

1/11/93
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DATA EVALUATION RECORD

STUDY TYPE: Chronic Feeding in the rodent; Guideline §83-1a
Carcinogenicity-Mouse; Guideline §83-2b

EPA PESTICIDE CHEMICAL CODE: 064103 (OPP); 064104 (SOPP)
TOX. CHEM NO: 623AA (OPP); 787 (SOPP)
MRID NO.: 00161577

TEST MATERIAL: *Ortho*-Phenylphenol

SYNONYMS: *o*-Phenylphenol

STUDY NUMBER: National Toxicology Program, TR 301

SPONSOR: U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

TESTING FACILITY: National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

ITILE OF REPORT: NTP Technical Report on the Toxicology and
Carcinogenesis of *Ortho*-Phenylphenol (CAS No. 90-43-7) Alone and
with 7,12-Dimethylbenz(a)anthracene (CAS No. 57-97-6) in Swiss CD-
1 Mice (Dermal Studies)

AUTHOR(S): Michael P. Luster, Ph.D., Chemical Manager

REPORT ISSUED: March 1986

CONCLUSIONS: Based on the available data from this NTP report,
very little information can be obtained on the systemic and
carcinogenic activity of OPP as only one dose was tested producing
very little toxicity and only non-neoplastic lesions in the form
of skin irritation.

Core Classification: Core-Supplementary Data; this study
does not satisfy the Guideline requirements for chronic
feeding study in rodents (§83-1a) or for a carcinogenicity
study in the mouse (§83-2b).

A. MATERIALS and METHODS: A copy of the Materials and Methods section from the investigators report is appended.

1. Test compound: o-Phenylphenol

Description - none given
Lot # - MM09157
Purity - 99 %

DMBA (7,12-Dimethylbenz(a)anthracene)
Description - none given
Lot 3 - C8H
Purity - not provided

TPA (12-O-Tetradecanoylphorbol-13-acetate)
Description - none given
Lot # - unknown
Purity - not provided

2. Test animals:

Species: Albino Mouse
Strain: Swiss Crl:CD-1(ICR)BR(Swiss CD-1)
Age: 5 weeks old
Weight: not provided
Source: Charles River Breeding Laboratories,
Portage, Michigan

3. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose applied dermally	Dose applied	
		male	female
Dose Given 3 days a week for 102 weeks to dorsal interscapular region			
1 Control	0.1 ml acetone	50	50
2 Low (LDT)	55.5 mg/0.1 ml acetone	50	50
Dose given single application of DMBA (0.05 mg/0.1ml acetone) and 1 week later the following doses for 102 weeks (except TPA group 85 wk male, 74 wk female)			
3 Control	0.1 ml acetone	50	50
4 OPP	55.5 mg/0/1 ml acetone	50	50
5 TPA	0.005 mg/0.1 ml acetone	50	50

The animals received the above doses dermally in acetone on the dorsal interscapular region, 3 days a week for 102 weeks except for the TPA group as mentioned above. Dose selection was based on 4 week studies. ONLY OPP RESULTS WILL BE DISCUSSED IN THIS REVIEW.

4. Diet preparation

Doses of OPP were prepared every 2 weeks and stored at room temperature in the dark. Samples of mixtures were analyzed for stability and concentration periodically.

5. Animal Husbandry

Animals were kept under standard NIH animal care conditions and received NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA) and water *ad libitum*.

6. Observations:

Animals were inspected twice daily for signs of toxicity and mortality along; dermal masses were recorded twice a week for the first 3 months and then monthly thereafter. Clinical observations were recorded daily for the first 17 months and then monthly thereafter.

7. Body weight

Animals were weighed weekly for the first 13 weeks and then monthly thereafter.

8. Food consumption and compound intake

Food consumption was not determined.

9. Ophthalmological examination

Ophthalmological examinations were not performed.

10. Hematology and clinical analysis

Blood was not collected for hematology and clinical analysis.

11. Urinalysis

Urine was not collected.

12. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. **No tissues were weighed.**

Digestive system	Cardiovas/Hemat.	Neurologic
Tongue	Aorta*	X Brain*+
X Salivary glands*	X Heart*	Periph. nerve*
X Esophagus*	X Bone marrow*	Spinal cord (3levels)*
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen	X Eyes (optic n.)*
X Jejunum*	X Thymus*	Glandular
X Ileum*	Urogenital	X Adrenal gland*
X Cecum*	X Kidneys*+	Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
Rectum*	X Testes*+	X Parathyroids*
X Liver *+	Epididymides	X Thyroids*
X Gall bladder*	X Prostate	Other
X Pancreas*	Seminal vesicle	X Bone*
Respiratory	X Ovaries*+	Skeletal muscle*
X Trachea*	X Uterus*	X Skin*
X Lung*		X All gross lesions
X Bronchi		and masses*
Nose		
Pharynx		
Larynx		

* Required for subchronic and chronic studies.

