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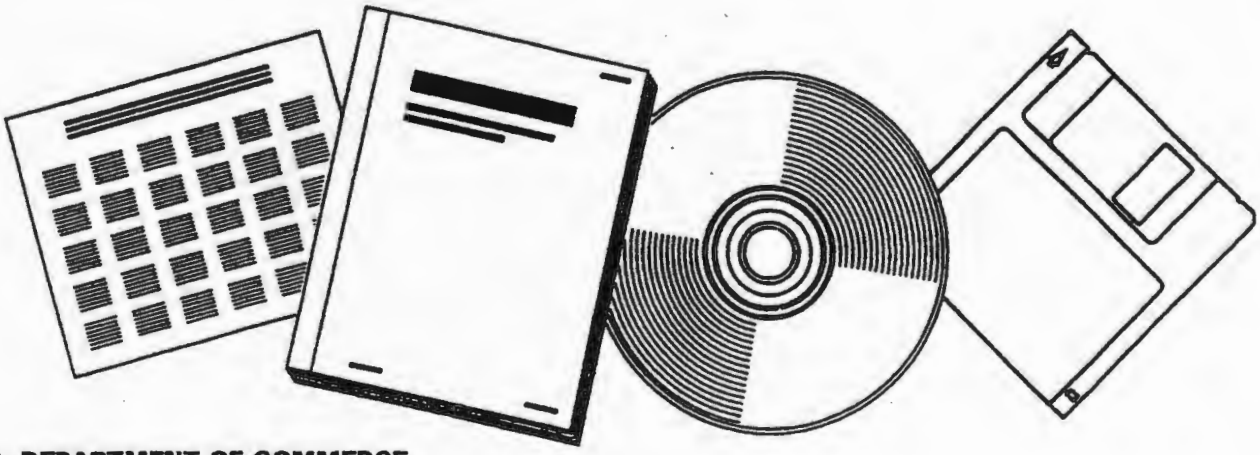

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RTI Project No. 31U-1287
RTI Master Protocol No. 27
RTI Study Code No. Mi80-2D
NIEHS Contract No. NO1-ES-6-2127

Date: July 29, 1983

FINAL REPORT

TERATOLOGIC EVALUATION OF PHENOL (CAS No. 108-95-2) IN CD-1 MICE

Laboratory Study: September 18, 1980 to January 12, 1981

Prepared for:

National Institute of Environmental
Health Sciences
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Research Triangle Park, NC 27709

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Phenol, a widely used industrial chemical and antimicrobial agent, was evaluated for toxic and teratogenic effects in timed-pregnant CD-1 mice. Phenol (0, 70, 140 or 280 mg/kg/day, po) in distilled water was administered in a volume of 10 ml/kg of body weight on gestational days (gd) 6-15. Females were weighed daily during treatment and observed for clinical signs of toxicity.

Dams in the high-dose group exhibited statistically significant signs of maternal toxicity (i.e., reduced maternal body weight and reduced weight gain), increased maternal mortality, and clinical signs including tremors and ataxia during phenol treatment. Examination of gravid uteri at term (gd 17) revealed no significant differences among treatment groups in the incidence of resorptions, dead fetuses or malformed fetuses. Gravid uterine weight, as well as average live fetal body weight per litter was decreased in a dose-related manner, with the high-dose group significantly below controls for both measures. Although no statistically significant evidence for a teratogenic effect of phenol in CD-1 mice was observed under the conditions of this study, data from the preliminary investigation suggest that increased malformations (primarily cleft palate) may occur with high-dose exposure to phenol in conjunction with compromised maternal status.

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QUALITY ASSURANCE REPORT

RTI Master Protocol No. RTI-27/31U-1287

TITLE: Teratologic Evaluation of Phenol (CAS No. 108-95-2) in CD-1 Mice.

SPONSOR: National Institute of Environmental Health Sciences

STUDY CODE NUMBER: Mi 80-2D

NIEHS NUMBER: N01-ES-6-2127

STUDY DATES: September 18, 1980 to January 12, 1981

STUDY DIRECTOR: Dr. C. Jones-Price

CD-1 (COBS, CD-1® (1CR)BR) outbred albino mice were obtained from the Charles River Breeding Laboratory, Inc., Kingston, N.Y., and were quarantined and examined upon arrival.

Phenol was obtained from Fluka AG, Chemische Fabrik CH9490 BUCHS (#77610; Lot No. 187130-280) supplied via Tredom Chemical Inc., Hauppauge, N.Y.

Listed below are the inspection dates and the dates reports were sent to management. Inspections were performed by Dr. V. Petrow.

Inspection Dates	Dates Findings were sent to Management
September 23, 1980	September 23, 1980
November 12, 1980	November 18, 1980
January 14, 1981	January 16, 1981

The Master Protocol and one amendment are included as Appendix I.

The Final Report Audit and Data Record Audits were performed by Mr. R. S. DeWoskin on April 1, 1983.

To the best of my knowledge this Final Report accurately describes the study methods and procedures used and the reported results accuratel reflect the raw data.

4-1-83
Date

R. S. DeWoskin
Robert S. DeWoskin
Quality Assurance Unit

RDS/sp

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Abstract

Phenol, a widely used industrial chemical and antimicrobial agent, was evaluated for toxic and teratogenic effects in timed-pregnant CD-1 mice. Phenol (0, 70, 140 or 280 mg/kg/day, po) in distilled water was administered in a volume of 10 ml/kg of body weight on gestational days (gd) 6-15. Females were weighed daily during treatment and observed for clinical signs of toxicity. A total of 22-29 females/group were confirmed to be pregnant at sacrifice on gd 17. The gravid uterus of each dam was weighed and the uterine contents examined for implantation sites and fetuses (live, dead or resorbed). Each live fetus was weighed and examined for external, visceral and skeletal malformations.

Dams in the high-dose group exhibited statistically significant signs of maternal toxicity (i.e., reduced maternal body weight and reduced weight gain), increased maternal mortality, and clinical signs including tremors and ataxia during phenol treatment. Examination of gravid uteri at term (gd 17) revealed no significant differences among treatment groups in the incidence of resorptions, dead fetuses or malformed fetuses. Gravid uterine weight, as well as average live fetal body weight per litter was decreased in a dose-related manner, with the high-dose group significantly below controls for both measures. Although no statistically significant evidence for a teratogenic effect of phenol in CD-1 mice was observed under the conditions of the present study, data from the preliminary investigation suggest that increased malformations (primarily cleft palate) may occur with high-dose exposure to phenol in conjunction with compromised maternal status.

1.0 Introduction

The use of phenolic compounds as antimicrobials began in 1867 with Lister's selection of phenol as the germicide of choice for surgical procedures (Marouchoc, 1979; Harvey, 1980). Phenol is bacteriostatic in a concentration of approximately 0.2%, bactericidal above 1% and fungicidal above 1.3% (Harvey, 1980). The presumed mode of germicidal action involves denaturing of protein, an action which also underlies the toxic effect of phenol on mammalian tissues (Marouchoc, 1979; Harvey, 1980). Less toxic agents have largely replaced phenol as a topical antiseptic and local anesthetic in modern medical practice, but several prescription and over-the-counter (OTC) products containing phenol (<1.5%) are still used in the treatment of topical fungal infections, psoriasis, oropharyngeal infections and chapped lips; OTC throat lozenges containing up to 32.5 mg phenol per lozenge are also available (PDR, 1981); phenol has also been found to be an effective cauterizing agent for dogbites, snakebites and other small wounds (Esplin, 1970). As a disinfectant for environmental surfaces, phenol is found in commercial products recommended for sanitation of floors, drains, stables, toilets, cesspools and other excrement-contaminated surfaces (Windholz, 1976; Harvey, 1980). Phenol is also widely used as an industrial chemical employed in the manufacture of dyes, phenolic resins, bisphenol-A, alkylphenols, halogenated phenols, caprolactam and others (Kirk-Othmer, 1968; Dean, 1978). Phenol has been ranked 38th among U.S. chemicals by volume with production of 2.38 billion pounds in 1978 (NCI, 1980). Many phenolic derivatives are marketed as antimicrobial agents, accounting for approximately 32% (75.3 million pounds) of all antimicrobials marketed in the U.S. in 1973 (Marouchoc, 1979).

Actual exposure of the public to phenol is difficult to assess, but NIOSH (1980) has estimated that approximately 1,018,442 persons in 206 occupations (not specified) are exposed to phenol in the U.S. As an additional consideration, an estimated 1,495,706 people are exposed to benzene (NIOSH, 1980) which can be metabolized to yield detectable plasma levels of phenol in man (Lehnert et al., 1978; Dean, 1978). An estimate of exposure to phenol for pregnant women is even more difficult to obtain. The Collaborative Perinatal Project conducted between 1958 and 1965 has reported a total of 23 out of 50,282 (0.05%) mothers exposed to phenol via topical antimicrobial preparations during the first four lunar months, but other potential sources of exposure to phenol during pregnancy were not examined (Heinonen et al., 1977).

The toxic nature of phenol is widely recognized and exposure limits have been imposed by governmental regulatory agencies (NIOSH/OSHA, 1980). The permissible workshift time-weighted average (TWA) exposure for humans is 5 ppm (19 mg/m^3). NIOSH has recommended a TWA of 20 mg/m^3 and a maximum 15-minute exposure limit of 60 mg/m^3. The "immediately dangerous to life and health" (IDLH) concentration in air is 100 ppm (NIOSH/OSHA, 1980). Clinical and laboratory data indicate that local tissue injury at the site of exposure occurs at concentrations exceeding 2%. With cutaneous exposure, the severity of this response varies with exposure conditions from mild surface irritation to deep necrosis followed by gangrene (Esplin, 1970); corrosion of the gastrointestinal tract occurs following ingestion, accompanied by pain, nausea, vomiting and diarrhea (Arena, 1963). Phenol is absorbed readily through intact, as well as abraded surfaces and onset of systemic toxicity is rapid

(Conning and Hayes, 1970). Severe renal damage with smokey colored urine, haemoglobinuria or anuria may result. With high doses, central nervous system (CNS) stimulation is soon followed by CNS depression, respiratory depression and unconsciousness. Cardiovascular collapse, hypotension, hypothermia and shock quickly develop. At dosages above 1 gram, death usually results from respiratory failure, but massive dosages may cause death via cardiac paralysis (Conning and Hayes, 1970; Esplin, 1970; Windholz, 1976; Arena, 1963; Harvey, 1980; Liao and Oehme, 1980). The estimated range for oral lethal dosages in humans is 50-500 mg/kg (Gosselin et al., 1976), but ingestion of as little as 1 g has resulted in a reported fatality (ILO, 1971).

Chronic exposure to low concentrations of phenol vapor or mist, results in digestive disturbances (vomiting, difficulty in swallowing, excessive salivation, diarrhea, loss of appetite), nervous disorders (headache, fainting, dizziness, mental disturbances) and skin eruptions. Chronic poisoning may terminate fatally in cases where there has been extensive damage to the kidneys or liver. Dermatitis resulting from contact with phenol or phenol-containing products is common in industry (Sax, 1968).

The evaluation of phenol for toxic effects including carcinogenicity, mutagenicity, reproductive toxicity and teratogenicity is of importance due to the potential for acute and chronic exposure related to industrial processing and/or use of consumer products. Phenol has previously been recognized as a primary carcinogen when applied to the skin of laboratory rodents and as a promoter of dimethylbenzanthracene-induced tumors in mice and rats (Boutwell and Bosch, 1959). Bioassay of

phenol (0, 2500 or 5000 ppm in drinking water for 103 weeks) failed to provide conclusive evidence for a carcinogenic effect in F344 rats or B6C3F1 mice although the incidence of leukemia and lymphomas was significantly increased for low-dose male rats (NCI, 1980). The mutagenic potential of phenol has been demonstrated in several in vitro assays (Dean, 1978). Clinical evidence has been presented which suggests that phenol, per se, (Heinonen et al., 1977) as well as organic solvents, in general, (Holmberg and Nurminen, 1980) may be associated with an elevated malformation rate. Nevertheless, very little laboratory data relevant to the reproductive toxicity or teratogenicity of phenol has been presented to date. Phenol (0.25%) plus glycerine (1.6%) injected into the egg yolk failed to increase the incidence of abnormalities in creeper fowl embryos above that of untreated controls (Landauer, 1945). Contaminated sea water, containing organic and heavy metal pollutants, (including phenol 2.1-2.3 ppm) produced embryotoxicity in white leghorn eggs, but the effect of phenol, per se, was not ascertained (Hatano and Ashida, 1975). Phenol is toxic to preimplantation (~30 hr) mouse embryos exposed in culture ($>10^{-4}$ M for 24 hrs) (Müller et al., 1981) and to rat fetuses exposed in utero (Minor and Becker, 1971), but has not been shown to be teratogenic to Sprague-Dawley rats exposed to phenol (20, 63 or 200 mg/kg/day, ip) on gd 9-11 or 12-14 (gd 1 = copulation evidence) (Minor and Becker, 1971).

In order to more clearly define the effects of gestational exposure to phenol, CD-1 mice (present study) and CD rats (Jones-Price et al., 1983) were examined in this laboratory following maternal exposure throughout organogenesis.

