I
4

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0053796

Note: The PC code for Orthophenylphenol (OPP) is 064103 and the PC code for Sodium Orthophenylphenol (SOPP) is 064104. There is only one document for both.

MEMORANDUM

DATE: October 12, 2005

SUBJECT: **ORTHO-PHENYLPHENOL AND SODIUM ORTHO-PHENYLPHENOL:**
Second Report of the Cancer Assessment Review Committee
PC Code: 064103 (ortho-phenylphenol) & 064104 (sodium ortho-phenylphenol)

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Jessica Kidwell

TO: Tim McMahon, Toxicologist (IO)
Michelle Centra, Risk Assessor (RMB2)
Antimicrobials Division (7510C)

The Cancer Assessment Review Committee met on June 8, 2005 to re-evaluate the carcinogenic potential of Ortho-phenylphenol and Sodium Ortho-phenylphenol. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

NOV 0 1 2005

1

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *ORTHO-PHENYLPHENOL AND SODIUM ORTHO-PHENYLPHENOL* **(Second Evaluation)**

PC codes 064103 and 064104

Final
October 12, 2005

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Timothy F. McMahon, Ph.D.

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke

Lori Brunsman, Statistician

William Burnam, Chair

Marion Copley

Vicki Dellarco

Abdallah Khasawinah

Esther Rinde

Jess Rowland

Linda Taylor

Yin-Tak Woo — See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist — See attached sheet

OTHER ATTENDEES: A. Najm Shamim (AD), Michelle Centra (AD), Theresa Schoenborn (Versar/AD)

OPP AND SOPP CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: Timothy F. McMahon, Ph.D.

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

Lori Brunsman, Statistician _____

William Burnam, Chair _____

Marion Copley _____

Vicki Dellarco _____

Abdallah Khasawinah _____

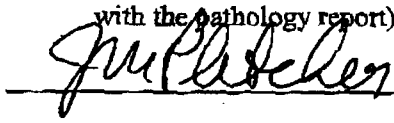
Esther Rinde _____

Jess Rowland _____

Linda Taylor _____

Yin-Tak Woo _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist 

OTHER ATTENDEES: A. Najm Shamim (AD), Michelle Centra (AD), Theresa Schoenborn (Versar/AD)

OPP AND SOPP CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: Timothy F. McMahon, Ph.D.

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke

Lori Brunsman, Statistician

William Burnam, Chair

Marion Copley

Vicki Dellarco

Abdallah Khasawinah

Esther Rinde

Jess Rowland

Linda Taylor

Yin-Tak Woo

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist

OTHER ATTENDEES: A. Najm Shamim (AD), Michelle Centra (AD), Theresa Schoenborn (Versar/AD)

TABLE OF CONTENTS

EXECUTIVE SUMMARY

On June 8, 2005, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of ortho-phenylphenol (OPP) and sodium ortho-phenylphenol (SOPP).

Tim McMahon of the Antimicrobials Division presented the chronic toxicity/carcinogenicity studies in CDF rats and albino mice as well as mode of action information. In the chronic toxicity/carcinogenicity study in rats, OPP was administered to CDF rats (50/sex/dose groups) in the diet for 24 months at dose levels of 0, 800, 4000 and 8000 ppm in males (39, 200, and 402 mg/kg/day, respectively) and 0, 800, 4000, and 10000 ppm in females (49, 248, and 647 mg/kg/day, respectively). An interim sacrifice group of twenty animals/sex for control and high dose groups and ten animals/sex for the low and mid dose groups were sacrificed at 12 months. In the mouse carcinogenicity study, B6C3F1 albino mice (50/sex/dose group) received ortho-phenylphenol (99.88% a.i.) in the diet for 24 months at dose levels of 0 250, 500 and 1000 mg/kg/day. A satellite group of ten animals/sex/dose group were sacrificed at 12 months.

The CARC concluded the following:

Carcinogenicity

Rat

- In male rats, the incidences of urinary bladder papillomas, transitional cell carcinomas, and/or combined papillomas and/or transitional cell carcinomas for the control, 800, 4000, and 8000 ppm dose groups (0, 39, 200, and 402 mg/kg/day), respectively, were as follows:

  Papillomas: 0/69 (0), 1/60 (2%), 0/60 (0), 11/68 (16%)
  Transitional cell carcinomas: 0/70 (0), 0/60 (0), 2/60 (3%), 37/70 (53%)
  Combined: 0/70 (0), 1/60 (2%), 2/60 (3%), 48/70 (69%)

  Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls, for urinary bladder papillomas, transitional cell carcinomas, and papillomas and/or transitional cell carcinomas combined, all at $p < 0.01$. Although historical control data from the testing laboratory were not provided, urinary bladder tumors are considered to be a rare tumor. There is a marginal tumor response for transitional cell carcinomas (3%, not statistically significant, vs 0%, controls) at the mid-dose of 4000 ppm (200 mg/kg/day) which is supported by hyperplasia at this dose as well as the tumors at the high dose. The CARC considered the urinary bladder tumors at the high dose to be treatment-related.

