statistically significant decrease in erythrocytes in workers exposed to a mixture

containing phenol, chlorinated benzenes, and other compounds. Due to the mixed nature of the exposure, the effect, if any, cannot be clearly attributed to phenol.

The results of Hsieh et al. (1992) also indicate that phenol can be immunotoxic to mice. The investigators observed a clear dose-related decrease in two related measures of antibody formation (the PFC assay and a direct measure of antibody titer using ELISA), along with some evidence of a decreased cell-mediated response at the high dose. Confirmation of their results in a repeat assay conducted according to EPA test guidelines would be useful in light of the small number of animals used and the limited number of risk assessments that have been based on in vivo/in vitro immunotoxicity assays.

No effect on spleen weight, cellularity, or antibody-forming cells (in the PFC assay) were observed in a two-generation study of Sprague-Dawley rats exposed to phenol in drinking water at much higher doses (Ryan et al., 2001; reported in unpublished form as IIT Research Institute, 1999), but qualitative and quantitative differences in effects of chemicals on the immune response of rats and mice are not unusual (e.g., see dioxin, as described in U.S. EPA, 2000c). "Necrosis or atrophy in the spleen or thymus" (not further described) was observed in a 14-day screening study of rats gavaged with phenol (Berman et al., 1995), supporting the immune organs as targets of phenol.

Baj et al. (1994) reported in an epidemiology study of Polish workers that exposure to Ksylamit® vapor resulted in immune effects, but it is unclear whether phenol is the causative agent because Ksylamit® contains a number of different aromatic compounds. Overall, the data indicate that phenol by itself may cause immunotoxicity in humans, but more data are needed to address this possibility. Interaction between phenol and benzene metabolites may also cause immune effects, as described below.

Benzene (which is metabolized to phenol) among other compounds, causes immunological effects, including lymphopenia and leukopenia (reviewed in ATSDR, 1998). However, although benzene is a leukemogen in humans, it has not been shown to induce leukemias in experimental animals. For example, in the NTP gavage studies of benzene (NTP, 1986), it was carcinogenic to both male and female F344 rats and B6C3F_1 mice, inducing tumors at multiple sites. There was a statistically significant increase in lymphomas in male and female mice but not in rats of either sex. No significant increase in leukemias was noted in either species.

One proposed mechanism by which this immunotoxicity is induced involves the interaction between phenol and hydroquinone, in which phenol stimulates the metabolism of hydroquinone. Eastmond et al. (1987) observed decreased bone marrow cellularity in male mice dosed intraperitoneally with phenol and hydroquinone but not with phenol alone at doses of up to 300 mg/kg-day for 12 days and only minimally with hydroquinone. These results appear to contradict those seen

at much lower doses (Hsieh et al., 1992), although the target tissue examined by Eastmond et al. (1987) was bone marrow, whereas Hsieh et al. examined the spleen. The former study was also conducted via the i.p. rather than oral route, but toxicity might be expected to be higher via the i.p. route because first-pass metabolism would be lower.

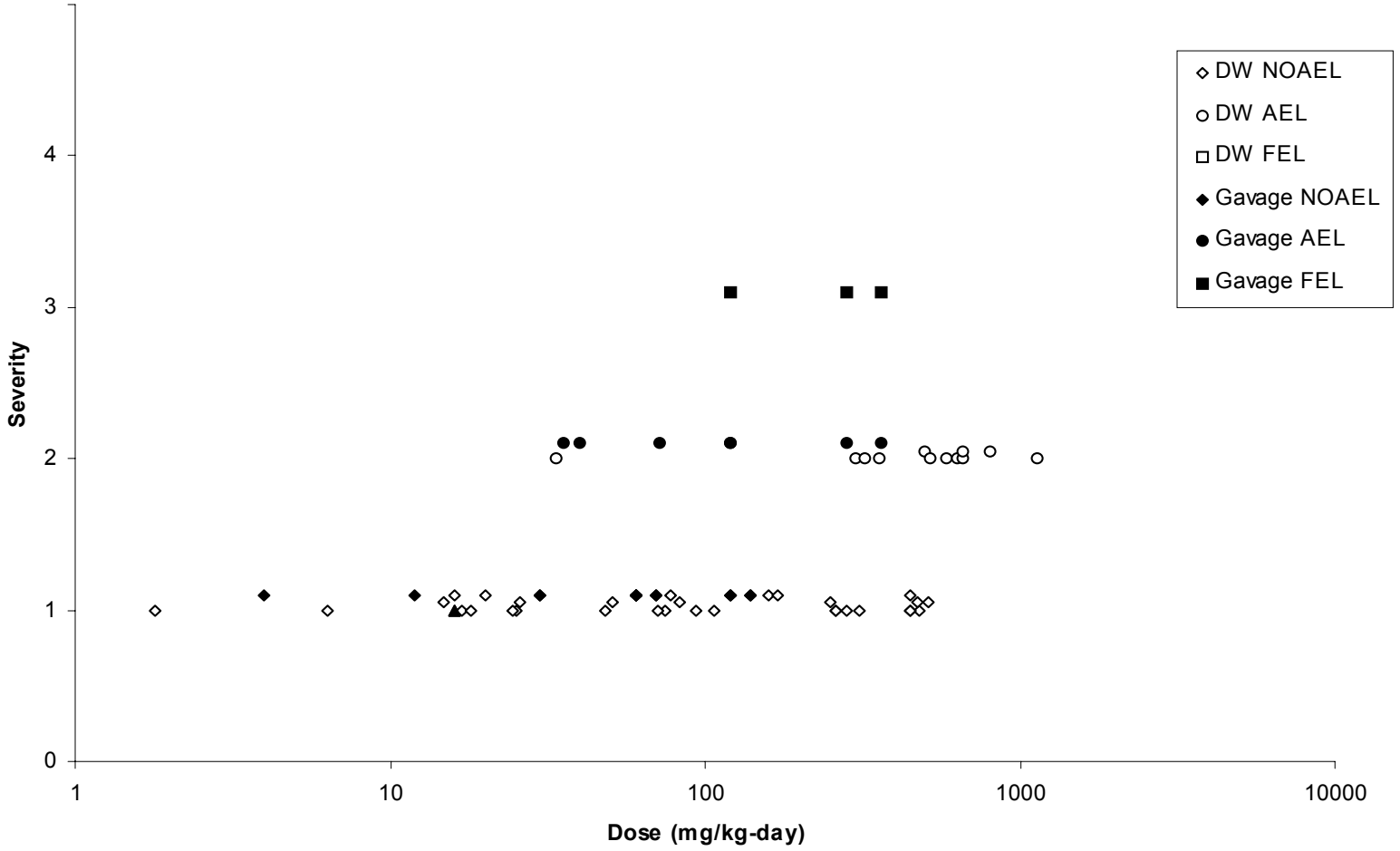
It is not known with certainty whether the toxic effects of phenol are due to the parent compound or to its metabolites. Distinguishing between effects of each is complicated by the lack of adequate data on concurrent blood levels of parent compound and metabolites. Phenol and metabolite levels in blood would be expected to rise in parallel as portal-of-entry metabolism becomes saturated. However, several lines of reasoning suggest that many of the toxic effects are due to the oxidative metabolites of phenol. In an in vitro study of the dysmorphogenic and embryotoxic effects of benzene and phenol and their metabolites on whole rat conceptuses, Chapman et al. (1994) found that phenol toxicity was much higher in the presence of S9.

The target tissues of phenol toxicity (kidney, liver, lung, and possibly bone marrow) are also those in which phenol can be oxidatively metabolized. In a 2-week inhalation study, Dalin and Kristoffersson (1974) observed altered balance and twitches in the absence of increased amounts of phenol in the blood, suggesting that a phenol metabolite rather than phenol itself is the toxic agent. Alternatively, the analytical method used may not have been sensitive enough or specific enough to detect any changes in blood levels of phenol. Phenol could produce portal-of-entry and systemic toxicity as a result of its ability to react with and to denature protein.

A key point with regard to the evaluation of the toxicity of orally administered phenol is whether gavage studies accurately represent the toxicity under environmental exposure conditions. Gavage studies are typically done using a single bolus dose per day, whereas environmental exposure is more likely to involve exposure distributed over the course of the day. Although laboratory animals consume drinking water in a few larger doses primarily during the active period rather than in continuous small sips, the toxicokinetics of environmental exposure are more closely modeled by a drinking water study or a gavage study using divided dosing, than by a study using a single gavage dose per day.

Figure 2 compares the doses and observed severity of effects in drinking water and gavage

Figure 2. Plot of severity with dose for drinking water (DW) (open symbols) or gavage (filled-in symbols). Values of 1, 2, 3 correspond with NOAEL, AEL, or FEL, respectively.



studies. Of particular interest is the number of gavage studies in which death, a frank effect, was observed, whereas drinking water studies at comparable or higher daily doses produced only tremors, kidney inflammation, and effects secondary to decreased water intake. The gavage NOAELs that occur at the same doses as gavage adverse effect levels are for maternal and developmental toxicity. The sole exception to the large difference between gavage and drinking water studies in doses that cause effects is the 28-day drinking water study by Hsieh et al. (1992), which reported hematological and immune effects at doses comparable to NOAELs in drinking water studies. As noted above, it would be useful to obtain independent confirmation in mice of the results reported by Hsieh et al. (1992).

Toxicokinetic data support this difference between gavage and drinking water studies and indicate that toxicity is correlated with peak blood concentrations rather than the area under the curve. Dow Chemical Co. (1994) observed "phenol twitching behavior" (including tremors and eye blinking) in rats gavaged with 150 mg/kg phenol; the behavior disappeared in less than an hour post-dosing, as phenol blood levels declined below peak values. By contrast, no twitching behavior was observed following a similar daily dose of phenol in drinking water. Unfortunately, blood levels of phenol or its metabolites were not determined in the drinking water phase of the study, but they are likely much lower than in the gavage phase, in light of the rapid blood clearance.

The higher systemic toxicity of gavaged phenol when it is administered in smaller volumes (NTP, 1983a) also supports the idea that toxicity is related to peak blood concentrations, because smaller dosing volumes would be expected to enhance the absorption rate. An unpublished GLP range-finding study for maternal toxicity (International Research and Development Corp., 1993) also found higher systemic toxicity for phenol when it was administered in smaller dosing volumes.[8] Data on the relationship between dosing volume and peak phenol blood concentrations are not available. Data on the relationship between peak blood concentrations and effects also are not available for the endpoints relevant to the critical effect.

The inhalation data on the effects of phenol are very limited (Table 7). Only one study conducted according to modern toxicological methods was located (Hoffman et al., 2001; available in unpublished form as Huntingdon, 1998), and the exposures in this study were for only 2 weeks.

[8]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

Other studies ranged from 2 weeks (Dalin and Kristoffersson, 1974) to 90 days (Deichmann et al., 1944; Sandage, 1961), but they included incomplete documentation of the study results, and they did not use modern methods for controlling exposure levels. In addition, the authors of some of the studies (e.g., Sandage, 1961) appear to have been looking for marked effects and thus dismissed statistically significant incidences of organ pathology of lesser severity. Nonetheless, the studies are fairly consistent with regard to the target organs and the effects observed. Exposure to high concentrations produced nervous system effects, and liver, kidney, and lung pathology occurred at lower concentrations. Rats were reported to be much less sensitive than rabbits or guinea pigs (Deichmann et al., 1944). The systemic targets observed following inhalation exposure to phenol are supported by data from the oral exposure route.

Information on the mode of action of inhaled phenol toxicity is also quite limited, but some extrapolation from other routes is possible. On the basis of the irritative and corrosive effects seen following dermal exposure to phenol, respiratory tract effects are likely due to direct contact of phenol with the respiratory tract tissue. As noted in Section 3, phenol is extensively absorbed following inhalation exposure. The lung can metabolize phenol prior to absorption, but the efficiency of metabolism in the lung is lower than that for the gut or liver (Cassidy and Houston, 1984). After the inhaled phenol (and its metabolites) reaches the blood stream, the same points described above for the oral route are relevant. In brief, it is not known whether the systemic toxic effects of inhaled phenol are due to phenol itself or to its metabolite(s), but at least some of the toxic effects appear to be attributable to phenol metabolite(s) (Chapman et al., 1994). Systemic toxicity appears to be related to peak concentrations in blood rather than to total daily intake.

4.6. WEIGHT OF EVIDENCE EVALUATION AND CANCER CHARACTERIZATION–SYNTHESIS OF HUMAN, LABORATORY ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

Chronic drinking water bioassays of phenol have been conducted in rats and mice (NCI, 1980). In these studies, NCI concluded that phenol was “not carcinogenic” in male or female F344 rats or B6C3F1 mice. However, the report also noted that leukemia and lymphoma were statistically significantly increased in low-dose male rats, although there was no significant increase at the high dose. The increases in leukemia are of particular interest in light of the leukemogenic effects of benzene (for which phenol is a metabolite) in humans. (In experimental animals, benzene has not

been shown to induce leukemia, although increases in lymphoma have been observed [e.g., NTP, 1986].)

The MTD was clearly reached in the rat study (NCI, 1980), on the basis of decreased body weight compared to controls and on kidney histopathology. Although the only sign of toxicity in the mouse study was decreased body weight (compared to the controls) secondary to decreased water consumption, higher doses probably could not have been tested, because of the decreased water consumption. Higher toxicity probably could have been achieved in a gavage study in mice at lower doses. These considerations suggest that an MTD was also reached in mice, although a definitive conclusion is difficult.

No other long-term oral carcinogenicity studies of phenol are available. No inhalation studies of phenol were of a sufficient duration to assess phenol carcinogenicity. The only long-term study that has assessed the carcinogenicity of phenol applied dermally without initiation was that of Boutwell and Bosch (1959), in which increased papillomas were seen at a dose that also caused ulceration.

In contrast with these negative results for oral carcinogenicity, dermally administered phenol has been consistently observed to be a promoter. Several authors (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959; Wynder and Hoffmann, 1961) observed that dermally applied phenol promoted DMBA-initiated skin tumors. These studies have generally reported significant skin ulceration at all phenol doses tested. The exception is Wynder and Hoffman (1961), who reported that 5% phenol promoted DMBA-initiated tumors in mice in the absence of any toxic reactions. When the same phenol dose was administered in different volumes, higher promotion activity was exhibited by the more concentrated solution, which also produced severe skin ulceration, suggesting that some of the promotion activity may have been related to the rapid cell division in the repairation of skin damage (Salaman and Glendenning, 1957). The observed response was dose-related (Boutwell and Bosch, 1959), but marked systemic toxicity was also observed at these doses. Co-carcinogenesis with dermally administered benzo[a]pyrene has also been observed (Wynder and Hoffmann, 1961). Because the benzo[a]pyrene was co-administered with the phenol, this assay cannot be classified as a true initiation/promotion assay. Production of papillomas by dermally administered phenol (in the absence of an initiator) was observed only at a concentration that caused ulceration, and hence was above the MTD.

Genotoxicity studies have found that phenol tends not to be mutagenic in bacteria (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983), but positive or equivocal results have been

obtained in gene mutation assays in mammalian cells (McGregor et al., 1988a, 1988; Paschin and Bahitova, 1982; Tsutsui et al., 1997). Increases were larger in the presence of S9 activation. Phenol tended to induce micronuclei in mice when administered intraperitoneally (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b), but it produced negative (or positive only at very high doses) results when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in in vitro micronucleus tests with human lymphocytes (Yager et al., 1990) and CHO cells (Miller et al., 1995), and it caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Phenol has been observed to act synergistically with hydroquinone in the production of genotoxic effects (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a).

Epidemiology data do not shed further light on the carcinogenic potential of phenol. Some studies (Kauppinen et al., 1986; Dosemeci et al., 1991) have reported elevated risks in phenol-exposed workers, whereas others have observed no effect (Wilcosky et al., 1984). However, the usefulness of each of these studies for risk assessment is limited by (depending on the study) an absence of an effect when latency was considered, a lack of a dose-response, and potential for confounding.

Although phenol was negative in oral bioassays conducted in rats and mice (NCI, 1980), questions remain regarding its carcinogenic potential in light of the positive results in initiation/promotion assays (albeit at exposures typically above the MTD), the increases in leukemia in low-dose male rats in the oral bioassay, and the observation of gene mutations in mammalian cells in vivo and micronuclei in vivo following i.p. dosing. No inhalation studies of sufficient duration to assess phenol carcinogenicity have been conducted. Dermal carcinogenicity or initiation/promotion studies with phenol at exposures below the MTD have not been conducted. The carcinogenic potential of phenol via inhalation exposure has not been evaluated at all. Under the draft revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), the data regarding the carcinogenicity of phenol via the oral, inhalation, and dermal exposure routes *are inadequate for an assessment of human carcinogenic potential*. Under the current guidelines (U.S. EPA, 1986a), phenol falls in Category D: not classifiable as to human carcinogenicity.

Similar conclusions have been reached by other groups in recent assessments of the carcinogenicity of phenol. IARC (1999) concluded that there is *inadequate evidence* in humans and in experimental animals for the carcinogenicity of phenol. Overall, IARC concluded that phenol

is *not classifiable as to its carcinogenicity to humans (Group 3).* Phenol is not included in the *9th Report on Carcinogens* (NTP, 2000a), and it is not listed as being considered for inclusion in the *10th Report on Carcinogens* (NTP, 2000b). This report contains only chemicals and substances that have been reviewed and classified as known human carcinogens or as reasonably anticipated to be carcinogens.

4.7. SUSCEPTIBLE POPULATIONS

People with decreased ability to conjugate and eliminate phenol, such as those with low activities of PST or glucuronyltransferase, may be more susceptible to phenol toxicity. If the toxicity of phenol is due to oxidative metabolites such as hydroquinone or catechol, individuals with increased oxidative activity would also be more sensitive to phenol toxicity. The activity of CYP2E1, the enzyme responsible for the oxidation of phenol, can be increased by exposure to a variety of agents, including ethanol and chlorinated solvents, so people with high exposures to these agents may be more sensitive to phenol.

4.7.1. Possible Childhood Susceptibility

As discussed in Section 4.3, a gavage study in rats (NTP, 1983a) reported decreased fetal body weight at a dose below that at which maternal toxicity occurred. In contrast, maternal toxicity occurred at a lower dose than did decreased fetal body weight in a gavage developmental toxicity study that used a divided-dosing protocol (Argus Research Laboratories, 1997). The observation of a fetal effect at a dose as low as 120 mg/kg-day (NTP, 1983a) suggests that the developing fetus is a possible susceptible population. The strength of this conclusion is weakened, however, by the small magnitude of the fetal weight decrease together with the increased litter size, which led to the high dose being identified as an *equivocal* LOAEL (see Section 4.3). The strength of the conclusion is also weakened by the observation in another gavage rat study (Argus Research Laboratories, 1997) that decreased fetal weight occurs only at doses above those that cause decreased maternal weight gain, and the observation of decreased fetal weight in a drinking water study (Ryan et al., 2001) only at concentrations that also resulted in reduced water consumption.

Only one study was located that specifically addressed age-related differences in the systemic toxicity of phenol. Deichmann and Witherup (1944) compared the lethality of an oral dose of 600 mg/kg phenol (administered as a 5% aqueous solution) in 10-day-old, 5-week-old, and adult rats. Mortality was 90%, 30%, and 60% in the neonates, young rats, and adult rats, respectively. Although the young and adult rats died within 1.5 hours of dosing, the neonates died 12–24 hours

after dosing. The data from this study suggest that neonates are more sensitive than adults and young rats may be less sensitive than adults. Alternatively, the age-related differences observed in this study could reflect inter-individual variability that was not a consequence of age. This study has not been replicated; therefore, definitive conclusions are not possible.