2.0 Objectives

The present study was designed to determine the toxic and teratogenic effects of phenol administered orally (po) to timed-pregnant CD-1 mice on gestational days (gd) 6 through 15. In order to select an appropriate range of doses for the teratologic evaluation, a preliminary toxicity study was performed using pregnant CD-1 mice. A high dose was selected for the teratology study which was expected to produce statistically significant signs of maternal and/or fetal toxicity, while allowing 90% or greater maternal survival in pregnant CD-1 mice treated on gd 6 through 15 and observed through sacrifice on gd 17.

3.0 Materials and Methods

3.1 Animals

Caesarean-originated, Barrier-sustained (COBS) CD-1® (1CR)BR outbred albino mice (CD-1 mice) were purchased from the Charles River Breeding Laboratory, Inc. (Kingston, NY). A total of 89 females were assigned to the preliminary toxicity study; 133 females were assigned to the teratology study. A total of 61 breeder males were used in the preliminary toxicity study; in the teratology study 71 male breeders were used and no male was represented more than three times in any given dose group. Males were 10-12 weeks and females were 6-7 weeks of age at the time of arrival at RTI. The weight range for plug-positive females assigned to the preliminary toxicity study was 28.0-46.2 g and to the teratology study 24.0-32.4 g. In the teratology study, two females were eliminated due to dosing errors, 4 treatment-related deaths occurred in the high-dose group prior to scheduled sacrifice and 25 females were found to be nonpregnant at sacrifice on gd 17 (Table 10). Gestational day 0 body weights (g) for the 102 confirmed-pregnant females (dams) evaluated in the teratology study were as follows:

		Body Weight (g)		
	n	Minimum	Median	Maximum
0 mg/kg	29	24.0	28.0	31.7
70 mg/kg	26	24.6	28.1:28.3	32.4
140 mg/kg	25	25.2	28.2	32.4
280 mg/kg	22	24.8	28.4:29.7	32.4

The Charles River CD-1 mouse was selected as the experimental subject since this strain has been the subject of choice on NIEHS contracts in teratology at RTI since 1976, thus providing an historical data base for reproductive performance and incidence of spontaneous malformations in control mice. In addition, historical data on the

incidence of malformations in Charles River CD-1 controls has been summarized in the recent literature (Palmer, 1972; Perraud, 1976; Fritz et al., 1979).

3.2 Animal Husbandry

Animals were randomly assigned to cages, and individually coded ear tags were affixed to each mouse during a seven-day quarantine period. Males were housed singly in solid bottom polycarbonate cages (5" x 11 1/2" x 7") and females were group housed (10 per cage) on Ab-Sorb-Dri® cage litter (Laboratory Products, Garfield, NJ) in solid bottom polypropylene or polycarbonate cages (6" x 19" x 10 1/2") with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Cages were placed on galvanized-steel racks (24" w x 60" l x 66" h) with adjustable shelves (Laboratory Products, Rochelle Park, NJ). Purina Certified Rodent Chow (5002) and deionized/filtered water were available ad libitum throughout the study. Animal holding rooms (8' x 12') were equipped with individual temperature controls (72 ± 2°F) and automatic adjustable light cycles (lights on 7:00 a.m. to 7:00 p.m.).

Prior to breeding, females were "primed" in order to increase the rate of successful mating (Whitten, 1956). A single male mouse was placed in a wire mesh cage (5" x 4" x 4" test tube basket) inside the home cage of 10 group-housed females. Forty-eight hours later females were placed overnight in the home cages of singly housed males (one male and two females per cage). On the following morning, each female was examined for copulation plugs (Hafez, 1970). Females which were negative for plugs were returned to group cages with other nonpregnant females. On the day of plug detection [gestational day (gd) 0], each plug-positive

female was assigned to a dose group in either the preliminary toxicit study or the teratology study; assignment to dose groups in the tera tology study was based upon body weight. Females were group house (maximum 10 per cage) through terminal sacrifice on gd 17. In additic to an individually-coded ear tag affixed to each mouse upon entry int the RTI Animal Facility, an identification number was assigned on gd to each plug-positive female.

3.3 Test Chemical

3.3.1 Description

Generic Name: Phenol

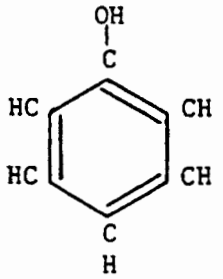
Chemical Names: Carbolic acid; hydroxybenzene; monohydroxybenzene oxybenzene; phenic acid; phenyl hydrate; phenyl hydroxide; phenyli acid; phenylic alcohol; benzenol; monophenol; phenyl alcohol.

CAS Registry No.: 108-95-2 (also 8002-07-1; 14534-23-7; 50356-25-7

Molecular Formula: C_6H_6O

Molecular Weight: 94.12

Structure:



Source: Fluka AG, Chemische Fabrik CH9490 BUCHS (#77610; Lot no 187130-280). Supplied via Tridom Chemical Inc. (TCI), 255 Oser Avenue Hauppauge, New York 11787.

Purity and Stability: See Appendix III.

Vehicle: Distilled water (Grand Island Biological Company, Grand Island, NY).

3.3.2 Safety Procedures

Phenol carries an Interstate Commerce Commission (ICC) and Department of Transportation (DOT) label of "Poison B" (Kirk-Othmer, 1968), and classification codes of carcinogen-neoplastigen and primary irritant (RTECS, 1981). The following safety procedures were recommended for all personnel involved in formulating solutions or dosing animals for this study in order to avoid contact with phenol.

1. Phenol can cause burns to the skin and be absorbed through intact skin. Therefore, double (two pair) Flexam® floor/exam latex gloves were worn by all personnel using phenol. Workers were advised if holes or tears were noted or suspected, to replace both pairs of gloves at once. Personnel also wore a lab coat, eye goggles and closed shoes.

2. Phenol is incompatible with strong oxidizers, therefore, no oxidizers were present in the hood while solutions were being prepared.

3. All phenolic formulations were made in a laminar flow hood with an activated charcoal filter vented to the outdoors. Weighing of the reagent was performed on a balance within this hood.

4. All personnel were advised to immediately and thoroughly wash any skin areas exposed to phenol with liberal quantities of 70 percent ethanol solution followed by soap and water. A large squeeze bottle containing 70 percent ethanol was immediately available in the hood in the dosing room for this purpose.

5. In the event of an accident or spill, personnel were advised to (1) remove contaminated clothing at once and immediately neutralize

the phenol-wetted areas with 70 percent ethanol followed by soap and water; (2) notify the nearest supervisor as soon as possible; (3) dry spill areas with paper towels, then wash with 70 percent ethanol and finally water; (4) promptly bag, label and dispose of all litter in metal cans provided for toxic wastes.

3.3.3 Dosage Formulations and Analysis

Phenol was dissolved in distilled water (Grand Island Biological Co., Grand Island, NY) with concentration (see Tables 8 and 15) determined by the following formula:

$$\text{Concentration (mg/ml)} = \frac{\text{dose level (mg/kg)}}{\text{dose volume (10 ml/kg)}}$$

On any given formulation date, each concentration was formulated independently. Phenol was weighed in a beaker under a laminar flow hood, distilled water was added and the mixture transferred to a graduatec cylinder. The beaker was rinsed twice with distilled water and the washings added to the cylinder. Distilled water was added to the cylinder to the appropriate total volume. Solutions were agitated with a glass rod to produce a visually uniform solution. Dosing solutions were stored at 5°C except at the time of dosing when they were warmed to room temperature. Each concentration was labeled with the date of preparation, a study code (Mi802D) and a random 5-digit concentration code number (see Tables 8 and 15). Personnel involved in dosing, animal care or teratologic evaluation were not informed of the formulation concentrations until all laboratory work had been completed.

Phenol solutions in distilled water were assayed by high performanc liquid chromatography (HPLC) in the reverse phase mode using a Water

Model 6000A pump with UK6 injector, or WISP 710 automatic injector and Waters Data Module (Waters Associates, Milford, MA). The specific HPLC conditions were as follows:

Column: 250 x 4.0 mm i.d. containing Lichrosorb RP-18 (C_{18}-bonded reverse phase packing)

Mobile phase: 20% CH_3CN/distilled H_2O containing 1% HOAc (k' phenol 4.7)

Flow Rate: 2.0 ml/min

Detector: UV absorbance at 254 nm (.032-.256 AUFS)

Injection Vol.: 10 µl

Samples were quantified from the absolute peak height of phenol (measured manually) and results calculated from an external standard calibration curve. Samples were prepared for HPLC analysis by diluting a 1.0 ml aliquot of the sample to 50 ml with distilled water in a volumetric flask. One or more 10 µl aliquots of diluted solution were injected directly. All sample concentrations (in distilled H_2O) fell within the limits of the standard curve.

The external calibration curve was prepared by single analysis of phenol standards at five concentrations (5-90 mg/ml) in distilled water. A new external standard calibration curve was prepared for each day of analysis to account for any changes in response of the system from day to day. Least-squares linear regression analysis of the calibration data gave a correlation coefficient of 0.9997 for each external standard calibration curve prepared.

3.3.4 Treatment

Timed-pregnant CD-1 dams were dosed daily with phenol or vehicle between 8:00 a.m. and noon on gd 6 through 15. The doses employed for the preliminary toxicity study (Substudies I-IV, inclusive) were 0, 100, 200, 230, 260, 275, 300 and 400 mg/kg/day (see also Table 2). Doses employed in the teratology study were 0, 70, 140 and 180 mg/kg/day. Treatment was administered by oral gavage (po) using an 18-gauge, 1 inch, stainless steel dosing tube (Perfektum®, Popper and Sons, Inc., New Hyde Park, NY). Treatment was administered in a volume of 10 ml/kg.

3.4 Toxicological and Teratological Evaluation

Dams were weighed on gd 0, 6 through 15 (prior to daily dosing) and 17 (immediately following sacrifice), and were also observed during treatment for clinical signs of toxicity. Subjects which died between gd 0 and sacrifice were necropsied. At sacrifice on gd 17, dams were evaluated for body weight, liver weight, gravid uterine weight and status of uterine implantation sites (i.e., implantation sites, resorptions, dead fetuses, live fetuses). The uteri of dams with no apparent implants were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites (Salewski, 1964). Live fetuses were dissected from the uterus and each live fetus was weighed, sexed and examined for gross morphological abnormalities. All live fetuses were examined for visceral malformations employing the Staples' fresh tissue dissection method (Staples, 1974). Half of the fetuses were decapitated prior to dissection and the heads were fixed in Bouin's solution for free hand sectioning and examination (Wilson, 1965). All fetal carcasses (50% without heads) were prepared with Alizarin Red S

stain and examined for skeletal malformations (Peltzer and Schardein, 1966; Crary, 1962). Malformations which were rare or difficult to describe were photographed and/or sketched. Further details concerning the evaluation of dams and fetuses at sacrifice are presented in Appendix II.

3.5 Experimental Design and Statistical Analysis

Nonparametric statistics (Table 1) were applied to data from the preliminary toxicity study (Substudies I-IV, inclusive; Table 2) or the teratology study (Breedings I-IV, inclusive; Table 9) in order to identify dose-response trends, differences among treatment groups, and differences between vehicle control and phenol-treated groups. Nonparametric statistical procedures were performed utilizing an unpublished statistical program developed by Dr. J. Haseman and Dr. T. Clemmer (National Institute of Environmental Health Sciences) and revised by Dr. D. Rosenthal and Mr. W. Hargrove, Research Triangle Institute. Whenever possible, the data have been presented as mean $\pm$S.E.M. using the dam or litter as the experimental unit for measures of toxicity and teratogenicity. Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956) was used to test for differences among dose groups. When Kruskal-Wallis was significant ($p<0.05$), the Mann-Whitney U test (two-tailed) was utilized to make pairwise comparisons between vehicle and each phenol-treated group (Siegel, 1956). Jonckheere's test for k independent samples (Jonckheere, 1954) was employed to identify significant dose-response trends. Nominal scale data were analyzed using a two-tailed Fisher's Exact Test for pairwise comparisons between vehicle and each phenol-treated group (Siegel, 1956). The alpha level

of each nonparametric statistical comparison was 0.05 and significance levels for nonparametric statistical comparisons have been reported as $p<0.05$, $p<0.01$, or $p<0.001$, whichever was less.

