

US EPA ARCHIVE DOCUMENT



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

SEP 5 1995

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

*please
06/2/95*

SUBJECT: Carcinogenicity Peer Review of o-Benzyl-p-chloro-phenol
(o-BCP)

FROM: Pamela M. Hurley, Ph.D. *Pamela M. Hurley 8/2/95*
Review Section I
Toxicology Branch I
Health Effects Division (7509C)
and
Esther Rinde, Ph.D. *E. Rinde*
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

TO: Ruth G. Douglas
Product Manager #32
Antimicrobial Branch
Registration Division (7505C)
and
Veronica Dutch
Accelerated Reregistration Branch
Special Review and Reregistration Division (7598W)

THROUGH: Stephanie R. Irene Ph.D. *Stephanie R. Irene*
Acting Director, Health Effects Division (7509C) *8/24/95*

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on March 29, 1995 to discuss and evaluate the weight-of-the-evidence on o-BCP with particular reference to its carcinogenic potential. The CPRC concluded that o-BCP should be classified as Group C - possible human carcinogen - and recommended that for the purpose of risk characterization, the Reference Dose (RfD) approach should be used for quantitation of human risk. This was based on increases in renal tubule combined adenomas/carcinomas in the male B6C3F1 mouse and in renal transitional cell carcinomas in the female F344/N rat. Renal tubular carcinomas in the mouse and renal transitional cell tumors in the rat are rare. Since these tumors could not be attributed to a specific mechanism, there were no available data for structural analogs, and little apparent genotoxicity concern, the CPRC recommended the RfD approach for estimating risk.

SUMMARY

Administration of o-BCP in corn oil by gavage to F344/N rats resulted in renal rare transitional cell carcinomas in both mid- and high dose female rats (one in each group); there were none in concurrent controls of either sex or in any of 1,068 historical control females. There was also one renal tubule adenoma in a high dose female and one renal tubular carcinoma in a high dose male that were within the range of historical controls for this tumor type in rats. Dosing in this study was considered to be adequate for carcinogenicity testing in both male and female rats.

Administration of o-BCP in corn oil by gavage to B6C3F1 mice resulted in statistically significant increases in renal tubule tumors; combined adenoma/carcinoma, with carcinomas contributing a substantial portion (33-50%) of the total incidence, at all doses in male mice. There were no renal tumors in controls and the historical control incidence for renal tubule adenomas and combined renal tubule adenoma/carcinoma is 4/949 (0.4%), and for carcinomas it is 0/949. Tumors noted in the lung and liver of female mice were not considered to be biologically significant by the CPRC. The high dose in the mouse study was considered to have been excessive in both sexes, based on clinical signs and kidney nephropathy. The mid dose was considered to be adequate for carcinogenicity testing for both sexes.

There were no available structural analogs for o-BCP, and from the submitted data there appears to be little concern for mutagenicity; however, there is a data gap for a mammalian cells in culture forward gene mutation assay (a mouse lymphoma assay with small and large colonies, is recommended).

The classification of Group C was based on the increases in rare kidney tubule tumors in the male mouse. The presence of a rare kidney transitional cell carcinoma in both mid- and high dose female rats (one in each group), with none in the concurrent controls of either sex or in any historical controls, was considered to be supportive of the mouse findings. Since the tumors could not be attributed to a specific mechanism, there were no available data for structural analogs, and there was no apparent genotoxicity concern, the CPRC recommended the RfD approach for estimating risk.

A. **Individuals in Attendance at the meetings:**

1. **Peer Review Committee:** (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene

Stephanie Irene

William Burnam

William Burnam

Karl Baetcke

Karl Baetcke

Marcia Van Gemert

Marcia Van Gemert

Kerry Dearfield

Kerry Dearfield

Elizabeth Doyle

Elizabeth A. Doyle

Marion Copley

Marion Copley

Hugh Pettigrew

Hugh Pettigrew

Esther Rinde

Esther Rinde

Yin Tak Woo

Yin Tak Woo

2. **Reviewers:** (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Pamela Hurley¹

Pamela M. Hurley 5/2/95

Roger Gardner

Roger Gardner 1/2/95

Lucas Brennecke²
(PAI/ORNL)

Lucas Brennecke

3. **Other Attendees:**

Bernice Fisher, Albin Kocialski, Kathryn Boyle, and Raymond Locke (HED)

¹Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

²Signature indicates concurrence with pathology report.

B. Material Reviewed

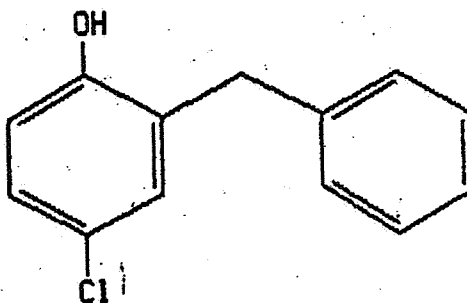
The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Pamela Hurley, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information:

o-Benzyl-p-chlorophenol (o-BCP), also known as Chlorophen, is an antimicrobial with widespread use. Some of these include control of viruses, fungi, bacteria, fleas and silverfish on domestic, zoo and household animals, farm equipment, household areas, hospitals, swimming pool areas, drinking water systems, commercial and private transportation facilities, food processing plants, eating establishments and many other commercial and residential areas. It is not considered to be a food use pesticide.