+ Organ weight required in subchronic and chronic studies.

a. Microscopic pathology

"Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin."

13. Statistics

The following procedures were utilized in analyzing the numerical data:

Survival Analysis by the product-limit procedure of Kaplan and Meier (1958)

Dose-relationship on survival by Cox (1972)

Testing of group equality by Tarone's (1975)

Tumor incidence analysis by Mantel and Haenszel (1959)

Fisher pair-wise (exact) test

Cochran-Armitage linear trend test (Armitage, 1971; Gart et.al. 1979)

14. Compliance

A statement regarding QA's and GLP's was provided.

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B. RESULTS:**1. Observations:**

Dose (mg/kg)	Toxicity/Mortality		(survival)	
	Control	OPP	Control	OPP
	Males		Females	
# of animals	50	50	50	50
Deaths on study	31	34	39	35
Terminal Sacrifice	18	16	11	15
Died during term sac	1	0	0	0

The investigators determined that the survival of the OPP group was not significantly different than that of the control. Attached Figure 2 presents the survival curves presented in the report.

2. Body weight

Attached Figure 1 from the investigators report presents the growth curves for male and female rats. According to the investigators, the mean body weights of the OPP treated males were 5 to 10% lower than the control after week 44. Females were unaffected. The following table presented selected weights:

Week	Mean Body Weight (% vehicle control) (Body weight gain) grams			
	Males		Females	
	Control	OPP	Control	OPP
0	29.3	29.9 (102)	24.0	23.9 (100)
13	34.9 (5.6)	33.9 (97) (4)	29.0 (5)	28.7 (99) (4.8)
27	37.7 (8.4)	35.6 (94) (5.7)	31.5 (7.5)	31.9 (101) (8)
53	40.6 (11.3)	38.3 (94) (8.4)	35.4 (11.4)	35.6 (101) (11.7)
79	41.3 (12)	39.1 (95) (9.2)	37.8 (13.8)	35.7 (94) (11.8)
103	39.4 (10.1)	39.0 (99) (9.1)	36.2 (12.2)	38.1 (105) (14.2)

Data extracted from NTP TR 301, Table 5 and calculations by the reviewer (body weight gain)

3. Sacrifice and Pathology

a. Microscopic pathology

1) Non-neoplastic

The investigators reported an increase in ulcers, active chronic inflammation, hyperkeratosis and acanthosis at the site of application; results on table below:

	Skin Lesions at Site of Application (out of 50 animals per sex)			
	Males		Females	
	Control	OPP	Control	OPP
Ulcer	5	19	1	11
Active chronic inflammation	10	25	7	20
Hyperkeratosis	7	27	4	16
Acanthosis	13	44	4	36

Data extracted from NTP TR 301, Table 8.

The investigators further noted an increase in the dilation of the kidney tubules in both sexes associated with hydronephrosis and nephropathy but according to them, was not compound related. Also thyroid follicular cysts in treated females; however, they felt that these were not compound related. They also reported an increase in lipoid degeneration of the adrenal gland zona fasciculata in both sexes but the biological relevance was unknown according to the investigators. There was also an increase in focal necrosis of the liver but it was not considered compound related.

2) Neoplastic

The investigators reported incidences of squamous cell papillomas and carcinomas with metastasis to the lungs with the combined treatment group (not reviewed here); however, no squamous cell papillomas or carcinomas were found with the single dose OPP treatment.

C. DISCUSSION:

The following is an abstract of the study investigators discussion and conclusions:

In the 2-year studies, dermal application of...*o*-phenylphenol to Swiss CD-1 mice caused nonneoplastic lesions at the site of application; these lesions consisted of inflammation, ulceration, and acanthosis. Increased incidences of these lesions were found in *o*-phenylphenol...groups compared with the incidences in the...acetone vehicle controls. No marked toxicity was observed in dosed groups at areas other than site of application. No marked effects on the kidney were seen in mice dosed with *o*-phenylphenol...although male and female mice dosed with *o*-phenylphenol had slight increases in the frequency of dilation of kidneys tubules, and females had a slight increase of lymphocytic infiltration.

Further:

...Other notable observations in the current 2-year studies included an increased incidence of follicular cysts (20/46, 43%) in the thyroid gland of female mice dosed with *o*-phenylphenol compared with that in the acetone vehicle controls (6/47, 13%) and increased incidences of lipoid degeneration in the zona fasciculata of the adrenal gland in...dose groups compared with those in acetone vehicle controls. The slight organ system toxicity observed in *o*-phenylphenol-dosed mice in these studies may be due in part, to the relatively poor absorption of *o*-phenylphenol through the skin (less than 1% of the administered dose [approximately 6 mg] is absorbed in human) (Harke and Klein, 1981). Alternatively, systemic exposure to *o*-phenylphenol may have occurred as a result of preening.