Table 1. Nonparametric Statistical Procedures in the Teratologic Evaluation of Phenol in CD-1 Mice

	Jonckheere's	Kruskal-Wallis	Mann-Whitney U	Fisher's Exact
MATERNAL TOXICITY				
Maternal Body Weight[a,j]	+	+	+	
Maternal Weight Gain[a]				
gestation	+	+	+	
treatment	+	+	+	
absolute[b]	+	+	+	
Gravid Uterine Weight[a]	+	+	+	
Maternal Liver Weight[a]	+	+	+	
EMBRYOTOXICITY				
All Litters[a,c]				
Implantation sites per litter	+	+	+	
Resorptions per litter	+	+	+	
% Resorptions per litter	+	+	+	
No. litters with resorptions				+
% Litters with resorptions				
Dead per litter	+	+	+	
% Dead per litter	+	+	+	
No. litters with dead				+
% Litters with dead				
Nonlive per litter[d]	+	+	+	
% Nonlive per litter	+	+	+	
No. litters with nonlive				+
% Litters with nonlive				
Affected per litter[e]	+	+	+	
% Affected per litter	+	+	+	
No. litters with affected				+
% Litters with affected				

(continued)

Table 1 (continued)

	Jonckheere's	Kruskal-Wallis	Mann-Whitney U	Fisher's Exact
Live Litters[f]				
Live fetuses per litter	+	+	+	
Average fetal body weight (g) per litter	+	+	+	
Males per litter	+	+	+	
Females per litter	+	+	+	
% Males per litter	+	+	+	
MALFORMATIONS[g]				
Gross Malformations				
Fetuses[h]				
Litters[i]				+
Visceral Malformations				
Fetuses[h]				
Litters[i]				+
Skeletal Malformations				
Fetuses[h]				
Litters[i]				+
All Malformations				
Males malformed per litter	+	+	+	
% Males malformed per litter	+	+	+	
Females malformed per litter	+	+	+	
% Females malformed per litter	+	+	+	
Fetuses malformed per litter	+	+	+	
% Fetuses malformed per litter	+	+	+	
No. fetuses malformed[h]				
% Fetuses malformed[h]				
No. litters with malformations[i]				+
% Litters with malformations[i]				

Table 1 (continued)

[a]Includes all dams pregnant at sacrifice.

[b]Weight gain during gestation minus gravid uterine weight.

[c]Litter size = no. implantation sites per dam.

[d]Nonlive = dead plus resorbed.

[e]Affected = nonlive plus malformed.

[f]Includes only dams with live fetuses; litter size = no. live fetuses per dam.

[g]Only live fetuses were examined for malformations; includes only litters with live fetuses.

[h]Fetuses with one or more malformations.

[i]Litters with one or more malformed fetuses.

[j]Maternal body weights for the following gestational days for rats (gd 0, 6, 11, 15 & 17).

3.6 Personnel

Project Officer:	John A. Moore, D.V.M. (NIEHS)
Project Advisor:	Dr. Carole A. Kimmel (NCTR)
Study Directors:	Dr. Jerry R. Reel (Initiation through May 31, 1981)
	Dr. Thomas A. Ledoux (Initiation through March 31, 1981)
	Dr. Catherine N. Jones-Price (April 1, 1981 through completion)
Senior Chemist:	Dr. Robert W. Handy
Junior Chemists:	Dr. John W. Hines Ms. Julia C. Albert
Statistical Advisors:	Dr. Joseph H. Haseman (NTP) Dr. Tyler D. Hartwell Mr. Philip V. Piserchia, M.S.
Laboratory Supervisors:	Mrs. Loretta Langhoff-Paschke (August 1, 1980 through June 19, 1981)
	Mrs. Melissa C. Marr (June 19, 1981 through completion)
Laboratory Personnel:	Mrs. Frieda S. Gerling Mrs. Lynn Smith King Mr. Burnes L. Ray Ms. Vickie I. Wilson Ms. Betty P. King Mr. Steven H. Pachman Mrs. Ellen B. Hahn

3.7 Storage of Records

All original data sheets for this study have been stored in the Research Triangle Institute Archives under the control of the RTI Quality Assurance Officer. Biological specimens have been placed in secure storage at the Research Services Building, Research Triangle Institute. Work sheets and computer printouts from the Triangle Universities Computation Center (TUCC) which were generated in the statistical analysis of data have been stored at Research Triangle Institute. Copies of this study report are on file with the Chemistry and Life Sciences Group, Research Triangle Institute as well as with the contracting agency, the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, and the National Center for Toxicological Research, Jefferson, Arkansas. In accordance with Sections 58.190 and 58.195 of the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1978), all records, data, and reports will be maintained in storage at RTI for a minimum of two years; chemical and biological samples will be maintained for a minimum of two years or for as long as the quality of the preparation affords evaluation, whichever is less.

4.0 Results

4.1 Preliminary Toxicity Study

4.1.1 Toxicological and Teratological Evaluation

Phenol (0, 100, 200, 230, 260, 275, 300 or 400 mg/kg/day, po) was administered to timed-pregnant females (5-27 per treatment group) on gd 6-15 in Substudies I-IV (inclusive) of the preliminary toxicity study (Table 2). Within each substudy, approximately equal numbers of plug-positive females (n = 5-7) were assigned to each phenol-treated group (1-4 phenol-treated groups per substudy), and a vehicle control group (n = 6-8) was included in each substudy (Table 2). The vehicle control, 275 and 300 mg/kg/day phenol treatments were used in multiple substudies, thus accounting for the large difference in number of animals per treatment group. Mortality rates for all treated females were 0% (0/27), 0% (0/6), 0% (0/6), 0% (0/6), 15% (2/13), 25% (5/20) and 40% (2/5) for the control through high-dose groups, respectively (Table 3). The primary clinical signs observed in phenol-treated females were tremor and weight loss (i.e., $\geq$1 g/24 hr) at dosages $\geq$200 mg/kg/day, phenol. Maternal body weight on gd 0 was matched across all treatment groups and no statistically significant treatment-related effects on maternal body weight were observed during dosing (gd 6, 11 or 15) or at sacrifice (Table 3). However, measures of maternal weight gain (i.e., gestational weight gain, weight gain during treatment and absolute weight gain) each revealed a statistically significant trend toward decreased weight gain with increasing dose ($p<0.05$ or 0.01, Jonckheere's Test; Table 3). In addition, maternal weight gain during treatment showed statistically significant reductions below vehicle control for the phenol, 230, 260,

275 and 400 mg/kg/day, groups (Table 3). At sacrifice on gd 17, neither gravid uterine weight nor maternal liver weight showed any statistically significant differences among treatment groups (Table 3).

Examination of uterine contents at sacrifice on gd 17 revealed no differences among treatment groups in the number of implantation sites per dam (i.e., based upon all confirmed-pregnant females at gd 17 sacrifice) or in the number of live fetuses per live litter (i.e., based only upon dams with one or more live fetuses). However, evidence for treatment-related fetal growth retardation, decreased prenatal viability and abnormal structural development was observed at dosages of phenol within the maternally toxic range (i.e., $\geq$200 mg/kg/day) (Tables 5, 6 and 7).

Based upon the results of the preliminary toxicity study, dosages of phenol (0, 70, 140 and 280 mg/kg/day, po) were selected for the teratology study. The high-dose was expected to produce both fetal and maternal toxicity (approximately 10-15% maternal mortality), while the low-dose was expected to produce no observable signs of maternal or fetal toxicity within the conditions of the present investigation.

4.1.2 Analysis of Dosage Formulations

Formulations of phenol from Substudies I-IV of the preliminary investigation were analyzed by HPLC approximately 10 months after formulation (see Section 3.3.3). Analysis verified that these formulations contained between 83.8-105% of the theoretical concentration of phenol.

Table 2. Assignment of Plug-Positive CD-1 Mice to Substudy and Dose Group in the Preliminary Investigation of Phenol Toxicity and Teratogenicity

Substudy (gd 0)	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
I (9/19/80)	6	6	6	0	0	0	6	5
II (9/26/80)	6	0	0	0	0	6	7	0
III (10/3/80)	7	0	0	6	6	0	7	0
IV (10/17/80)	8	0	0	0	0	7	0	0
Total	27	6	6	6	6	13	20	5

Table 3. Preliminary Toxicity Study: Maternal Toxicity in CD-1 Mice Exposed to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
SUBJECTS (Dams)								
Total treated	27	6	6	6	6	13	20	5
Removed	0	1[e]	1[e]	0	0	0	0	0
Deaths	0	0	0	0	0	2	5	2
Nonpregnant at sacrifice	1	0	0	2	0	1	3	0
Pregnant (%) at sacrifice	26 (96.3)	5 (100)	5 (100)	4 (66.7)[f]	6 (100)[f]	10 (90.9)	12 (80.0)	3 (100)
MATERNAL BODY WEIGHT (g)[a]								
Gestational day 0	34.25 ±0.73	34.64 ±0.99	33.46 ±1.72	33.65 ±1.21	33.63 ±0.81	34.26 ±1.02	35.52 ±1.06	34.03 ±2.15
Gestational day 6	36.51 ±0.80	36.28 ±1.00	35.04 ±1.39	37.00 ±1.30	35.93 ±0.95	37.55 ±0.99	38.03 ±0.98	36.50 ±2.25
Gestational day 11	40.81 ±0.87	38.26 ±1.03	37.44 ±1.33	37.33 ±1.63	37.47 ±1.48	40.41 ±0.81	40.37 ±1.01	38.00 ±2.41
Gestational day 15	49.03 ±0.93	47.24 ±0.96	46.32 ±2.12	41.35 ±4.37	43.37 ±2.54	47.64 ±1.09	48.40 ±1.42	43.40 ±2.95
Gestational day 17	54.69 ±1.09	52.94 ±1.29	51.38 ±2.42	46.08 ±5.60	47.77 ±3.56	53.39 ±1.21	53.00 ±1.65	48.33 ±3.00

(continued)

Table 3 (concluded)

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
MATERNAL WEIGHT GAIN (g)[a]								
Gestation period	20.44§§ ±1.01	18.30 ±1.40	17.92 ±2.36	12.43 ±5.35	14.13 ±3.74	19.13 ±1.16	17.48 ±1.62	14.30 ±0.86
Treatment period	12.52†§§ ±0.57	10.96 ±0.87	11.28 ±1.78	4.35* ±3.88	7.43* ±2.83	10.09* ±1.13	10.37 ±1.38	6.90** ±0.89
Absolute weight gain[b]	3.14[c]§ ±0.53	1.59 ±1.13	3.14 ±1.15	0.44 ±1.81	2.20 ±1.00	3.07 ±0.64	1.24 ±0.80	0.80 ±1.33
GRAVID UTERINE WEIGHT (g)[a]	17.49[c] ±0.76	16.71 ±1.38	14.78 ±1.77	11.98 ±3.94	11.94 ±2.87	16.06 ±0.81	16.25 ±1.15	13.50 ±2.01
MATERNAL LIVER WEIGHT (g)[a]	2.78† ±0.05	2.52 ±0.13	2.77 ±0.16	2.27* ±0.18	2.44* ±0.14	2.79 ±0.09	2.74[d] ±0.10	2.43 ±0.21

[a] Includes all dams pregnant at sacrifice; mean ± S.E.M.

[b] Weight gain during gestation minus gravid uterine weight.

[c] Gravid uterine weight omitted for two dams; n = 24

[d] Liver weight omitted for one dam; n = 11.

[e] Removal due to dosing error.

[f] One dam with all resorptions.

† $p<0.05$ Kruskal-Wallis.

* $p<0.05$ Mann-Whitney U.

** $p<0.01$ Mann-Whitney U.

§ $p<0.05$ Jonckheere's.

§§ $p<0.01$ Jonckheere's.

Table 4. Preliminary Toxicity Study: Clinical Signs in CD-1 Mice Exposed to Phenol on Gestational Days 6 Through 15

Gestational Day	Clinical Signs	Phenol (mg/kg/day, po) 0 Dam #	0 Wt.	100 Dam #	100 Wt.	200 Dam #	200 Wt.	230 Dam #	230 Wt.	260 Dam #	260 Wt.	275 Dam #	275 Wt.	300 Dam #	300 Wt.	400 Dam #	400 Wt.
6	* tremors after dosing					all		all		all		all		all		all	
7	* weight loss[a]			6	1.6												
	* tremors after dosing					all		all		all		all		all		all	
	* death													3			
8	* weight loss[a]			21	1.4	12	1.1	52	1.2	51	1.8			23	1.7		
								56	2.1					28	1.0		
								72	1.6								
	* tremors after dosing					all		all		all		all		all		all	
	* rough coat							52									
	* death													54			
9	* weight loss[a]							72	1.1	67	4.0			23	2.6		
														28	1.7		
														66	2.3		
														70	5.2		
	* tremors after dosing					all		all		all		all		all		all	
	* rough coat									67				66			
10	* weight loss[a]							72	3.1	67	1.9			66	1.0	5	1.0
	* tremors after dosing					all		all		all		all		all		all	
	* rough coat									55				58			
										67				66			
														70			
	* death															10	
																15	
11	* weight loss[a]											47	5.4	58	4.1		
	* tremors after dosing					all		all		all		all		all		all	
	* rough coat											47		70			
12	* weight loss[a]									51	1.2	47	1.2	58	1.5		
	* tremors after dosing					all		all		all		all		all		all	
	* vaginal bleeding	75										76					
		79										80					
		81										82					
		83															
	* hyperactive											32					
	* rough coat									67							
13	* weight loss[a]							56	4.9								
	* tremors after dosing					all		all		all		all		all		all	
	* vaginal bleeding					27											
	* rough coat							52						70			
								72									
	* deaths													33			
														74			
14	* weight loss[a]	89	5.0					56	1.9					36	1.1		
														42	1.1		
	* tremors after dosing					all		all				all		all		all	
	* respiratory distress									71							
	* rough coat									51				58			
														66			
														70			
	* death											41					

(continued)

Table 4. (continued)

Gestational Day	Clinical Signs	Phenol (mg/kg/day, po) 0		100		200		230		260		275		300		400	
		Dam #	Wt.	Dam #	Wt.	Dam #	Wt.	Dam #	Wt.	Dam #	Wt.	Dam #	Wt.	Dam #	Wt.	Dam #	Wt.
15	* weight loss[a]	89	2.2									32	1.4				
												47	3.0				
	* tremors after dosing					all		all				all		all		all	
	* rough coat											47					
	* death													45			
16	* death											84					
17	* large cyst surrounding left ovary	57								55							
	* pale yellow gall bladder	57						56		71							

[a]Weight loss is defined as a loss equal to or greater than 1 g in 24 hours.