The Caswell (or Tox. Chem.) Number for o-BCP is 083.
The Chemical Abstracts Registry Number (CAS No.) is 120-32-1 and the PC Code is 062201.

The structure of o-BCP is:



o-Benzyl-p-chlorophenol

D. Evaluation of Carcinogenicity Evidence:

1. 2-Year Chronic/Carcinogenicity Gavage Study in Rats

Reference: Biotechnical Services, Inc. (1994) Toxicology and Carcinogenesis Studies of o-Benzyl-p-Chlorophenol in F344/N Rats and B6C3F₁ Mice (Gavage Studies): Study Number NTP TR 424, Publication Number 94-3155. Testing Facility: Battelle Columbus Laboratories; Submitted by: U.S. Public Health Service, National Toxicology Program, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709. MRID Nos. NR-433842-01, 422793-01.

a. Experimental Design

Technical o-benzyl-p-chlorophenol (o-BCP, 97%) was tested in a two-year gavage study with corn oil as the vehicle in male and female F344/N rats at the following dose levels: males: 0, 30, 60 or 120 mg/kg/day; females: 0, 60, 120 or 240 mg/kg/day. Each dose was administered in a volume of 5 ml/kg. Eighty rats/sex/dose level were scheduled for the study from which 10/sex/dose level were sacrificed at 3 months and 20/sex/dose level sacrificed at 15 months (10 of these were used for clinical chemistry only). Other parameters examined and/or recorded were body weights, clinical observations, food consumption, hematology, selected clinical chemistries, urinalysis and histopathology.

b. Discussion of Tumor Data

There were no statistically significant increases in any neoplasms for any of the treated groups when compared to the control groups for either sex, according to statistical analyses conducted by the National Toxicology Program (NTP). In females, one renal tubule adenoma was observed in the high dose group (2%). Two of these tumors were observed in 1,068 historical control females (std. dev. 0.6%, range 0-2%). Therefore, the 1 renal tubule adenoma was not outside of the historical control range. In males, one renal tubule carcinoma was observed in the high dose group (2%) and one renal tubule adenoma was observed in the control group (2%). The historical control incidence of renal tubule adenomas for males was 8/1,069 animals (std. dev. 1.0%, range 0-2%) and the historical control incidence of renal tubule carcinomas for males was 4/1,069 (std. dev. 1.0%, range 0-4% animals). Therefore again, the one carcinoma was not outside of the historical control range. The following table summarizes the kidney neoplasms for male and female rats in the study along

with the historical control data. The historical control data were only provided as indicated in the table. Individual dates and numbers of animals for each of the studies were not provided. The Health Effects Division (HED) did not conduct a statistical analysis on the kidney tumor data because these are rare tumors, they appeared in very small numbers and we did not believe they would be statistically significantly increased.

However, two transitional cell carcinomas of the kidneys were observed in females, one in the mid-dose group and one in the high dose group. These are considered to be rare tumors. None were observed in either the control group or in any of the males. None of these tumors were observed in any of 1,068 historical control females either.

Kidney Neoplasms for Male and Female Rats in 2-Year Gavage
 Study With o-Benzyl-p-Chlorophenol

Organ	Control	Low Dose	Mid-Dose	High Dose
Males				
Kidney				
Renal tubule adenoma	1/50	0/49	0/50	0/50
Renal tubule carcinoma	0/50	0/49	0/50	1/50
Historical Incidences				
<u>Renal Tubule Neoplasms</u>	<u>Adenoma</u>	<u>Carcinoma</u>	<u>Adenoma or Carcinoma</u>	
Total ^a	8/1069	4/1069	12/1069	
Standard deviation	1.0%	1.0%	1.4%	
Range	0%-2%	0%-4%	0%-4%	
Females				
Kidney				
Renal tubule adenoma	0/50	0/50	0/51	1/50
Renal Transitional epithelium carcinoma	0/50	0/50	1/51	1/51
Historical Incidences				
<u>Renal Tubule Neoplasms</u>	<u>Adenoma</u>	<u>Carcinoma</u>	<u>Adenoma or Carcinoma</u>	
Total ^a	2/1068	0/1068	2/1068	
Standard deviation	0.6%	-	0.6%	
Range	0%-2%	-	0%-2%	
<u>Renal Transitional Cell Neoplasms</u>				
Total ^a	-	0/1068	0/1068	
Standard deviation	-	-	-	
Range	-	-	-	

^aData as of August 20, 1992

c. Non-Neoplastic Effects and Other Considerations

Mortality

Survival appeared to be similar in all dose groups. At termination 26, 26, 26 and 24 males and 27, 31, 28 and 29 females in control, low, mid- and high dose groups, respectively had survived to week 104.

Clinical Signs and Other Parameters

Yellow staining of the urogenital area hair coat was observed in most treated females, which appeared to be related to treatment. The incidences were as follows: 9/80, 66/80, 69/80 and 77/80 for the 0, 60, 120, and 240 mg/kg/day dose groups, respectively. No other treatment-related clinical findings were observed.

Mean body weights of treated animals were similar to that of the vehicle controls and food consumption was not affected by treatment.

No consistent treatment-related changes were found in any of the hematological or the clinical chemistry values.