Conclusions:

Very little information can be obtained on the systemic and carcinogenic activity of OPP from this study as only one dose was tested producing very little toxicity and only non-neoplastic lesions in the form of skin irritation.

This study is classified as Core-Supplementary Data and does not satisfy the Guideline requirements for chronic feeding study in rodents (§83-1a) or for a carcinogenicity study in the mouse (§83-2b).

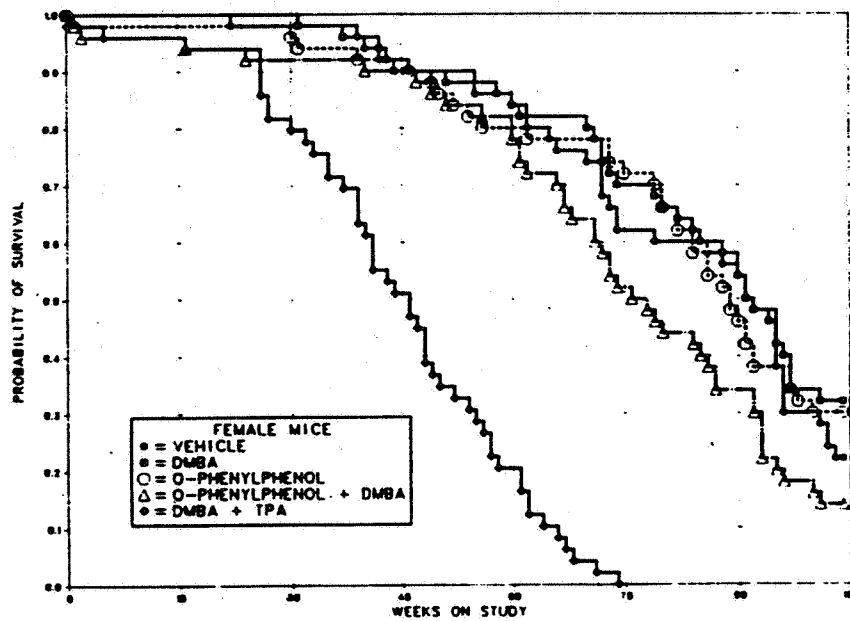
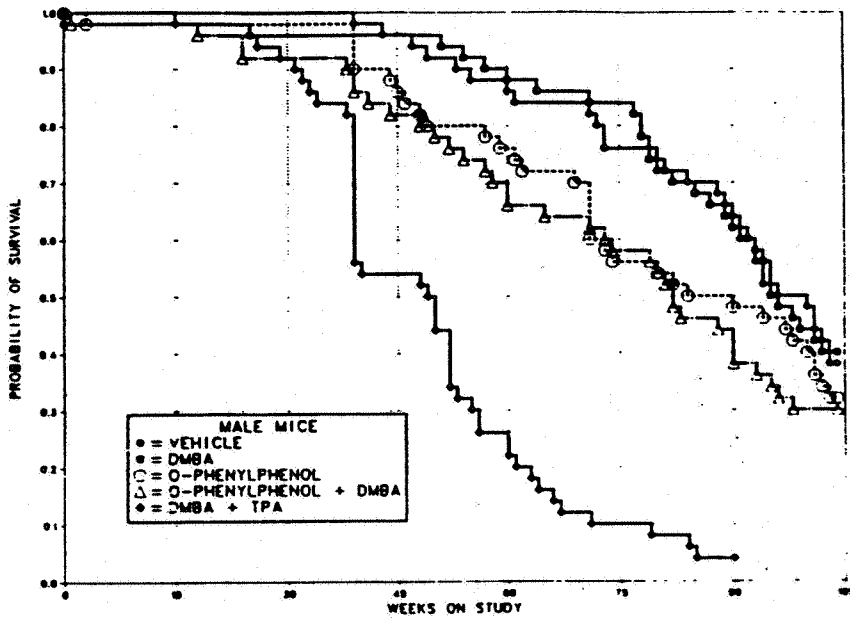