- There were no compound-related increases in tumors in female rats.

◂ Dosing at the high dose of 8000 ppm was considered to be adequate and not excessive in both sexes. This was based on significantly decreased body weight gains (males, 15% and females, 22%) during the first 13 weeks of the study at the high dose; decreased body weight of 11% and 15% in males and females at week 104 at the high dose respectively; increased incidence of urinary bladder masses in high dose males at 24 months, and an increased incidence of several non-neoplastic lesions in high dose males at 24 months, including bladder calculus, congestion, hemorrhage, nodular hyperplasia, mineralization, and necrosis.

Mouse

◂ In male mice, the incidences of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 250, 500, and 1000 mg/kg/day dose groups, respectively, were as follows:

Adenomas: 29/60 (48%), 34/58 (59%), 41/59 (69%), 46/60 (77%)
Carcinomas: 11/60 (18%), 5/58 (9%), 14/59 (24%), 12/60 (20%)
Combined: 34/60 (57%), 36/58 (62%), 46/59 (78%), 48/60 (80%)

Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the controls, for liver adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 500 mg/kg/day dose group with the controls for liver adenomas and adenomas and/or carcinomas combined, both at $p < 0.05$. Historical control data from the testing laboratory were not provided. However, the incidence of liver adenomas (77%) and combined adenomas and/or carcinomas (80%) at the 500 and 1000 mg/kg/day exceeded the historical control average (30%, adenomas, 42%, combined) and range (4-60%, adenomas; 10-68%, combined) from the National Toxicology Program (NTP). The CARC considered the male mouse liver tumors (adenoma driven) at 500 and 1000 mg/kg/day to be treatment-related.

◂ Female mice had a significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls for liver carcinomas at $p < 0.05$. There were no other statistically significant findings for female mice. The CARC did not consider the liver tumors in female mice to be treatment-related.

◂ The CARC considered the mid-dose of 500 mg/kg/day dose to be adequate, and not excessive, in both sexes for assessing the carcinogenic potential of OPP based on decreases in body weight gain. At 12 and 24 months, a decrease of 14-25% in body weight gain in males and females at the mid dose of 500 mg/kg/day was observed, and a decrease of 27-38% in body weight gain was observed at the high dose of 1000 mg/kg/day. Accentuated lobular pattern of the liver was observed in male mice at the 500

and 1000 mg/kg/day dose levels at 12 months, and in male and female mice at 24 months. The 1000 mg/kg/day dose, a limit dose, was considered to be excessive by the CARC due to the decreases in body weight gain.

Mutagenicity

Based on the available data regarding the mutagenicity of OPP, there is no clear evidence of mutagenicity. Positive results generally seen in cytogenetic assays were associated with excessive cytotoxicity and not related to direct damage to DNA. The proposed mechanism for severe cytotoxicity is oxidative damage which is supported by the evidence showing the non-linearity of the response for urinary bladder tumors observed in rats. Thus, the tumor response observed in the rat studies with OPP is consistent with a threshold effect involving oxidative damage leading to cytotoxicity and not a direct DNA damaging effect. An MOA for the liver tumors seen in mice was not determined at this time.

Mode of Action

•In summary, the data are sufficient to support a mode of action for development of **urinary bladder tumors** in male rats from administration of OPP only at high doses. The key events are as follows:

- High doses of OPP lead to saturation of phase II detoxification enzyme pathways, resulting in increased oxidative metabolites PHQ and/or PBQ. The amount of these oxidative metabolites in the urine of male rats is sufficient to cause cytotoxicity but not direct genotoxicity. Cytotoxicity occurs through oxidative damage to cells.

- Oxidative damage to the urinary bladder epithelium (including macromolecular binding to cellular proteins) caused by the quinone metabolites elicits a hyperplastic regenerative response in urinary bladder. Continued presence of these quinone metabolites results in the progression of the hyperplastic response to papillary and nodular hyperplasia, and eventually to the urinary papillomas and carcinomas observed in long-term feeding studies. Evidence suggests that there are not sufficient oxidative metabolites generated *in vivo*, but that a non-genotoxic mode of action is operative.

Liver tumors observed in mice after administration of high doses of OPP have been postulated to be the result of depletion of cellular glutathione and oxidative damage; however, convincing evidence in support of this as a mode of action are limited and, therefore, no definitive conclusions can be reached regarding the mode of action for liver

9

tumor development. It should be noted, however, that in the review of the toxicology of OPP by Bomhard et al. (2002), it is stated with respect to the mouse liver tumors that "Very high spontaneous incidences of hepatocellular tumors in this substrain (up to ca. 60% after two years) may be considered as a confounding factor rendering, in conjunction with the extraordinary sensitivity of especially male mice to liver tumor development following high dose treatment with many non-genotoxic substances, the human relevance of the tumorigenicity seen in the one [mouse] study at least questionable." Nonetheless, the incidence of liver adenoma and adenoma/carcinoma combined at the mid and high dose in male mice exceeded historical control data incidence for this tumor type from the NTP database (Haseman et al., 1998) and the CARC considered the statistically significant increases in male mouse liver tumors to be treatment-related at 2 doses.