Table 5. Preliminary Toxicity Study: Embryotoxicity in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
ALL LITTERS[a]								
(Pregnant Dams)	(26)	(5)	(5)	(4)	(6)	(10)	(12)	(3)
Implantation sites per litter[b]	13.38 ±0.43	14.00 ±0.84	11.80 ±1.02	15.25 ±1.18	14.50 ±1.43	13.60 ±0.56	14.83 ±0.78	12.00 ±1.15
Resorptions per litter[b]	1.50† ±0.60	1.80 ±0.80	0.80 ±0.49	7.25** ±2.75	4.17* ±1.51	0.90 ±0.28	2.00 ±0.91	0.33 ±0.33
% Resorptions per litter[b]	10.4 ±3.7	13.1 ±6.3	7.3 ±4.6	52.3[f] ±22.1	34.6[f] ±15.2	6.6 ±1.9	14.4 ±7.4	2.4 ±2.4
No. litters with resorptions	15	3	2	4	6	7	6	1
% Litters with resorptions	57.7	60.0	40.0	100	100	70.0	50.0	33.3
Dead per litter[b]	0.12††§§ ±0.08	0	0	0	0.83*** ±0.17	0.20 ±0.13	0.33 ±0.14	0.67** ±0.33
% Dead per litter[b]	0.7††§§ ±0.5	0	0	0	5.5*** ±1.2	1.6 ±1.1	2.1 ±0.9	5.7** ±3.0
No. litters with dead	2	0	0	0	5¶¶	2	4	2
% Litters with dead	7.7	0	0	0	83.3	20.0	33.3	66.7

(continued)

Table 5. (continued)

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
Nonlive per litter[b,c]	1.62† ±0.60	1.80 ±0.80	0.80 ±0.49	7.25** ±2.75	5.00** ±1.41	1.10 ±0.28	2.33 ±0.89	1.00 ±0.58
% Nonlive per litter[b]	11.1† ±3.7	13.1 ±6.3	7.3 ±4.6	52.3* ±22.1	40.0** ±14.3	8.2 ±1.9	16.5 ±7.2	8.1 ±4.2
No. litters with nonlive	16	3	2	4	6	8	9	2
% Litters with nonlive	61.5	60.0	40.0	100	100	80.0	75.0	66.7
Affected per litter[b,d]	1.62††§ ±0.60	1.80 ±0.80	1.40 ±0.60	9.25** ±2.14	5.17** ±1.35	1.80 ±0.84	2.67* ±0.92	3.67* ±0.33
% Affected per litter[b]	11.1††§ ±3.7	13.1 ±6.3	12.3 ±5.3	64.1** ±17.6	41.2* ±13.9	13.6 ±6.4	18.8 ±7.7	30.6* ±1.4
No. litters with affected	16	3	3	4	6	8	11	3
% Litters with affected	61.5	60.0	60.0	100	100	80.0	91.7	100
LIVE LITTERS[d]								
(No. litters with live fetuses)	(26)	(5)	(5)	(3)	(5)	(10)	(12)	(3)
Live fetuses per litter[b]	11.77 ±0.59	12.20 ±1.16	11.00 ±1.18	10.67 ±3.84	11.40 ±1.94	12.50 ±0.62	12.50 ±1.23	11.00 ±1.00
Avg. fetal body weight (g) per litter[b]	0.964§§§ ±0.025†††	0.929 ±0.020	0.885 ±0.055	0.752 ±0.124	0.690** ±0.020	0.831** ±0.038	0.777*** ±0.032	0.715* ±0.081
Males per litter[b]	6.38 ±0.44	6.60 ±0.93	6.60 ±1.47	5.33 ±2.19	3.80 ±0.73	6.80 ±0.96	5.17 ±0.53	6.67 ±1.20
% Males per litter[b]	56.1 ±3.7	54.2 ±4.7	58.5 ±10.1	45.7 ±6.9	34.6 ±5.0	53.1 ±5.8	46.9 ±6.1	60.2 ±7.6
Females per litter[b]	5.38 ±0.49	5.60 ±0.75	4.20 ±1.02	5.33 ±1.76	7.60 ±1.69	5.60 ±0.62	7.33 ±0.95	4.33 ±0.88
% Females per litter[b]	43.9 ±3.7	45.8 ±4.7	40.1 ±9.7	54.3 ±6.9	65.4 ±5.0	46.2 ±5.8	53.1 ±6.1	39.8 ±7.6

Table 5. (continued)

[a]Includes all dams pregnant at sacrifice; litter size = no. implantation sites per dam.

[b]Reported as mean ± S.E.M.

[c]Nonlive = dead plus resorbed.

[d]Affected = nonlive plus malformed.

[e]Includes only dams with live fetuses; litter size = no. live fetuses per dam.

[†]$p<0.05$ Kruskal-Wallis.

[††]$p<0.01$ Kruskal-Wallis.

[†††]$p<0.001$ Kruskal-Wallis.

[§]$p<0.05$ Jonckheere's.

[§§]$p<0.01$ Jonckheere's.

[§§§]$p<0.001$ Jonckheere's.

[¶¶]$p<0.01$ Fisher's Exact.

[*]$p<0.05$ Mann-Whitney U.

[**]$p<0.01$ Mann-Whitney U.

[***]$p<0.001$ Mann-Whitney U.

Table 6. Preliminary Toxicity Study: Teratological Defects in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15[a]

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
Fetuses examined[b]	(306)	(61)	(55)	(32)	(57)	(125)	(150)	(33)
Litters examined[c]	(26)	(5)	(5)	(3)	(5)	(10)	(12)	(3)
GROSS MALFORMATIONS								
Cleft palate: small or stunted fetus						5		
normal or full sized fetus				4		2	1	
Micromelia (disproportionately short limb)								1
Adactyly (missing all digits)								1
Anophthalmia right				1				
VISCERAL MALFORMATIONS								
Missing subclavian right								1
SKELETAL MALFORMATIONS								
Abnormal skull fusion				2				
Fused cervical arches (to each other)			1					
Fused thoracic arches								1
Thoracic centra misaligned								1
Ribs fused to each other			2	1	1		2	5
Branched rib								2
Missing rib							1	
VARIATIONS								
Undescended testes: bilateral (normal size fetus)	2	2	1					
Wavy rib	2					1		
Misaligned sternebrae	9	2	2				1	
Doubled centra						1		1
Undescended testes - stunted fetus						2	1	2

Table 6. (continued)

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
Clubbed limb without bone change								1
White spots left ventricle			1					
Incomplete ossification	1							
Enlarged nasal sinus(es)	1							

[a]A single fetus may be represented more than once in listing individual defects.

[b]Only live fetuses were examined for malformations.

[c]Includes only litters with live fetuses.

Table 7. Preliminary Toxicity Study: Summary of Malformations in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
Fetuses examined[a]	(306)	(61)	(55)	(32)	(57)	(125)	(150)	(33)
Litters examined[b]	(26)	(5)	(5)	(3)	(5)	(10)	(12)	(3)
GROSS MALFORMATIONS								
Fetuses[c]	0	0	0	5	0	7	1	1
Litters[d]	0	0	0	2¶	0	1	1	1
VISCERAL MALFORMATIONS								
Fetuses[c]	0	0	0	0	0	0	0	1
Litters[d]	0	0	0	0	0	0	0	1
SKELETAL MALFORMATIONS								
Fetuses[c]	0	0	3	3	1	0	3	6
Litters[d]	0	0	1	1	1	0	2	3¶¶¶

(continued)

Table 7. (continued)

	Phenol (mg/kg/day, po)							
	0	100	200	230	260	275	300	400
ALL MALFORMATIONS								
Males malformed per litter[e]	0†††§§	0	0.20* ±0.20	2.00*** ±1.53	0	0.40 ±0.40	0.25* ±0.18	2.00** ±0.58
% Males malformed per litter[e]	0†††§§	0	2.0* ±2.0	25.6*** ±18.9	0	5.7 ±5.7	10.7* ±8.5	28.9*** ±4.3
Females malformed per litter[e]	0†§	0	0.40* ±0.40	0.67** ±0.67	0.20* ±0.20	0.30 ±0.30	0.08 ±0.08	0.67*** ±0.33
% Females malformed per litter[e]	0†§	0	20.0* ±20.0	11.1** ±11.1	3.3* ±3.3	7.5 ±7.5	0.8 ±0.8	19.4*** ±10.0
Fetuses malformed per litter[e]	0†††§§§	0	0.60* ±0.60	2.67*** ±2.19	0.20* ±0.20	0.70 ±0.70	0.33** ±0.19	2.66*** ±0.67
% Fetuses malformed per litter[e]	0†††§§§	0	5.0* ±5.0	18.9*** ±15.7	1.7* ±1.7	6.4 ±6.4	9.9** ±8.3	24.1*** ±4.9
No. fetuses malformed[c]	0	0	3	8	1	7	4	8
% Fetuses malformed[c]	0	0	5.5	25.0	1.8	5.6	2.7	24.2
No. litters with malformations[d]	0	0	1	2¶	1	1	3	3¶¶¶
% Litters with malformations[d]	0	0	20.0	66.7	20.0	10.0	25.0	100

[a] Only live fetuses were examined for malformations.

[b] Includes only litters with live fetuses.

[c] Fetuses with one or more malformations.

[d] Litters with one or more malformed fetuses.

[e] Reported as mean ± S.E.M. for all live litters.

† $p<0.05$ Kruskal-Wallis.

††† $p<0.001$ Kruskal-Wallis.

¶ $p<0.05$ Fisher's Exact.

¶¶¶ $p<0.001$ Fisher's Exact.

* $p<0.05$ Mann-Whitney U.

** $p<0.01$ Mann-Whitney U.

*** $p<0.001$ Mann-Whitney U.

§ $p<0.05$ Jonckheere's.

§§ $p<0.01$ Jonckheere's.

§§§ $p<0.001$ Jonckheere's.

Table 8. Preliminary Toxicity Study: Analysis of Phenol Dosage Formulations[a]

Treatment, mg/kg/day (Formulation Code)	Substudy and dosing period		Date of formulation	Date of analysis	Theoretical concentration (mg/ml)	Measured concentration[b] (mg/ml)	% Theoretical concentration
Phenol, 100 (42223)	I	9/25 to 10/4/80	9/24/80	7/19/81	10	10.5	105
Phenol, 200 (86241)	I	9/25 to 10/4/80	9/24/80	7/19/81	20	19.5	97.5
Phenol, 230 (88572)	III	10/9 to 10/18/80	10/8/80	7/19/81	23	22.2	96.5
Phenol, 260 (70445)	III	10/9 to 10/18/80	10/8/80	7/19/81	26	23.0	88.5
Phenol, 275 (20565)	II	10/2 to 10/11/80	10/1/80	7/20/81	27.5	24.4	88.7
	IV	10/23 to 11/1/80	10/22/80	7/18/81		25.6	93.1
Phenol, 300 (72237)	I	9/25 to 10/4/80	9/24/80	7/19/81	30	27.4	91.3
	II	10/2 to 10/11/80	10/1/80	7/20/81		27.1	90.3
	III	10/9 to 10/18/80	10/8/80	7/19/81		25.8	86.0
Phenol, 400 (14756)	I	9/25 to 10/4/80	9/24/80	7/19/81	40	33.5	83.8

[a]Samples of phenol formulations in distilled water were analyzed by HPLC (see Section 3.3.3).

[b]"Measured concentration" represents the average calculated concentration of three independent aliquots for each formulation.