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED o-PHENYLPHENOL BY DERMAL APPLICATION FOR TWO YEARS

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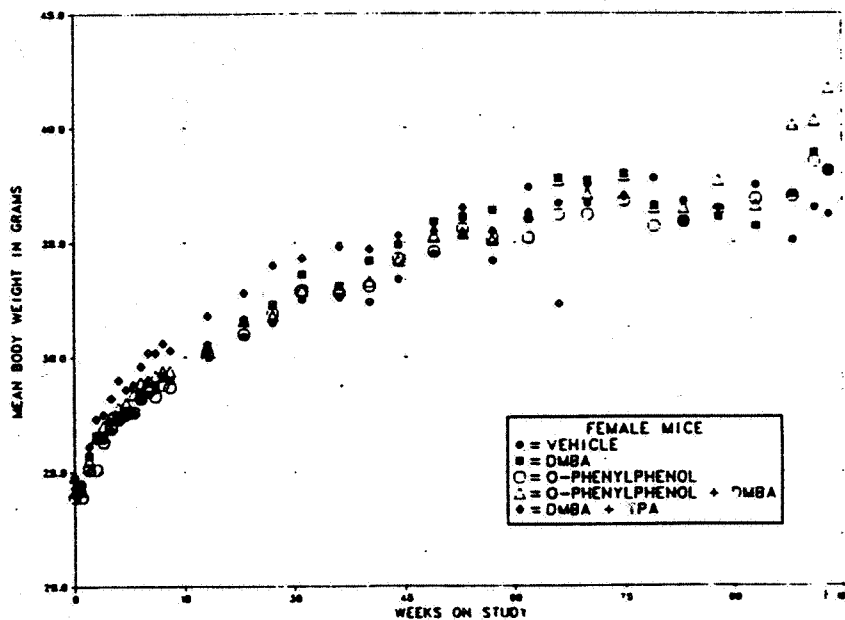
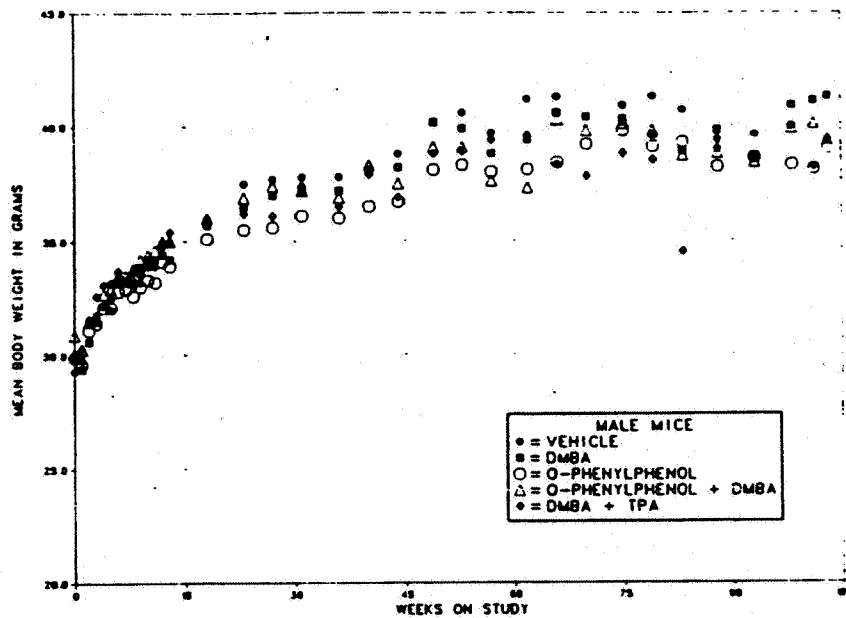


FIGURE 1. GROWTH CURVES FOR MICE ADMINISTERED o-PHENYLPHENOL BY DERMAL APPLICATION FOR TWO YEARS

II. MATERIALS AND METHODS

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PROCUREMENT AND CHARACTERIZATION OF *o*-PHENYLPHENOL

o-Phenylphenol, manufactured by Dow Chemical USA, was obtained from Callahan Chemical Company in one batch (lot no. MM09157). The identity of *o*-phenylphenol was confirmed by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of *o*-phenylphenol was determined by elemental analysis, Karl Fischer water analysis, and gas chromatography (Appendix D). The cumulative data indicated that the *o*-phenylphenol test material was greater than 99% pure and that the major impurity identified was water (0.21%).

o-Phenylphenol was stable at temperatures up to 60° C for 2 weeks. *o*-Phenylphenol was stored in the dark at 23° C. Results of periodic analysis of the bulk chemical by infrared spectroscopy and gas chromatography indicated no notable degradation of the chemical throughout the studies (Appendix D).

7,12-Dimethylbenz(α)anthracene (DMBA), lot no. C8H, was obtained from Fisher Scientific and purified at Midwest Research Institute by

column chromatography and recrystallization (Appendix E). The DMBA was stored at -20° C. 12-O-Tetradecanoylphorbol-13-acetate (TPA) in individual 10-mg flame-sealed vials was obtained from Consolidated Midland Corporation, Brewster, New York. The TPA was stored in brown bottles (wrapped in aluminum foil) at -20° C in portions sufficient for 1 day of dosing.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

The appropriate amount of *o*-phenylphenol was weighed and mixed with acetone (Burdick and Jackson). *o*-Phenylphenol at 840 mg/ml in acetone was stable for at least 2 weeks at room temperature in the dark (Table 1; Appendix F).