Data from humans and rats are consistent in showing very little fetal expression of CYP2E1, which is rapidly induced shortly after birth and rises though childhood (reviewed in Hakkola et al., 1998). The age after parturition at which CYP2E1 levels peak has been studied in laboratory animals, with inconsistent results. Some studies suggest that peak levels are reached during childhood, with a subsequent decrease to adult levels (Schenkman et al., 1989), whereas others have shown a rapid rise in CYP2E1 levels during childhood to a maximum level that is sustained during adulthood (Song et al., 1986). If the toxic moiety is a metabolite, decreased P450 metabolism could be expected to result in decreased toxicity. However, sulfate conjugation is also lower early in life (Iwasaki et al., 1993), so more of the dose is available for oxidative metabolism. Indeed, Heaton and Renwick (1991) found higher production of oxidative metabolites in young rats. This difference was higher in males, with the percentage of the administered dose recovered as hydroquinone conjugates decreasing from 38% of the administered dose in 3-week-old males to 8.2% in 16-week-old rats. Smaller decreases with age (from 17.8% of the administered dose in 4-week-old rats to 10.5% in 15-week-old rats) were observed in females. These data suggest the potential for children to be more sensitive than adults to the systemic effects of phenol.

4.7.2. Possible Gender Differences

Kenyon et al. (1995) (in mice) and Heaton and Renwick (1991) (in rats) reported higher excretion of hydroquinone conjugates in males than in females, suggesting higher levels of hydroquinone production in males. By contrast, Meerman et al. (1987) reported only slightly faster metabolism in male rats. These data would tend to suggest that if hydroquinone is the toxic moiety, phenol would be more toxic in males. However, few differences in phenol toxicity between males and females were identified; differences in NOAELs reflect differences in water consumption per unit weight, resulting in differences in estimated intake. Acute oral lethality data do suggest that phenol is more toxic to males (Thompson and Gibson, 1984).

5. DOSE RESPONSE ASSESSMENTS

5.1 Oral Reference Dose (RfD)

5.1.1. Choice of Principal Study and Critical Effect

An extensive database for the effects of orally administered phenol is available. The studies relevant to the development of the RfD are summarized in Table 2. Two-year drinking water studies conducted in rats and mice are available (NCI, 1980). Hematology and serum biochemical evaluations were not included in those chronic studies, but they were included in a recent two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). The only study evaluating hematological effects in mice is a 28-day drinking water study (Hsieh et al., 1992). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch, 1998). A number of developmental toxicity studies have been conducted in rats and mice, all using the gavage route (Argus Research Laboratories, 1997; NTP, 1983a; NTP, 1983b, Narotsky and Kavlock, 1995).

As shown in Table 2, the study with the lowest NOAEL/LOAEL boundary is the 28-day drinking water study in mice by Hsieh et al. (1992). In this study, the NOAEL was 6.3 mg/kg-day, and the LOAEL was 33.6 mg/kg-day, based on decreased antibody response, supported by decreases in hematocrit and red blood cells. Statistically significant decreases in erythrocyte counts were observed at the low and mid doses, but these results were considered biologically questionable in the absence of effects on hematocrit, in the absence of data addressing whether the apparent inconsistency was due to macrocytosis, and in light of the lack of support from other studies.

As noted in Section 4.5, this study is useful for hazard identification. However, confirmation of the study results in an independent assay in mice would be useful before using the data for dose-response assessment, considering the small number of animals tested and the limited number of risk assessments that have been based on in vivo/in vitro immunotoxicity assays. In addition, although qualitative differences between rats and mice in immune assays are not unusual (e.g., dioxin, see U.S. EPA, 2000c), it is of interest that Hsieh et al. (1992) observed immune effects in mice at very low doses, but the only other study evaluating similar immune parameters (Ryan et al., 2001; IIT Research Institute, 1999) did not observe effects in rats at 10-fold higher doses. No other studies in mice have directly evaluated effects on antibody forming cells.

In another study that evaluated immune effects of phenol on mice (Eastmond et al., 1987), no effect on bone marrow cellularity was observed at phenol doses of up to 300 mg/kg-day in mice dosed intraperitoneally for 12 days. Similarly, Corti and Snyder (1998) evaluated the effects of benzene metabolites on CFU-e cells (an erythroid progenitor cell sensitive to benzene) harvested from

mice and found that the cytotoxicity of phenol was much lower than that of other benzene metabolites. The effects in mice and rats were not compared in that study.

In light of these issues, and in the absence of other data supporting the observed effects at such low doses, the results of Hsieh et al. (1992) are too preliminary to be used as the basis for the phenol RfD. However, this study does raise concerns regarding the potential of phenol to cause hematological and immune effects, and it would be worthwhile to address these questions in a mouse immunotoxicity study conducted according to modern methods. The uncertainties regarding these endpoints and the use of a database uncertainty factor to address these uncertainties are further addressed in Section 5.1.3.

The next lowest NOAEL/LOAEL combination was observed in a 14-day gavage study in rats conducted as part of a large-scale screening study of a number of chemicals (Berman et al., 1995; Moser et al., 1995). Tremor, kidney tubular degeneration, and increased rearing in an FOB were observed at the high dose of 40 mg/kg-day but not at the next lower dose of 12 mg/kg-day. Although the incidence of kidney histopathology was not statistically significant, the high dose can be considered a LOAEL in light of the low statistical power of the study (only eight female rats per group) and the rarity of these lesions in short-term studies. The corresponding NOAEL is 12 mg/kg-day.

The relevance of this NOAEL to environmental exposures is questionable, however, due to the markedly higher toxicity observed in gavage studies than in drinking water studies, as discussed in Section 4.5, and the absence of supporting toxicity in drinking water studies of much longer duration. In particular, drinking water studies found no kidney histopathology in rats exposed to 260 mg/kg-day for 2 years (NCI, 1980) (although kidney inflammation was observed at higher doses), in mice exposed to doses up to 660 mg/kg-day for 2 years (NCI, 1980), or in parental rats in a two-generation reproduction study (Ryan et al., 2001; IIT Research Institute, 1999).

The only other study reporting kidney histopathology at low doses was a poorly documented and unpublished 6-month gavage study in rats (Dow Chemical Co., 1945). The very small dosing volume used by Berman et al. (1995) also may have contributed to the high toxicity, considering the findings of NTP (1983a).

The principal study for development of the RfD is Argus Research Laboratories (1997), in which decreased maternal weight gain was observed in rats gavaged on GD 6–15 with 120 mg/kg-day phenol; the maternal NOAEL was 60 mg/kg-day, based on decreased body weight gain, and the developmental NOAEL was 120 mg/kg-day. The BMDL was 93 mg/kg-day. No effect on body

weight was reported at 120 mg/kg-day in systemic toxicity studies using drinking water as the exposure route (NCI, 1980; ClinTrials BioResearch, 1998), but it is not surprising that maternal toxicity following 9 days of dosing occurs at a lower dose than does chronic systemic toxicity, considering the different physiological status during pregnancy. Although the principal study was conducted via the gavage route, measures were taken to decrease the impact of bolus dosing by dividing the daily dose into three administrations per day.

It is of interest that rats consume drinking water not in many little sips, but in a few larger doses primarily associated with food consumption during the active period of the day. Therefore, the toxicokinetic profile of the divided-dose gavage study may actually be fairly similar to the toxicokinetic profile that would be observed with drinking water exposure. In addition, a more precise measurement of administered dose is possible in gavage studies, because spillage can occur in drinking water studies.

The NOAEL identified in the Argus Research Laboratories (1997) study is supported by a developmental toxicity study (NTP, 1983a) in which decreased fetal weight was observed in CD rats gavaged on GDs 6–15 with 120 mg/kg-day; the NOAEL was 60 mg/kg-day. The high dose of 120 mg/kg-day was considered an *equivocal* LOAEL for developmental effects, in light of the small magnitude of the weight decrease, the increased litter size (which can result in decreased fetal weight), and the absence of an effect on fetal weight at a maternally toxic dose in another gavage developmental study in rats (Argus Research Laboratories, 1997). In the NTP (1983a) study, the maternal toxicity NOAEL was the high dose, 120 mg/kg-day.

Because of the uncertainties regarding identification of the critical effect level for the NTP (1983a) study, it was not considered to be an appropriate co-principal study. BMD modeling could not be conducted on the fetal weight endpoint, because fetal weights were reported only as an average across both sexes for all litters; no individual animal data were available. Because fetal weights of male fetuses tend to be heavier than those of females, and because the number of fetuses per litter affects the fetus weight, it was not appropriate to model the pooled data.

Although the decreased maternal weight gain (Argus Research Laboratories, 1997) was a mild effect and was possibly confounded by the gavage dosing, these results are supported by a drinking water study. Decreased motor activity was seen in female rats consuming the high concentration of phenol (5000 ppm, corresponding to 360 mg/kg-day) in the 13-week neurotoxicity study (ClinTrials BioResearch, 1998). The NOAEL in females was 107 mg/kg-day; no adequate fit could be obtained using BMD modeling. As discussed in Section 4.2, the authors considered the

decreased motor activity to be secondary to dehydration, but an analysis of the individual animal data and comparison with the literature could not confirm this assumption.

Ryan et al. (2001) conducted a two-generation drinking water study (also available in unpublished form as IIT Research Institute, 1999) in rats in which decreased parental and pup weight occurred at a LOAEL of 301 mg/kg-day, with a NOAEL of 71 mg/kg-day. However, these lower body weights, compared with control, are likely to be secondary to decreased water consumption and not an indication of phenol toxicity.

5.1.2. Method of Analysis: Benchmark Dose

The RfD was derived by the BMD approach using BMDS Version 1.3, which downloaded from the National Center for Environmental Assessment's web site. The BMR was defined as the default of a change of one standard deviation (U.S. EPA, 2000d). A BMDL of 93 mg/kg-day was derived for decreased maternal weight gain (Argus Research Laboratories, 1997) using the polynomial model. Similar BMDL values of 125 and 129 mg/kg-day were calculated using the power and Hill models, respectively, although the fit (based on the Akaike Information Criterion [AIC]) was slightly better using the polynomial model, and a more conservative BMDL was obtained using this model.

An alternative BMDL for this endpoint could be calculated using the geometric mean of the BMDLs from all three models, 114 mg/kg-day, on the rationale that the small difference in AICs was not meaningful. Other measures of fit (based on the goodness-of-fit p value and on visual fit) also indicated that all three models are comparable. However, in this case the slightly more conservative approach was used, in part as an added degree of protection because of the uncertainties regarding immunotoxicity. Details of the model results are presented in Appendix B.

5.1.3. RfD Derivation

The data on the within-human variability in the toxicokinetics and toxicodynamics of ingested phenol are insufficient to adjust the default uncertainty factor for intraspecies variability (UF_A). In a sample of liver fractions from 10 people, Seaton et al. (1995) found that the kinetics of phenol sulfation and hydroquinone conjugation varied by up to approximately threefold. Much larger variability in CYP2E1 has been found, particularly between neonates and adults (Vieira et al., 1996). These data on inter-individual variability in enzymatic metabolism are not adequate to move

from the default UF_H of 10, because they do not reflect potential variability in portal-of-entry metabolism of phenol or uncertainty regarding the identity of the toxic moiety. Furthermore, variability in CYP2E1 does not necessarily translate directly into variability in tissue dose of because metabolism by CYP2E1 may be limited by blood flow to the liver.

The absorption, distribution, and metabolism of ingested phenol in rats and humans appear to be generally qualitatively similar, although the data are insufficient for a quantitative comparison. Comparison of laboratory animal and human phenol toxicokinetics is also limited by incomplete information regarding the identity of the toxic moiety. As discussed in Section 4.5, the data suggest that most of the toxic effects of phenol can be attributed to its oxidative metabolites, but the data are insufficient to rule out the possibility that some effects may be attributable to phenol itself. In the absence of adequate data on which to based a toxicokinetic or toxicodynamic comparison of rodents and humans, the default UF_A of 10 is used for interspecies extrapolation. However, it may be possible to reduce this default value of 10 after review and evaluation of data (perhaps supplemented by a PBPK model) that compare the toxicokinetics of phenol and its metabolites in the placenta and fetus of rats and humans, if such data become available.

The BMDL was based on an effect of minimal severity (decreased maternal weight *gain*), and a higher BMDL and NOAEL were obtained for effects on maternal weight. The BMDL is also within 50% of the NOAEL identified for the decreased maternal weight endpoint. Therefore, no uncertainty factor (UF) is required for extrapolation from a NOAEL to a LOAEL. No UF for extrapolation across duration is needed because this developmental study is supported by chronic bioassays in two species in which toxicity was observed only at higher doses.

The database for phenol by the oral route can be considered complete. It includes 2-year drinking water studies conducted in rats and mice (NCI, 1980), a two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), and gavage developmental toxicity studies in rats (Argus Research Laboratories, 1997; NTP, 1983a; Narotsky and Kavlock, 1995) and mice (NTP, 1983b). However, the range of endpoints evaluated in the chronic toxicity studies was limited and did not include hematological or serum biochemistry evaluations. Immunological and hematological effects in mice were observed by Hsieh et al. (1992) in a 28-day drinking water at low doses. These endpoints were evaluated, and no significant hematological or serum biochemistry effects were observed at doses of up to >300 mg/kg-day in the two-generation rat study (IIT Research Institute, 1999; Ryan et al., 2001). The difference in these results suggest species differences between mice and rats, but confirmation of the

immunological and hematological effects in an assay done according to modern test methods would be useful.

An i.p. study of the effects of phenol on bone marrow cellularity in mice at doses of up to 300 mg/kg-day (Eastmond et al., 1987) and an in vitro study with mouse bone marrow cells (Corti and Snyder, 1998) also do not indicate that mouse blood cells are highly susceptible to effects of phenol. However, these studies did not evaluate the same parameter measured by Hsieh et al. (1992), and significant interspecies differences in immunotoxicity are not unusual. It is of interest that the endpoints affected in the Hsieh et al. (1992) study (two measures of effects on antibody-forming cells, PFC and ELISA) are the immune endpoints most highly predictive of effects on host resistance (Luster et al., 1992; Luster et al., 1993). Therefore, to account for the uncertainties regarding the immunological and hematological effects in mice, a database uncertainty factor of 3 is used.

An additional degree of public health protection may also be provided by the use of a gavage study rather than the more environmentally relevant route of drinking water. This is because gavage administration results in a higher peak blood level–presumably even using a divided-dosing protocol–than does ingestion of the same daily dose in drinking water. Because at least some toxic effects of phenol are related to peak blood levels rather than to total intake, toxicity would be expected to be higher following gavage exposure than drinking water exposure.

A composite UF of 300 results. No modifying factor is applied because the existing uncertainties have been addressed with the standard uncertainty factors.

RfD = 93 mg/kg-day /300 = 0.3 mg/kg-day, or 3E-1 mg/kg-day.

Note that this RfD is applied to ingested phenol *in addition to* the normal daily endogenous production of phenol, as discussed in further detail in Section 6.1.2.

An additional uncertainty factor for sensitive populations such as infants and children is not needed for phenol because sufficient studies of reproductive and developmental toxicity have been performed.

5.2. Inhalation Reference Concentration (RfC)

5.2.1. Choice of Principal Study and Critical Effect

The minimal database needed for the development of an RfC is a well-conducted subchronic inhalation study that has adequately evaluated a comprehensive array of endpoints, including the respiratory tract, and established a NOAEL and a LOAEL (U.S. EPA, 1994b). This criterion was not met for phenol. Neither of the two available subchronic studies (Deichmann et al., 1944; Sandage, 1961) are adequate for exposure-response assessment, because neither included adequate documentation of the histopathology results, and neither used modern methods for generating or monitoring exposure levels. These studies can, however, be used for hazard identification, and they identify the respiratory tract, liver, and kidney as targets of inhalation exposure to phenol.

The phenol database also includes a well-conducted 2-week inhalation study with rats that used modern exposure methods, evaluated a wide array of endpoints, and included a thorough histopathology evaluation of the respiratory tract (Hoffman et al., 2001; the full unpublished study report is available as Huntingdon, 1998). The only treatment-related effect observed was a red nasal discharge in male rats, which was observed with a statistically significant duration-related and concentration-related incidence in the mid- and high-concentration groups. However, because the red nasal discharge was likely due to a nonspecific response to stress, this response is not considered adverse.

In the absence of an inhalation study of sufficient duration, no RfC for phenol can be derived. A route-to-route extrapolation is not appropriate, because phenol can be a direct-contact irritant, and so portal of entry effects are a potential concern.

5.2.2. RfC Derivation

No RfC could be derived, due to insufficiencies of the database.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, the data regarding the carcinogenicity of phenol are *inadequate for assessment of human carcinogenic potential*. Phenol was negative in oral carcinogenicity studies in rats and mice, but questions remain regarding increased leukemia in male rats in the bioassay as well as the positive gene mutation data and the positive results in dermal initiation/promotion studies at doses at or above the MTD. No inhalation studies of an appropriate duration exist. Therefore, no quantitative assessment of carcinogenic potential via any route is possible.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Oral Noncancer

In most studies of phenol administered in drinking water, water consumption was markedly decreased at the highest dose, presumably due to poor palatability. A number of toxic effects secondary to the decreased water consumption have been observed, including decreased body weight compared to controls, decreased pup weight, and decreased pup survival pre-culling. Other effects that may not have been secondary to decreased water consumption were kidney inflammation (NCI, 1980) and decreased motor activity (ClinTrials BioResearch, 1998). Gavage studies found more severe effects and reported these effects at lower doses. Observed effects included lung, liver, and kidney pathology; tremors and other nervous system effects; and, at sufficiently high doses, death.

These data suggest that the toxicity of phenol is higher via gavage dosing than via administration in drinking water. The suggestion is supported by the finding that a series of behaviors termed "phenol twitching behavior" correlate with peak blood levels rather than area under the curve (Dow Chemical Co., 1994). For a given daily dose, peak blood levels would be much higher following gavage dosing than following continuous administration in water. A direct comparison of the toxicity of phenol when administered via these two routes could determine definitively whether route-specific differences exist. Nonetheless, the data supporting the higher toxicity of phenol administered by gavage were considered sufficiently strong to consider it inappropriate to use the Berman et al. (1995) study (which also used small dosing volumes) as the principal study.

Developmental toxicity studies have been conducted only via the gavage route. In the principal study (Argus Research Laboratories, 1997), decreased maternal weight gain was observed in rats administered 120 mg/kg-day in a divided-dosing protocol. The BMDL for this study was 93 mg/kg-day and the NOAEL was 60 mg/kg-day. Although exposure in this study was for only 9 days, comparison with the entire database for phenol via the oral route indicates that this study appropriately identifies the critical effect. Because a maternal effect (decreased maternal body weight gain during gestation) is considered the critical effect for phenol (i.e., the first adverse effect or its known precursor that occurs to the most sensitive species as the dose rate of an agent increases),

protection from this effect would also be protective from systemic effects of chronic exposure, which occur at higher doses. In light of the different physiological status during pregnancy, it is not unreasonable for maternal toxicity following 9 days of dosing to occur at a lower dose than does chronic systemic toxicity.

Although a NOAEL of 60 mg/kg-day was identified for decreased fetal weight in the NTP (1983a) study, this study was not considered adequate to be a co-principal study. The high dose of 120 mg/kg-day was considered an *equivocal* LOAEL for developmental effects, in light of the small magnitude of the weight decrease and the absence of an effect on fetal weight at a maternally toxic dose in another gavage developmental study in rats (Argus Research Laboratories, 1997). In addition, although the observed decrease in fetal weight was small (but statistically significant), increased litter size was also seen at this dose. It is possible, therefore, that the dams were near the limit of what they could carry in terms of pup burden (total fetal weight).