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC used multiple descriptors for the classification of ortho-Phenylphenol and sodium ortho-phenylphenol.

OPP and SOPP were classified as "Not Likely to be Carcinogenic to Humans" based on convincing evidence that carcinogenic effects are not likely below a defined dose range (i.e, below 200 mg/kg/day). This classification is based on the following: convincing evidence that a non-linear mode of action for bladder tumors was established in rats. High doses of OPP lead to saturation of phase II detoxification enzyme pathways, resulting in increased amounts of the oxidative metabolites PHQ and/or PBQ. The generation of PBQ is considered dose-dependent, appearing in increased quantity only at higher doses of OPP (>200 mg/kg/day). The shift in biotransformation products with increased dose of OPP has been postulated to be associated with the non-linear response observed in tumorigenicity of the urinary bladder, involving oxidative damage to cells and subsequent regenerative hyperplasia. With continued exposure, this process leads to development of tumors. Evidence suggests that a non-genotoxic mode of action is operative.

OPP and SOPP were also classified as "Likely to be Carcinogenic to Humans," based on the presence of urinary bladder tumors in rats and the presence of liver tumors in mice at doses above 200 mg/kg/day. This classification is based on the fact that insufficient data were provided to support a mode of action for the mouse liver tumors. Although the tumors were benign and observed only in one sex at high doses, more data are required for any conclusion to be drawn regarding the mode of action for these tumors.

The CARC noted that although both chemicals are classified as "Likely to be Carcinogenic to Humans" above a defined dose range, quantification of cancer risk is not required since the NOAEL selected for the chronic Reference Dose would address the concerns for the precursor events leading to development of bladder and liver tumors. The non-cancer

assessment for OPP established a chronic Reference Dose using a NOAEL of 39 mg/kg/day from the combined chronic toxicity/carcinogenicity study in rats (MRIDs 43954301, 44852701, 44832201) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity at the LOAEL of 200 mg/kg/day. The NOAEL of 39 mg/kg/day selected for the chronic RfD is sufficiently protective of the key events involved in the carcinogenic mode of action, which are not present at doses below 200 mg/kg/day in the rat and is also protective of liver adenomas occurring at ≥500 mg/kg/day in the mouse. Thus, the precursor events leading to development of bladder and liver tumors are not likely to occur using the NOAEL selected for the chronic RfD value and this value is thus protective against development of tumors.

I. INTRODUCTION

On June 8, 2005, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of ortho-phenylphenol (OPP) and sodium ortho-phenylphenol (SOPP).

II. BACKGROUND INFORMATION

Ortho-phenylphenol (OPP) is a bacteriostat, microbiostat, menaticide, fumigant, and bacteriocide chemical. As a fungicide, tolerances have been established (40 CFR 180.129) for combined residues of OPP and SOPP from postharvest application on apples, cantaloupes, carrots, cherries, citrus, cucumbers, grapefruits, kiwifruits, kumquats, lemons, limes, nectarines, sweet oranges, bell peppers, peaches, pears, pineapples, plums and prunes, sweet potatoes, tangerines, and tomatoes. In addition, OPP is used in applications to hard surfaces (walls, floors, barns) and agricultural premises and equipment, wood preservation for control of sapstain and mold, food handling surfaces and equipment, air deodorization, commercial and institutional premises, medical premises, residential and public access premises (carpet, hard surfaces, crack and crevice treatment), material preservatives (stains and paints, metalworking fluids, textiles, paper slurries, cement mixtures, glues and adhesives, and consumer, household and institutional cleaning products).

Structure:

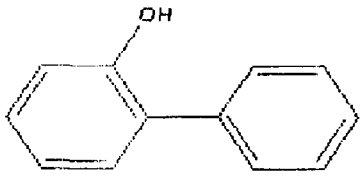


Figure 1: OPP

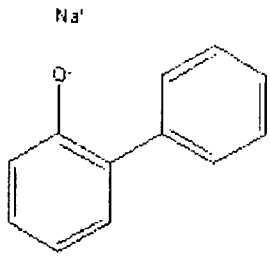


Figure 2: SOPP

On January 5, 1994, the Health Effects Division's Carcinogenicity Peer Review Committee (CPRC) met to discuss and evaluate the weight of evidence with respect to carcinogenicity of OPP and SOPP. These two chemicals are considered to be the same. Both OPP and SOPP were classified as B2 (probable) human carcinogens on the strength of evidence showing malignant urinary bladder tumors as well as malignant tumors of the kidney in male rats, and papillomas of the urinary bladder and uterine endometrium in female rats. These neoplastic lesions were observed in several chronic studies conducted with rats, and are summarized in the August 24, 1994 document entitled "Carcinogenicity Peer Review of Orthophenylphenol (OPP) and Sodium Orthophenylphenol (SOPP)" (HED Doc. No. 011196).