Dose mixtures of *o*-phenylphenol in acetone were periodically analyzed at the testing and analytical laboratories (Appendix G). The results of analyses at the testing laboratory indicated that none of the mixtures analyzed differed from the target concentration by more than 10% (Table 2; Appendix H). In the 2-year studies, formulated mixtures of *o*-phenylphenol were stored at room temperature for no longer than 2 weeks.

TABLE 1. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE DERMAL STUDIES OF *o*-PHENYLPHENOL

	Four-Week Studies	Two-Year Studies
Preparation	Weighed portions of <i>o</i> -phenylphenol mixed with the appropriate volume of technical-grade acetone and placed on a magnetic stirrer for 5-10 min	Weighed portions of <i>o</i> -phenylphenol or TPA mixed with the appropriate volume of acetone; mixing column inverted 21 times until the chemical appeared to be in solution, then inverted an additional 21 times
Maximum Storage Time	9 d	2 wk
Storage Conditions	23° C	4° C in foil-wrapped vials

TABLE 2. SUMMARY OF RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR DERMAL STUDIES OF *o*-PHENYLPHENOL

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	Concentration of <i>o</i> -Phenylphenol in Acetone
Mean (mg/ml)	556
Range (mg/ml)	527-587
Standard deviation	15.7
Coefficient of variation (percent)	2.82
Number of samples	15

FOUR-WEEK STUDIES

Four-week studies were conducted to evaluate the cumulative toxic effects of repeated dermal administration of *o*-phenylphenol and to determine the doses to be used in the 2-year studies.

Seven-week-old male and female Swiss Webster CFW mice (CrI:CFW[SW]BR) were received from Charles River Breeding Laboratories and observed for 18 days before the studies began. Mice were housed individually in polycarbonate cages. Feed and water were available ad libitum. Groups of 10 mice of each sex were given dermal applications to the dorsal interscapular region of 0, 5.95, 11.4, 20.8, 35.7, or 55.5 mg/0.1 ml in acetone, 3 days per week (Wednesday, Friday, and Monday) for 4 weeks. To facilitate the application of the *o*-phenylphenol, the hair on the application site was clipped weekly with an Oster® clipper that had a number 40 clipping head. Further experimental details are summarized in Table 3.

Animals were monitored twice daily for clinical signs of ill health; moribund animals were killed. Animal weights were recorded weekly. At the end of the 4-week studies, survivors were killed. A necropsy was performed on all animals, except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 3.

TWO-YEAR STUDIES

Study Design

In contrast to the 4-week studies in which Swiss Webster (CrI:CFW[SW]BR) mice were used,

Swiss CrI:CD-1(ICR)BR(Swiss CD-1) were used in the 2-year studies. Groups of 50 Swiss CD-1 mice of each sex were dosed for 74-102 weeks according to five different protocols. Vehicle controls were given dermal applications to the dorsal interscapular region of 0.1 ml acetone 3 days per week. One dose group was given dermal applications of *o*-phenylphenol (55.5 mg/0.1 ml acetone) 3 days per week for 102 weeks. Three additional groups were given a single dermal application to the dorsal interscapular region of 0.1 ml DMBA (0.05 mg/0.1 ml acetone). Starting 1 week later, the latter three groups were given dermal applications of either acetone (vehicle), *o*-phenylphenol (55.5 mg/0.1 ml), or TPA (0.005 mg/0.1 ml) 3 days per week for the remainder of the studies at the original site of DMBA application. A 2-cm² area of skin was covered with these materials. All groups were dosed for 102 weeks except the male (85 weeks) and female (74 weeks) DMBA/TPA groups, which were killed before the end of the studies because of the high number of deaths. The exposure regimens are given in Table 3.

Source and Specifications of Animals

The male and female Swiss CD-1 mice used in these studies were obtained from Charles River Breeding Laboratories, Portage, Michigan, from their cesarean-originated, barrier-sustained production colony. Animals were shipped to the testing laboratory at 5 weeks of age and quarantined for 17 days. A complete necropsy was performed on five animals of each sex to assess their health status. The mice were placed on study at 7 weeks of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix I).