Treatment-related changes were observed in several of the items measured during the urinalysis examinations. These changes were observed at various times, but for some parameters were not consistent. The changes indicated renal damage and impairment; however, urine concentrating ability was not significantly altered. They included increases in urinary protein (251-285% in males, 213-306% in females), and alkaline phosphatase (185-225% in males, 133-200% in females) and decreases in N-acetyl- β -glucose amidase (54-62% in males; 32-59%, not dose-related in females) and in galactosidase (30-51% in males; 9-42%, not dose-related in females). Most of these changes were observed in both the mid- and high dose animals. In addition, dose-related increases in urinary coproporphrin were also observed at all dose levels in males (179-321% with increasing dose) and in the mid- and high dose groups in females (157-214%).

Non-Neoplastic Effects

Changes in several mean organ weights were observed throughout the study in both sexes. These included increases in liver, heart and kidney weights and decreases in thymus weights. The increases in kidney weights were the most consistent and were

observed in all treated male groups at least once during the study and in the mid- and high dose female groups at least twice during the study (104-118%).

Significant increases in the incidences and severity of nephropathy were observed as early as 3 months in females in the high dose group ($p < 0.01$ for both incidences and severity). By 15 months, although nephropathy was observed in nearly all rats including controls, it increased in severity with dose and was more severe in males than in females ($p < 0.05$ for all treated male groups and $p < 0.01$ for high dose females only). At termination, again, nephropathy was present in most animals of both sexes including controls and the severity was dose-related and was greater in males ($p < 0.05$ for low dose males and < 0.01 for mid- and high dose males; $p < 0.05$ for mid-dose females and < 0.01 for high dose females). The histologic changes were similar in both treated and control rats and were similar to those observed in aged F344/N rats. In addition to nephropathy, numerical increases in some other lesions were observed, once or twice in both the mid- and high dose groups (males), but particularly in the high dose group. With the exception of some of the effects on the kidney which were summarized separately, it was difficult to tell from the tables in the report as to whether or not any of these were statistically significant because the tables for nonneoplastic lesions did not include statistical significance. These are listed below:

males: mineralization of the stomach, hyperplasia of the parathyroid gland, fibrosis of the spleen, fibrous osteodystrophy of the cranium and femur, renal tubule hyperplasia and hyperplasia of the transitional epithelium of the kidney.

females: hyperplasia of the adrenal gland cortex, mineralization of the renal pelvis and transitional cell hyperplasia of the kidney.

The Pathology Working Group which analyzed this study stated that the "small foci of mineralization found at the corticomedullary junction and in the renal pelvis were not considered treatment related and were not severe enough to obstruct urine flow or induce proliferation of renal pelvic epithelium." The increased incidence of hyperplasia of the parathyroid gland in males was attributed to renal secondary hyperparathyroidism. The increased incidences of fibrous osteodystrophy were "ascribed to and correlated with the increased severity of the nephropathy and the development of secondary renal hyperparathyroidism, with lesions

primarily restricted to male rats, in which the nephropathy was more severe." The authors also stated that "transitional cell hyperplasia occurs in the renal pelvis epithelium as a component of some cases of severe nephropathy." A specific review of these was conducted on both the 15-month and terminal sacrifices with the vehicle control and the high dose groups because of the appearance of the two rare transitional cell carcinomas in females. Increases in transitional cell hyperplasia were found in both sexes (5/59 and 26/59 in control versus high dose males, 4/60 and 17/59 in control versus high dose females).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of o-BCP, based on treatment-related increases in incidences in selected microscopic lesions (nephropathy and severity of nephropathy at all dose levels, hyperplasia of the kidney tubule, transitional cell hyperplasia of the kidney, hyperplasia of the parathyroid gland and fibrous osteodystrophy of the cranium and femur); increases in kidney weights; increases in supporting urinalysis values (coproporphyrin, protein and alkaline phosphatase); decreases in supporting urinalysis values (N-acetyl- β -glucose amidase and galactosidase activities) and an increase in yellow staining of the urogenital area.

2. 2-Year Carcinogenicity Gavage Study in Mice

Reference: Biotechnical Services, Inc. (1994) Toxicology and Carcinogenesis Studies of o-Benzyl-p-Chlorophenol in F344/N Rats and B6C3F₁ Mice (Gavage Studies): Study Number NTP TR 424, Publication Number 94-3155. Testing Facility: Battelle Columbus Laboratories; Submitted by: U.S. Public Health Service, National Toxicology Program, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709. MRID Nos. NR-433842-01, 422793-02.

a. Experimental Design

Technical o-Benzyl-p-chlorophenol (o-BCP, 97%) was tested in a two-year gavage study with corn oil as the vehicle in groups of 70 male and female B6C3F₁ mice at the following dose levels: 0, 120, 240 or 480 mg/kg/day. Three and 15 month evaluations were included in the study design in which 10/sex/dose were sacrificed for evaluation. Other parameters examined and/or recorded were body weights, clinical observations, food consumption and

histopathology.

b. Discussion of Tumor Data

There were three target organs of interest in the mouse study: the kidney, liver and lung. The kidney is of particular interest because a few kidney tumors (although of a different type) appeared in the female rat as well.