The CPRC concluded at that time that "the evidence for OPP and SOPP is sufficient for classification as Group B2 – probable human carcinogen, based on evidence of multiple tumor types in multiple studies. However, in consideration of what is known about the metabolism of these compounds and anticipated human exposures, the CPRC felt that it was inappropriate to apply a low-dose extrapolation methodology (Q*) to the animal data. Therefore, the CPRC recommended the use of the Margin of Exposure (MOE) methodology to be applied for estimation of human risk, for the time being."

Metabolism studies available to the CPRC at that time indicated a dose-dependent metabolic profile for OPP and SOPP with the possibility that the tumors induced are based upon an altered metabolic profile. Specifically, lower doses of OPP and SOPP are metabolized primarily to glucuronide and sulfate conjugates. Increasing doses lead to formation of free oxidative metabolites, particularly the 2,5-dihydroxybiphenyl metabolite, which can be converted to the corresponding quinine metabolite in aqueous media. In addition to this general metabolic scheme, it has been observed that male rats excrete as much as 7 times the 2,5-hydroxybiphenyl metabolite (present as the glucuronide conjugate). Significant activity of glucuronidase in the bladder could easily convert the conjugate back to the aglycone generating the free hydroxyl/quinine metabolite.

On this basis, the CPRC felt that it was inappropriate to apply linear low-dose extrapolation to the animal data and instead applied the Margin of Exposure Methodology (MOE) for estimation of human risk. The NOAEL value of 40 mg/kg/day from the two-generation rat reproduction study was originally chosen as the point of departure on the basis that at the time, it represented the lowest NOAEL value in the database and also contained endpoints from the study relevant to the carcinogenicity assessment, which included increased urinary bladder and transitional cell hyperplasia, renal hemorrhage, urinary bladder calculi, and increased kidney weights. However, since that time, the HED RfD Peer Review Committee recommended that the NOAEL value of the 2-generation reproduction study be revised to 140 mg/kg/day and not 40 mg/kg/day. The presence of calculi at the 140 mg/kg/day dose was discounted by the committee. In fact, a more recent publication (Niho et al., 2002) provided evidence that there is no relation between calculi and neoplastic lesions of the urinary tract. All other effects in the study were significant only at

490 mg/kg/day. This result is additionally supported by the results of a second 2-generation reproduction toxicity study reviewed by the Agency (MRID 43928801) in which the parental NOAEL was determined to be 100 mg/kg/day and the LOAEL 500 mg/kg/day, based on similar effects in the kidneys.

Since the time of the CPRC determination, two additional carcinogenicity studies have been submitted to the Agency for review (MRIDs 43545501 [mouse study] and 43954301 [rat study].) In addition, there are some additional open literature sources regarding the carcinogenicity and mutagenicity of OPP and SOPP that were not considered previously by the CPRC in 1994.

The current CARC was asked to review these additional data and to consider what influence the newer data have on the carcinogenicity classification of OPP and SOPP.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity in F344 Rats

Reference: Wahle, B.S. and Christenson, W.R. (1996): Technical Grade ortho-phenylphenol: A combined Chronic Toxicity/Oncogenicity Study in the Rat. Study conducted for Bayer Corporation Pittsburgh, PA by Bayer Corporation Agriculture Division, Stillwell KS and submitted under MRID 43954301. Unpublished.

A. Experimental Design

In a combined chronic toxicity/carcinogenicity study (MRID 43954301) CDF rats from SASCO, Inc., Madison, WI received ortho-phenylphenol, Technical Grade (99.5-100% a.i.; Batch #S-01-93, Mixture of Bayer AG, Leverkusen, Germany and Dow, Midland, Michigan) in the diet for 24 months at dose levels of 0, 800, 4000 and 8000 ppm in males (39, 200, and 402 mg/kg/day, respectively) and 0, 800, 4000, and 10000 ppm in females (49, 248, and 647 mg/kg/day, respectively). An interim sacrifice group of twenty animals/sex for control and high dose groups and ten animals/sex for the low and mid dose groups were sacrificed at 12 months.

B. Discussion of Mortality and Tumor Data

Survival Analysis

Male rats had a statistically significant increasing trend for mortality with increasing doses of ortho-phenylphenol (Table 1) (L. Brunsman, 5/19/05, TXR No. 0053394). There were no significant differences in the pair-wise comparisons of the dosed groups with the controls for mortality.