The NOAEL was supported quantitatively by the NOAEL of 107 mg/kg-day for decreased motor activity in a 90-day drinking water neurotoxicity study (ClinTrials BioResarch, 1998). A NOAEL of 71 mg/kg-day for decreased parental and pup body weights was also identified in a drinking water two-generation reproduction study (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), although these effects are likely secondary to decreased water consumption. There is, however, some uncertainty in the identification of the NOAEL for this latter study. As described in Section 4.3, a statistically significant decrease in uterine weight was observed at all three doses in this study. The decrease was not considered adverse for a number of reasons, including the absence of a dose-response and the small number of animals outside the control range. This consideration, however, is rather subjective and is based on considerable professional judgement.

A key uncertainty in the development of the RfD is the interpretation of the study by Hsieh et al. (1992). Immunotoxicity (decreased response of antibody-forming cells) and hematotoxicity (decreased red blood cells and hematocrit) were observed in this 28-day drinking water study in mice at doses much lower than the doses than produced toxicity in other studies. No immunological or hematotoxic effects were seen at much higher doses in a two-generation drinking water study in rats (Ryan et al., 2001; IIT Research Institute, 1999). These differing results suggest species differences between mice and rats, but confirmation of the immunological and hematological effects in an assay done in mice according to modern test methods would be needed before using the data for dose-response assessment, considering the small number of animals tested and the limited number of risk

assessments that have been based on in vivo/in vitro immunotoxicity assays.

Similarly, in the absence of hematotoxicity in monkeys, rats, and mice following inhalation exposure to phenol levels resulting in comparable or higher systemic doses of phenol (Sandage, 1961; Dalin and Kristoffersson, 1974), confirmation of the reported hematological effects would also be useful. A database uncertainty factor of 3 was used to account for the uncertainty regarding immunotoxicity. This factor could be removed if an immunotoxicity study conducted according to U.S. EPA (1998c) guidelines became available.

Although it does not directly affect the determination of the RfD, uncertainty also exists regarding whether the decreased motor activity in females reported by ClinTrials BioResearch, (1998) was due to dehydration only or whether phenol exposure also contributed to the effect. The NOAEL from this study was used as supporting data for the principal study. The study authors attributed the decreased motor activity to dehydration, because of the marked decrease in water intake and the absence of supporting changes in the FOB. By contrast, this assessment concluded that phenol at least contributed to the effect, because there was no clear correlation between individual animals with dehydration and those with decreased activity and because the limited literature on the topic reports no effect on motor activity of water deprivation for several days. A neurotoxicity study in which the controls were allowed only limited access to drinking water would also address this issue.

6.1.2. Inhalation Noncancer

The database for inhalation toxicity of phenol is very limited. A well-conducted 2-week study is available (Hoffman et al., 2001; available in unpublished form as Huntingdon, 1998), but the duration is less than that appropriate for serving as the basis for the RfC. Longer-term studies have been conducted (Deichmann et al., 1944; Sandage, 1961), but they are limited by inadequate control of exposure levels, unclear sensitivity of the evaluation, and limited reporting.

However, the inhalation toxicity studies are sufficient however, to identify the respiratory tract, liver, kidney, and nervous system as targets of inhaled phenol toxicity. A significant uncertainty exists regarding which species is the most appropriate for extrapolation to humans. Deichmann et al. (1944) reported marked systemic toxicity in rabbits and deaths in guinea pigs at exposure concentrations that caused no histopathology in rats. No inhalation studies in guinea pigs or rabbits have been conducted to confirm these findings. In addition, it is unclear which of these species is most like humans.

The primary data need for developing an RfC is a 90-day inhalation study that includes a thorough examination of the respiratory tract. Pharmacokinetic studies of inhaled phenol would also aid in the extrapolation from experimental animals to humans.

6.1.3. Cancer

Several epidemiology studies have evaluated the carcinogenesis of phenol, but they have not found a consistent dose-related association. Because all of the subjects were also exposed to other chemicals and there was no correction for smoking, these studies are not adequate to reach conclusions on the carcinogenic potential of phenol.

Phenol was negative in drinking water bioassays with rats and mice (NCI, 1980), although an increased incidence of leukemias was observed in low-dose male rats. No inhalation studies of sufficient duration to assess carcinogenicity were found. In short-term dermal assays, tumorigenicity (production of papillomas in the absence of treatment by an initiating agent) was observed only at a dose/concentration combination that produced ulceration and thus was well above the MTD (Salaman and Glendenning, 1957). Similarly, although phenol was a promoter when tested in initiation/promotion studies, the doses tested typically caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959) and death (Boutwell and Bosch, 1959). There were two exceptions. First, the low concentration tested by Salaman and Glendenning) caused promotion as well as "transient light crusting." Because the site of the weekly treatment was rotated across four sites on the body, it is unclear whether more severe effects would have been observed if the same site had been treated for the entire study. The second exception was that the low concentration tested by Wynder and Hoffmann (1961) was reported as causing no toxicity, although the sensitivity of the evaluation is unclear. On the basis of the high observed toxicity, it is not clear whether the promoting activity observed for phenol in several studies was secondary to the repeated injury and healing of the skin. From these considerations, the data regarding the carcinogenic potential of phenol are *inadequate for an assessment of human carcinogenic potential.*

6.2. DOSE-RESPONSE

No human data that are adequate for the derivation of a phenol RfD were located. Therefore, laboratory animal data were used.

The RfD of 0.3 mg/kg-day was based on a BMDL of 93 mg/kg-day for decreased maternal body weight gain in a gavage rat developmental toxicity study that used a divided-dosing protocol

(Argus Research Laboratories, 1997). There was a corresponding NOAEL of 60 mg/kg-day and a LOAEL for maternal toxicity of 120 mg/kg-day. A composite UF of 300 was used. This factor is based on a default factor of 10 for extrapolation from laboratory animals to humans, a default factor of 10 to account for intrahuman variability, and a factor of 3 to account for database insufficiencies.

Although the database for phenol can be considered "complete," there are uncertainties regarding the immunotoxicity potential of phenol in light of the immunotoxicity (decreased antibody forming cells) reported by Hsieh et al. (1992). The database factor may be reconsidered with results from an immunotoxicity study in mice that is compliant with EPA immunotoxicity test guidelines (U.S. EPA, 1998**c**). This RfD is at least twice the endogenous rate of phenol formation in humans, estimated as 0.014–0.14 mg/kg-day (Bone et al., 1976; Lawrie and Renwick, 1987; Renwick et al., 1988), based on total phenol (free plus conjugated) levels in urine. This means that endogenous production is approximately 5–50% of the RfD.

Note also that the RfD is meant to apply to ingested phenol *in addition to* the endogenous formation of phenol. Endogenous phenol is produced by bacteria in the gut, so endogenous phenol and ingested phenol would have similar toxicokinetics. Both humans and laboratory animals efficiently conjugate and excrete phenol at low doses, resulting in only a small degree of systemic exposure to free phenol (or any of its oxidative metabolites) at these low levels. The primary difference between endogenous and exogenous phenol would result, because endogenous phenol is formed in the intestines and some phenol may reach the colon and rectum, where some will escape the hepatic portal circulation and be absorbed directly without conjugation. By contrast, a smaller amount of ingested phenol would be expected to reach the colon and rectum.

The data are insufficient to determine the degree of conjugation of endogenously formed phenol in humans because the available data are based on analysis of daily urinary excretion of total phenol (i.e., phenol conjugates plus any trace amounts of free phenol) (Lawrie and Renwick, 1987; Renwick et al., 1988); oxidative metabolites of phenol were not measured. The phenol conjugation capacity of the liver is an important determinant of the ingested dose that would result in toxicity, but there is no information on the degree of phenol conjugation by humans at doses in the range of the RfD. Human variability exists in both the levels of endogenous phenol production and in the conjugative capacity of the liver.

In the absence of more detailed information, it is reasonable to assume that humans have adapted by having adequate conjugation capacity for the range of endogenous phenol production. Therefore, the default total uncertainty factor of 10 for human variability in toxicokinetics and

toxicodynamics is considered adequate. Determining whether oxidative metabolites are formed in individuals who have high endogenous levels of phenol formation would enhance the confidence in determining the intraspecies uncertainty factor.

The principal study (Argus Research Laboratories, 1997) used an adequate number of animals and evaluated an appropriate array of endpoints for a developmental toxicity study. Although gavage dosing was used, the divided-dosing protocol provided a significant enhancement that made the gavage dosing more closely resemble an environmentally relevant route of exposure. The principal study is judged to have medium confidence. Although the use of gavage dosing lowers the confidence in the study, the dosing frequency may be fairly similar to that in drinking water studies, in which rodents typically consumed water in a few larger doses and often in association with food consumption.

Confidence in the supporting database is medium to high. Although the oral toxicity database meets the minimal criteria for a high-confidence database (chronic studies in two species, developmental toxicity studies in two species, and a multigeneration reproduction study), the chronic studies did not evaluate a sufficient array of endpoints. In particular, the chronic mouse study (NCI, 1980) did not evaluate hematological and immunological effects, making interpretation of the results of the Hsieh et al. (1992) study difficult. Consideration of the above issues results in medium to high confidence in the RfD.

The RfD developed in this document can be compared with other limits on phenol exposure, partially as a test of the reasonableness of the RfD. Phenol is used in a number of industrial products, as well as in over-the-counter medicines such as cough drops, throat sprays, and mouthwashes (e.g., Cepastat® and Chloraseptic® brands). Use of these consumer products can result in short-term, high-level phenol exposures, but prolonged exposure (more than a week) at these levels is not recommended. The short duration of exposures to the cough medicines suggests that safe exposure levels would be higher than those for lifetime exposure. On the other hand, at least some aspects of phenol toxicity appear to be related to peak concentrations in blood, and higher peak blood concentrations could result from the consumer product exposure.

ATSDR (1998) estimated that intake of the maximum recommended dosage of 300 mg phenol/day would result in an approximate dose of 4–8 mg/kg-day. No documentation of this maximum recommended dosage could be located. Use of these products on a daily basis over the course of a lifetime would result in a dose approximately 10-fold higher than the RfD derived in this document; however, these products are not intended for use over a prolonged period. Evaluation of

potential health effects in individuals who do consume these products in large amounts or over long periods of time could provide additional information about human health effects of phenol and safe exposure levels.

The use of a higher dose of phenol than the RfD in over-the-counter medicines suggests that this RfD provides an adequate degree of public health protection. However, the maximum recommended dosage may not be protective of pregnant women and fetuses, as consumers are advised "as with any drug, if you are pregnant or nursing a baby, seek the advice of a medical professional before using this drug."

Although a substantial amount of data on phenol toxicokinetics are available, they are not sufficient to move away from the default UFs for interspecies extrapolation and intraspecies variability (IPCS, 2001). Data on how blood levels of phenol and its metabolites relate to doses in rats and humans would be useful in addressing the interspecies UF, as would data on the potential for phenol to cross the placenta. Similarly, data on how differences in enzyme activities relate to phenol and metabolite blood levels would be useful in addressing intrahuman variability. Finally, a drinking water study compliant with EPA test guidelines (U.S. EPA, 1998c) that evaluated hematological effects and immunological effects in mice could address the uncertainties associated with the Hsieh et al. (1992) study and lead to reconsideration for the need for a database UF.

The available data are inadequate to derive an RfC. As noted above, a 90-day inhalation study that evaluated the respiratory tract would be necessary for development of an RfC.

Because the data were considered inadequate to assess the carcinogenicity of phenol, no quantitative assessment was conducted.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

The support document and IRIS summary for phenol have undergone both internal peer review by scientists within EPA and a more formal external peer review by scientists outside EPA in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The three external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

A. Data Presentation

All three reviewers agreed that the document is well organized. Two reviewers recommended specific changes to increase the clarity of certain sections.

Response to comments: The specific changes requested by the reviewers to clarify the text were made.

B. Are there additional data/studies that should be included?

One reviewer was not aware of any other data/studies that should be included. Another reviewer recommended a developmental toxicity study but noted that it uses an unconventional study design. A third reviewer criticized EPA for superficially exploring human health literature and relying heavily on animal experiments. This third reviewer also suggested that EPA expand its discussion of studies looking at phenol usage in over-the-counter drugs.

Response to comments: We reviewed the developmental toxicity study (Minor and Becker, 1971) recommended by one reviewer and concluded that it should not be included in the document because it would not contribute any significant information to the database. The specific reasons for the exclusion are: (1) this study used intraperitoneal (i.p.) dosing, a route of exposure of limited relevance for human environmental exposure; because several well-conducted oral developmental toxicity studies of phenol are available, it is not useful or necessary to supplement the database with an i.p. study; (2) phenol was administered on gestation days 9–11 or days 12–14; such studies of short windows of exposure can be useful for mechanistic purposes, but they are inadequate to fully assess developmental toxicity; (3) this study is presented only as an abstract published 30 years ago, and the full study was never published, raising questions about the reliability of the results, and in addition, the abstract provides insufficient information for evaluation of the study.

In response to the request for additional human data, additional reviews of literature databases and secondary review articles were conducted, but no additional data were identified. Trade associations were also contacted, with the same results. However, information relating the effects observed in animals to effects observed in the available human studies was added to Section 4.5. Due to limitations such as confounding exposures and uncertain exposure estimation, the available human

data are useful for hazard identification but not for dose-response assessment.

Manufacturers of over-the-counter drugs containing phenol, the relevant trade associations were contacted, and reference books were consulted, in order to obtain information on the basis for the recommended maximal dosage, but the information was not available.

C. For the RfD, has the most appropriate critical effect (decreased fetal body weight in the NTP (1983a) study and decreased maternal weight gain in the Argus Research Laboratories (1997) study) been chosen?

One reviewer agreed with the selection of decreased fetal body weight as the critical effect, with a NOAEL of 60 mg/kg-day. This reviewer noted that although this is a relatively nonspecific toxic effect, it could be appropriately considered adverse. In contrast, a second reviewer stated that the reduction in body weight was a weak basis for the RfD because it was nonspecific and because of other changes affecting body weight (e.g., increased litter size). On the basis of these considerations, the reviewer did not consider the observed reduction in body weights to be an adverse effect and suggested that the corresponding dose might be considered a NOAEL. Neither of these reviewers commented on the appropriateness of the co-critical effect of decreased maternal weight gain endpoint, also with a NOAEL of 60 mg/kg-day (and a BMDL of 93 mg/kg-day).

A third reviewer asserted that the critical effect occurs at a lower dose and that the RfD should be based on a NOAEL lower than 60 mg/kg-day. The reviewer stated that the data from the NTP (1983a) study are consistent with a LOAEL of 30 mg/kg-day, based on statistically significant increases in the number of litters with nonlive fetuses in the low- and mid-dose groups and a trend comparison of the fetal weight data. The reviewer also stated that data from the Argus Research Laboratories (1997) study support the conclusion that 60 mg/kg-day is a LOAEL, based on an increase in the percent litters with fetuses with alterations. The reviewer also recommended that the LOAEL in the IIT Research Institute (1999) study should be 20 mg/kg-day (with no NOAEL identified), based on decreased uterine weight and decreased prostate weight, rather than the NOAEL of 70.0 mg/kg-day identified in the Toxicological Review. The reviewer asked for additional discussion of the interpretation of these results but did not recommend a specific critical effect. This reviewer also recognized the uncertainties in the Hsieh et al. (1992) study but suggested that macrocytosis could explain the inconsistency in that study between erythrocyte count and hematocrit.

Response to comments: The relationship between litter size and fetal weight was noted, and the analysis of the NTP (1983a) study notes that 120 mg/kg-day is an *equivocal* LOAEL, with a NOAEL of 60 mg/kg-day. Due to the uncertainties in the identification of the NOAEL/LOAEL boundary for this study, the NTP (1983a) study was then judged not appropriate as a co-principal study. If the NOAEL in the NTP (1983a) study were changed to 120 mg/kg-day, there would not be a significant effect on the RfD because the Argus Research Laboratories (1997) study identified a NOAEL of 60 mg/kg-day and a LOAEL of 120 mg/kg-day, with a BMDL of 93 mg/kg-day. In addition, the NOAEL identified in the principal study (Argus Research Laboratories, 1997) is supported by a NOAEL of 107 mg/kg-day in the ClinTrials BioResearch (1998) study, a value that is very close to the BMDL used to derive the RfD.

Additional information about the litters with nonlive fetuses, decreased uterine weight, and other findings mentioned by the third reviewer were added to the document (Section 4.3). The former endpoint was not considered treatment-related due to the absence of a dose-response. The data on percent litters with fetuses with alterations were independently analyzed using the chi-square test, and no significant effect was observed. Text was also added to Section 6.1.1 regarding uncertainties in the interpretation of the uterine weight data.

D. Has the noncancer assessment been based on the most appropriate studies?

Two of the reviewers agreed that the noncancer assessment is based on the most appropriate studies, although, as indicated in the previous question, one of the reviewers raised some concerns about the interpretation of those studies. Two of the reviewers also agreed with EPA's assessment of the deficiencies of the study by Hsieh et al. (1992). One reviewer agreed with the selection of significant endpoints in the phenol document; however, this reviewer disagreed with the use of gavage studies for the derivation of the RfD. This reviewer asserted that EPA should have used the two-generation drinking water study by IIT Research Institute (1999) to derive the RfD.

Response to comments: As described in the Toxicological Review, the effects at the LOAEL (the high dose) of the IIT Research Institute (1999) study appear to be secondary to decreased water consumption due to poor palatability, and so do not appropriately reflect phenol toxicity. Text was added to the Toxicological Review noting that rats consume water only intermittently during the day, and so a divided-dose gavage protocol is fairly similar to drinking water consumption.

E. For the noncancer (RfD) assessment, are there other data that should be considered in developing uncertainty factors or the modifying factor? Do the data support the use of different values from those proposed?

All three reviewers agreed with the uncertainty factors presented in the phenol document and EPA's rationale for selecting these factors.

Response to comments: None.

F. Do the confidence and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments?

The reviewers agreed that the confidence statements are carefully reasoned and clearly stated. Two of the reviewers agreed that the weight-of-evidence statements are appropriate, and a third reviewer noted that no weight-of-evidence statement is used for noncarcinogenicity.

Response to comments: None needed.

(2) Chemical-Specific Comments

A. When endogenously produced phenol is taken into account, can the RfD be supported? Note that the RfD is applied to ingested phenol in addition to the normal daily endogenously

produced phenol. Are there differences in endogenous phenol production between rats and humans that should be taken into account in the development of the RfD?

The reviewers agreed that the RfD applied to ingested phenol in addition to the normal daily endogenously produced phenol is appropriate. One reviewer noted that endogenous production is a relatively small fraction (~2–20%) of the RfD. (This fraction became 5–50% after addition of the database uncertainty factor during consensus review.) Another reviewer noted that both the experimental animals and humans would have similar baseline levels of endogenous phenol production.

Response to comments: Additional supporting information provided by the reviewers was incorporated into the text. Toxicokinetic considerations regarding quantitatively accounting for endogenous production were also incorporated into the text.

B. Do you agree/disagree with the recommendation that there are not sufficient data to generate a scientifically defensible RfC and cancer slope factor?