4.2 Teratology Study

4.2.1 Toxicological and Teratological Evaluation

Due to practical considerations in processing large numbers of dams and fetuses, an experimental design with multiple breeding dates was employed (Table 9). A total of 31-36 plug-positive CD-1 females were assigned to each of four treatment groups (phenol 0, 70, 140 and 280 mg/kg/day). Two unscheduled deaths due to dosing error occurred, one each in the mid- and high-dose groups. Four additional deaths in the high-dose group appeared to be treatment-related, resulting in mortality rates for all treated females of 11% (4/35) for the high-dose and 0% for the vehicle, low- and mid-dose groups (Table 10). Maternal body weight (i.e., pertaining to females confirmed to be pregnant on gd 17) did not differ significantly among treatment groups on gd 0 or prior to the initiation of dosing on gd 6. During treatment (i.e., gd 11 and 15) and at sacrifice (gd 17), dose-related trends ($p<0.05$, Jonckheere's Test) were observed for decreased maternal body weight as dosage increased. Differences among treatment groups were also significant on gd 17 ($p<0.05$, Kruskal-Wallis) with the high-dose group significantly below controls ($p<0.05$, Mann-Whitney U) on this measure (Table 10). Significant dose-related trends ($p<0.01$ or 0.001, Jonckheere's Test) were also observed for measures of maternal weight gain (i.e., gestational weight gain, weight gain during treatment and absolute weight gain), with the high-dose group exhibiting lower weight gain than controls ($p<0.01$ or 0.001, Mann-Whitney U) for all three measures (Table 10). Gravid uterine weight was decreased in a dose-related manner ($p<0.05$, Jonckheere's Test) with the high-dose significantly below

controls. Maternal liver weight (g) also decreased as dosage increased ($p<0.05$, Jonckheere's Test), but no significant differences among individual treatment groups were found ($p>0.05$, Kruskal-Wallis). Clinical signs observed primarily in the high-dose group during treatment included clinical weight loss ($\geq$1 g/24 hr), tremors, ataxia, lethargy and irritability; mid-dose females exhibited mild tremors after dosing on gd 6-8, but this response was not observed on subsequent days of treatment. Four females exhibited vaginal bleeding during treatment with pregnancy outcome for these females as follows. (1) Female number 107 in the 70 mg/kg/day phenol group exhibited vaginal bleeding on gd 9, and was found to have twelve live fetuses at sacrifice on gd 17. (2) Female number 176 in the 70 mg/kg/day phenol group exhibited vaginal bleeding on gd 14 and was found to have six resorption sites; this female was also found to have neoplastic loci in the kidneys. (3) Female number 110 exhibited vaginal bleeding on gd 9 in conjunction with a generally debilitated condition at dosing; this female died within two hours post-dosing on gd 9. (4) Female number 181 exhibited heavy vaginal bleeding on gd 15 and was found to have eleven resorption sites at sacrifice on gd 17; the pattern of weight gain and loss for this female suggested that spontaneous abortion of the entire litter probably occurred between dosing on gd 15 and sacrifice on gd 17.

When uterine contents were examined on gd 17, treatment groups were found to be matched for the numbers of implantation sites per dam and no statistically significant evidence of altered prenatal viability or structural development was observed (Table 12). A dose-related decrease ($p<0.001$, Jonckheere's Test) in average fetal body weight per litter was

observed, with high-dose fetal weights significantly below vehicle controls ($p<0.001$, Mann-Whitney U; Table 12). An apparent, though not statistically significant, increase in the incidence of cleft palate was observed: 0/308, 1/290, 1/280 and 8/214 in the vehicle through high-dose group, respectively. By comparison, the historical control incidence of cleft palate from this laboratory is 1/1580 CD-1 mouse fetuses (Jones-Price, unpublished data). The apparent response to phenol treatment may reflect the compromised status of high-dose females which exhibited clinically and statistically significant signs of toxicity during treatment and at sacrifice on gd 17 (see above).

4.2.2 Analysis of Dosage Formulations

Samples of each phenol formulation in distilled water were stored (5°C) prior to analysis. According to HPLC analysis conducted approximately 7 to 8 months after formulation, the percent theoretical concentration for all phenol dosage formulations fell within an acceptable range of 90 to 110% (Table 15).

Table 9. Distribution of Plug-Positive CD-1 Mice Across Dose Groups and Breeding Dates in the Teratologic Evaluation of Phenol

Breeding (gd 0)	Phenol (mg/kg/day, po)			
	0	70	140	280
I (11/1/80)	14	14	14	14
II (11/8/80)	9	9	9	10
III (11/25/80)	7	8	8	7
IV (12/5/80)	5	0	0	5
Total	35	31	31	36

Table 10. Maternal Toxicity in CD-1 Mice Exposed to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)			
	0	70	140	280
SUBJECT (Dams)				
Total treated	35	31	31	36
Removed	0	0	1[c]	1[c]
Deaths	0	0	0	4
Nonpregnant at sacrifice	6	5	5	9
Pregnant (%) at sacrifice	29 (82.9)	26 (83.9)	25 (83.3)	22 (71.0)
MATERNAL BODY WEIGHT (g)[a]				
Gestational day 0	28.26 ±0.38	28.51 ±0.39	28.66 ±0.41	29.09 ±0.52
Gestational day 6	31.08 ±0.32	31.12 ±0.38	30.66 ±0.42	31.58 ±0.50
Gestational day 11	34.69§ ±0.45	34.38 ±0.63	34.32 ±0.36	32.91 ±0.72
Gestational day 15	42.86§ ±0.78	43.01 ±0.98	42.75 ±0.51	39.70 ±1.08
Gestational day 17	48.03†§ ±1.03	48.65 ±1.24	48.00 ±0.63	43.28* ±1.70

(continued)

Table 10. (continued)

	Phenol (mg/kg/day, po)			
	0	70	140	280
MATERNAL WEIGHT GAIN (g)[a]				
Gestation period	19.76††§§ ±0.99	20.15 ±1.22	19.34 ±0.64	14.19** ±1.63
Treatment period	11.79†††§§ ±0.66	11.89 ±0.84	12.09 ±0.43	8.12** ±0.93
Absolute weight gain[b]	4.18†††§§§[d] ±0.50	4.02 ±0.52	3.02 ±0.44	1.42*** ±0.67
GRAVID UTERINE WEIGHT (g)[a]	15.80†§[d] ±0.82	16.13 ±0.87	16.32 ±0.62	12.77* ±1.16
MATERNAL LIVER WEIGHT (g)[a]	2.49§§ ±0.06	2.46 ±0.06	2.33 ±0.03	2.26 ±0.08

[a]Includes all dams pregnant at sacrifice; mean ± S.E.M.

[b]Weight gain during gestation minus gravid uterine weight.

[c]Death due to dosing error.

[d]One gravid uterine weight not recorded; n=28.

†$p<0.05$ Kruskal-Wallis.

††$p<0.01$ Kruskal-Wallis.

†††$p<0.001$ Kruskal-Wallis.

§$p<0.05$ Jonckheere's Test.

§§$p<0.01$ Jonckheere's Test.

§§§$p<0.001$ Jonckheere's Test.

*$p<0.05$ Mann-Whitney U.

**$p<0.01$ Mann-Whitney U.

***$p<0.001$ Mann-Whitney U.

Table 11. Clinical Signs in CD-1 Mice Exposed to Phenol on Gestational Days 6 Through 15

Gestational Day	Clinical Signs	Phenol (mg/kg/day, po) 0 Dam #	0 Wt.	70 Dam #	70 Wt.	140 Dam #	140 Wt.	280 Dam #	280 Wt.
6	* weight loss from day 0			186	1.3				
	* slight spasmodic tremors after dosing					all			
	* tremors after dosing, unable to walk							all	
7	* weight loss[a]							222	1.0
	* tremors after dosing			112					
	* very slight tremors, lethargy					all			
	* tremors after dosing, unable to walk							all	
8	* weight loss[a]							110	5.2
								219	1.5
								218	4.5
	* tremors barely perceptible, lethargy					all			
	* tremors after dosing, unable to walk							all	
	* body swollen							110	
	* death							192	
9	* weight loss[a]							110	1.6
								197	4.7
								222	1.0
	* tremors after dosing, unable to walk							all	
	* vaginal bleeding			107				110	
	* moving slowly, coat ruffled							110	
	* death							110	
10	* weight loss[a]							184	1.5
	* tremors, ataxia							all	
	* very jumpy, mouth bleeding							174	
11	* weight loss[a]	98	2.3					134	3.8
								174	2.5
								222	2.2
	* tremors (variable severity); ruffled fur							all	
	* respiratory distress							134	
								141	
12	* weight loss[a]			176	1.4			205	3.4
	* tremors							all	
	* tremors; unable to walk							197	
	* respiratory distress							200	
	* death							134	
13	* weight loss[a]			176	3.0			173	4.6
								174	2.3
	* tremors							all	
	* severe tremors; unable to walk							406	

(continued)

Table 11. (continued)

Gestational Day	Clinical Signs	Phenol (mg/kg/day, po) 0 Dam #	0 Wt.	70 Dam #	70 Wt.	140 Dam #	140 Wt.	280 Dam #	280 Wt.
14	* weight loss[a]			176	1.2			174	1.0
	* tremors, mild very sluggish							all	
	* vaginal bleeding			176					
15	* weight loss[a]							181	4.2
	* tremors, mild very sluggish							all	
	* severe vaginal bleeding							181	
	* respiratory distress							208	
	* death							174	
17	* (Day of sacrifice)								
	* neoplasms in kidneys (bilateral)			176					
	* ovarian cyst					116			

[a]Weight loss is defined as a loss greater than 1.0 g in 24 hours.

Table 12. Embryotoxicity in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)			
	0	70	140	280
ALL LITTERS[a]				
(Pregnant Dams)	(29)	(26)	(25)	(22)
Implantation sites per litter[b]	12.14 ±0.44	11.96 ±0.50	12.28 ±0.27	12.41 ±0.49
Resorptions per litter[b]	1.48 ±0.38	0.69 ±0.25[f]	0.92 ±0.36	2.45 ±0.79[g]
% Resorptions per litter[b]	12.7 ±3.2	7.4 ±3.8	7.6 ±3.0	21.4 ±6.9
No. litters with resorptions	16	11	12	14
% Litters with resorptions	55.2	42.3	48.0	63.6
Dead per litter[b]	0.03 ±0.03	0.11 ±0.06	0.16 ±0.07	0.23 ±0.11
% Dead per litter[b]	0.3 ±0.3	0.9 ±0.5	1.4 ±0.7	1.6 ±0.9
No. litters with dead	1	3	4	4
% Litters with dead	3.4	11.5	16.0	18.2

(continued)

Table 12 (continued)

	Phenol (mg/kg/day, po)			
	0	70	140	280
Nonlive per litter[b,c]	1.52 ±0.38	0.81 ±0.25	1.08 ±0.37	2.68 ±0.78
% Nonlive per litter[b]	12.9 ±3.3	8.3 ±3.8	9.0 ±3.1	23.0 ±6.8
No. litters with nonlive	16	13	14	15
% Litters with nonlive	55.2	50.0	56.0	68.2
Affected per litter[b,d]	1.55 ±0.38	0.92 ±0.26	1.16 ±0.40	3.09 ±0.81
% Affected per litter	13.2 ±3.2	9.2 ±3.8	9.6 ±3.4	26.0 ±7.0
No. litters with affected	17	14	15	15
% Litters with affected	58.6	53.8	60.0	68.2
LIVE LITTERS[e]				
(No. litters with live fetuses)	(29)	(25)	(25)	(20)
Live fetuses per litter[b]	10.62 ±0.57	11.60 ±0.42	11.20 ±0.46	10.70 ±0.79
Avg. fetal body weight (g) per litter[b]	1.026†††§§§ ±0.022	0.989 ±0.022	0.996 ±0.013	0.842*** ±0.028
Males per litter[b]	6.28 ±0.47	5.68 ±0.34[h]	5.84 ±0.40[i]	5.30 ±0.65
% Males per litter[b]	57.7 ±3.0	49.1 ±2.4	52.6 ±2.9	49.9 ±4.7

(continued)

Table 12. (continued)

	Phenol (mg/kg/day, po)			
	0	70	140	280
Females per litter[b]	4.34† ±0.35	5.84** ±0.38	5.32 ±0.41	5.40 ±0.63
% Females per litter[b]	42.6 ±3.1	50.1 ±2.6	47.1 ±2.9	50.1 ±4.7

[a] Includes all dams pregnant at sacrifice; litter size = no. implantation sites per dam.

[b] Reported as mean ± S.E.M.

[c] Nonlive = dead plus resorbed.

[d] Affected = nonlive plus malformed.

[e] Includes only dams with live fetuses; litter size = no. live fetuses per dam.

[f] One dam had all resorptions.

[g] Two dams had all resorptions.

[h] The sex of two fetuses was not determined.

[i] The sex of one fetus was not determined.

† $p<0.05$ Kruskal-Wallis.

††† $p<0.001$ Kruskal-Wallis.

§§§ $p<0.001$ Jonckheere's Test.

** $p<0.01$ Mann-Whitney U.

*** $p<0.001$ Mann-Whitney U.

Table 13. Teratological Defects in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15[a]

	Phenol (mg/kg/day, po)			
	0	70	140	280
Fetuses examined[b]	(308)[d]	(290)	(280)	(214)
Litters examined[c]	(29)	(25)	(25)	(20)
GROSS MALFORMATIONS				
Exencephaly		1		
Open eye: Bilateral		1		
Cleft palate - normal or full sized fetus		1	1	8
VISCERAL MALFORMATIONS				
No aortic arch			1	
Missing subclavian: Left			1	
SKELETAL MALFORMATIONS				
Lumbar centra off center				1
Branched rib				1
Missing rib	1	1		
VARIATIONS				
Hematoma - Face				1
Small lung lobe				1
Misaligned sternebrae	7	6	14	3
Doubled centra				1
Undescended testes - stunted fetus		1		
Small kidney		1		
Incomplete ossification				1

[a]A single fetus may be represented more than once in listing individual defects.