According to the statistical analysis conducted by HED, male mice had a significant dose-related increasing trend and significant pair-wise comparisons of all dose groups with the controls, for kidney renal tubule adenomas and/or adenocarcinomas combined, all at $p < 0.05$. There was also a significant difference in the pair-wise comparison of the 120 mg/kg/day dose group with the controls for kidney renal tubule adenomas at $p < 0.05$. There were no apparent increases in kidney neoplasms in any of the female mice, including controls.

The following table summarizes the HED statistical analyses on renal tubule adenomas, carcinomas and combined adenoma/carcinoma in male mice.

α -Benzyl-p-Chlorophenol - B₆C₃F₁ Mouse Study

Male Kidney Renal Tubule Tumor Rates[†] and
 Peto's Prevalence Test Results (p values).

	<u>Dose (mg/kg/day)</u>			
	0	120	240	480
Adenomas (%)	0/48 (0)	2/37 (5)	2/42 (5)	2 ^a /35 (6)
p =	0.132	0.046*	0.066	0.092
Adenocarcinomas (%)	0/45 (0)	0/32 (0)	2 ^b /40 (5)	1/30 (3)
p =	0.094	-	0.066	0.110
Combined (%)	0/48 (0)	2/37 (5)	4/42 (10)	3/35 (9)
p =	0.046*	0.046*	0.015*	0.036*

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aFirst adenoma observed at week 99, dose 480 mg/kg/day.

^bFirst carcinoma observed at week 104, dose 240 mg/kg/day.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then p < 0.05. If **, then p < 0.01.

The historical control incidences for kidney renal tubule tumors in male B6C3F1 mice were only provided as totals (individual studies not listed) and are as follows:

Adenomas: 4/949 (0.4%) with a standard deviation of 0.8% and a range of 0-2%.

Carcinomas: 0/949 (0.0%).

Adenomas or Carcinomas: 4/949 (0.4%).
(combined adenoma/carcinoma)

The incidences of kidney renal tubule combined adenoma/carcinoma in male mice in the study were above the historical control incidences for all dose levels (5%, 10% and 9% for the low, mid- and high dose groups, respectively versus 0.4% in the male historical controls for combined adenoma/carcinoma).

In addition to the kidney neoplasms, hepatocellular adenomas and/or carcinomas and lung alveolar/bronchiolar adenomas (particularly in females) were considered for further examination. Although in the NTP evaluation, the liver and lung tumor incidences were not significantly increased by pairwise comparison to controls, these tumors were selected by HED on the basis of dose-related numerical increases in liver and lung tumors in the NTP adjusted tumor rates for females. Also, the first one of these tumors was discovered at an earlier time in the high dose group than in the control group. Since NTP did not consider these tumors to be of biological significance, historical control data were not provided in the report.

According to the statistical analysis conducted by HED, female mice had a significant dose-related increasing trend in hepatocellular adenomas and/or carcinomas combined at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls. No significant compound-related increases for liver or lung tumors were found in male mice, or for lung tumors in female mice.

The statistical analyses of the female mice were based upon Peto's prevalence test since there was a statistically significant positive trend for mortality in female mice with increasing doses of o-Benzyl-p-Chlorophenol.

The following table presents the tumor analysis results for liver tumors in female mice.

σ -Benzyl-p-Chlorophenol - B₆C₃F₁ Mouse Study

Female Hepatocellular Tumor Rates⁺ and Peto's
 Prevalence Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0	120	240	480
Adenomas (%)	11/50 (22)	14/47 (30)	15/47 (32)	14 ^a /44 (32)
p =	0.051	0.191	0.142	0.071
Carcinomas (%)	2/50 (4)	1/46 (2)	3/45 (7)	3 ^b /40 (8)
p =	0.177	0.751 ^d	0.302	0.248
Combined (%)	13/50 (26)	15/47 (32)	17 ^c /47 (36)	16 ^c /44 (36)
p =	0.048 [*]	0.281	0.147	0.078

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aFirst adenoma observed at week 63, dose 480 mg/kg/day.

^bFirst carcinoma observed at week 80, dose 480 mg/kg/day.

^cOne animal each in the 240 and 480 mg/kg/day dose groups had both an adenoma and a carcinoma.

^dNegative change from control.

Note: Significance of trend denoted at control.
 Significance of pair-wise comparison with control denoted at dose level.
 If *, then p < 0.05. If **, then p < 0.01.

The HED Carcinogenicity Peer Review Committee concluded that the incidences of hepatocellular adenomas and/or carcinomas and lung alveolar/bronchiolar adenomas were not affected by treatment in either sex. They believed that these tumors were observed at an earlier time in the high dose groups (considered to be an excessive dose) than in the control groups because the high dose animals were dying from other causes at an earlier time and these tumors were incidentally discovered at the time of death. In addition, these tumors are considered to be common tumors in the B6C3F1 mouse.

c. Non-Neoplastic Effects and Other Considerations

Mortality

The statistical evaluation of mortality indicated significant increasing trends with increasing doses of o-Benzyl-p-Chlorophenol in both male and female mice.

Clinical Signs and Other Parameters

Clinical findings that were considered to be related to treatment were emaciation, abnormal posture, rough hair coat and hypoactivity. These findings were more frequent in the highest dose group, but also occurred as a dose-related trend. No other treatment-related effects were observed.

Mean final body weights of all treated males and mid- and high dose females were significantly lower than the vehicle controls. However, only the decreases in the high dose groups were considered to be biologically significant. By study termination, the mean body weight value for the high dose males was 66% of the control value and for the high dose females it was 70% of the control value.