Two reviewers agreed that data are insufficient to generate an RfC and cancer slope factor. One of these reviewers also commented on statements made by EPA related to red nasal discharge identified in study animals. This reviewer stated that the secretion/discharge is not directly suggestive of, or a precursor to, a nasal or ocular lesion but is simply the animal's response to stress. A third reviewer did not comment on this question because it was beyond this person's area of expertise.

Response to comments: The information on red nasal discharge provided by the reviewer was incorporated into the text.

C. Was the interpretation of the decreased fetal body weight in rats in the National Toxicology Program (NTP) study (NTP, 1983a) appropriate?

One reviewer raised some issues regarding the adversity of the effect but generally agreed with EPA's decision to designate the decreased fetal body weight finding at 120 mg/kg-day as an equivocal LOAEL, resulting in a NOAEL of 60 mg/kg-day. A second reviewer disagreed that decreased fetal body weight is an adverse effect and asserted that 120 mg/kg-day should be classified as a NOAEL instead. A third reviewer supported interpreting the data using trend analysis rather than pairwise comparisons, resulting in a LOAEL of 30 mg/kg-day for fetal body weight reduction.

Response to comments: On the basis of the weight of evidence, the weight of the reviewers' comments, and the supporting data from the Argus Research Laboratories (1997) study, the high dose in the NTP (1983a) study was retained as an equivocal LOAEL, but the study was removed from being a co-principal study for the derivation of the RfD. Although there was a dose-response trend at the low and mid doses, the decreases in the fetal body weight at these doses were marginal (1%–2%) and were not considered biologically significant. Only the response (7%) at the high dose was significantly different from the control. A meaningful benchmark dose could not be calculated for these data, because fetal body weight by sex were not in the published study and not available in

NTP's archives.

D. Please comment on the choice of gavage developmental toxicity studies as the co-critical studies in light of the differences between phenol toxicity when administered in drinking water and by gavage.

One reviewer agreed with EPA's willingness to use gavage studies but suggested that the IIT Research Institute (1999) drinking water study represents a more relevant exposure scenario and supports a lower LOAEL, based on decreased absolute and relative uterine weight. A second reviewer asserted that the drinking water study is more appropriate because a divided gavage dose is not equivalent to more extended intake during *ad libitum* water consumption. A third reviewer supported the use of the gavage study and asserted that the divided dose administered in the Argus Research Laboratories study (1997) is not unrealistic because animals in drinking water studies tend to drink when they eat, not continuously throughout the day. This reviewer believed that the NOAEL and the LOAEL are not overly conservative.

Response to comments: Additional information about the interpretation of the decreased uterine weight in the IIT Research Institute (1999) drinking water study and associated uncertainties was added Section 4.3. In particular, even though the decrease in relative uterine weight was statistically significant at all dose levels, there was no dose-response. Information was also added to the text noting that rats drink water in a small number of periods during the day rather than continuously through the day, so a divided-dose gavage study is fairly similar to drinking water exposure.

E. Was the interpretation of decreased motor activity in the 13-week oral neurotoxicity study appropriate?

One reviewer agreed with EPA's interpretation of decreased motor activity in the 13-week oral neurotoxicity study. A second reviewer found the interpretation difficult to comment on, given the confounded results of the study, and a third reviewer did not respond to this question because it was beyond this person's area of expertise.

Response to comments: The issues potentially confounding the neurotoxicity were noted in the document.

OVERALL RECOMMENDATION

All three reviewers stated that the document is acceptable with revisions.

New Reference:

Minor, JL; Becker, BA. (1971). A comparison of the teratogenic properties of sodium salicylate, sodium benzoate, and phenol. Toxicol Appl Pharmacol 19:373.

Appendix B. Benchmark Dose Modeling Results

Benchmark dose (BMD) modeling was performed to identify potential critical effect levels for derivation of the RfD for phenol. The modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000d) using Benchmark Dose Software Version 1.3 (BMDS), which is available from EPA (U.S. EPA, 2001). The BMD modeling results are summarized in Table B-1, and the output is attached as Appendix C. A brief discussion of the modeling results for each endpoint is presented below.

Because all the following endpoints are continuous variables, the continuous models available with BMDS (power, polynomial, and Hill models) were used. The hybrid model was not used, because the hybrid model software in BMDS is still undergoing Beta-testing and was not considered sufficiently validated to provide a BMDL as the basis for the quantitative dose-response assessment. (The hybrid modeling approach defines the benchmark response [BMR] directly in terms of risk, whereas the standard approach, defines the BMR in terms of a change in the mean.) For all of the modeling conducted, the BMR was defined as a 1.0 SD change in the mean because this is the default measure recommended by the EPA (U.S. EPA, 2000d) in the absence of a clear biological rationale for selecting an alternative response level.

Argus Research Laboratories, 1997

Two endpoints were modeled from this study: decreased maternal body weight gain and the related endpoint of decreased maternal body weight. The decrease in weight gain was the more sensitive endpoint, with a NOAEL of 60 mg/kg/day and a LOAEL of 120 mg/kg/day.

As summarized in Table B-1, the BMD and BMDL estimates for the endpoint of decreased maternal body weight gain was similar for all three models. The model fit was also generally similar. A visual analysis of the data fit in the regions of the BMDLs indicated that the data fitting from the three model was adequate and comparable across all models. The goodness-of-fit *p*-values calculated for the power and polynomial models were very similar, but no *p*-value could be computed for the Hill model because there were no degrees of freedom for the calculation. (This was because the number of parameters included in the model was equal to the number of data points.) The Akaike Information Criterion (AIC), a measure of goodness of fit that takes into account the number of degrees of freedom, was very similar for all three models but was marginally better (i.e., lower) for the polynomial model. The polynomial model was chosen as the basis for the BMDL for this endpoint, based on the slightly better fit and as a slightly more health-protective value. An alternative BMDL for this endpoint could be calculated using the geometric mean of the BMDLs from all three models, 114 mg/kg-day, based on the rationale that the small difference in AIC observed was not meaningful.

For the endpoint of decreased maternal body weight, all three continuous models gave a similar BMD estimate and provided adequate data fits, with goodness-of-fit *p*-values larger than 0.1. However, the best data fit was obtained with the polynomial model, which had a *p*-value of 0.92, compared with a *p*-value of 0.30 obtained with the power model. The *p*-value for the Hill model was not computed due to insufficient degrees of freedom. A visual analysis of the data fit in the region of

the BMDLs also indicated that good fit was obtained with all three models, with the best fit obtained using the polynomial model. In addition, the AIC analysis also indicated that the best data fit was obtained with the polynomial model. Comparable BMDLs of 143 and 147 mg/kg/day were obtained using the polynomial and Hill models, respectively. Thus, the BMDL chosen for this endpoint was 143 mg/kg-day, obtained with the polynomial model, with a corresponding BMD of 345 mg/kg. This BMDL is higher than the BMDL for maternal body weight gain, the other endpoint modeled for this study.

ClinTrials BioResearch (1998)

Only one endpoint from this study was modeled: decreased total activity counts in a motor activity assay in females at week 4. Acceptable fits (p=0.35 and p=0.17) were obtained with the polynomial and power models, respectively. The visual fit for both models in the region of the BMDL was acceptable but not as good as would be desired. Much better fit was obtained with the Hill model, based on visual fit, but no BMDL could be calculated for this model. The same BMDL of 219 mg/kg-day was calculated with both the power and polynomical models and was chosen as the study BMDL.

Hsieh et al. (1992)

Three related endpoints were modeled for this study: plaque-forming cells, plaque-forming cells/10^6 spleen cells, and antibody titer. The study NOAEL was 6.2 mg/kg-day, with a LOAEL of 33.6 mg/kg-day.

As summarized in Table B-1, unacceptable fits were obtained with the power and polynomial models for the endpoint of plaque-forming cells/10^6 spleen cells. No p-value could be obtained for the Hill model due to insufficient degrees of freedom, but a visual analysis of the results indicated that the Hill model provided an acceptable fit. Based on the Hill model, the BMD and BMDL for decreased plaque-forming cells/10^6 spleen cells were 1.26 mg/kg-day and 0.38 mg/kg-day, respectively.

Similar results were obtained for plaque forming cells/spleen. Inadequate fits were obtained with the power and polynomial models, and the Hill model provided no p-value at all due to insufficient degrees of freedom. A visual analysis of results indicated that the Hill model provided an overall adequate fit, but not a very good fit in the range of the BMD. In addition, this model failed to estimate BMD or BMDL. Therefore, no BMD and BMDL can be identified for this particular endpoint.

For the endpoint of decreased antibody titer, the power and polynomial models both had marginal fit, based on the goodness-of-fit p-values; visual inspection of the data indicated that these models had inadequate fit. No p-value could be calculated for the Hill model, due to insufficient degrees of freedom, but a visual analysis of the model results indicated an adequate fit. Therefore, based on the Hill model, the BMD and BMDL for decreased antibody titer were 3.51 mg/kg-day and 0.73 mg/kg-day, respectively.

The lowest BMDL for this study was 0.38 mg/kg-day, calculated for plaque-forming cells/10^6

spleen cells. However, this BMDL is not used for risk assessment due to uncertainties in the appropriate BMR for this in vivo/in vitro study design.

Table B-1. Benchmark Dose Modeling Results for Phenol

Model	*p*-value	AIC	BMD[a]	BMDL[a]
Argus Research Laboratories (1997)				
Maternal Body Weight Gain				
Power	0.3165	545	152	125
Polynomial	0.3191	543	157	93
Hill	N/A	545	151	129
Maternal Body Weight				
Power	0.3013	731	354	244
Polynomial	0.9188	729	345	**143**
Hill	N/A[b]	733	345	147
ClinTrials BioResearch Ltd. (1998)				
Motor Activity - Total Activity Counts in Females in Week 4				
Power	0.1701	629	337	219
Polynomial	0.3477	625	336	**219**
Hill	N/A	630	246	---
Hsieh et al., 1992				
Plaque-Forming Cells				
Power	0.008	236	15.7	10.5
Polynomial	0.003	227	14.3	9.7
Hill	NA	229	1.26	0.38
Plaque-Forming Cells/Total Spleen				
Power	0.054	469	23	15.3
Polynomial	<0.0001	509	23.4	5.87
Hill	N/A	467	6.14	--- [c]

Antibody Titer				
Power	0.102	-66.2	21.4	13.1
Polynomial	0.102	-70.2	21.4	13.1
Hill	N/A	-66.9	3.51	0.73
a. BMD and BMDL are based on benchmark response of 1.0 SD. Results are presented in units of mg/kg/day.				
b. NA: the information is not available because there are insufficient degrees of freedom for the test				
c. ---: failed to estimated this value.				

Appendix C. Benchmark Dose Modeling Output

maternal body weight gain

```
            ============================================================
               Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
               Input Data File: F:\BMDS\MA.(d)
               Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                     Fri May 10 11:56:51 2002
                          ================================================
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	101.864	
rho =	0	Specified
control =	64	
slope =	-43.1882	
power =	-0.0984037	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.01	-0.049	-0.053
rho	-1	1	-0.011	0.049	0.054
control	0.01	-0.011	1	-0.77	-0.74
slope	-0.049	0.049	-0.77	1	1
power	-0.053	0.054	-0.74	1	1

Parameter Estimates

Variable	Estimate	Std. Err
alpha	98.6453	328.851

rho	0	0.834088
control	63.4328	2.12406
slope	-0.0653203	0.11847
power	1	0.30005

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.4	9.93	0.0571
60	25	58	9.4	59.5	9.93	-0.152
120	23	56.8	10.8	55.6	9.93	0.121
360	25	39.8	9.5	39.9	9.93	-0.0118

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.393473	4	544.786945
R	-297.755244	2	599.510487

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	60.506	6	<.00001
Test 2	0.779177	3	0.8544
Test 3	1.00324	1	0.3165

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .05 A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

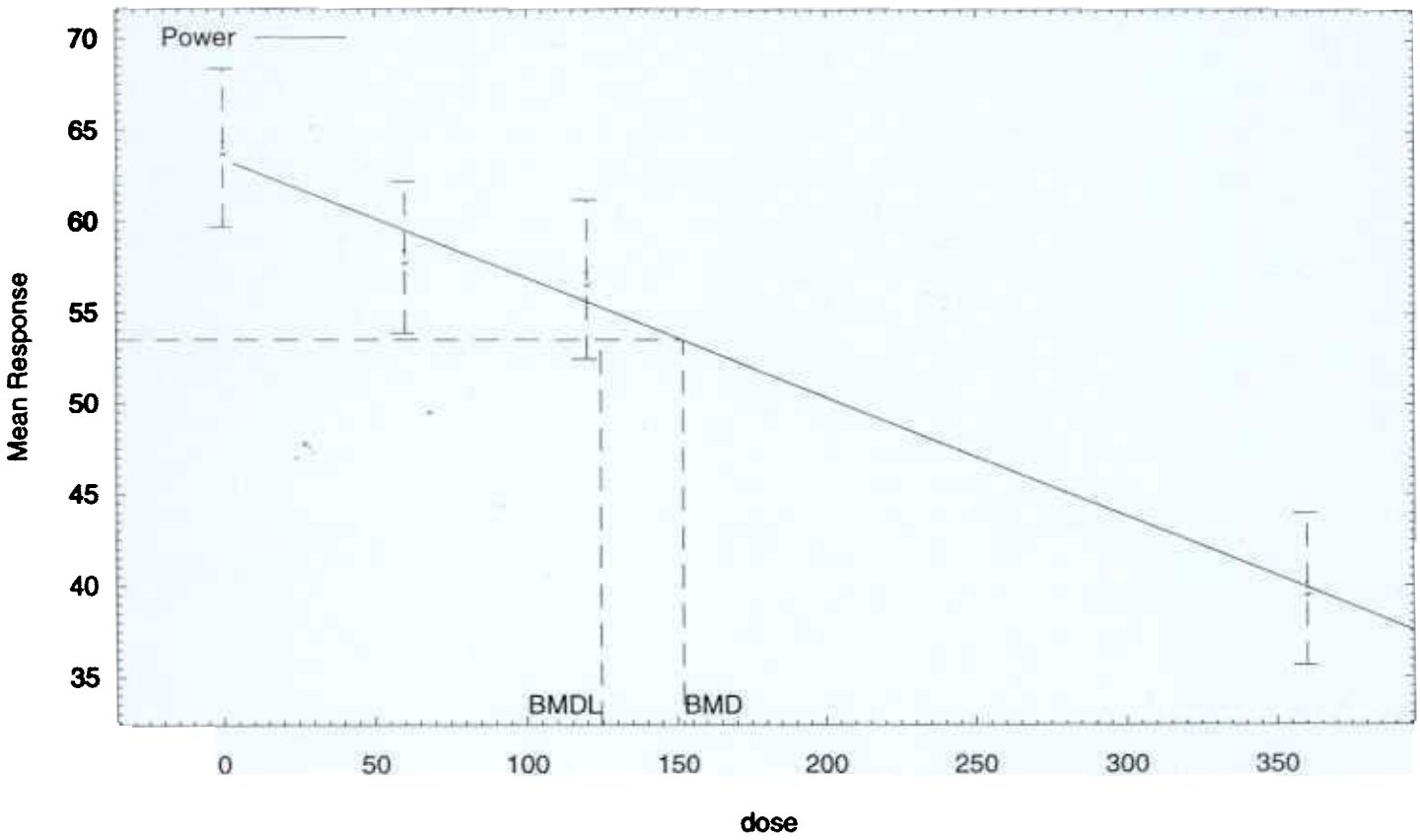
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 152.051

BMDL = 124.591

Power Model with 0.95 Confidence Level



11:56 05/10 2002

maternal body weight gain

```
          Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
          Input Data File: F:\BMDS\MA.(d)
          Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                    Fri May 10 11:55:32 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	101.864	
rho =	0	Specified
beta_0 =	63.3326	
beta_1 =	-0.0615798	
beta_2 =	-1.00086e-005	

Parameter Estimates

Variable	Estimate	Std. Err
alpha	98.6343	14.2366
beta_0	63.2966	1.94612
beta_1	-0.0618515	0.0343059
beta_2	-8.99169e-006	8.68663e-005

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-1.2e-006	1.9e-006	-2e-006
beta_0	-1.2e-006	1	-0.77	0.68
beta_1	1.9e-006	-0.77	1	-0.98
beta_2	-2e-006	0.68	-0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.3	9.93	1.63
60	25	58	9.4	59.6	9.93	-3.91
120	23	56.8	10.8	55.7	9.93	2.44
360	25	39.8	9.5	39.9	9.93	-0.163

Model Descriptions for likelihoods calculated

Model A1 $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2 $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.388117	3	542.776234
R	-297.755244	2	599.510487

Test 1 Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2 Are Variances Homogeneous (A1 vs A2)
Test 3 Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.506	6	<.0001
Test 2	0.779177	3	0.8544
Test 3	0.992529	1	0.3191

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than 05 The model

chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

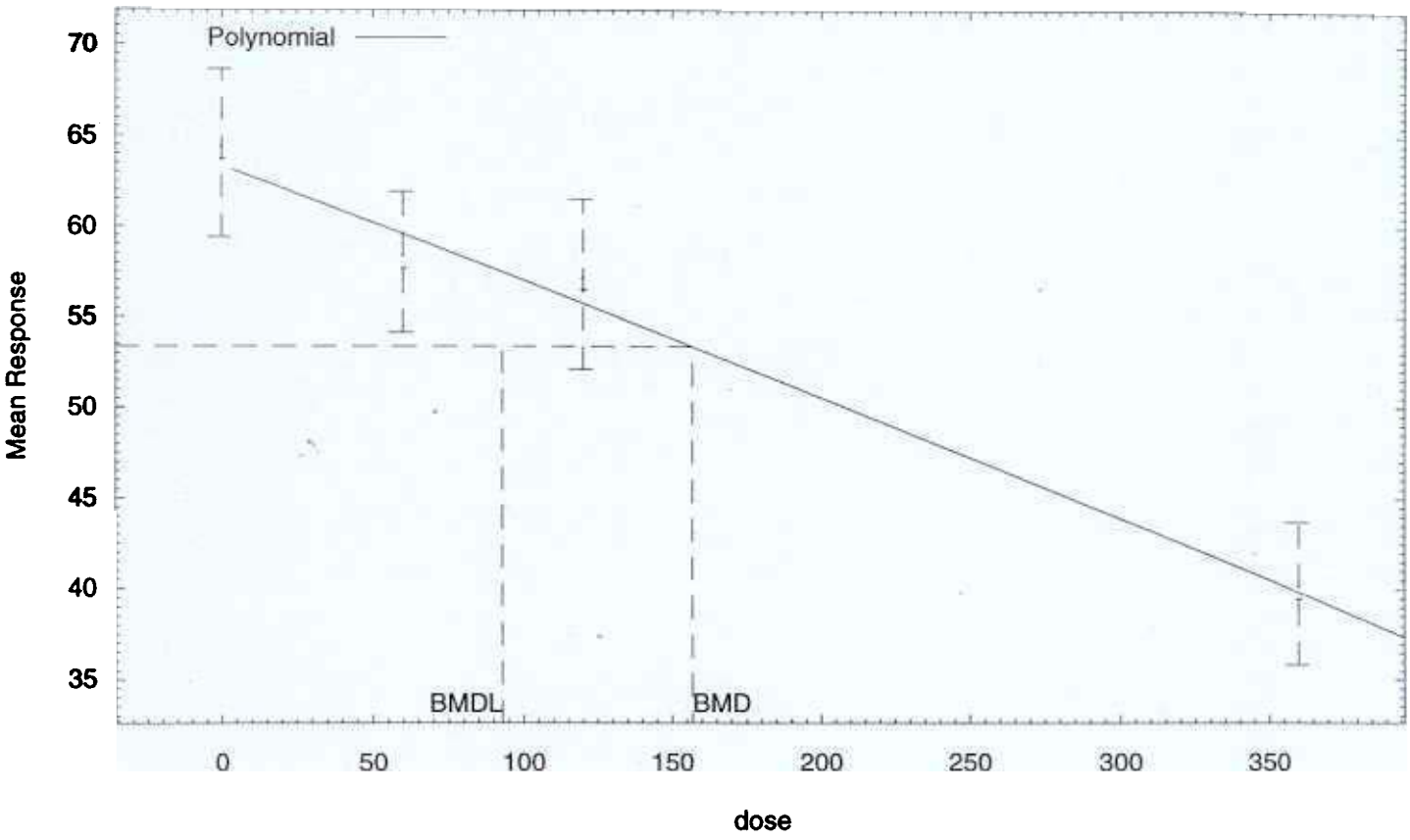
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 156.987