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TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DERMAL STUDIES OF o-PHENYLPHENOL

	Four-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Test Groups	10 male and 10 female mice	50 male and 50 female mice
Doses	0.1 ml of 0, 5.95, 11.4, 20.8, 35.7, or 55.5 mg o-phenylphenol in 0.1 ml of acetone by dermal application	Vehicle control: 0.1 ml acetone 3 × wk; complete carcinogen: 0.1 ml o-phenylphenol (55.5 mg/0.1 ml acetone) 3 × wk; promotion test: 0.1 ml 7,12-dimethylbenz(a)anthracene (DMBA) (0.05 mg/0.1 ml acetone), then 0.1 ml o-phenylphenol 3 × wk; initiator control: 0.1 ml DMBA, then 0.1 ml acetone 3 × wk; positive control: 0.1 ml DMBA, then 0.1 ml 12-O-tetradecanoylphorbol-13-acetate (TPA) (0.005 mg/0.1 ml acetone) 3 × wk
Date of First Dose	6/20/79	7/28/80
Date of Last Dose	7/16/79	7/12/82
Duration of Dosing	3 d/wk for 4 wk	DMBA--once; o-phenylphenol--102 wk; TPA--85 wk (male), 74 wk (female)
Type and Frequency of Observation	Observed 2 × d; clipped 1 × wk, 24 h before topical application	Observed 2 × d for clinical signs of ill health; dermal masses recorded 2 × wk for the first 3 mo and 1 × mo thereafter; clinical observations recorded daily for the first 17 mo and 1 × mo thereafter. Weighed 1 × wk for 13 wk, 1 × mo thereafter
Necropsy and Histologic Examination	Necropsy performed on all animals; histologic examinations were performed on all 55.5 mg o-phenylphenol and vehicle control animals; tissues examined microscopically: heart, kidneys, liver, lungs, ovaries, skin, and thyroid gland	Necropsy performed on all animals; histologic examination performed on all animals; the following tissues were examined microscopically: gross lesions and tissue masses (and regional lymph nodes, if possible), mandibular lymph node, salivary gland, femur including marrow, thyroid gland, parathyroids, small intestine, colon, liver, gallbladder, prostate/testes or ovaries/uterus, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, skin (application site and unspecified sites), lungs and mainstem bronchi, kidneys, adrenal glands, urinary bladder, pituitary gland, eyes, and mammary gland
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species	Swiss Webster CFW mice	Swiss CD-1 mice
Animal Source	Charles River Breeding Laboratories (Portage, MI)	Same as 4-wk studies
Testing Laboratory	Battelle Columbus Laboratories	Same as 4-wk studies
Time Held Before Test	18 d	17 d

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TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DERMAL STUDIES OF o-PHENYLPHENOL (Continued)

	Four-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Age When Placed on Study	54 d	52 d
Age When Killed	87-88 d	112 wk
Necropsy Dates	7/23/79-7/24/79	7/27/82-7/28/82
Method of Animal Distribution	Animals randomized from weight classes into cages by a table of random numbers; cages randomized to test and control groups by another table of random numbers	Same as 4-wk studies; animals housed individually after randomization of cages to dose groups
Animal Identification	Toe clipping	Toe clipping
Feed	Purina 5001 Pelleted Lab Chow® (Ralston Purina Co., St. Louis, MO); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA) (Appendix J); available ad libitum
Bedding	Absorb-Dri® hardwood chips (Absorb-Dri Inc., Garfield, NJ); changed 2 × wk	Heat-treated hardwood chips (Absorb-Dri Inc., Rochelle Park, NJ)
Water	Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 4-wk studies
Cages	Polycarbonate (Lab Products Inc., Rochelle Park, NJ); changed 1 × wk	Same as 4-wk studies
Cage Filters	Spun-bonded polyester (DuPont 2024) (Snow Filtration Co., Cincinnati, OH)	Same as 4-wk studies
Animals per Cage	5 for 2 d, then individually	1
Other Chemicals on Test in the Same Room	None	None
Animal Room Environment	Temp--21°-23° C; hum--40%-60%; fluorescent light 12 h/d; 15 room air changes/h	Same as 4-wk studies; DMBA administration conducted under yellow light

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II. MATERIALS AND METHODS

Animal Maintenance

All animals were clipped with an Oster® clipper once per week, 24 hours before dermal application. The mice were housed individually in polycarbonate cages. Feed and water were available ad libitum. Details of animal maintenance are summarized in Table 3.

Clinical Examinations and Pathology

All animals were observed twice daily; clinical signs were recorded daily for the first 17 months and once per month thereafter. The number of dermal growths per animal and physical descriptions of the growths were recorded. Dermal masses were recorded twice per week for the first 3 months and once per month thereafter. Body weights were recorded once per week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Moribund animals were killed, as were animals that survived to the end of the studies. A necropsy was performed on all animals, including those found dead unless they were excessively autolyzed or cannibalized. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 3.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, target tissues, and tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Chairperson of

the Pathology Working Group (PWG) for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent evaluations, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Nonneoplastic lesions are not examined routinely by the quality assurance pathologist or PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and PWG if they are considered part of the toxic response to a chemical or if they are deemed of special interest.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for survival used the method of Cox (1972) for testing two groups for equality. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of

animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of dose groups with the acetone vehicle controls and with DMBA/vehicle controls.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. All reported P values for tumor analyses are one-sided.