Table 1. Orthophenylphenol - CDF(F-344)/BR Rat Study (MRID 43954301)

Male Mortality Rates[a] and Cox or Generalized K/W Test Results

Weeks

Dose (ppm)	1-26	27-52	52[i]	53-78	79-105[f]	Total
0	0/70	1/70	20/69	0/49	18/49	19/50 (38)*
800	0/60	0/60	10/60	4/50	13/46	17/50 (34)
4000	0/60	0/60	10/60	3/50	15/47	18/50 (36)
8000	0/70	2/70	20/68	5/48	17/43	24/50 (48)

[a]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 52.
[f]Final sacrifice at week 105.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analysis

Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls, for urinary bladder papillomas, transitional cell carcinomas, and papillomas and/or transitional cell carcinomas combined, all at $p < 0.01$ (Table 2). The statistical analyses of the male rats were based upon Peto's Prevalence Test (L. Brunsman, 5/19/05, TXR No. 0053394). No historical control data were provided.

There were no compound-related increases in tumors in female rats.

Table 2. Orthophenylphenol - CDF(F-344)/BR Rat Study (MRID 43954301)

Male Urinary Bladder Tumor Rates[1] and Peto's Prevalence Test Results

Dose (ppm)

	0	800	4000	8000
Papillomas (%)	0/69 (0)	1/60 (2)	0/60 (0)	11[a]/68 (16)
p =	0.00000**	0.16622	-	0.00016**
Transitional Cell Carcinomas (%)	0/70 (0)	0/60 (0)	2/60 (3)	37[b]/70 (53)
p =	0.00000**	-	0.07410	0.00000**
Combined (%)	0/70 (0)	1/60 (2)	2/60 (3)	48/70 (69)
p =	0.00000**	0.16622	0.07410	0.00000**

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

[a]First papilloma observed in an interim sacrifice animal at week 52, dose 8000 ppm.
[b]First transitional cell carcinoma observed at week 37, dose 8000 ppm.

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 $p < 0.01$.

C. Non-neoplastic Lesions

As shown in Table 3, several non-neoplastic lesions were observed in increased incidence in males and females.

Table 3. Non-neoplastic lesions at 24 months in rats treated with OPP

Dose (males/females)	0 ppm (0/0 mg/kg/day)	800 ppm (39/49 mg/kg/day)	4000 ppm (200/248 mg/kg/day)	8000 ppm (402/647 mg/kg/day)
MALES				
kidney:				
cyst	4/50	7/50	5/50	17/50* (34%)
hyperplasia	4/50	3/50	3/50	7/50 (14%)
infarct	2/50	0/50	0/50	7/50 (14%)
urinary bladder:				
hyperplasia (nodular)	1/50	0/50	0/50	43/50* (86%)
hyperplasia (simple)	2/50	2/50	6/50	42/50* (84%)
mineralization	3/50	6/50	5/50	18/50* (36%)
necrosis	0/50	0/50	0/50	20/50* (40%)
FEMALES				
kidney:				
cyst	14/50	8/50	5/50	37/50* (74%)
hyperplasia	3/50	0/50	3/50	30/50* (60%)
infarct	3/50	0/50	3/50	29/50* (58%)
urinary bladder:				
hyperplasia (nodular)	0/50	0/49	0/50	1/50 (2%)
hyperplasia (simple)	0/50	0/49	0/50	6/50* (12%)
mineralization	0/50	0/49	2/50	1/50 (2%)
necrosis	0/50	0/49	0/50	2/50 (4%)

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing at the high dose of 8000 ppm was considered to be adequate and not excessive in both sexes. This was based on significantly decreased body weight gains (males, 15% and females, 22%) during the first 13 weeks of the study at the high dose and decreased body weight of 11% and 15% in males and females, respectively, at week 104 at the high dose. In addition, there was an increased incidence of calculus in the kidneys in the high dose males (at 12 and 24 months), increased hyperplasia of the urinary bladder in males (at 12 and 24 months) and females (at 24 months) along with an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in the high dose males. High dose males and females also had an

increase in cysts of the kidney at 24 months. High dose females also had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney.

2. Carcinogenicity Study in Mice

Reference: Quast, J.F. and McGuirk, R.J. (1995): Ortho-phenylphenol: Two Year Dietary Chronic Toxicity/Carcinogenicity Study in B6C3F1 mice. Study conducted by Dow Chemical Company, Midland, Michigan and Freeport Texas for Dow Chemical Company, Midland, Michigan and Miles Inc., Stillwell, KS. Submitted under MRID 43545501. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID# 43545501) B6C3F1 albino mice (50/sex/dose group) from Charles River Laboratory, Portage, MI received ortho-phenylphenol (99.88% a.i.; Lot# 8800005-24, mixture of Dow Chemical Company and Miles, Inc. products) in the diet for 24 months at dose levels of 0 250, 500 and 1000 mg/kg/day. A satellite group of ten animals/sex/dose group were sacrificed at 12 months.