BMDL = 92.9272

Polynomial Model with 0.95 Confidence Level



11:55 05/10 2002

maternal body weight gain

```
            ============================================================
             Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
             Input Data File: F:\BMDS\MA.(d)
             Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                    Fri May 10 11:57:36 2002
 ========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~

   The form of the response function is

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   Power parameter restricted to be greater than
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   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
                        alpha =      101.585
                          rho =            0   Specified
                    intercept =           64
                            v =        -24.2
                            n =      1.42687
                            k =      189.176


           Asymptotic Correlation Matrix of Parameter Estimates

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

                     alpha          rho    intercept            v            k

         alpha            1            0            0            0            0

           rho            0            1            0            0            0

     intercept            0            0            1            0            0

             v            0            0            0            1            0

             k            0            0            0            0            1
```

Parameter Estimates

Variable	Estimate	Std Err
alpha	98.6445	1
rho	0	1
intercept	63.4595	1
v	-2388.7	1
n	1	NA
k	36185.3	

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.5	9.93	0.0544
60	25	58	9.4	59.5	9.93	-0.152
120	23	56.8	10.8	55.6	9.93	0.124
360	25	39.8	9.5	39.9	9.93	-0.013

Model Descriptions for likelihoods calculated

Model A1: $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Y_i = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.395812	4	544.791623
R	-297.755244	2	599.510487

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.506	6	<.0001
Test 2	0.779177	3	0.8544
Test 3	1.00792	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0 The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

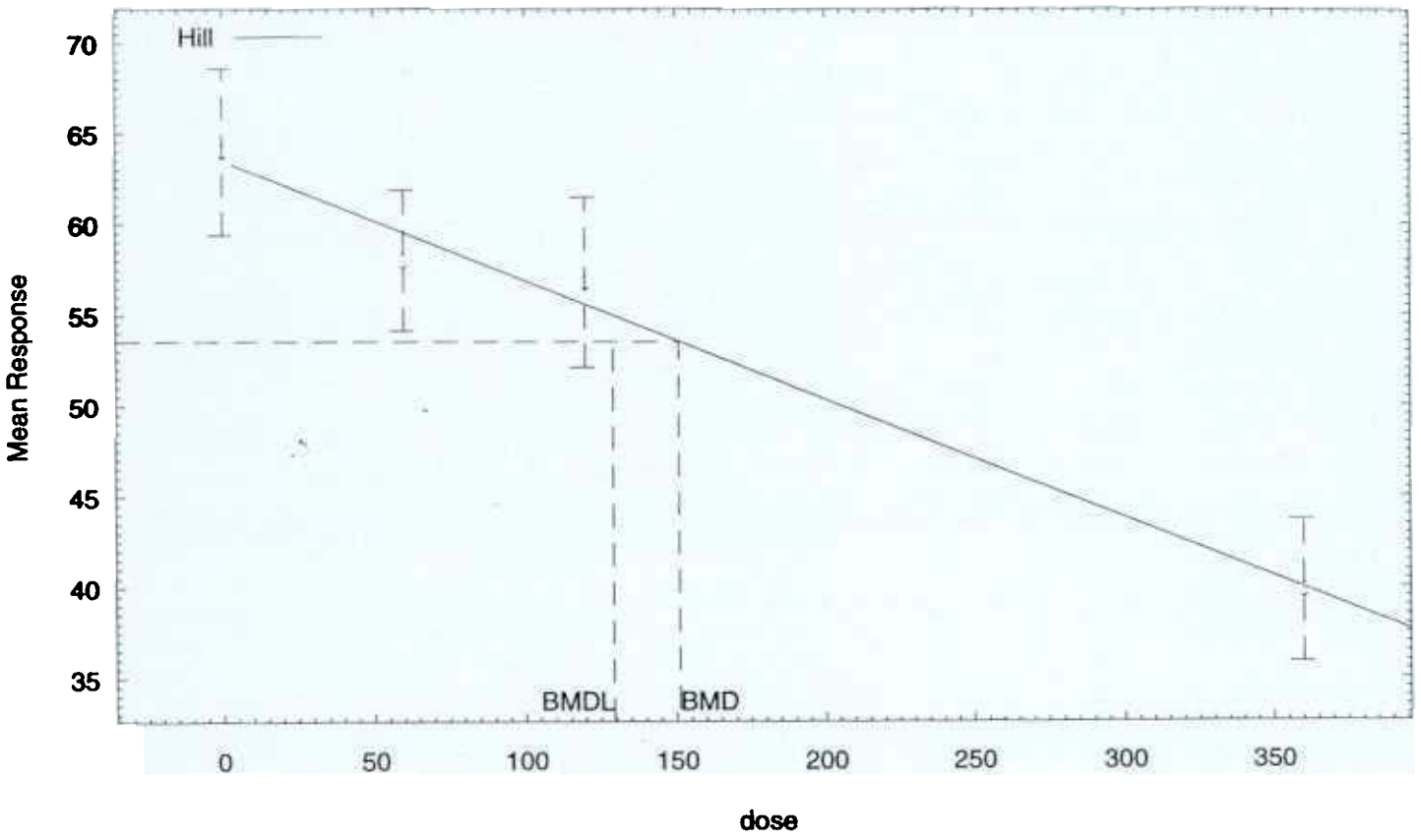
BMD = 151.083

BMDL = 129.177

Warning optimum may not have been found Bad completion code in Optimization routine.

BMDL computation failed for one or more point on the BMDL curve
The BMDL curve will not be plotted

Hill Model with 0.95 Confidence Level



11:57 05/10 2002

BMR=1.0 SD

```
            Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
            Input Data File: C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.(d)
            Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.plt
                                                  Thu Jan 10 16:36:20 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	779.391	
rho =	0	Specified
control =	440.4	
slope =	-357.319	
power =	-0.432011	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.095	0.16	0.16
rho	-1	1	0.094	-0.16	-0.16
control	-0.095	0.094	1	-0.78	-0.76
slope	0.16	-0.16	-0.78	1	1
power	0.16	-0.16	-0.76	1	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	747.101	25868.4
rho	0	5.71247
control	439.654	6.06278

slope	-0.0770251	0.338099
power	1	0.724264

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	440	29	440	27.3	0.0273
60	25	435	28.2	435	27.3	0.00247
120	23	429	25.1	430	27.3	-0.0443
360	24	412	29.1	412	27.3	0.0137

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Warning: Likelihood for model A1 larger than the Likelihood for model A2.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.236054	5	732.472108
A2	-361.411149	8	738.822299
fitted	-361.769541	4	731.539082
R	-368.360689	2	740.721377

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	13.8991	6	0.003046
Test 2	-0.350191	3	<.00001
Test 3	1.06697	1	0.3016

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

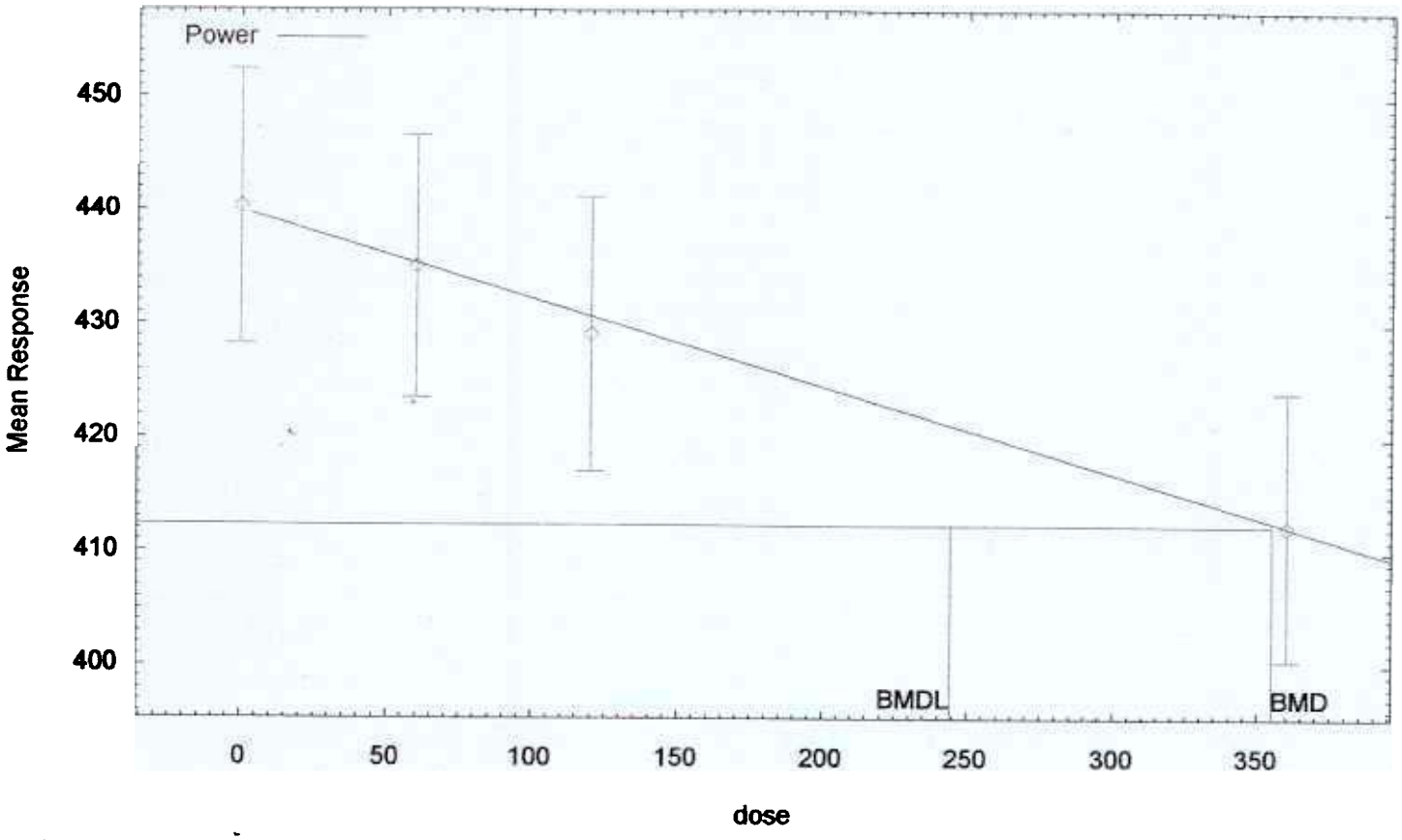
The p-value for Test 2 is less than .05. Consider running a non-homogeneous variance model

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	354.861
BMDL	=	244.116

Power Model with 0.95 Confidence Level



16:37 01/10 2002

Maternal body weight

```
        Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
        Input Data File: F:\BMDS\Argus maternal bw.(d)
        Gnuplot Plotting File:  F:\BMDS\UNSAVED1.plt
                                                    Fri May 10 10:18:09 2002
```

BMDS MODEL RUN

The form of the response function is

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	779.391	
rho =	0	Specified
beta_0 =	440.188	
beta_1 =	-0.0971452	
beta_2 =	5.21988e-005	

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	746.657	108.336
beta_0	440.199	5.35447
beta_1	-0.0970673	0.0943987
beta_2	5.19026e-005	0.000239328

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-5.5e-008	7.6e-008	-7.5e-008
beta_0	-5.5e-008	1	-0.77	0.68
beta_1	7.6e-008	-0.77	1	-0.98
beta_2	-7.5e-008	0.68	-0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	440	29	440	27.3	-0.167
60	25	435	28.2	435	27.3	0.401
120	23	429	25.1	429	27.3	-0.251
360	24	412	29.1	412	27.3	0.0167

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.736054	5	733.472108
A2	-361.411149	8	738.822299
fitted	-361.741255	3	729.482510
R	-368.365774	2	740.731548

Test 1 Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	13.9092	6	0.003031
Test 2	0.649809	3	0.8849
Test 3	0.010402	1	0.9188

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than 05 The model

chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

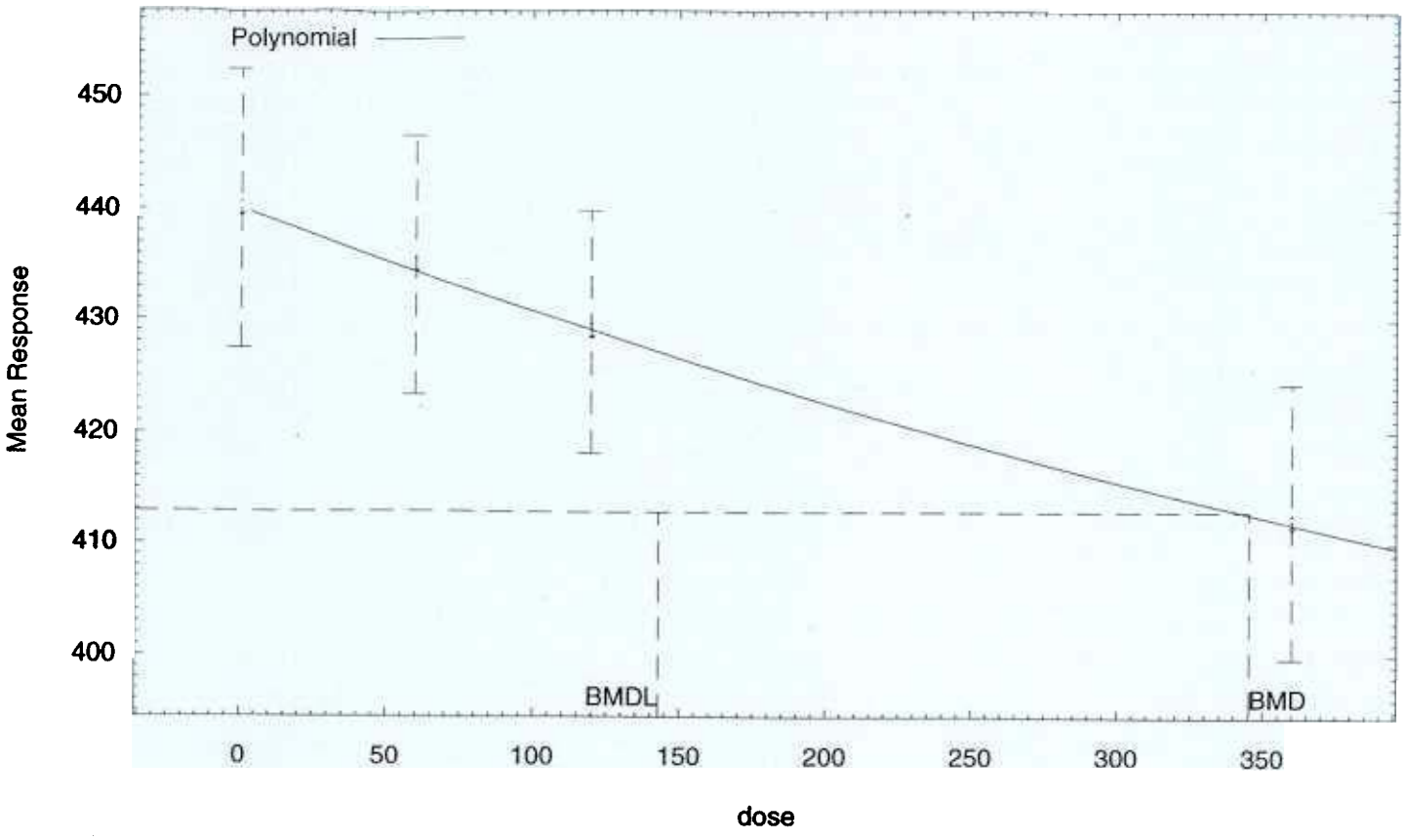
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 345.237

BMDL = 142.794

Polynomial Model with 0.95 Confidence Level



10:18 05/10 2002

BMR=1.0 SD

```
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.plt
                                                    Thu Jan 10 16:38:54 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	772.871	
rho =	0	Specified
intercept =	440.4	
v =	-28.1	
n =	1.44931	
k =	160.473	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	0	0	0	0	0
rho	0	1	0	0	0	0
intercept	0	0	1	0	0	0
v	0	0	0	1	0	0
n	0	0	0	0	1	0
k	0	0	0	0	0	0

Parameter Estimates

Variable	Estimate	Std Err.
alpha	746.575	1
rho	0	1
intercept	440.4	1
v	-57.271	1
n	1.25328	1
k	370.906	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res
0	23	440	29	440	27.3	5.28e-008
60	25	435	28.2	435	27.3	3.13e-007
120	23	429	25.1	429	27.3	-3.6e-007
360	24	412	29.1	412	27.3	9.88e-007

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Warning: Likelihood for fitted model larger than the Likelihood for model A1

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.736054	5	733.472108
A2	-361.411149	8	738.822299
fitted	-361.736054	5	733.472107
R	-368.860689	2	741.721377

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	14.8991	6	0.001905
Test 2	0.649809	3	0.8849
Test 3	1.01313e-007	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