Life Table Analyses: The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and acetone vehicle control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates

time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses: The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and acetone vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals on which a necropsy was actually performed during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses: Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons (Gart et al., 1979) are given in the appendix containing the analyses of primary tumor incidence. This test is based on the overall proportion of tumor-bearing animals and does not adjust for survival differences.

Analysis of Time to Skin Tumor Appearance: Following histopathologic confirmation of skin tumor development, statistical differences in time to first visible appearance of tumor were determined with life table analyses as described above.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984) are included for those tumors appearing to show compound-related effects.

V. REFERENCES

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1. Abrahamson, S.; Lewis, E. (1971) The detection of mutations in *Drosophila melanogaster*. Hollaender, A., Ed.: Chemical Mutagens: Principles and Methods for Their Detection, Vol. 2. New York: Plenum Press, pp. 461-487.
2. Berenblum, I., Ed. (1969) Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2. Geneva: International Union Against Cancer.
3. Boorman, G.; Montgomery, C., Jr.; Hardisty, J.; Eustis, S.; Wolfe, M.; McConnell, E. (1985) Quality assurance in pathology for rodent toxicology and carcinogenicity tests. Milman, H.; Weisburger, E., Eds.: Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications, pp. 345-357.
4. Boutwell, R.; Bosch, D. (1959) The tumor-promoting action of phenol and related compounds for mouse skin. *Cancer Res.* 19:413-423.
5. Clive, D.; Johnson, K.; Spector, J.; Batson, A.; Brown, M. (1979) Validation and characterization of the L5178Y/TK^{+/-} mouse lymphoma mutagen assay system. *Mutat. Res.* 59:61-108.
6. Cox, D. (1972) Regression models and life tables. *J. R. Stat. Soc.* B34:187-220.
7. Dreisbach, R. (1974) Handbook of Poisoning: Diagnosis and Treatment, 8th ed. Los Altos, CA: Lang Medical Publications, pp. 321-322.
8. Gaches, C. (1975) Woodworm and the bladder. *Proc. R. Soc. Med.* 68:525-527.
9. Gart, J.; Chu, K.; Tarone, R. (1979) Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* 62(4):957-974.
10. Gosselin, R.; Hodge, H.; Smith R.; Gleason, M. (1976) Clinical Toxicology of Commercial Products, 4th ed. Baltimore, MD: The Williams and Wilkins Co., p. 126.
11. Goto, K.; Maeda, S.; Kano, Y.; Sugiyama, T. (1978) Factors involved in differential Giemsa-staining of sister chromatids. *Chromosoma* 66:351-359.
12. Hagiwara, A.; Shibata, M.; Hirose, M.; Fukushima, S.; Ito, N. (1984) Long-term toxicity and carcinogenicity study of sodium o-phenylphenate in B6C3F₁ mice. *Food Chem. Toxic.* 22(10):809-814.
13. Harke, H.; Klein, H. (1981) Absorption of o-phenylphenol from hand washing disinfectants. *Zentralbl. Bakteriol. Mikrobiol. Hyg.* 174:274-278.
14. Haseman, J. (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* 58:385-392.
15. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutagen.* 5(Suppl.1):3-142.
16. Hiraga, K.; Fujii, T. (1981) Induction of tumours of the urinary system in F344 rats by dietary administration of sodium o-phenylphenate. *Food Cosmet. Toxicol.* 19:303-310.
17. Hodge, H.; Maynard, E.; Blanchet, H.; Spencer, H.; Rowe, V. (1952) Toxicological studies of orthophenylphenol (Dowicide 1). *J. Pharmacol. Exp. Therap.* 104:202-210.
18. Innes, J. (1968) Evaluation of carcinogenic, teratogenic and mutagenic activities of selected pesticides and industrial chemicals. Vol. 1, Carcinogenic Study. U.S. Dept. of Commerce, National Technical Information Service.
19. John, J.; Murray, F.; Rao, K.; Schwetz, B. (1981) Teratological evaluation of orthophenylphenol in rats. *Fundam. Appl. Toxicol.* 1:282-285.
20. Kaneda, M.; Teramoto, S.; Shingu, A.; Shirasu, Y. (1978) Teratogenicity and dominant-lethal studies with o-phenylphenol. *J. Pest. Sci.* 3:365-370.
21. Kaplan, E.; Meier, P. (1958) Nonparametric estimation of incomplete observations. *J. Am. Stat. Assoc.* 53:457-481.