B. Discussion of Mortality and Tumor Data

Survival Analysis

There were no statistically significant incremental changes in mortality with increasing doses of ortho-phenylphenol in male or female mice (L. Brunsman, 5/19/05, TXR No. 0053394).

Tumor Analysis

As shown in Table 4, male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the controls, for liver adenomas and adenomas and/or carcinomas combined, all at $p<0.01$ (L. Brunsman, 5/19/05, TXR No. 0053394). There was also a significant difference in the pair-wise comparison of the 500 mg/kg/day dose group with the controls for liver adenomas and/or carcinomas combined, at $p < 0.05$.

Table 4. Orthophenylphenol - B6C3F1 Albino Mouse Study (MRID 43545501)

Male Liver Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Test Results

	Dose (mg/kg/day)			
	0	250	500	1000
Adenomas (%)	29[a]/60 (48)	34/58 (59)	41/59 (69)	46/60 (77)
p =	0.00045**	0.17484	0.01519*	0.00119**
Carcinomas (%)	11/60 (18)	5/58 (9)	14/59 (24)	12[b]/60 (20)
p =	0.2191	0.96629	0.30968	0.50000
Combined (%)	34[c]/60 (57)	36[d]/58 (62)	46[e]/59 (78)	48[f]/60 (80)
p =	0.00120**	0.34115	0.01098*	0.00512**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54.

[a]First adenoma observed in an interim sacrifice animal at week 54, dose 0 mg/kg/day.
[b]First carcinoma observed at week 61, dose 1000 mg/kg/day.
[c]Six animals in the control group had both an adenoma and a carcinoma.
[d]Three animals in the 250 mg/kg/day dose group had both an adenoma and a carcinoma.
[e]Nine animals in the 500 mg/kg/day group had both an adenoma and a carcinoma.
[f]Ten animals in the 1000 mg/kg/day group had both an adenoma and a carcinoma.

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 $p < 0.01$.

As shown in Table 5, female mice had a significant difference in the pair-wise comparison of the 250 ppm dose group with the controls for liver carcinomas at $p< 0.05$. There were no other statistically significant findings for female mice (L. Brunsman, 5/19/05, TXR No. 0053394).

The statistical analyses of male and female mice were based upon Fisher's Exact Test and the Exact Test for Trend.

Historical control data from the NTP database (Hasemen et al., 1998) indicated a range of 4-60% for liver adenoma (mean of 30%), a range of 6-29% for liver carcinoma (mean of 18%), and a range of 10-68% for combined liver adenoma/carcinoma (mean of 42%). The incidences of adenomas and combined adenomas and/or carcinomas at the 500 and 1000 mg/kg/day dose levels exceeded the historical control range. Historical control data from the testing laboratory were not provided.

C. Non-Neoplastic Lesions

An increased incidence of accentuated lobular pattern of the liver was observed in males and females of the 500 and 1000 mg/kg/day dose groups at 12 and 24 months. A slight increase in ovarian cysts was observed in females of the 1000 mg/kg/day dose group at 12 months.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The CARC considered the mid-dose of 500 mg/kg/day dose to be adequate, and not excessive, in both sexes for assessing the carcinogenic potential of OPP based on decreases in body weight gain. At 12 and 24 months, a decrease of 14-25% in body weight gain in males and females at the mid dose of 500 mg/kg/day was observed, and a decrease of 27-38% in body weight gain was observed at the high dose of 1000 mg/kg/day. Accentuated lobular pattern of the liver was observed in male mice at the 500 and 1000 mg/kg/day dose levels at 12 months, and in male and female mice at 24 months. The 1000 mg/kg/day dose, a limit dose, was considered to be excessive by the CARC due to the decreases in body weight gain.

Table 5. Orthophenylphenol - B6C3F1 Albino Mouse Study (MRID 43545501)

Female Liver Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Test Results

	Dose (mg/kg/day)			
	0	250	500	1000
Adenomas (%)	13/58 (22)	16[a]/59 (27)	17/58 (29)	20/60 (33)
p =	0.1040	0.35408	0.26257	0.13211
Carcinomas (%)	2/58 (3)	8[b]/59 (14)	6/58 (10)	5/60 (8)
p =	0.3520	0.04997*	0.13578	0.23364
Combined (%)	14[c]/58 (24)	23[c]/59 (39)	18[d]/58 (31)	22[e]/60 (37)
p =	0.1631	0.06298	0.26677	0.10047

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54.

[a]First adenoma observed in an interim sacrifice animal at week 54, dose 250 mg/kg/day.
[b]First carcinoma observed at week 73, dose 250 mg/kg/day.
[c]One animal in each of the control and 250 mg/kg/day dose groups had both an adenoma and a carcinoma.
[d]Five animals in the 500 mg/kg/day dose group had both an adenoma and a carcinoma.
[e]Three animals in the 1000 mg/kg/day group had both an adenoma and a carcinoma.