[b]Only live fetuses were examined for malformations.

[c]Includes only litters with live fetuses.

[d]One skeleton not evaluated due to improper processing.

Table 14. Summary of Malformations in CD-1 Mice Following Maternal Exposure to Phenol on Gestational Days 6 Through 15

	Phenol (mg/kg/day, po)			
	0	70	140	280
Fetuses examined[a]	(308)[f]	(290)	(280)	(214)
Litters examined[b]	(29)	(25)	(25)	(20)
GROSS MALFORMATIONS				
Fetuses[c]	0	2	1	8
Litters[d]	0	2	1	3
VISCERAL MALFORMATIONS				
Fetuses[c]	0	0	1	0
Litters[d]	0	0	1	0
SKELETAL MALFORMATIONS				
Fetuses[c]	1	1	0	1
Litters[d]	1	1	0	1

(continued)

Table 14. (continued)

	Phenol (mg/kg/day, po)			
	0	70	140	280
ALL MALFORMATIONS				
Males malformed per litter[e]	0.03 ±0.03	0.12 ±0.07	0.08 ±0.06	0.25 ±0.12
% Males malformed per litter[e]	0.38 ±0.38	1.8 ±1.0	2.5 ±2.0	9.7 ±5.7
Females malformed per litter[e]	0	0	0	0.2 ±0.2
% Females malformed per litter[e]	0	0	0	2.5 ±2.5
Fetuses malformed per litter[e]	0.03 ±0.03	0.12 ±0.07	0.08 ±0.06	0.45 ±0.30
% Fetuses malformed per litter[e]	0.2 ±0.2	1.0 ±0.6	1.6 ±1.4	5.5 ±3.3
No. fetuses malformed[c]	1	3	2	9
% Fetuses malformed[c]	0.3	1.0	0.7	4.2
No. litters with malformations[d]	1	3	2	4
% Litters with malformations[d]	3.4	12.0	8.0	20.0

[a]Only live fetuses were examined for malformations.

[b]Includes only litters with live fetuses.

[c]Fetuses with one or more malformations.

[d]Litters with one or more malformed fetuses.

[e]Reported as mean ± S.E.M. for all live litters.

[f]One skeleton did not process properly and was not evaluated.

Table 15. Analysis of Phenol Dosage Formulations[a]

Treatment, mg/kg/day (Formulation Code)	Replicate and dosing period		Date of formulation	Date of analysis	Theoretical concentration (mg/ml)	Measured concentration[b] (mg/ml)	% Theoretical concentration
Phenol, 70 (13596)	I & II	11/7 to 11/16/80 and 11/14 to 11/23/80	11/7/80	7/18/81	7	7.3	104.3
	III	12/1 to 12/10/80	12/1/80	7/18/81		7.1	101.4
Phenol, 140 (63471)	I & II	11/7 to 11/16/80 and 11/14/ to 11/23/80	11/7/80	7/18/81	14	13.7	97.9
	III	12/1 to 12/10/80	12/1/80	7/18/81		13.6	97.1
Phenol, 280 (25179)	I & II	11/7 to 11/16/80 and 11/14 to 11/23/80	11/7/80	7/18/81	28	26.5	94.6
	III	12/1 to 12/10/80	12/1/80	7/18/81		27.8	99.3
	IV	12/11 to 12/20/80	12/10/80	7/19/81		26.5	94.6

[a]Samples of phenol formulations in distilled water were analyzed by HPLC (see Section 3.3.3).

[b]"Measured concentration" represents the average calculated concentration of three independent aliquots for each formulation.

5.0 Discussion

In the present study, maternal and fetal toxicity, but no significant teratogenic effects were observed in CD-1 mice treated with phenol (0, 70, 140, or 280 mg/kg/day, po, on gd 6-15). A statistically significant increase in the incidence of fetal malformations was observed in the preliminary investigation, and an apparent, but nonsignificant, increase was observed in the teratology study at dosages within the maternally toxic range (i.e., $\geq$200 mg/kg/day, phenol). Since maternal stress from a variety of sources can affect prenatal viability, fetal growth, and fetal structural development in laboratory animals (Fraser, 1977), these effects are not necessarily attributable to the test compound *per se*, but are more likely the result of compromised maternal status. In a concurrent study from this laboratory, phenol (0, 30, 60 or 120 mg/kg/day, po, gd 6-15) produced dose-related signs of fetal toxicity even at dosages below the maternally toxic range, but failed to significantly increase the incidence of malformations in CD rats (Jones-Price et al., 1983). These results appear to be in agreement with those of Minor and Becker (1971) which revealed fetal toxicity, but no teratogenic effect following exposure of Sprague-Dawley rats to phenol (20, 63 or 200 mg/kg/day, ip) on gd 9-11 or 12-14 (gd 1 = copulation evidence).

6.0 References

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APPENDIX I

Protocol and Amendment:

Teratologic Evaluation of Phenol

Protocol Amendment

Research Triangle Institute

Title: Teratogenic Assessment of Phenol

Sponsor: National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Safety Procedures:

Changed from:

Safety Procedures:

Ingestion of even small amounts of phenol may cause nausea, vomiting, circulatory collapse, tachypnea, paralysis, convulsions, coma, grennish or smoky-colored urine, and death from respiratory or cardiac arrest. Average fatal dose is 15 grams but death from as little as one gram has been reported. It has a human threshold limit value of 5 ppm and a human oral LDLo of 140 mg/kg.

Obviously phenol is toxic and carries a Department of Transportation label of poison B, and classification codes of carcinogen-neoplastigen, primary irritant and germicide. Phenol is mutagenic in cultures of *E. coli* and in Drosphilia. As of 1978 no *in vivo* mutagenicity studies have been reported with phenol.

In order to keep direct contract with phenol to a minimum, the formulations of this compound will be prepared in a laminar flow hood (vented outdoors), weighing on a balance located within this hood. The technician dosing the test solutions will wear gloves and be thoroughly aware of the dangers of this compound.

Changed to:

Safety Procedures:

Ingestion of even small amounts of phenol may cause nausea, vomiting circulatory collapse, tachypnea, paralysis, convulsions, coma, grennish or smoky-colored urine, and death from respiratory or cardiac arrest. Average fatal dose if 15 grams but death from as little as one gram has been reported. It has a human threshold limit value of 5 ppm and a human oral LDLo of 140 mg/kg.

Obviously phenol is toxic and carries a Department of Transportation label of poison B, and classification codes of carcinogen-neoplastigen, primary irritant and germicide. Phenol is mutagenic in cultures of *E. coli* and in Drosphilia. As of 1978 no *in vivo* mutagenicity studies have been reported with phenol.

The following procedures will be followed by all personnel either formulating solutions or dosing animals in order to avoid contact with phenol.

1. Phenol can cause burns to the skin and be absorbed through intact skin. Therefore, double (2 pair) Flexam® floor/exam latex gloves must be worn by all personnel using phenol. If any holes or tears are noted or suspected both pairs of gloves should be replaced at once. Personnel will also wear a lab coat, eye goggles and closed shoes.
2. Phenol is incompatable with strong oxidizers, therefore, no oxidizers may be present in the hood while the phenol solutions are being prepared. This will be checked by another biologist prior to preparing the drug.

3. All phenolic formulations will be made in a laminar flow hood with an attached activated charcoal filter vented to the outdoors. Weighing of the reagent will be performed on a balance within this hood.

4. All personnel must immediately and thoroughly wash any skin areas exposed to phenol with liberal quantities of 70 percent ethanol solution followed by soap and water. A large squeeze bottle containing 70 percent ethanol will be immediately available in the hood and the dosing room for this purpose and be appropriately labeled and reserved solely for this purpose.

5. In the event of an accident or spill personnel must remove AT ONCE any wet clothing and IMMEDIATELY neutralize the phenol wetted areas first with the 70 percent ethanol solution then soap and water. The nearest supervisor will be notified as soon as possible. Spill areas will be dried with paper towels, then washed with 70 percent ethanol solution and finally water. All litter should be promptly bagged, labeled and disposed of in metal cans provided for toxic wastes.

6. Thorough initial directions and procedures will be given by T. Ledoux and timely inspections of the procedures will also be made by him.

Thomas A. Ledoux

Thomas A. Ledoux, Ph.D.
Co-study Director

7-11-80
Date

Jerry R. Reel

Jerry R. Reel, Ph.D.
Co-study Director

7/11/80
Date

PROTOCOL

RESEARCH TRIANGLE INSTITUTE

Teratogenic Potential of Environmental Agents

Contract No.: N01-ES-6-2127

Title:

Teratogenic Assessment of Phenol

Objective:

To determine if and to what extent phenol is teratogenic to mice.

Test Material: Phenol

Molecular Formula: C_6-H_6-0

Molecular Weight: 94.12

The test agent will be supplied by the sponsor (NIEHS)

Chemistry:

The purity and stability of the compound will be determined by the sponsor (NIEHS).

Formulations - dissolved in sterile distilled water.

Concentration - the initial formulation for each dose level studied will be checked by analytical methods (GLC) at RTI. Samples of each batch prepared will be taken and stored (-20°C). If the initial concentrations are different from what is expected, additional formulations will also be checked.

Personnel:

Co-study Director: Dr. Thomas Ledoux

Co-study Director: Dr. Jerry R. Reel

Laboratory Supervisor: Ms. Patricia Fisher

Biologist I: Ms. Loretta Langhoff

Biologist I: Ms. Melissa Marr

Biologist I: Ms. Frieda Gerling

Technician III: Mr. Burnes Ray

Technician II: Ms. Betty King

Technician II: Ms. Vickie Wilson

Biologist I: Mr. Thomas Mangum

Biologist I: Ms. Vera Lynn Smith

Sponsor:

National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709

Testing Facility:

Research Triangle Institute, Chemistry and Life Sciences Group, P. O. Box 12194, Research Triangle Park, N.C. 27709

Date of Study:

The Preliminary toxicity tests will be performed starting about June, 1980.

The teratology investigation will be initiated as soon as the maximum tolerated dose has been established, most likely in July, 1980.

Animals Used:

Charles River Caesarean-Originated, Barrier-Sustained (COBS) CD-1 (ICR)BR (outbred albino) mice.

Justification of Test System:

Desire for small laboratory animal which is easy to handle and dose, as well as being economical for a study requiring a large number of animals.

Diet:

The mice will be maintained on Purina Certified Rodent Chow (5002) and deionized water, ad libitum. The cage litter will be Ab-sorb-dri.

Identification of Animals:

Individually coded tags will be affixed to the left ear of each mouse upon entry into the animal facility.

Method of Avoiding Bias:

The mice selected by the supplier will be indiscriminately caged upon arrival at the RTI Animal Research Facility. Once mating has been confirmed, the female mice will be equally distributed across treatment groups by weight.

Route of Administration:

Phenol will be dissolved in sterile distilled water (4°C) and administered orally (gavage).

Dosage Interval:

Preliminary Toxicity Study: The mice will be dosed daily between 9:00 and 11:00 AM on days 6-15 of gestation. Teratology Study: The mice will be dosed daily between 9:00 and 11:00 AM on days 6-15 of gestation (day 0 will be the day that a vaginal plug has been detected).

Dosage Amount:

The mice will be dosed at 1% of their body weight; i.e., 0.01 ml of test solution will be administered per gram of body weight. In order to administer sufficient compound, for every mg/kg of phenol a 0.1 mg/ml

solution will be prepared; e.g., a 400 mg/kg solution would require a concentration of 40.0 mg/ml.

Dosage Level:

Preliminary Toxicity Study: Proper testing of compounds for teratogenicity requires a sufficiently high dose of the compound to cause significant toxicity in the mother or the fetus. Since LD_{50} values are usually determined in non-pregnant animals it is necessary to first establish the LD_{50} value in pregnant animals of the same strain and age as will be used in the subsequent teratology study. The oral LD_{50} for phenol is 300 mg/kg in nonpregnant mice. Therefore, pregnant mice will be dosed by gavage on days 6-15 of gestation with doses of 400, 300, 200 and 100 mg/kg. A vehicle (sterile water) control group will also be a part of this study. At least six pregnant mice will be inserted in each test group.

Formulation:

Each dose will be formulated independent of the other doses. Sufficient drug solutions will be made to last the entire administration period (10 days). The calculated amount of phenol will be added to a beaker (e.g., 40 mg/ml for the 400 mg/kg/day dose) and dissolved in sterile water. Then the formulation will be transferred to a graduate cylinder, the beaker will be rinsed twice with sterile water and the washings added to the cylinder. Enough sterile water will be added to the cylinder to bring the volume up to the proper amount. The formulation will then be stirred with a glass rod until any convection currents are eliminated. After removing approximately 60 ml of the phenol solution for administration, the remainder of the formulation will be given to the chemist for concentration verification. Sterile water alone will serve as the vehicle control.

Teratology Study: The highest dose in the toxicity investigation which allows 90% or greater maternal animal survival to day 17 of gestation will be termed the maximum tolerated dose (MTD). This dose and two lower (geometric) doses and a vehicle control will be given on days 6-15 of gestation.