Non-Neoplastic Effects

There were decreases in either absolute and/or relative kidney weights at various times in both sexes at all dose levels. These were not always dose-related and sometimes there was an increase in relative weight when there was a decrease in absolute weight. Absolute and relative liver weights in females were increased at various times in the mid- and high dose groups. Changes in other organ weights were not consistent.

At both 3 and 15 months, there were dose-related increases in incidence and severity of kidney nephropathy in both sexes. At termination, there were dose-related increases in fibrous osteodystrophy, lymphoid depletion of the spleen, ulcer of the small intestine, and severity of kidney nephropathy in both sexes. Dose-related increases were also observed for myocardial degeneration in males and coagulative necrosis of the liver, chronic inflammation of the liver, squamous hyperplasia and ulcer of the forestomach, mineralization of the glandular stomach mucosa and hematopoietic cell proliferation of the spleen in females. Some of the effects observed in females were observed in males but were not strongly dose-related. With the exception of some of the effects on the kidney which were summarized separately, it is difficult to tell from the tables whether or not any of these were statistically significant because the tables for nonneoplastic lesions did not include statistical significance. The report stated that the fibrous osteodystrophy was "ascribed to and correlated with the increased severity of the nephropathy and the development of secondary renal hyperparathyroidism." It also stated that the hyperplasia of the forestomach was "probably related to an irritant action of the dosed compound." In addition, the mucosal ulceration of the forestomach could also "have been related a response to the irritant action of the dosed compound or secondary to the nephropathy. Other lesions considered to be secondary to the combined disturbances of nephropathy and the associated secondary hyperparathyroidism included mineralization of the glandular mucosa with focal gastric and duodenal ulcers...; myocardial degeneration...; and focal coagulative necrosis of the liver...". The following table summarizes non-neoplastic effects on the kidney and liver. For the kidney, the statistical analyses were conducted by NTP.

Summary of Selected Liver and Kidney Nonneoplastic Lesions in Mice at 3 and 15 Months (Kidney Only) and at Termination

Organ	Control	Low Dose	Mid-Dose	High Dose
Males				
Liver				
Hyperplasia	5/50	4/50	5/50	3/50
Necrosis, coagulative	4/50	4/50	7/50	5/50
Kidney				
Nephropathy				
3 months	1/10 (0.1)	3/10 (0.1)	10/10 ^b (1.2) ^b	10/10 ^b (2.2) ^b
15 months	9/10 (0.9)	10/10(2.7) ^b	10/10 (2.7) ^b	10/10 (2.7) ^b
Termination	39/50(0.8)	48/50(2.0) ^b	50/50 ^b (2.4) ^b	49/50 ^b (2.4) ^b
Hyperplasia of renal tubule				
Single Section	0/50	0/50	3/50	6/50 ^b
Step Sections ^a	9/50	16/50 ^c	13/50	9/50
Single and Step Combined	9/50	16/50 ^c	14/50	13/50
Females				
Liver				
Hyperplasia	3/50	8/50	7/50	4/52
Coagulative necrosis	1/50	3/50	3/50	18/52
Chronic inflammation	0/50	0/50	1/50	5/52
Kidney				
Nephropathy				
3 Months	0/10	2/10 (0.2)	8/10 ^b (0.9) ^b	7/9 ^b (0.9) ^b
15 Months	0/10	9/10 ^b (1.0) ^b	10/10 ^b (1.5) ^b	9/9 ^b (2.6) ^b
Termination	19/50 (0.4)	38/50 ^b (1.1) ^b	48/50 ^b (1.8) ^b	50/52 ^b (2.2) ^b
Hyperplasia of Tubule				
Single Section	0/50	0/50	0/50	0/52
Step Sections ^a	0/50	-	-	1/52
Single and Step Combined	0/50	-	-	1/52

^aStep sections were more extended evaluations.

^bStatistically significant $p \leq 0.01$

^c Statistically significant $p \leq 0.05$

() = mean severity grade

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The high dose level was considered to be excessive based on increasing trends in mortality with increasing doses in both sexes; clinical signs of toxicity (emaciation, abnormal posture, rough hair coat and hypoactivity) and observed microscopic lesions at the high dose level (increases in incidences of nephropathy (including severity) and fibrous osteodystrophy in both sexes, coagulative necrosis of the liver in females and myocardial degeneration in males). In addition, the mean final body weights of the high dose groups were significantly lower than the respective control groups (32.2 grams versus 48.6 grams or 66% of the control group for males and 33.3 grams versus 47.6 grams or 70% of the control group for females). The dosing was considered to be adequate for assessing the carcinogenic potential of o-BCP at the mid-dose level, based on an increasing trend in mortality in both sexes with increasing dose, clinical signs of toxicity, changes in organ weights at all dose levels, increases in the incidences and severity of nephropathy with increasing dose and dose-related increases in the incidences of non-neoplastic lesions in a variety of organs at a lesser extent than in the high dose group.

3. Initiation/Promotion Study of o-Benzyl-p-Chlorophenol

Reference: Biotechnical Services, Inc. (undated) NTP Technical Report on the Initiation/Promotion Study of o-Benzyl-p-Chlorophenol in Swiss (CD-1®) Mice: Study Number NTP TR 444, Publication Number 93-3157. Testing Facility: Battelle Columbus Laboratories; Submitted by: U.S. Public Health Service, National Toxicology Program, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709. MRID No. NR-430569-01.