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 $p < 0.01$.

IV. TOXICOLOGY

1. Metabolism

The general scheme of biotransformation for OPP is shown below:

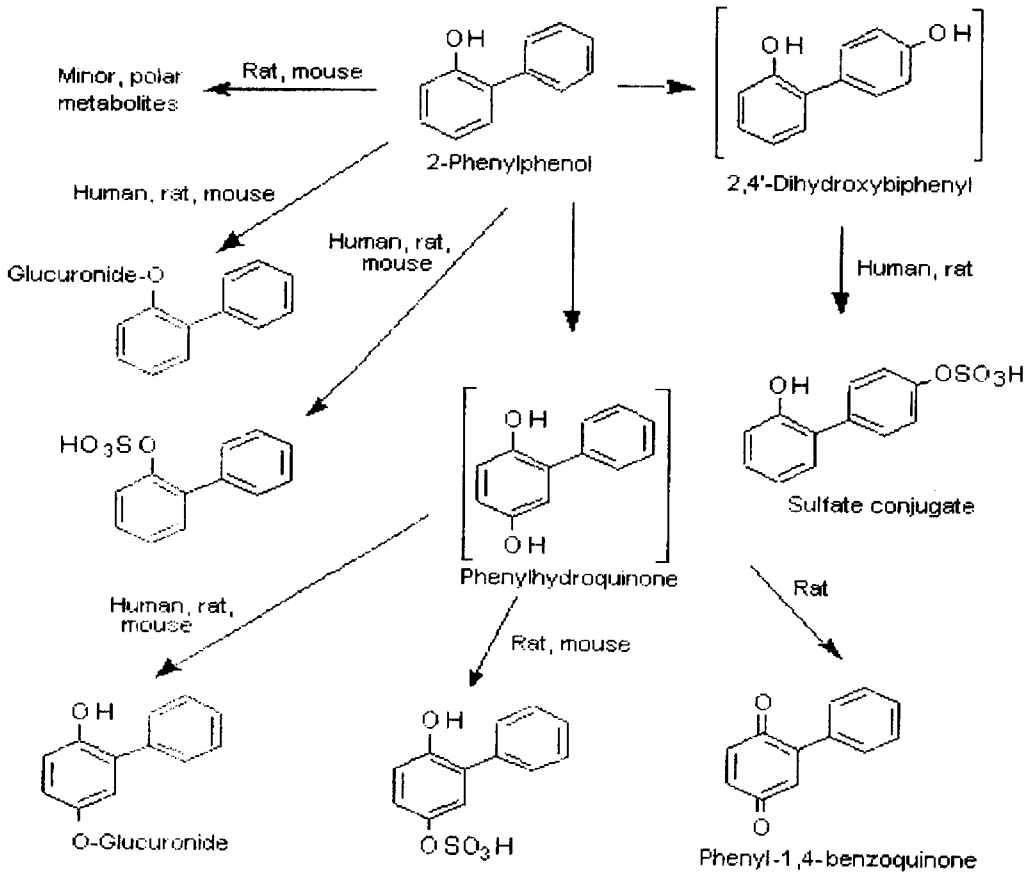


Figure 5 :Proposed pathways of metabolism of 2-phenylphenol in mice, rats, and humans
From: Bartels et al. (1998)

It has been postulated that with regard to the toxicity and carcinogenicity of ortho-phenylphenol, formation of phenolic metabolites occurs in the liver through the action of cytochrome P-450 (demonstrated by Ozawa et al., 2000, to be rat CYP2C11 and possibly CYP2E1, and human CYP1A2 through conversion of OPP to PHQ), followed by a secondary peroxidase-mediated activation in the kidney and/or bladder. Kolachana et al. 1991 proposed that the presence of prostaglandin (H) synthase (PHS) in the rat urinary bladder transitional epithelium and kidney medullary papilla was responsible for the activation of phenylhydroquinone to reactive intermediates in the bladder and kidney. To test this hypothesis, experiments were conducted to determine whether phenylhydroquinone could be metabolized by the peroxidase activity of PHS using arachidonic acid and hydrogen peroxide as co-factors. *In vitro* experiments showed that phenylhydroquinone was metabolized by PHS to a quinine-like product. Formation of phenylbenzoquinone was also demonstrated in the presence of arachidonic acid. Addition of indomethacin (inhibitor of co-oxidation) and glutathione (quinine scavenger) significantly reduced formation of phenylbenzoquinone from phenylhydroquinone. Use of myeloperoxidase and horseradish peroxidase in the presence of hydrogen peroxide also resulted in formation of phenylbenzoquinone. Thus, co-oxidation is a potential pathway for generation of phenylbenzoquinone.