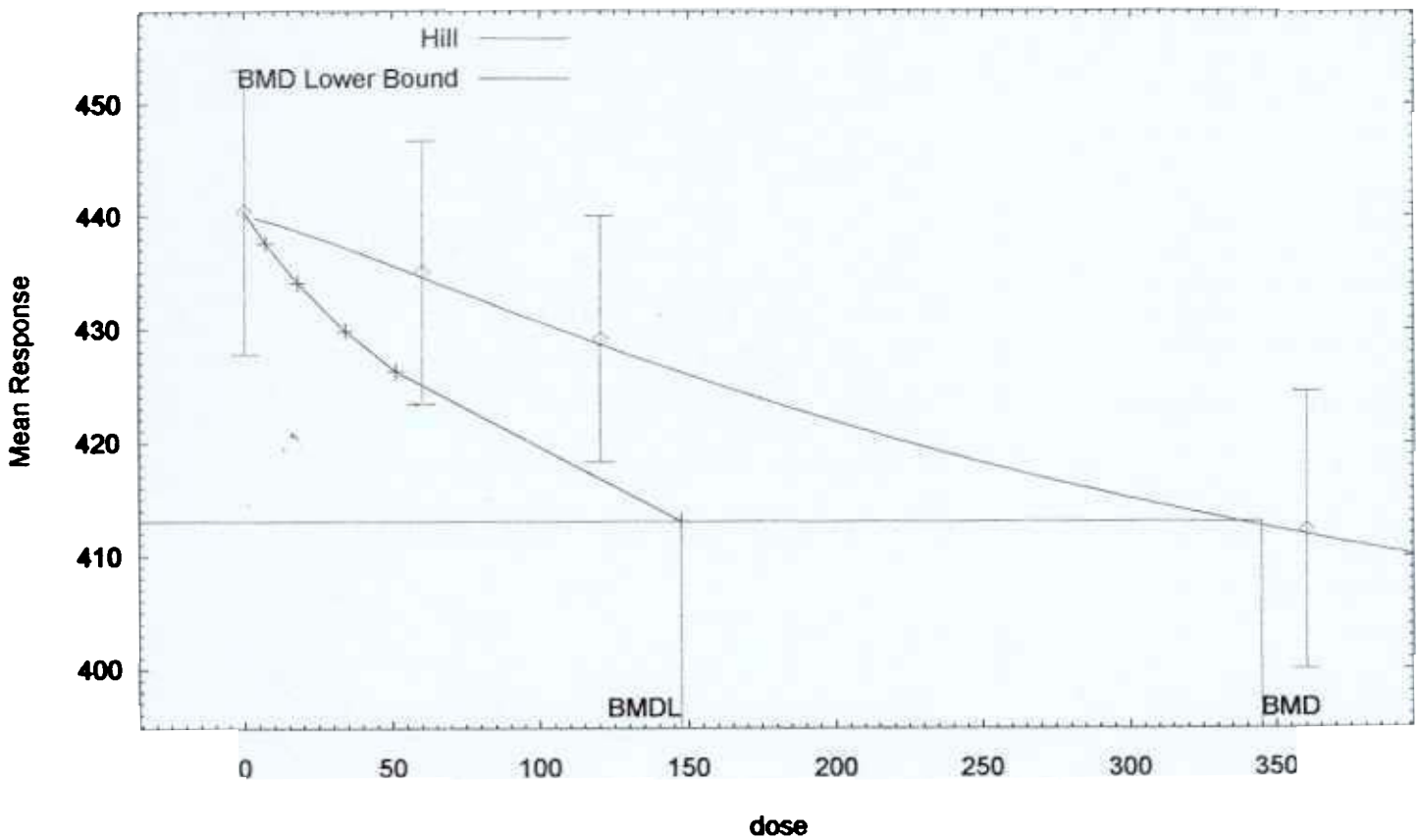
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	344.737
BMDL	=	147.428

Hill Model with 0.95 Confidence Level



16:38 01/10 2002

Clintrials motor activity. BMR=1 SD

Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
Input Data File: F:\BMDS\UNSAVED1.(d)
Gnuplot Plotting File: F:\BMDS\UNSAVED1.plt
Fri May 17 13:46:34 2002

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	14556.4	
rho =	0	Specified
control =	468	
slope =	-2101.71	
power =	-0.471502	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	NA	NA	NA
rho	-1	1	NA	NA	NA
control	NA	NA	NA	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	13773.9	81444
rho	0	0.995906
control	455.234	58.2246
slope	-0.348708	5.10257
power	1	2.40548

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	455	117	0.109
24.6	15	451	149	447	117	0.037
107	15	394	78	418	117	-0.204
360	14	337	127	330	117	0.0622

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var(e(ij)) = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var(e(ij)) = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var(e(i)) = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-309.709699	5	629.419399
A2	-307.217620	8	630.435240
fitted	-310.650690	4	629.301380
R	-315.381614	2	634.763229

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	16.328	6	0.0009712
Test 2	4.98416	3	0.173
Test 3	1.88198	1	0.1701

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

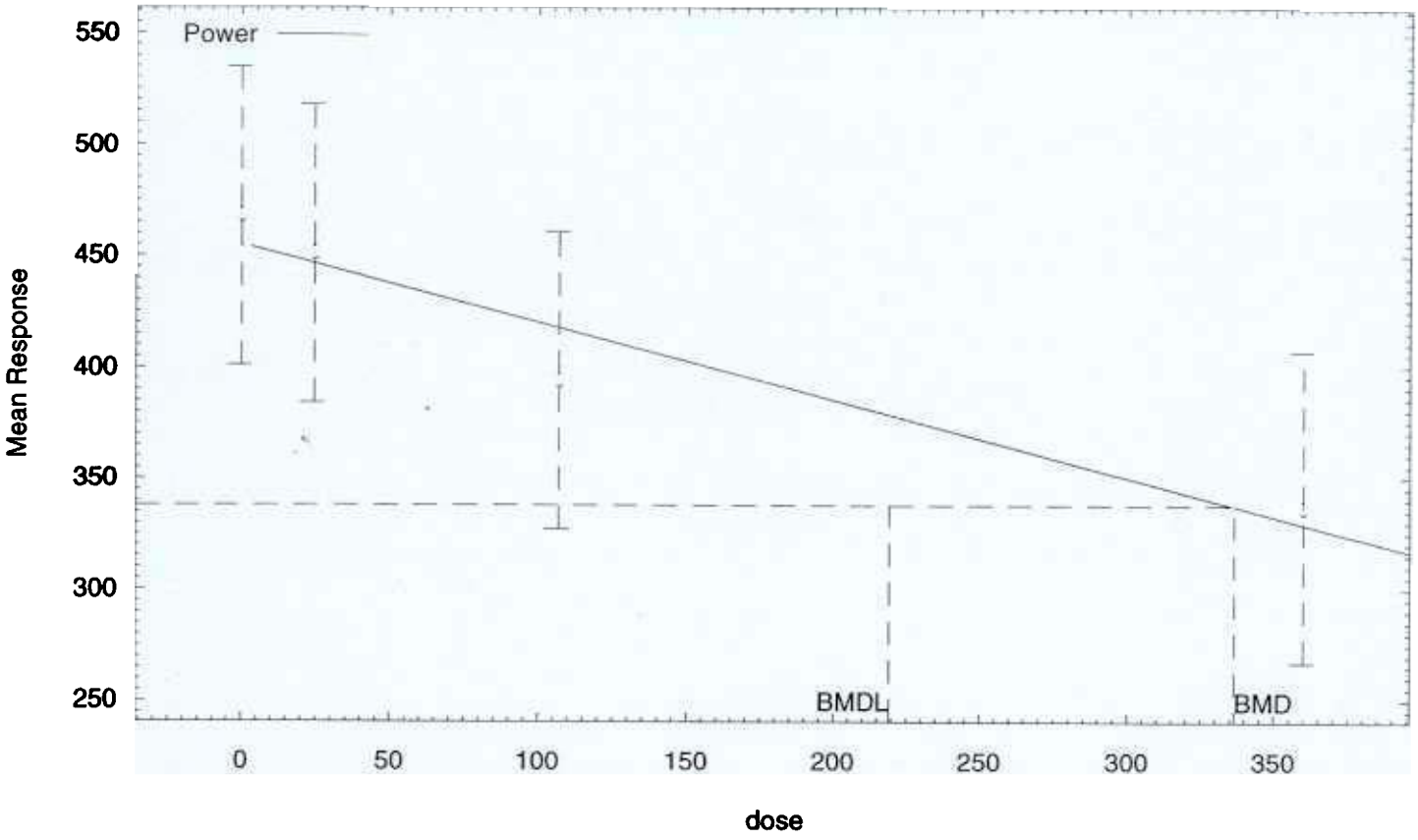
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

336.564

219.138

Power Model with 0.95 Confidence Level



13:46 05/17 2002

Clintrials motor activity in females at 4 weeks, BMR = 1 SD

Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
Input Data File: F:\BMDS\UNSAVED1.(d)
Gnuplot Plotting File: F:\BMDS\UNSAVED1.plt
Fri May 17 13:53:20 2002

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
The polynomial coefficients are restricted to be negative
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	1	
rho =	0	Specified
beta_0 =	469.177	
beta_1 =	-0.83776	
beta_2 =	0	

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	13763.4	2532.12
beta_0	455.234	20.0191

beta_1	-0.348708	0.108862
beta_2	0	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	7.1e-010	-2.3e-01
beta_0	7.1e-010	1	-0.65
beta_1	-2.3e-011	-0.65	1

The following parameter(s) have been estimated at a
boundary
point or have been specified. Correlations are not
computed:

beta_2

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	455	117	1.63
24.6	15	451	149	447	117	0.555
107	15	394	78	418	117	-3.06
360	14	337	127	330	117	0.871

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-310.209699	5	630.419399
A2	-307.217620	8	630.435240
fitted	-310.650699	2	625.301398
R	-315.381614	2	634.763229

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	16.328	6	0.0009712
Test 2	5.98416	3	0.1124
Test 3	0.881999	1	0.3477

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

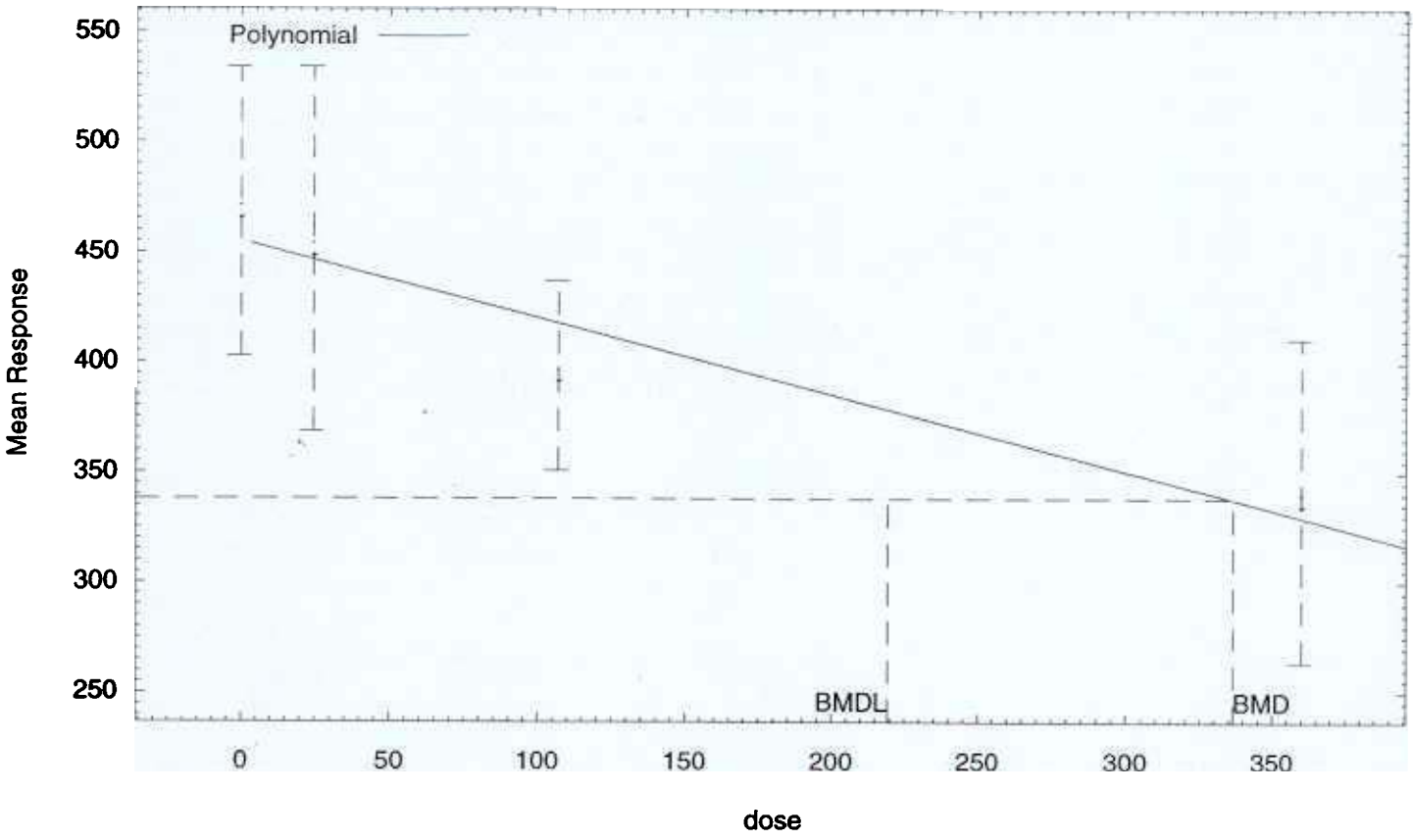
BMD = 336.435

BMDL = 219.137

BMDL computation failed for one or more point on the BMDL curve.

The BMDL curve will not be plotted

Polynomial Model with 0.95 Confidence Level



13:53 05/17 2002

Clintrials motor activity BMR=1 SD

```
        ================================================================
          Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
          Input Data File: F:\BMDS\UNSAVED1.(d)
          Gnuplot Plotting File:  F:\BMDS\UNSAVED1.plt
                                                    Fri May 17 13:48:20 2002
 ================================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   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
                            alpha =     13197.2
                              rho =           0   Specified
                        intercept =         468
                                v =        -131
                                n =     1.77566
                                k =     94.7123


             Asymptotic Correlation Matrix of Parameter Estimates

                      alpha          rho    intercept            v            n
      k

      alpha              1            0            0            0            0
       0

        rho              0            1            0            0            0
       0

  intercept              0            0            1            0            0
       0

          v              0            0            0            1            0
       0

          n              0            0            0            0            1
       0
```

k	0	0	0	0	0
1					

Parameter Estimates

Variable	Estimate	Std Err
alpha	13569.6	
rho		
intercept	468	1
v	-161.338	1
n	1.34226	1
k	121.061	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	468	116	1.17e-007
24.6	15	451	149	451	116	-3.09e-007
107	15	394	78	394	116	-2.44e-007
360	14	337	127	337	116	7.32e-007

Model Descriptions for likelihoods calculated

Model A1: $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2 $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R $Y_i = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-310.209699	5	630.419399
A2	-307.217620	8	630.435240
fitted	-310.209699	5	630.419399
R	-315.881614	2	635.763229

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2 Are Variances Homogeneous (A1 vs A2)
Test 3 Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.328	6	0.000605
Test 2	5.98416	3	0.1124
Test 3	2.93312e-011	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .05 A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

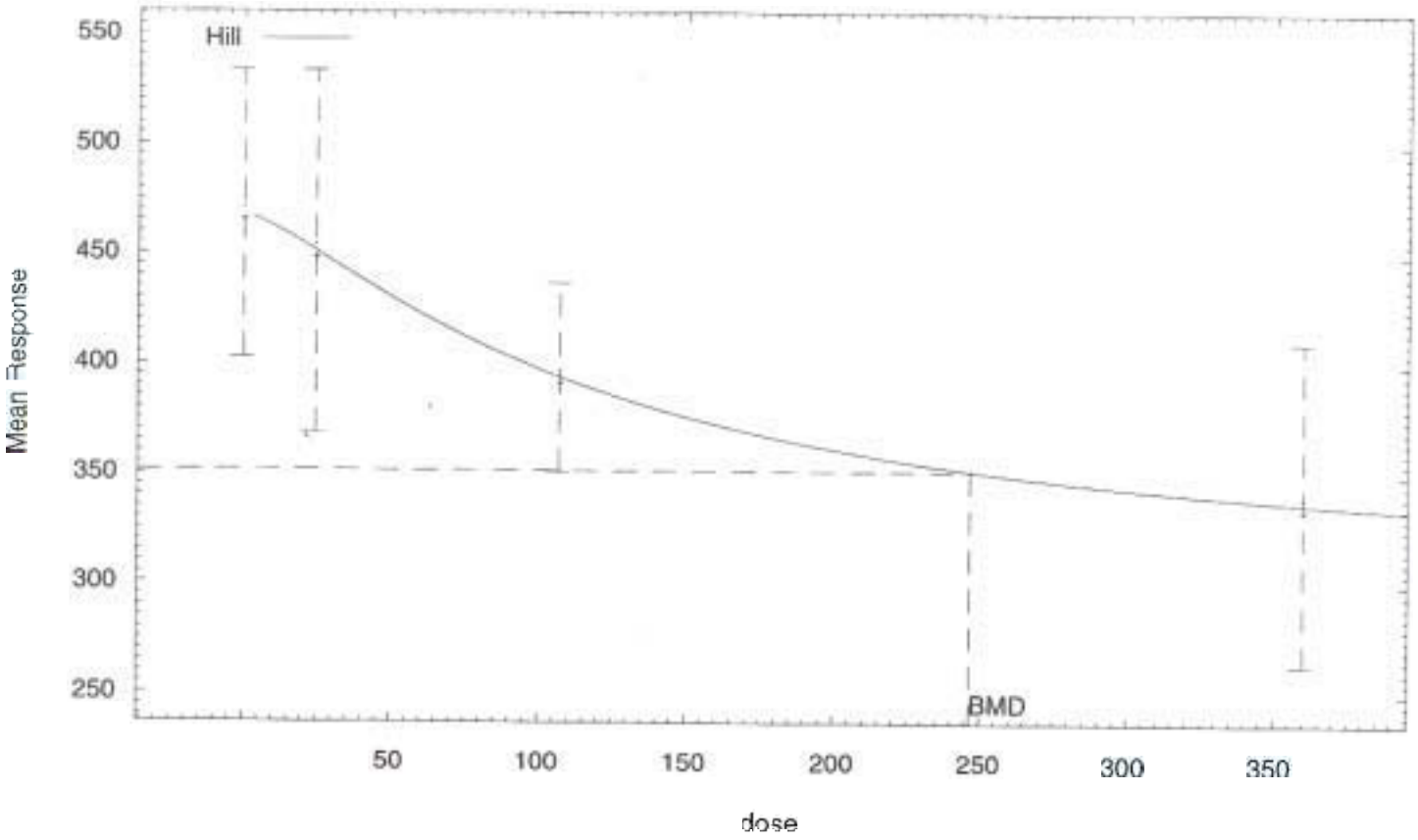
Confidence level = 0.95

BMD = 246.508

Warning optimum may not have been found Bad completion code in Optimization r outine.