In this study, o-BCP was tested as a potential initiator, promoter and complete carcinogen. The study was only conducted for 1 year and does not meet the Subdivision F Guideline requirements for a carcinogenicity study. The draft report has not been reviewed by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session and the results are not yet final.

E. Additional Toxicology Data on o-BCP

1. Metabolism

No metabolism studies are available for o-BCP at this time.

2. Mutagenicity

Minimum testing to satisfy the mutagenicity requirements have not been completed. Acceptable studies are available to fulfill the requirements for a Salmonella assay and a structural chromosomal aberration assay.

a. Gene Mutation

o-BCP was tested for potential to induce reverse mutations in Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100. The exogenous metabolic activation mixes were derived from either hamster or rat liver S9 homogenates (all strains were tested with each). The test material was tested up to levels of cytotoxicity in all tester strains, ranging from 0.3 to 33 $\mu\text{g}/\text{plate}$ without metabolic activation and from 1 to 100 $\mu\text{g}/\text{plate}$ with metabolic activation. o-BCP failed to induce a mutagenic response, either with or without metabolic activation. The study was classified as Acceptable (MRID 412875-01).

b. Structural Chromosomal Aberration

o-BCP was tested in a bone marrow micronucleus assay in mice at dose levels up to and including 2000 mg/kg. The animals showed clear signs of toxicity, although there was only slight evidence that the test chemical reached the target organ. o-BCP tested negatively under the conditions of the study. The study was classified as Acceptable (MRID 415728-01).

Non-activated and S9-activated cultures of CHO cells were exposed to o-BCP at concentrations of 4, 8, 15, 30 or 60 $\mu\text{g}/\text{ml}$ (-S9), or 1.3, 2.5, 5, 10 or 20 $\mu\text{g}/\text{ml}$ (+S9). They were then examined for structural chromosome aberrations 20 hours after initiation of treatment. The highest dose tested (HDT) caused excessive toxicity (complete inhibition), but at no doses were there increased cytogenetic effects. The study was classified as Acceptable (MRID 433229-01).

o-BCP was evaluated in a Chinese hamster ovary (CHO) cell chromosomal aberration study and in a sister chromatid exchange

(SCE) assay in two separate laboratories. In the first laboratory, o-BCP was tested up to levels of cytotoxicity (16.0 ug/mL for both assays). In the second laboratory, o-BCP was tested at dose levels up to 14,900 µg/mL in the chromosome assay and up to 801 µg/mL in the SCE assay. The test material precipitated for all nonactivated doses \geq 596 µg/mL and cytotoxicity was observed at 801 µg/mL in the SCE assay. The test material was not clastogenic, and it did not increase the frequency of SCE induction in CHO cells either in the presence or absence of S9 activation. These studies were classified as unacceptable because of the extreme differences in the dose ranges tested between the two testing laboratories and the discrepancies in cytotoxic levels. Other deficiencies were also reported which compromised the overall study (MRID 412875-02).

There is one data gap for mutagenicity testing. Under the new mutagenicity guidelines, a mammalian cells in culture forward gene mutation assay (specifically, a mouse lymphoma assay³) is needed in order to complete the mutagenicity testing requirements.

3. Structure-Activity Correlations

At the present time, HED has not been able to locate any structural analogues of sufficient similarity to o-BCP that have been tested for carcinogenic potential. Therefore, a comparison for potential carcinogenic effects could not be done.

4. Acute, Subchronic and Chronic Toxicity Studies

Acute Studies

An acute oral toxicity study in rats with o-BCP at dose levels up to 5000 mg/kg induced clinical signs of toxicity, abnormal gastrointestinal contents, superficial staining of several body surfaces, congestion or hemorrhage of the thymus, slight congestion or incomplete collapse of the lungs and congestion of the brain. In animals that survived until termination, fibrous adhesions between the stomach and adjacent tissues of the left lobe of the liver and abdominal wall were observed. In the lower dose rats, a low

³In a published study (Caspary et al., Mut Res: 61-81, 1988), O-BCP was reported positive in the mouse lymphoma assay without activation. Since there was some uncertainty about the toxicities in the cited paper, this assay needs to be performed to fulfill the data gap and to address the concern raised by the reported results.

incidence of abnormal gastro-intestinal contents, slight or moderate hydronephrosis and slight congestion of the lungs were observed. The acute oral median lethal dosages were calculated to be as follows: 4462 (4383 - 4545) mg/kg (males), 3852 (3128 - 4838) mg/kg (females) and 4147 (3783 - 4608) mg/kg (combined sexes). Toxicity Category: III. Classification: Unacceptable pending receipt of purity of test material (MRID No. 00131367).

o-BCP was tested in an acute dermal toxicity study in rats at 2000 mg/kg (limit test). There were no clinical signs of toxicity and no mortalities. The report stated that "occluded application of Chlorophen (another name for o-BCP) caused discoloration of the treated skin, loss of elasticity from the second or third day after dosing and complete loss of dermal flexibility from Day 8. Intact skin was revealed by the slough of a dark, hard inflexible layer from the dermal test site which began on Day 10 and was completed by one-half of the animals before termination." Small areas of ulceration, encrustation and slight sloughing were observed in some animals of both sexes at the treatment site. There were no significant internal lesions in any of the animals. The acute dermal LD₅₀ is greater than 2000 mg/kg. Toxicity Category: III. Classification: Unacceptable pending receipt of purity of test material (MRID No. 00130937).