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10

V. REFERENCES

22. Linhart, M.; Cooper, J.; Martin, R.; Page, N.; Peters, J. (1974) Carcinogenesis bioassay data system. *Comp. Biomed. Res.* 7:230-248.
23. Luster, M.; Dean, J.; Boorman, G.; Archer, D.; Lauer, L.; Lawson, L.; Moore, J.; Wilson, R. (1981) The effects of orthophenylphenol, tris(2,3-dichloropropyl)phosphate and cyclophosphamide on the immune system and host susceptibility of mice following subchronic exposure. *Toxicol. Appl. Pharmacol.* 58:252-261.
24. MacIntosh, F. (1945) The toxicity of diphenyl and *o*-phenyl-phenol. *Analyst* 30:334-335.
25. Mantel, N.; Haenszel, W. (1959) Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.* 22:719-748.
26. Margolin, B.; Collings, B.; Mason, J. (1983) Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* 5:705-716.
27. Maronpot, R.; Boorman, G. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* 10:71-80.
28. McConnell, E.; Solleveld, H.; Swenberg, J.; Boorman, G. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *J. Natl. Cancer Inst.* (in press).
29. McMahon, R.; Cline, J.; Thompson, C. (1979) Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Res.* 39:682-693.
30. Merck Index (1976) 9th ed. Windholz, M., Ed. Rahway, NJ: Merck and Co., Inc., p. 950.
31. Nakao, T.; Ushiyama, K.; Kabashima, J.; Nagai, F.; Nakagawa, A.; Ohno, T.; Ichikawa, H.; Kobayashi, H.; Hiraga, K. (1983) The metabolic profile of sodium *o*-phenylphenate after subchronic oral administration to rats. *Food Chem. Toxic.* 21(3):325-329.
32. National Cancer Institute (NCI) (1976) Guidelines for Carcinogen Bioassay in Small Rodents, Technical Report Series No. 1, February.
33. National Institutes of Health (NIH) (1978) NIH Specification NIH-11-133f, November 1.
34. Oehme, F. (1971) Comparative toxicity of *o*-phenylphenol and an *o*-phenylphenol-containing disinfectant. *Toxicol. Appl. Pharmacol.* 19:412.
35. Ogata, A.; Ando, H.; Kubo, Y.; Hiraga, K. (1978) Teratological tests of *o*-phenylphenol (OPP) and sodium *o*-phenylphenol (OPP-Na) in mice. *Annu. Rep. Tokyo Metrop. Res. Lab. Publ. Health* 29:89-96.
36. Perry, P.; Wolff, S. (1974) New Giemsa method for the differential staining of sister chromatids. *Nature (London)* 251:156-158.
37. Reiners, J., Jr.; Davidson, K.; Nelson, K.; Mamrack, M.; Slaga, T. (1983) Skin tumor promotion: A comparative study of several stocks and strains of mice. Langenback, R.; Resnew, S.; Rice, J., Eds.: *Basic Life Science. Organ and Species Specificity in Chemical Carcinogenesis*, Vol. 24. New York: Plenum Publishing Corp., pp. 173-186.
38. Reitz, R.; Fox, R.; Quast, J.; Hermann, E.; Watanabe, P. (1983) Molecular mechanisms involved in the toxicity of orthophenylphenol and its sodium salt. *Chem.-Biol. Interact.* 43:99-119.
39. Sadtler Standard Spectra, IR No. 1146, UV No. 341, NMR No. 6528M. Philadelphia: Sadtler Research Laboratories.
40. Sasaki, M.; Nakao, T. (1968) Effect of *o*-phenylphenol on immune response in vitro. *Annu. Rep. Tokyo Metrop. Res. Lab. Publ. Health* 29(2):109.
41. Savides, M.; Oehme, F. (1980) Urinary metabolism of orally administered *ortho*-phenylphenol in dogs and cats. *Toxicology* 17(30):355-363.

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V. REFERENCES

42

42. Shirasu, Y.; Moriya, M.; Kato, K.; Tezuka, H.; Henmi, R.; Shingu, A.; Kaneda, M.; Teramoto, S. (1978) Mutagenicity testing on *o*-phenylphenol. *Mutat. Res.* 54:227 (abstract).
43. Slaga, T. (1983) Overview of tumor promotion in animals. *Environ. Health Perspect.* 50:3-14.
44. Stahl, E., Ed. (1969) *Thin-Layer Chromatography*, 2nd ed. New York: Springer-Verlag, p. 876.
45. Takahashi, K. (1978) Statistical evaluation of mutagenicity of a citrus fungicide ortho-phenylphenol. *Mutat. Res.* 54:255 (abstract).
46. Taniguchi, Y.; Morimoto, J.; Okada, K.; Imai, S.; Tsubura, Y. (1981) Toxicological study of *o*-phenylphenol (OPP) in mice. I. Acute oral toxicity of ddy mice. *Nara Igaku Zasshi* 32:425-429.
47. Tayama-Nawai, S.; Yoshida, S.; Nakao, T.; Hiraga, K. (1984) Induction of chromosome aberrations and sister-chromatid exchanges in CHO-K1 cells by *o*-phenylphenol. *Mutat. Res.* 141:95-99.