BMDL computation failed

Hi Model



3:48 05/1 2002

PFC response, BMR=1.0 SD

```
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 14:01:03 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	28373.3	
rho =	0	Specified
control =	1123	
slope =	-1807.87	
power =	-0.361754	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.065	NA	NA
rho	-1	1	-0.056	NA	NA
control	0.065	-0.056	1	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero.

Parameter Estimates

Variable	Estimate	Std. Err

alpha	32160.5	346106
rho	0	1.59091
control	976.333	37.4625
slope	-11.4263	18.1954
power	1	0.423031

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res
0	5	1.12e+003	221	976	179	0.818
1.8	5	896	134	956	179	-0.333
6.2	5	795	110	905	179	-0.616
33.6	5	616	186	592	179	0.132

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-110.300585	5	230.601171
A2	-108.879712	8	233.759424
fitted	-113.784950	4	235.569899
R	-119.888342	2	243.776683

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	22.0173	6	6.469e-005
Test 2	2.84175	3	0.4167
Test 3	6.96873	1	0.008295

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is less than .05. You may want to try a

different model

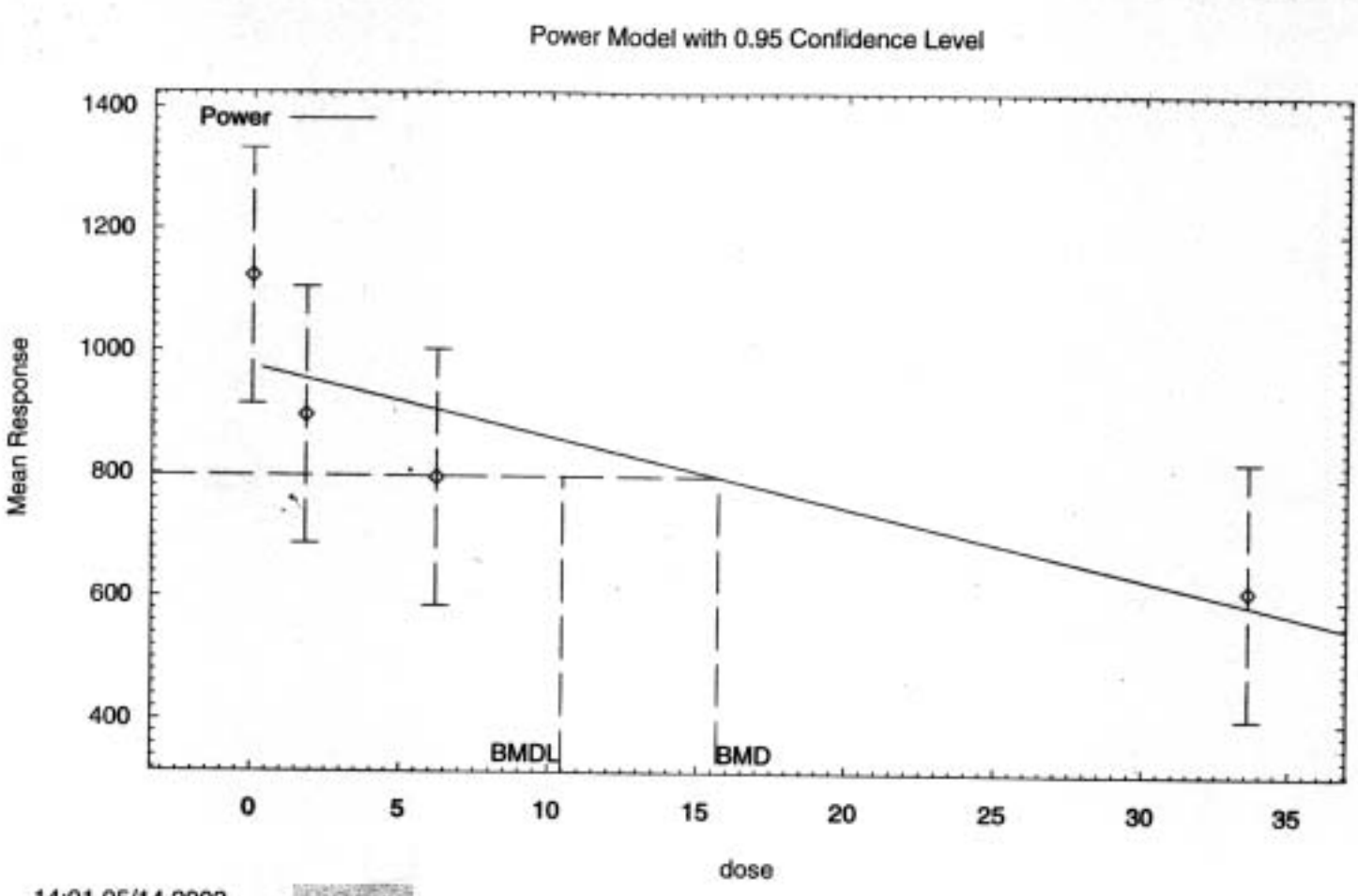
Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 15.6948

BMDL = 10.4544


Power Model with 0.95 Confidence Level
Power
Mean Response
1400
1200
1000
800
600
400
BMDL
BMD
0
5
10
15
20
25
30
35
dose
14:01 05/14 2002

PFC response BMR = 1 SD

Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
Input Data File: F:\BMDS\MA.(d)
Gnuplot Plotting File: F:\BMDS\MA.plt
Tue May 14 14:20:26 2002

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
The polynomial coefficients are restricted to be negative
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	1	
rho =	0	Specified
beta_0 =	1071.13	
beta_1 =	-56.7007	
beta_2 =	0	

Parameter Estimates

Variable	Estimate	Std. Err
alpha	26722.6	8451.07
beta_0	976.316	46.0371
beta_1	-11.4246	2.69108
beta_2	0	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-0.00012	0.00019
beta_0	-0.00012	1	-0.61
beta_1	0.00019	-0.61	1

The following parameter(s) have been estimated at a boundary
point or have been specified. Correlations are not computed:

beta_2

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	1.12e+003	221	976	163	4.49
1.8	5	896	134	956	163	-1.83
6.2	5	795	110	905	163	-3.38
33.6	5	616	86	592	163	0.72

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-107.560658	5	225.121317
A2	-105.022715	8	226.045431
fitted	-111.931872	2	227.863744
R	-118.871191	2	241.742383

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.697	6	<.0001
Test 2	5.07589	3	0.1663
Test 3	8.74243	1	0.003109

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the
dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05 A
homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is less than .05. You may want
to try a
different model

Benchmark Dose Computation
Specified effect = 1

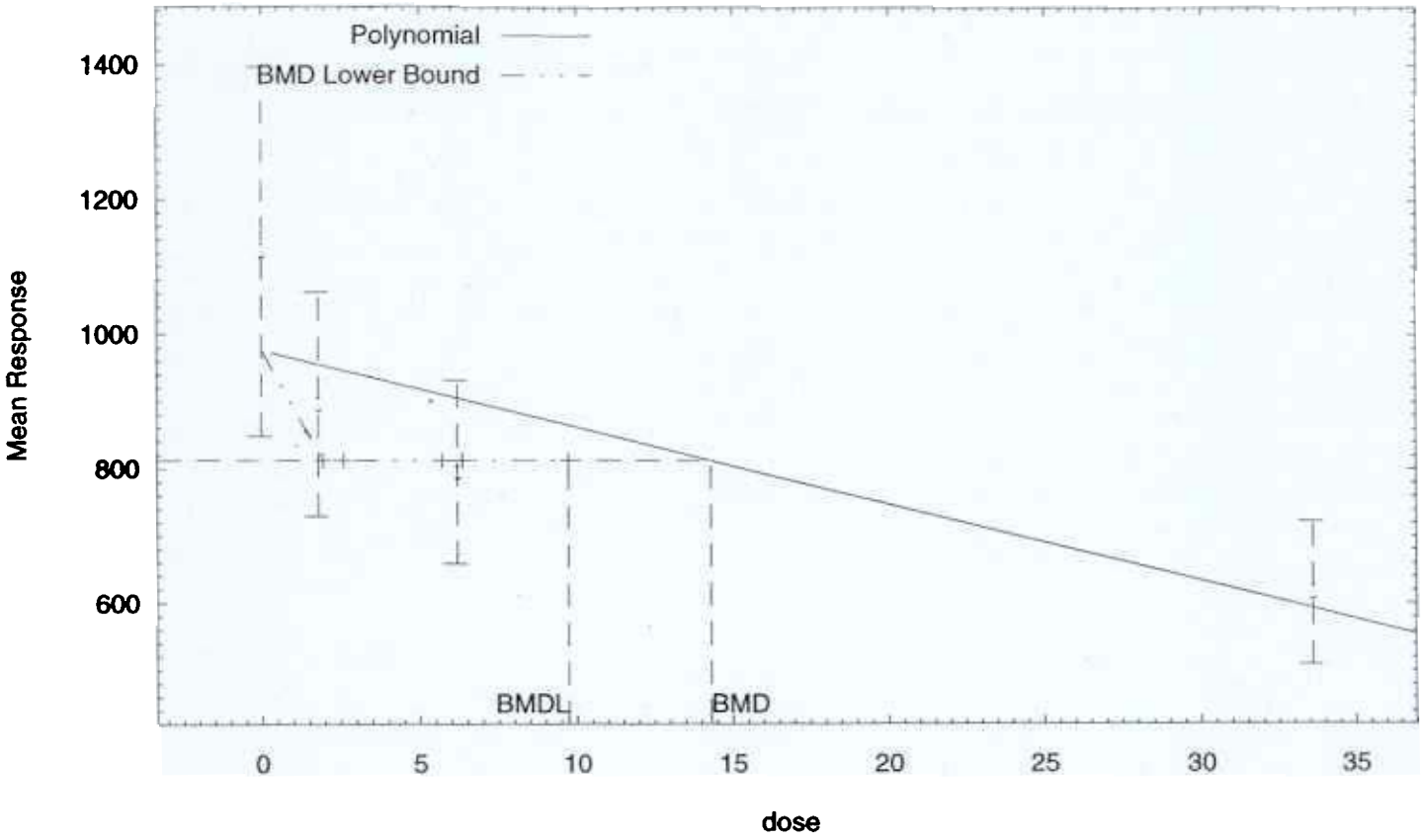
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 14.3086

BMDL = 9.73181

Polynomial Model with 0.95 Confidence Level



14:20 05/14 2002

PFC response, BMR=1.0 SD

```
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 14:02:22 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	24615.1	
rho =	0	Specified
intercept =	1123	
v =	-507	
n =	0.62026	
k =	2.95446	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	23185.3	1
rho	0	1
intercept	1115.7	1
v	-533.126	1
n	1	NA
k	3.17243	1

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	1.12e+003	221	1.12e+003	152	0.048
1.8	5	896	134	923	152	-0.175
6.2	5	795	110	763	152	0.21
33.6	5	616	186	629	152	-0.0825

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-110.300585	5	230.601171
A2	-108.879712	8	233.759424
fitted	-110.512739	4	229.025477
R	-119.888342	2	243.776683

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	22.0173	6	<.0001
Test 2	2.84175	3	0.4167
Test 3	0.424307	0	NA

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

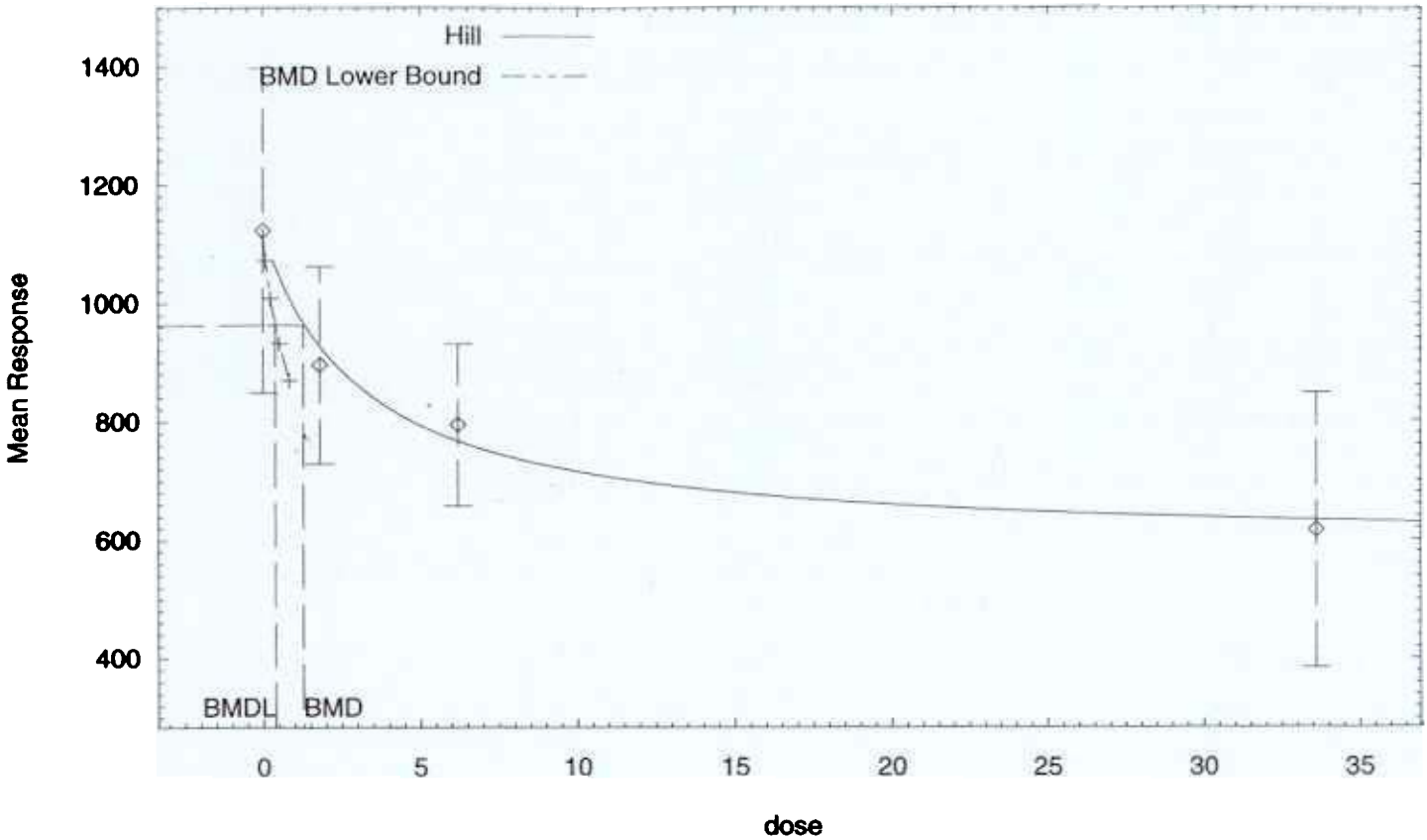
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1.26834

BMDL = 0.383495

Hill Model with 0.95 Confidence Level



14:02 05/14 2002

PFC/total spleen

```
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 15:50:01 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = Dose
The power is restricted to be greater than or equal to 1
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	4.76625e+009
rho =	0
control =	265947
slope =	-173704
power =	-0.0701236

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.39	NA	NA
rho	-1	1	-0.4	NA	NA
control	0.39	-0.4	1	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero.

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.00015149	0.0025216

rho	2.52122	1.36567
control	237600	17339.2
slope	-3207.96	6498.98
power	1	0.554928

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	2.66e+005	1.19e+005	2.38e+005	7.36e+004	0.385
1.8	5	2.15e+005	3.91e+004	2.32e+005	7.14e+004	-0.24
6.2	5	2.08e+005	4.22e+004	2.18e+005	6.59e+004	-0.152
33.6	5	1.3e+005	4.07e+004	1.3e+005	3.44e+004	0.0108

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model A3: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = alpha*(Mu(i))^{rho}$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759631	6	467.519262
fitted	-229.618859	5	469.237718
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75059	2	0.09299
Test 4	3.71846	1	0.05381

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

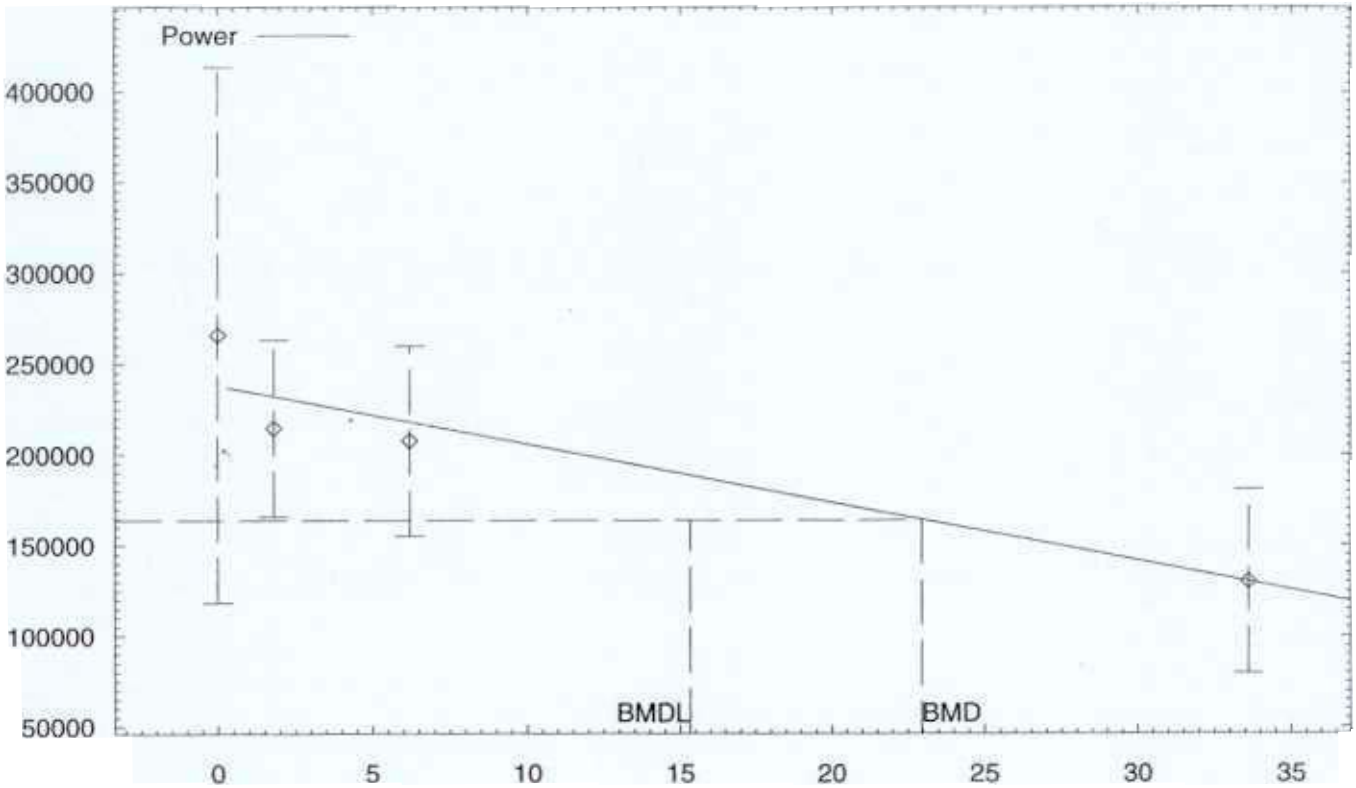
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

22.9511

15.3302

Power Model with 0.95 Confidence Level



Mean Response

dose

15:50 05/14 2002

PFC/total spleen

Polynomial Model. $Revision: 2.1 $ $Date 2000/10/11 17:51:39 $
Input Data File: F:\BMDS\MA.(d)
Gnuplot Plotting File: F:\BMDS\MA.plt
Tue May 14 16:19:41 2002

BMDS MODEL RUN

The form of the response function is

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2

Dependent variable = MEAN
Independent variable = dose
The polynomial coefficients are restricted to be negative
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	1
rho =	0
beta_0 =	251869
beta_1 =	-9322.72
beta_2 =	0

Parameter Estimates

Variable	Estimate	Std. Err
alpha	216454	344531
rho	0.982068	0.138912
beta_0	188775	63404
beta_1	-7724.65	20131.8
beta_2	-1.63675	555.248

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_1	beta_2
alpha	1	-1	0.14	-0.027	0.017
rho	-1	1	-0.053	0.021	0.0082
beta_0	0.14	-0.053	1	-0.79	0.71
beta_1	-0.027	0.021	-0.79	1	-1
beta_2	0.017	0.0082	0.71	-1	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2
Res.						
0	5	2.66e+005	1.19e+005	1.[illegible]9e+005	1.81e+005	2.13
1.8	5	2.15e+005	3.91e+004	1.[illegible]5e+005	1.75e+005	1.14
6.2	5	2.08e+005	4.22e+004	1.[illegible]1e+005	1.57e+005	2.13
33.6	5	1.3e+005	4.07e+004	-7.[illegible]6e+004	1.13e+005	8.94

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2 $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model A3: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = alpha*(Mu(i))^{rho}$

Model R: $Yi = Mu + e(i$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759622	6	467.519244
fitted	-249.295307	5	508.590613
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75057	2	0.09299
Test 4	43.0714	1	<.0001

The p-value for Test 1 is less than .05 There appears to be a

difference between response and/or variances among the
dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05 A
non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than 05. The
modeled variance appears
to be appropriate here

The p-value for Test 4 is less than 05 You may want
to try a different
model

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 23.3523

BMDL = 5.86859

PFC/total spleen