o-BCP was tested in an acute inhalation toxicity study in rats at dose levels up to 3.13 mg/l. Clinical signs observed were bradypnea, hyperpnea, decreased motor activity, ataxia, gasping, respiratory rales, hunched posture, piloerection, ungroomed appearance, pigmented orbital secretion and staining of the snout, corneal opacities, bloated abdomen and nasal secretions. Occasional observation of diarrhea and periorbital hair loss were also observed. Six males and 6 females died. Bodyweight gains appeared to be effected by treatment. Externally localized or general surface staining of the coat, incomplete collapse and congestion of the lungs, firm lungs and aerated fluid in the trachea together with gaseous and fluid stomach and intestinal contents were observed in addition to 3 cases of hydronephrosis, 4 cases of enlarged cervical lymph nodes and a hepatic nodule. In surviving animals there was distension of the stomach and intestinal tract and an enlarged mesenteric lymph nodes in 1 high dose female. There was also an increase in lung weights in the animals which died. The LC₅₀'s were calculated to be the following: males: 2.60 (1.92 - 3.52) mg/l; females: 2.43 (2.03 - 2.90) mg/l and combined: 2.50 (2.13 - 2.93) mg/l. Toxicity Category: III. Classification: Acceptable (MRID No. 00130938).

o-BCP was corrosive to the eye and a severe irritant (ocular discharge, a beefy-red appearance of the conjunctivae, everted eyelids due to moderate chemosis, opalescent cornea over the whole surface and marked congestion of the iris by 4 days). In addition, areas of ulceration had exposed part of the corneal stroma of 2/3 rabbits. The mean PIS was 79 at 72 hours (severely irritating). Toxicity Category: I. Classification: Unacceptable pending receipt of purity of test material (MRID No. 00131368).

o-BCP was mildly irritating to the skin. The maximum primary dermal irritation score (PDII) was 2.33 at 24 hours. Toxicity Category: IV. Classification: Unacceptable pending receipt of purity of test material (MRID No. 00131369).

Subchronic Studies

o-Benzyl-p-chlorophenol was tested in subchronic gavage studies in rats and mice. The animals were dosed 5 days/week for 13 weeks at the following dose levels: rats - 0, 30, 60, 120, 240 and 480 mg/kg/day and mice - 0, 500, 650, 800 and 1000 mg/kg/day. In rats, the NOEL was 120 mg/kg/day and the LOEL was 240 mg/kg/day based on clinical signs of toxicity, changes in clinical chemistries, decreases in thymus weights, increases in kidney weights, chronic nephropathy and thymic lymphoid depletion. In mice, the NOEL was not established because kidney lesions were present at the lowest dose level. Observed toxic effects included mortalities, clinical signs of toxicity, increases in liver weights, decreases in kidney weights, renal lesions (necrosis, casts, inflammation and regeneration of renal tubules) and necrosis of thymic lymphocytes. The study was classified as Supplementary because minimal summary data tables were provided to support the text of the report. In addition, these tables needed legends submitted with them for clarification. Also, no individual animal data were provided. For the mouse study, neither clinical biochemistry studies nor urinalysis studies were conducted (MRID 412482-02).

o-BCP was tested in a 21-day dermal study on New Zealand rabbits at dose levels of 1, 5 and 25 mg/kg/day. The NOEL for systemic effects was 25 mg/kg/day (HDT) and the NOEL for effects on the skin was 1 mg/kg/day. The LOEL for skin lesions was 5 mg/kg/day (acanthosis, hyperkeratosis, parakeratosis, dermatitis, and scabs with some ulcerated areas in the 25 mg/kg/day dose group). This study was classified as Core Guideline (MRID 412482-01).

F. Weight of Evidence Considerations:

The Committee considered the following observations from the toxicological data on o-BCP for a weight-of-the evidence determination on its carcinogenic potential:

1. Male and female F344/N rats were administered o-BCP in corn oil by gavage for two years at the following dose levels: males: 0, 30, 60 or 120 mg/kg/day; females: 0, 60, 120 or 240 mg/kg/day. The dosing was considered to be adequate for assessing the carcinogenic potential based on increases in incidences of several microscopic lesions, increases in kidney weights and decreases in supporting urinalysis values.

In female rats, although there were no statistically significant increases in any neoplasms for any of the treated groups when compared to the control groups, two rare transitional cell carcinomas of the kidney were observed, one in the mid-dose group and one in the high dose group. None were observed in either the concurrent or historical control groups or in any of the males.

In female rats, one renal tubule adenoma was observed in the high dose group (2%). None appeared in the concurrent control group. However, two of these tumors were observed in 1,068 historical control females (range 0-2%). Therefore, the 1 renal tubule adenoma was not outside of the historical control range.

In male rats, one renal tubule carcinoma was observed in the high dose animals (2%) and one renal tubule adenoma was observed in the control group (2%). The historical control incidence of renal tubule adenomas for males was 8/1,069 animals (std. dev. 1.0%, range 0-2%) and the historical control incidence of renal tubule carcinomas for males was 4/1,069 (std. dev. 1.0%, range 0-4%) animals. Therefore again, the one carcinoma was not outside of the historical control range.