Procedure:

The mice will be quarantined for at least seven days after arrival from the breeder. Two females will then be paired with one male CD-1 mouse overnight. The next morning the female mice will be checked for copulation plugs, which will be taken as evidence that mating has occurred (day 0 of gestation) and then uniformly distributed to control or experimental groups by weight.

Preliminary Toxicity Study: Phenol will be given by gavage (1.0% body weight) on days 6-15 of gestation into pregnant CD-1 mice at 100, 200, 300 or 400 mg/kg/day (at least six dams per dose group). Additional dose levels will be tested, if necessary, until the maximum tolerated dose (MTD) has been determined.

Teratology Study: Once the MTD has been determined, this dose as well as two geometrically lower doses will be administered to pregnant CD-1 mice. At least two replicates, each with at least 10 dams per test group, will be studied. As each replicate comes off study, the subsequent replicate will be put on test. If a total of at least 20 pregnant dams is not obtained for each dose level which does not produce maternal lethality, a third replicate will be placed on test.

Employing the Staples procedure (fresh tissue dissection method for examining fetal viscera), all live fetuses will be dissected for visceral observations. Half of the fetuses will be decapitated prior to dissection

(every other one) and the heads will be fixed in Bouin's solution for Wilson's razor-blade sectioning and examination. All fetal carcasses (half minus their head) will be prepared (Alizarin Red S) and examined for skeletal alterations.

Data Collection:

Data collected during the teratological examination will be compiled, checked, and analyzed statistically in order to determine whether there were differences between the vehicle control and treatment groups.

Statistical Analyses:

The litter will be considered to be the experimental unit for analysis of data regarding embryo toxicity and teratogenicity. Statistical significance of the differences between groups will be determined by the Mann-Whitney U Test. Jonckheere's test will be employed to test the significance of dose-response relationships. Two-tailed tests will be performed with $p<0.05$ selected as the level of significance.

Safety Procedures:

Ingestion of even small amounts of phenol may cause nausea, vomiting, circulatory collapse, tachypnea, paralysis, convulsions, coma, greenish or smoky-colored urine, and death from respiratory or cardiac arrest. Average fatal dose is 15 grams but death from as little as one gram has been reported. It has a human threshold limit value of 5 ppm and a human oral LDLo of 140 mg/kg.

Obviously phenol is toxic and carries a Department of Transportation label of poison B, and classification codes of carcinogen-neoplastigen, primary irritant and germicide. Phenol is mutagenic in cultures of *E. coli* and in Drosphilia. As of 1978 no *in vivo* mutagenicity studies have been reported with phenol.

In order to keep direct contact with phenol to a minimum, the formulations of this compound will be prepared in a laminar flow hood (vented outdoors), weighing on a balance located within this hood. The technician dosing the test solutions will wear gloves and be thoroughly aware of the dangers of this compound.

Compliance with FDA Regulations:

The Teratology Laboratory at RTI is presently in full compliance with Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory studies (Federal Register, December 22, 1978, pp 60013-60025). Thus it is fully anticipated that this study would be conducted in compliance with such regulations.

Thomas A. Ledoux, Ph.D. Date
Co-Study Director

Jerry R. Reel, Ph.D. 6/6/80 Date
Co-Study Director

Don Feldman, D.V.M., Manager Date
Animal Research Facility

Vladimir Petrow, D.Sc.,Ph.D. Date
F.R.I.C.
Chairman, Quality Assurance Unit

John A. Moore, D.V.M. 8/25/80 Date
Project Officer
N.I.E.H.S.

APPENDIX II

Sacrifice and Dissection of Dams and Fetal Examination

Sacrifice and Dissection of Dams. On gestational day 17, approximately one to one and a half days before expected parturition, pregnant dams were sacrificed by cervical dislocation. The sacrifice time, dam weight and clinical signs of toxicity prior to sacrifice were recorded for each dam. The abdomen of each dam was opened and both uterine horns exposed. Any internal abnormalities of the dam were noted and affected tissues were removed, labeled, and placed in neutral-buffered 10% formalin for future histopathological evaluation. The liver was dissected and weighed.

The uterus and right ovary were removed along with attached adipose tissue. The left ovary was dissected separately, thus, allowing identification of left and right uterine horns once the uterus was removed. The weight of the unopened gravid uterus was recorded to the nearest hundredth of a gram. The uterus was opened to expose the implantation sites which were categorized according to the following criteria:

Resorptions

"Implantation Site"	(I)	Evidence of implantation site visible after staining with ammonium sulfide (Salewski, 1964).
"Early"	(E)	Metrial glands; maternal placenta; maternal and fetal placenta.
"Middle"	(M)	Some fetal tissue, in addition to the above.
"Late"	(L)	Discernible limb buds.
"Full"	(F)	"Nonlive" fetus; discernible digits are present, but the weight is less than or equal to 0.3 g.

Dead		
"Dead"	Assigned a fetus number	"Nonlive", discernible digits, greater than 0.3 g.
Live		
"Live"	Assigned a fetus number	Observable fetal heartbeat and/or movement.

All implantations were classified and listed according to their position in the uterus. All "live" or "dead" fetuses were placed in order on a moist paper towel.

The uteri of dams with no apparent implants were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites (Salewski, 1964).

External Examination. External examination of each fetus was conducted under a magnifying lens. Initially, a check was made for disproportionate body shape, hematomas, hernias, abnormal swellings and/or depressions, as well as protrusion of meninges, spinal cord, and/or brain. The head was examined to detect if its shape was abnormal and whether or not the eyes, ears, nose, and mouth were normal in shape, location and size. The mouth was examined for the presence of an abnormally-sized lower jaw or for cleft lip of the upper jaw. Abnormalities of the tongue and palate were checked by depressing the tongue and opening the mouth with a pair of forceps inserted between the upper and lower jaw. The eyes were examined for size (macro- or microphthalmia), bulging (exophthalmia), open eye, or absence of eyes (anophthalmia). The extremities were checked for unusual size, shape, or location and the digits were examined for number as well as separation. The presence and proper location of the anus was checked. The

external genitalia were checked for general shape and size. The tail was examined for normal length and abnormal kinking or curling.

Each live or dead fetus and each "F" resorption was weighed to three significant figures. The weights of all live fetuses in a litter were used to derive a mean litter weight.

Approximately one-half of the fetuses were decapitated; in general, odd or even numbered fetuses were decapitated in every other litter. When only one fetus was found in a given uterine horn, that fetus was decapitated regardless of fetus number. This procedure insured that in the overall study some heads were taken from all uterine positions. The heads were placed in individual bottles of Bouin's solution for a head examination after fixation (see "Head Examination" below). Each bottle was identified by study code (Mi80-2D) dam number, fetus number, and date.

Visceral Examination. All fetuses were examined for visceral malformations, variations, and any other abnormalities under a stereomicroscope. Each fetus was opened for examination by making a small transverse incision anterior to the umbilicus. The second and third incisions extended from the first incision laterally and then posteriorly to the base of each hind limb. This skin flap was pulled down and the presence or absence of umbilical vessels was observed. A fourth incision was made through the skin above the sternum. The skin was teased away from the muscle and the presence or absence of a median fissure or cleft of the sternum was observed. The diaphragm was examined for herniation. The diaphragm was then cut bilaterally, the ribs were cut on the left of the sternum and the ribs were pinned back.

The thymus gland was removed and the greater vessels of the heart were then examined and any deviation from the norm was noted. Two specific heart cuts were made in order to examine the interior of the right and left ventricle, pulmonary artery, and aorta. The first cut was made to the right of the ventral midline surface at the apex and extended anteriorly and ventrally into the pulmonary artery. This incision, permitted examination of the tricuspid valve, papillary muscles, chordae tendineae, right side of the interventricular septum, and the semilunar valve of the pulmonary artery. The second heart cut was made slightly to the left of the ventral midline surface at the apex and extended anteriorly and ventrally into the aorta. The following structures were thus visible: bicuspid valve, papillary muscles, chordae tendineae, left side of the interventricular septum, and semilunar valve of the aorta.

The lungs were examined and the lobes counted, noting any abnormal color. The trachea and esophagus were checked for fusion as well as trachea-esophageal fistula. The liver lobes were counted and checked for splotches as well as fusion, texture, and normal coloration. The bile duct, gall bladder, stomach, spleen, pancreas, pyloric sphincter, and the intestines (small and large) were also examined and any variation from normal noted.

The reproductive organs were examined for proper size and location, and the sex of each fetus was recorded. Using forceps, the ureter was traced from the kidney to the bladder to insure that the ureters were attached. The adrenals and kidneys were also examined for proper size and location. Transverse kidney cuts were then made in order to expose

the renal papillae. Finally, the fetus was eviscerated and prepared for skeletal examination (see "Skeletal Examination" below).

Head Examination. Following a minimum fixation period of 48 hours in Bouin's solution, fetal heads were placed on a wax block and examined under a stereomicroscope to verify abnormalities detected earlier during the "external examination." Any external irregularities were recorded. The head was then sliced into six sections with a scalpel or razor blade. The internal structures were examined (Wilson, 1965) and abnormalities recorded. Additional slices were made through hematomas, swellings, or depressions in order to help ascertain the cause of such abnormalities.

A horizontal section was made beginning at the mouth which bisected both ears and exposed the tongue, palate, upper lip, and lower jaw. The tongue was removed, and the palate and upper lip were examined for incomplete closure (cleft). The head was turned over, dorsal side up, for the remaining sections which were made in the frontal plane.

The second slice was made slightly anterior to the eyes, exposing the nasal septum, nasal sinuses, and a cross-sectional view of the palate. Any abnormal closure or shape of the sinuses was recorded.

The third slice bisected the eyes and exposed the olfactory lobes of the brain, more posterior areas of the nasal septum, nasal sinus (nasopharyngeal cavity), and palate. The fetal eye consists of an optic cup, retina, vitreous chamber with a lens, and cornea. The vitreous chamber was removed to check for convoluted retina. All of these structures were examined for any improper alignment.

Slice 4, posterior to the eyes, exposed the cerebral hemispheres and lateral ventricles (ventricles I and II). The ventricles were closely examined for any abnormal constriction or distention (hydrocephaly).

The final slice exposed the third ventricle which communicates rostrally with the lateral ventricles via the interventricular foramina and caudally with the fourth ventricle via the cerebral aqueduct. Any constriction or distention in the third ventricle was noted.

All sections of any head having a malformation (except fetal heads with cleft palate and/or open eye) were stored in a vial of Bouin's fluid with the study code (Mi80-2D), sacrifice date, female number, fetus number, and malformation description labeled on the bottle. The vial was stored in an appropriately marked box for later examination, if necessary.

Skeletal Examination. Upon completion of the visceral exam, the fetuses were placed in partitioned plastic boxes and allowed to stand overnight in 70% ethanol in preparation for skeletal staining (Peltzer and Schardein, 1966).

Following dehydration, the ethanol was drained off and replaced with a 1% KOH solution with Alizarin Red S stain (125 mg/liter of 1% KOH) for six hours. After six hours, the KOH stain was poured off and replaced with a fresh solution of 1% KOH. Approximately 18 hours later the 1% KOH was replaced with a solution of "2:2:1" (70% ethanol: glycerin:benzyl alcohol). About six hours later the "2:2:1" was poured off and a solution of "1:1" (70% ethanol:glycerin) was added (modified from Crary, 1962).

After the skeletal staining process was complete, skeletons were removed from the ethanol:glycerin solution and placed in a small gauze-lined petri dish for examination under a stereomicroscope as described below.

The skull of the mouse fetus at gestational day 17 consists of the following bones: supraoccipital, exoccipital, interparietal, parietal, frontal, nasal, premaxillae, maxillae, zygomatics, squamosals, and mandibles. Each of these bones was checked for size, shape, and degree of ossification. Bones that were partially ossified, misshaped, or had any other deviations from normal were noted.

The six sternebrae were checked for fusion to other sternebrae, the presence of extra sternebrae, misalignment (displacement by at least one-third the antero-posterior length of the sternebra; sternebrae were classified as "scrambled" if all six were misaligned), and for bipartite sternebrae (one sternebra in two parts).

The complete vertebral column normally consisting of 7 cervical vertaebrae, 13 thoracic vertebrae, 6 lumbar vertebrae, 4 sacral vertebrae, and variable caudal vertebrae was examined for absent or extra arches, centra, and ribs, as well as ossification sites, fusion, branching, misalignment, or any abnormal shapes or length.

The appendicular skeleton which in the day 17 mouse fetus includes the pectoral girdle (clavicles, scapulae), forelimbs (humeri, radii, ulnae, and metacarpals), the pelvic girdle (ilia, ischia, pubes), hind-limbs (femora, tibiae, fibulae, and metatarsals) was also examined for degree of ossification, and any abnormal shape or size was noted.

Following examination, all skeletons were stored by litter in glycerin and 70% ethanol (1:1) in sealed bags labeled with the study code (Mi80-2D), sacrifice date, dam number and fetus numbers. All malformed skeletons were individually bagged for storage. Skeletons from the study were boxed together and stored in a secured area of the Research Services Building.