```
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 15:50:56 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	1
rho =	2.30623
intercept =	265947
v =	-135762
n =	0.475261
k =	9.59273

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	rho	intercept	v	k
alpha	1	-1	NA	-0.99	
rho	-1	1	NA	0.99	
intercept	NA	NA	NA	NA	NA
v	-0.99	0.99	NA	1	
k	0.21	-0.22	NA	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	9.6535e-006	1.06893e-008
rho	2.73759	8.76021e-005
intercept	265131	1.22396
v	-136181	2.5363
n	1	NA
k	4.00666	8.61636e-005

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	2.66e+005	1.19e+005	2.65e+005	8.24e+004	0.0099
1.8	5	2.15e+005	3.91e+004	2.23e+005	6.5e+004	-0.127
6.2	5	2.08e+005	4.22e+004	1.82e+005	4.94e+004	0.511
33.6	5	1.3e+005	4.07e+004	1.43e+005	3.56e+004	-0.373

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A3 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759620	6	467.519240
fitted	-228.438245	5	466.876489
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75057	2	0.09299
Test 4	1.35725	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

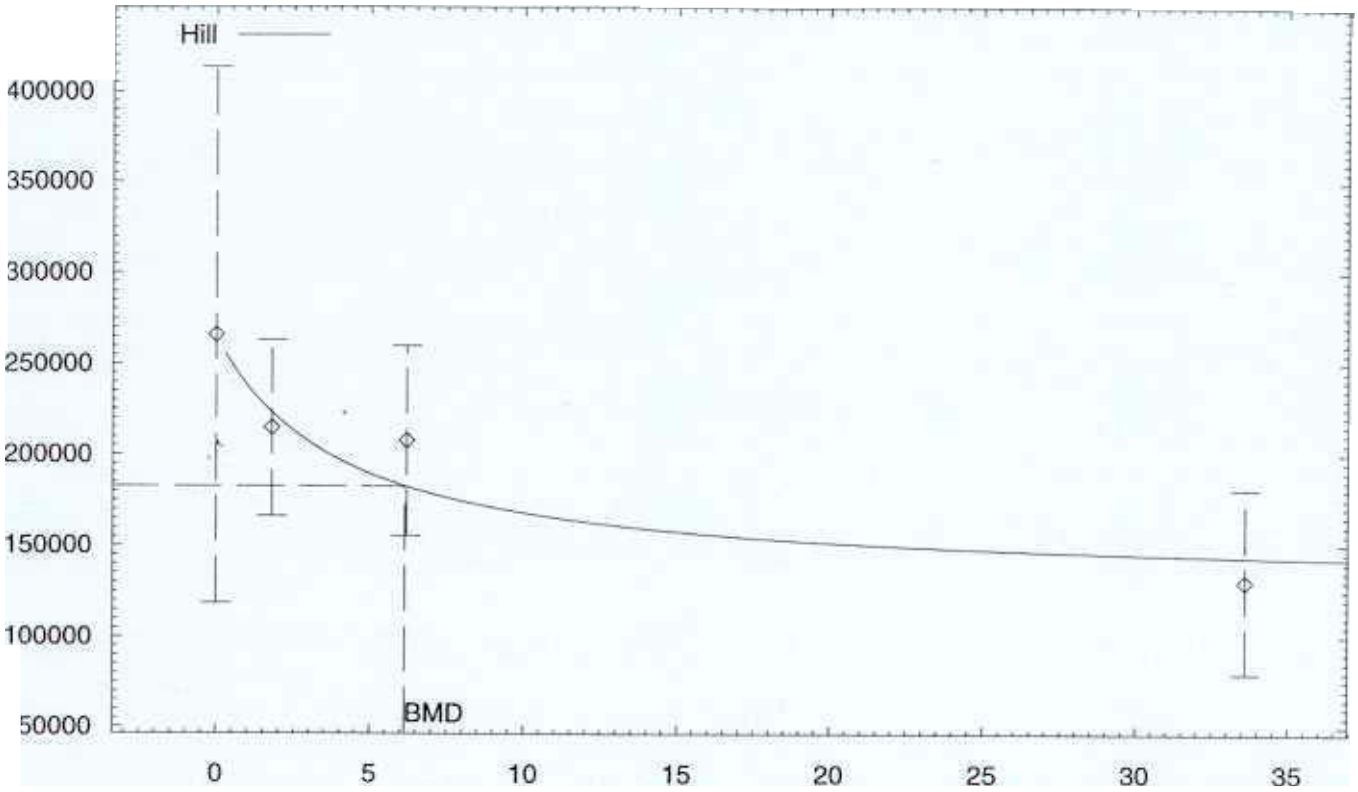
Confidence level = 0.95

BMD = 6.13974

Warning: optimum may not have been found. Bad completion code in Optimization routine.

BMDL computation failed.

Hill Model



Mean Response

dose

15:50 05/14 2002

Antibody titer

```
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 15:52:46 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	0.0098315	
rho =	0	Specified
control =	0.446	
slope =	-1.46784	
power =	-0.592416	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	0.99	0.091	NA	NA
rho	0.99	1	0.041	NA	NA
control	0.091	0.041	1	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero

Parameter Estimates

Variable	Estimate	Std. Err

alpha	0.00898754	0.018154
rho	0	1.9925
control	0.402624	0.0174706
slope	-0.00443496	0.0109976
power	1	0.658352

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.403	0.0948	0.458
1.8	5	0.392	0.152	0.395	0.0948	-0.0279
6.2	5	0.325	0.042	0.375	0.0948	-0.529
33.6	5	0.263	0.083	0.254	0.0948	0.0991

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Yij = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	37.119156	4	-66.238312
R	33.214605	2	-62.429209

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	2.66783	1	0.1024

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

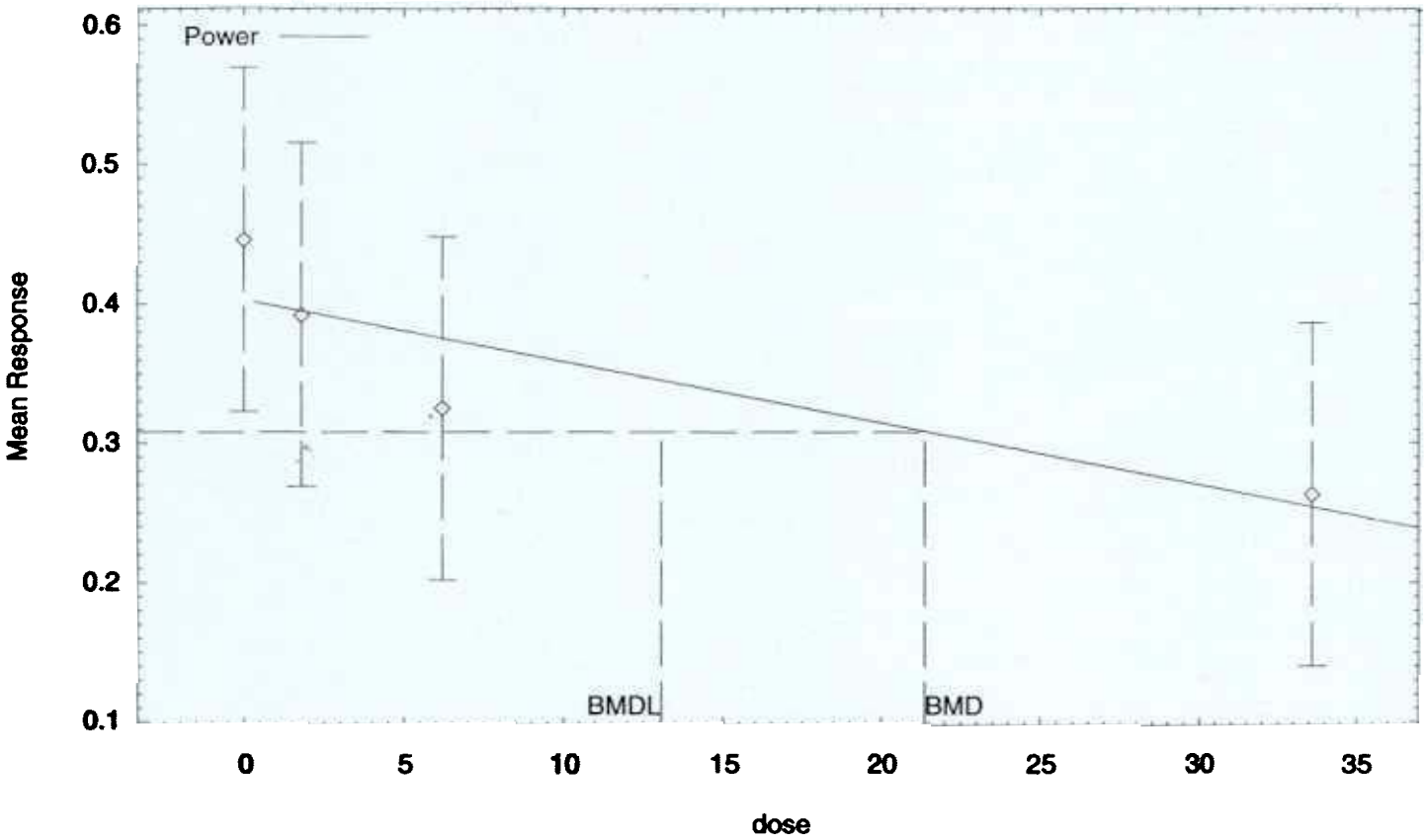
The p-value for Test 3 is greater than .05. The model chosen appears

to adequately describe the data

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	21.3762
BMDL	=	13.0626

Power Model with 0.95 Confidence Level



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antibody titer

```
            ============================================================
            Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
            Input Data File: F:\BMDS\MA.(d)
            Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                  Tue May 14 16:22:39 2002
                        ================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +


   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   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
                            alpha =    0.0098315
                              rho =            0   Specified
                           beta_0 =     0.439645
                           beta_1 =   -0.0221168
                           beta_2 =            0


                          Parameter Estimates

        Variable         Estimate          Std. Err
           alpha       0.00898754        0.00284211
         beta_0          0.402624         0.0266986
         beta_1       -0.00443496        0.00156066
         beta_2                 0                NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


          Asymptotic Correlation Matrix of Parameter Estimates

                   alpha        beta_0        beta_1
    alpha              1        -2e-008      5.4e-009
    beta_0       -2e-008              1         -0.61
    beta_1     5.4e-009          -0.61             1
```

The following parameter(s) have been estimated at a boundary
point or have been specified. Correlations are not computed:

beta

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.403	0.0948	2.29
1.8	5	0.392	0.152	0.395	0.0948	-0.139
6.2	5	0.325	0.042	0.375	0.0948	-2.64
33.6	5	0.263	0.083	0.254	0.0948	0.495

Model Descriptions for likelihoods calculated

Model A1 $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2 $Y_{ij} = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model R: $Y_i = Mu + e(i$
$Var\{e(i)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	37.119156	2	-70.238312
R	33.214605	2	-62.429209

Test 1 Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	2.66783	1	0.1024

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the
dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A
homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .05 The model
chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

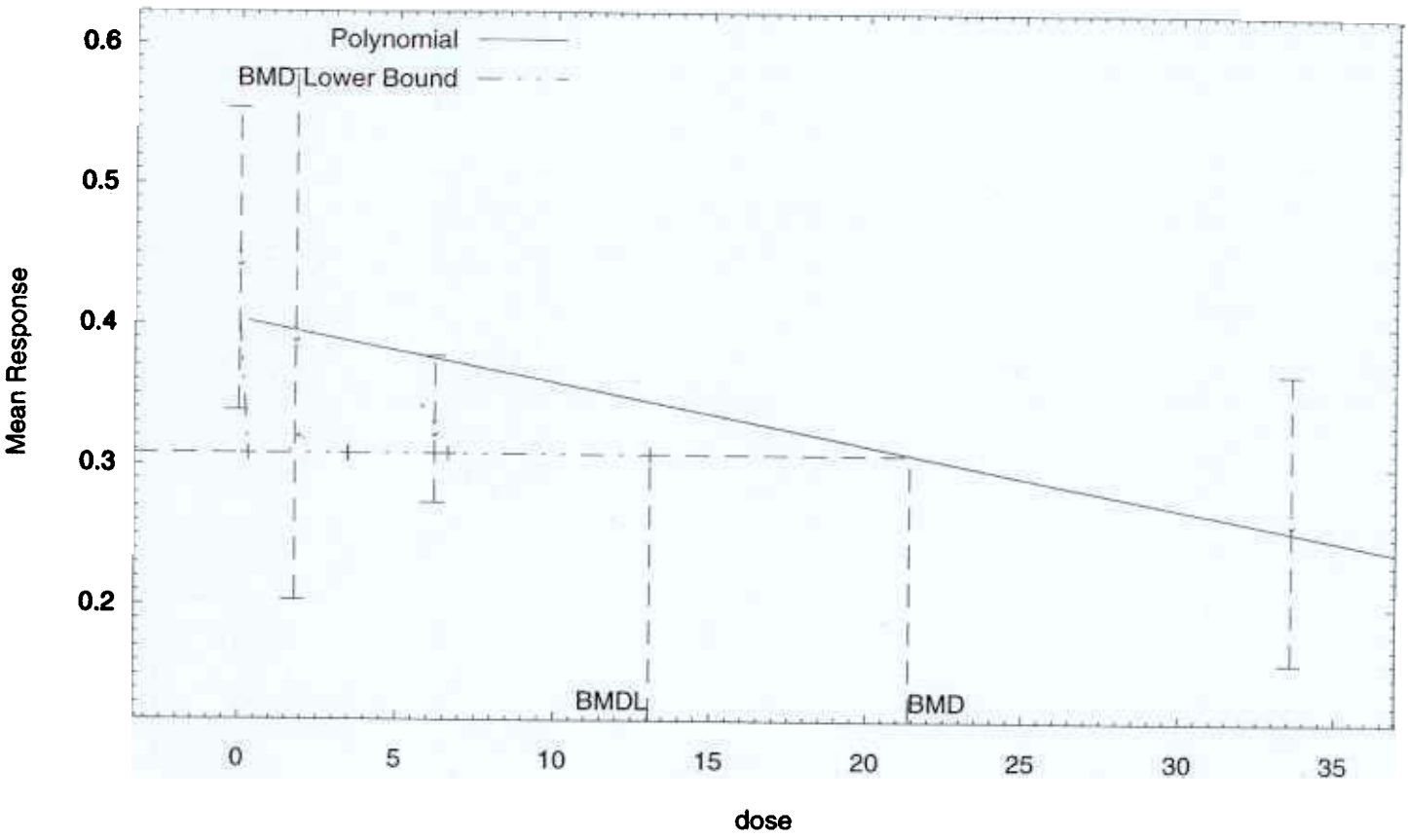
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 21.3762

BMDL = 13.0626

Polynomial Model with 0.95 Confidence Level



16:22 05/14 2002

Antibody titer

```
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                  Tue May 14 15:53:39 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
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

alpha =	0.00678961	
rho =	0	Specified
intercept =	0.446	
v =	-0.183	
n =	1.39743	
k =	4.26269	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	0	0	0	0	0
rho	0	1	0	0	0	0
intercept	0	0	1	0	0	0
v	0	0	0	1	0	0
n	0	0	0	0	1	0
k	0	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.0078652	1
rho	0	1
intercept	0.446	1
v	-0.200218	1
n	1.14792	1
k	4.2868	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.446	0.0887	-1.73e-008
1.8	5	0.392	0.152	0.392	0.0887	3.45e-007
6.2	5	0.325	0.042	0.325	0.0887	1.51e-007
33.6	5	0.263	0.083	0.263	0.0887	-1.34e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	38.453073	5	-66.906146
R	33.214605	2	-62.429209

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	5.46761e-007	0	NA

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the dose levels
It seems appropriate to model the data

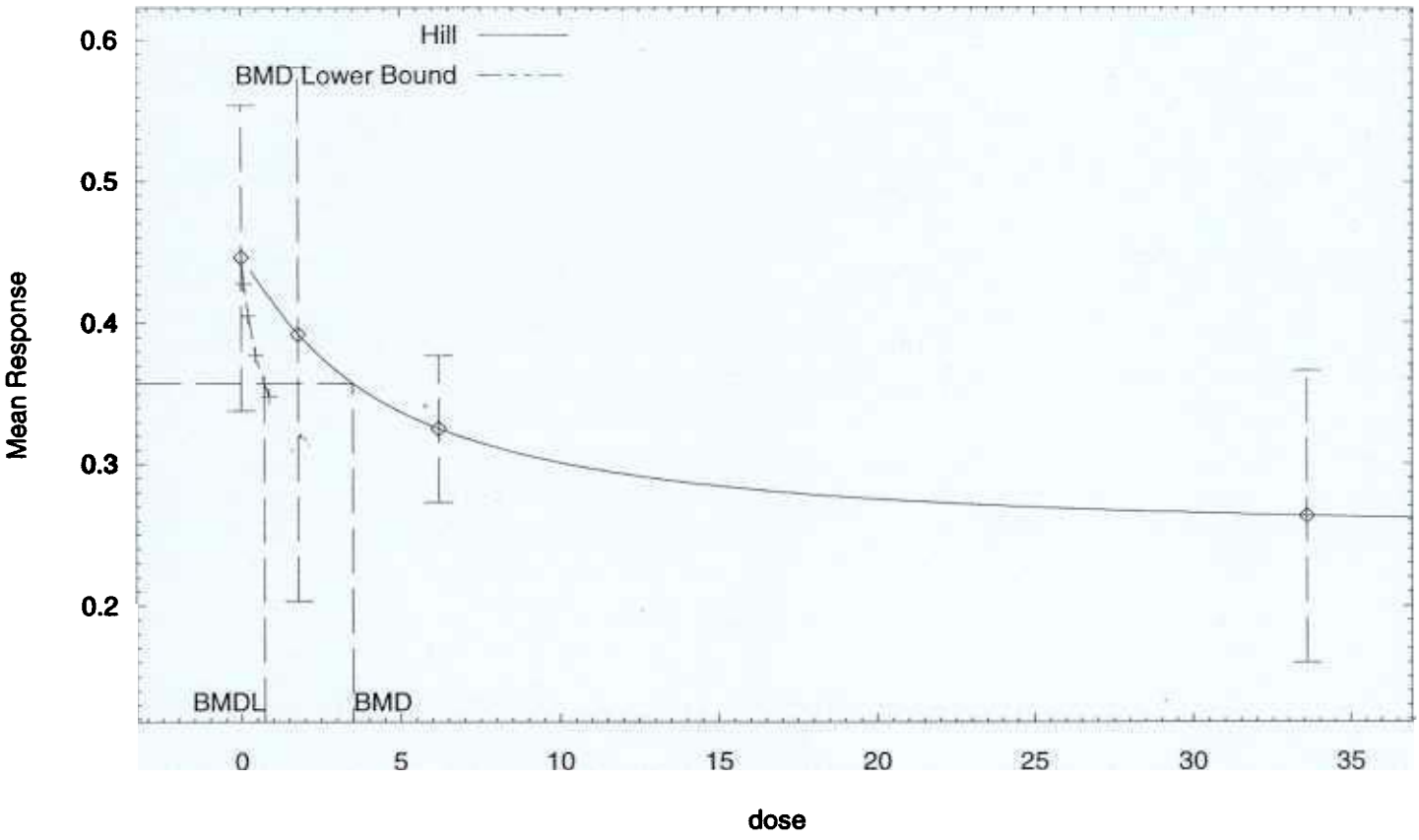
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	3.51089
BMDL	=	0.726794

Hill Model with 0.95 Confidence Level



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