In both male and female rats, treatment-related increases in the incidences and severity of nephropathy were observed in all dose groups for males and in the mid- and high dose groups for females. In addition, in a specific review conducted on both the 15-month and terminal sacrifices with the vehicle control and the high dose groups, increases in transitional

cell hyperplasia of the renal pelvis epithelium were found the high dose groups of both sexes (5/59 and 26/59 in control versus high dose males, 4/60 and 17/59 in control versus high dose females). The low and mid-dose groups were not examined in this special review. The transitional cell hyperplasia may have been related to the severe nephropathy.

2. o-BCP was tested in a two-year gavage study with corn oil as the vehicle in male and female B6C3F₁ mice at the following dose levels: 0, 120, 240 or 480 mg/kg/day. The high dose level was considered to be excessive based on increasing trends in mortality with increasing doses in both sexes; clinical signs of toxicity and microscopic lesions which were considered to be too severe (increases in incidences of nephropathy (including severity) and fibrous osteodystrophy in both sexes, coagulative necrosis of the liver in females and myocardial degeneration in males). In addition, the mean final body weights of the high dose groups were significantly lower than the respective control groups (66% of the control group for males and 70% of the control group for females). The dosing was considered to be adequate for assessing the carcinogenic potential of o-BCP at the mid-dose level, based on an increasing trend in mortality in both sexes with increasing dose, clinical signs of toxicity, changes in organ weights at all dose levels, increases in the incidences and severity of nephropathy with increasing dose and dose-related increases in the incidences of non-neoplastic lesions in a variety of organs at a lesser extent than in the high dose group.

Male mice had a significant dose-related increasing trend and significant pair-wise comparisons of all dose groups with the controls, for kidney renal tubule adenomas and/or adenocarcinomas combined, all at $p < 0.05$. There was also a significant difference in the pair-wise comparison of the 120 mg/kg/day dose group with the controls for kidney renal tubule adenomas at $p < 0.05$. The incidences of these tumors in the study were above the historical control incidences for all dose levels (5%, 10% and 9% for the low, mid- and high dose groups, respectively versus 0.4% in the male historical controls for combined adenoma/carcinoma).

In both male and female mice, dose-related increases in the incidences and severity of kidney nephropathy were observed in all dose groups.

In both male and female mice, dose-related decreases in either absolute and/or relative kidney weights were observed at

various times at all dose levels (62-90% of controls in males, 72-89% of controls in females). These were not always dose-related and sometimes there was an increase in relative weight when there was a decrease in absolute weight.

3. In a Salmonella assay, an in vivo bone marrow assay in mice and an in vitro CHO cell chromosomal assay, o-BCP tested negatively for potential to induce mutations or chromosomal aberrations. There is a data gap for a mammalian cells in culture forward gene mutation assay (specifically, a mouse lymphoma assay in order to address positive results reported in the literature).

4. At the present time, HED has not been able to locate any structural analogues of sufficient similarity to o-BCP that have been tested for carcinogenic potential. Therefore, a comparison for potential carcinogenic effects could not be done.

5. Carcinogenicity in animals -- o-Benzyl-p-chlorophenol (o-BCP)

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to o-BCP resulted in an increased compound-related incidence of rare kidney tubule tumors in the male B6C3F1 mouse. The tumor response was outside the historical range and attributable to both adenomas and carcinomas, with carcinomas contributing a substantial portion of the total incidence. Kidney transitional cell carcinomas observed in the female F344/N rat, also rare and also outside the historical range, provided additional support. There is no available data on structural analogs, and the submitted mutagenicity data appears to have a low concern (however, there is a data gap for the mutagenicity evidence).

The relevance of the tumor data to an evaluation of o-BCP's potential for human carcinogenicity is discussed elsewhere in this document.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that o-BCP should be classified as a Group C - possible human carcinogen and that for the purpose of risk characterization the RfD approach should be used for quantification of human risk.

The classification of Group C was based on the compound-related increases in rare kidney tubule tumors at all doses in the male B6C3F1 mouse (combined adenoma/carcinoma, with carcinomas contributing a substantial portion (33-50%) of the total incidence). The incidences of these tumors exceeded that of historical controls. The presence of a rare kidney transitional cell carcinoma at both the mid- and high doses (one in each group) in the female F344/N rat, with none in the concurrent controls or in any of 1,068 historical control females, was considered to be supportive of the mouse findings. While the highest dose in the mouse study was considered excessively toxic, the mid-dose was adequate for carcinogenicity testing in both male and female mice. Dosing in the rat study was considered to be adequate for carcinogenicity testing in both sexes.

There were no available structural analogs for o-BCP, and from the submitted data there appears to be little concern for mutagenicity; however, there is a data gap for a mammalian cells in culture forward gene mutation assay (a mouse lymphoma assay with small and large colonies, is recommended).

The Group C classification is thus based on the rare kidney tubule tumors in the male mouse, supported by the rare kidney transitional cell tumors (to a lesser degree) in the female rat. Since these tumors could not be attributed to a specific mechanism, there were no available data for structural analogs, and little apparent genotoxicity concern, the CPRC recommended the RfD approach for estimating risk.