APPENDIX III

Radian Corp. Special Report on Phenol

PURITY ANALYSIS REPORT

PRIMARY NAME Phenol

CAS REGISTRY NUMBER 108-95-2

RADIAN INVENTORY NUMBER NT-000310

CONTRACT 209-003

DCN 81-209-003-72

DATE 29 May 1981

PERCENT PURITY OF THE MAJOR COMPONENT: 99.9

Reportable Impurities:

Method of Analysis: Gas Chromatography

1) Column: 3% OV-17 on 80/100 Supelcoport, 6'x2mm ID

Injection Size: 0.5 µL, 1.0 µL, ____ µL

Input: 10
Attenuation: 16
Detector: FID

Carrier Gas Flow: 30 mL/min nitrogen
Make-Up Gas Flow: ____ mL/min ____

Sample Concentration:
/ / Neat
/X/ Solution
0.0208 grams sample/2.0 mL methanol

Detector Temperature: 230 °C
Inlet Temperature: 15 °C

| | Isothermal
|X| Program

Initial Temperature: 50 °C
Initial Hold: 2 minutes
Rate of Temperature Increase: 8 °C/minute
Final Temperature: 180 °C
Final Hold: 20 minutes

	Retention Time	Area Percent
Major Component:	615	99.9
Detectable Impurities:		

Comments: Chromatograms shown in Figures 1 and 2

2) Column: 1% SP 1240DA on 80/100 Supelcoport, 6'x2mm ID

Injection Size: 0.4 µL, 0.3 µL, 0.2 µL

Input: 10
Attenuation: 16
Detector: FID

Carrier Gas Flow: 30 mL/min nitrogen
Make-Up Gas Flow: ____ mL/min ____

Sample Concentration:
/ / Neat
/X/ Solution
0.0208 grams sample/2.0 mL methanol

Detector Temperature: 230 °C
Inlet Temperature: 150 °C

| | Isothermal
|X| Program

Initial Temperature: 50 °C
Initial Hold: 2 minutes
Rate of Temperature Increase: 8 °C/minute
Final Temperature: 180 °C
Final Hold: 15 minutes

	Retention Time	Area Percent
Major Component:	7.77	99.9
Detectable Impurities:		

Comments: Chromatogram shown in Figure 3

Analysis Performed By: [signature] Approved: [signature]

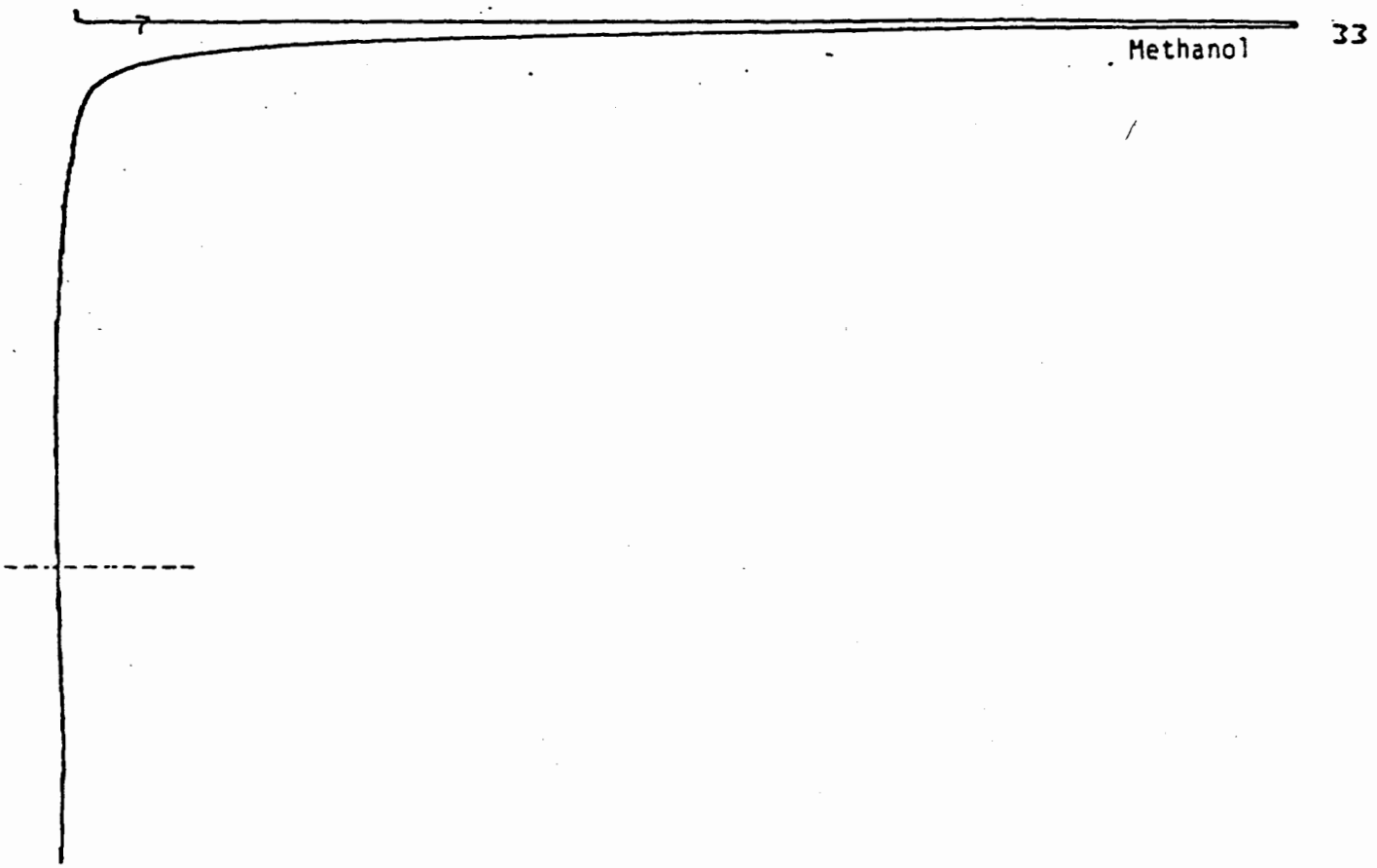

FIGURE 1.
CHROMATOGRAM OF METHANOL ON OV-17 COLUMN

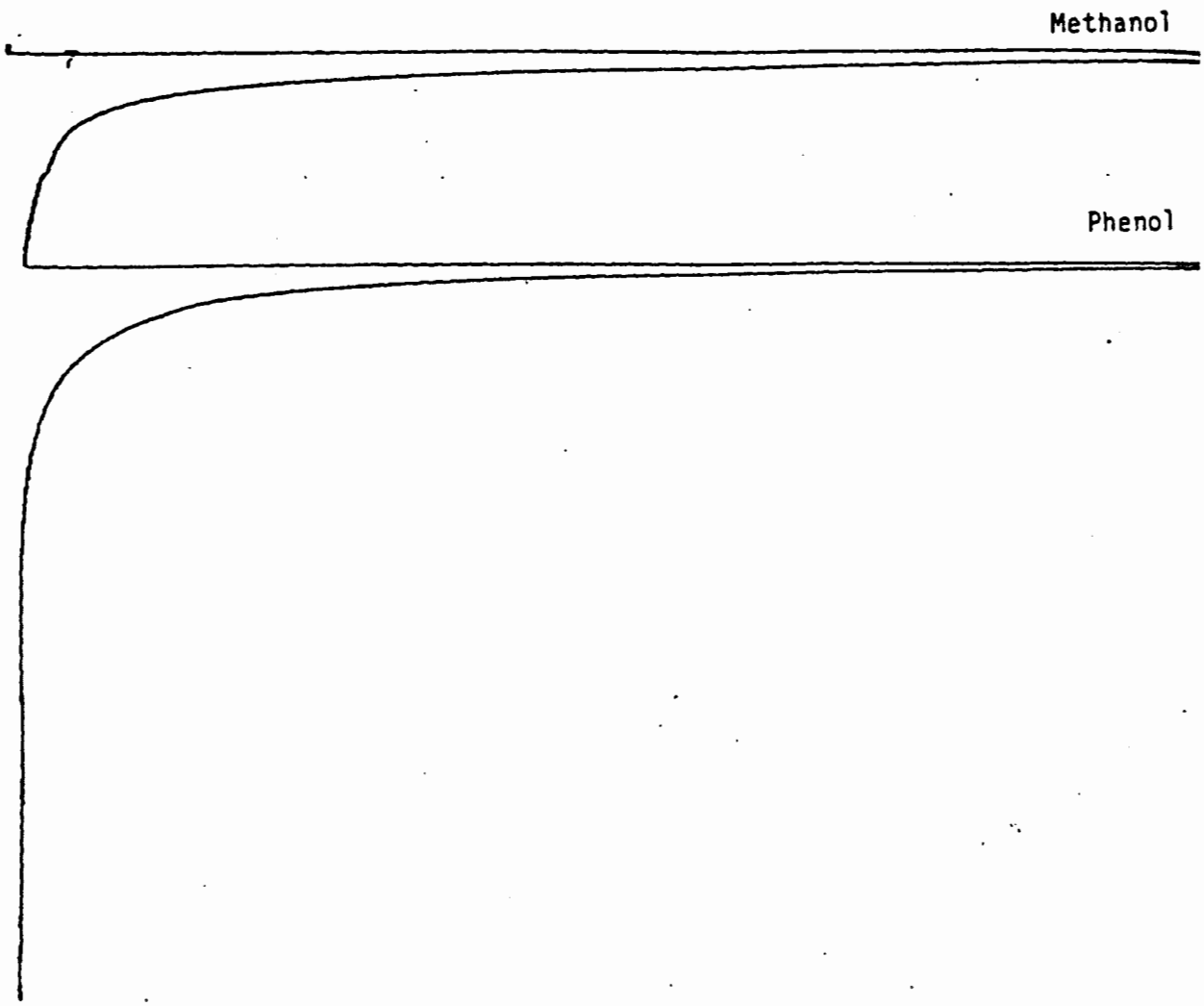

FIGURE 2.
CHROMATOGRAM OF PHENOL SOLUTION ON OV-17 COLUMN

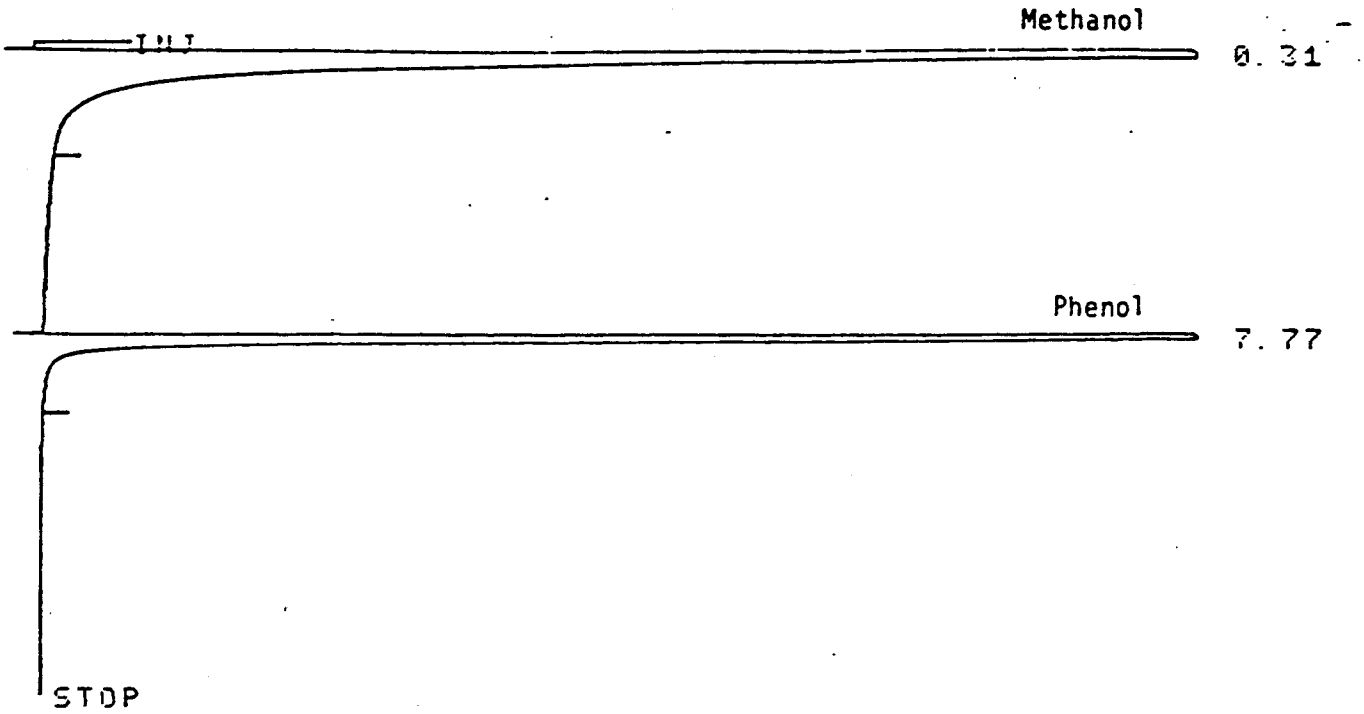

FIGURE 3.

CHROMATOGRAM OF PHENOL SOLUTION ON SP1240 DA COLUMN

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