In a study submitted to the Office of Pesticide Programs, *in vitro* incubations were conducted with microsomal PHS from ram seminal vesicles using ortho-phenylphenol (OPP) or the metabolites phenylhydroquinone (PHQ) and 2-phenyl-1,4-benzoquinone (PBQ). Both OPP and PHQ stimulated cyclooxygenase activity, while PBQ had no effect. Higher concentrations of OPP and PHQ were inhibitory to the cyclooxygenase activity. Conversion of PHQ to PBQ was demonstrated as was binding of PHQ and PBQ to native or heat-inactivated PHS protein (DP Barcode D203250, "Interactions of o-Phenylphenol (OPP) and its Metabolites with Microsomal Prostaglandin-H-Synthetase: Possible Implications for OPP_induced Tumor Formation in the Rat Urinary Bladder.").

The 1999 IPCS summary from the joint FAO/WHO meeting provides information on these studies.

Groups of four male Fischer 344 rats were given single oral doses of [14C]2-phenylphenol (purity, 99.8%; specific activity, 19mCi/mmol) or [^{14}C]sodium 2-phenylphenol (purity, 98.7%) by gavage at a dose of 500 mg/kg bw and were immediately placed in glass metabolism cages. About 90-95% of the administered radiolabel on both compounds was recovered in urine and 5-6% in feces, mainly during the first 24 hours. The rates of urinary excretion were virtually identical in the two groups. In a second experiment, animals were fed diets containing 13 000 ppm of 2-phenylphenol or 20 000 ppm of the sodium salt (equimolar amounts) for 2 weeks before administration of single oral doses of the labelled compounds. The animals still eliminated most of the radiolabel (88-94%) in urine and small amounts in feces (3-5%). Preconditioning did not greatly affect the disposition of radiolabel, although the sodium salt

appeared to have been eliminated somewhat more rapidly than 2-phenylphenol (Reitz et al., 1983).

Groups of four male Fischer 344 rats were given single oral doses of [^{14}C]2-phenylphenol (purity not given; specific activity, 1.6 mCi/mmol) at 160 mg/kg bw or [^{14}C]sodium 2-phenylphenol at 250 mg/kg bw (equimolar levels; purity not given; specific activity, 1.6 mCi/mmol). The animals were fasted overnight before and for 6 h after dosing. Urine and feces were collected daily for 7 days. The excretion patterns in the two groups did not differ significantly, and 82-98% of the dose was recovered in urine and only 2-5% in feces within 24 h of dosing. Two male rats received bile duct cannulae, and bile was collected for 3 days after a single oral dose of 250 mg/kg bw of radiolabelled sodium salt. Excretion of radiolabel in the bile began within the first hour of dosing, reached a peak within 3-6 h, and was almost complete by 8 hours. About one-fourth of the dose was recovered in bile over the 3-day collection period. The authors interpreted these results as indicating rapid absorption from the intestine and enterohepatic circulation of 2-phenylphenol metabolites. The pattern of distribution in organs and tissues, examined on days 1, 3, and 7 after administration of the sodium salt and on days 1 and 7 after administration of 2-phenylphenol, showed little difference. Little radiolabel was retained in organs and tissues, including the urinary bladder (Yamaha et al., 1983; Sato et al., 1988).

In a comparative study, [^{12}C/^{13}C/^{14}C]2-phenylphenol (purity, 99.5%; specific activity, 48 mCi/mmol) was given to 10 male B6C3F$_1$ mice as a single oral dose of 15 or 800 mg/kg bw, to 10 male and 10 female Fischer 344 rats as a single oral dose of 28 or 27 mg/kg bw, and to six male volunteers as a dermal dose of approximately 6 µg/kg bw on the forearm for 8 h. The compound was well absorbed in the mice, 84% and 98% of the two doses being recovered in urine collected over 48 h. Extensive absorption and rapid elimination were also seen in the rats, 89 and 86% of the dose being found in the urine of males and females, respectively, within 24 h. 2-Phenylphenol was also rapidly eliminated by the volunteers, 99% of the absorbed dose being collected in urine within the first 48 h of exposure (Bartels et al., 1998).

Urinary metabolites were analyzed in groups of 10 male B6C3F1 mice given single oral doses of 25 or 1000 mg/kg or five daily doses of 1000 mg/kg and killed 48 hours later. Groups of two male and female F344 rats were given single oral doses of labeled compound at 25 or 125 mg/kg and killed 24 hours after dosing. The excretion of [^{14}C]2-phenylphenol in mice was rapid and was complete by 12-24 h after dosing, with 74-98% of the recovered radiolabel in urine and 6-13% in feces; < 1% was recovered in the tissues and carcass. Eight radiolabelled metabolites were detected in the urine of both mice and rats, with no major differences in distribution by species, by sex in the rats, or single or repeated dosing in mice. A small amount (0.4%) of free 2-phenylphenol was detected only in urine of female rats given the single dose of 125 mg/kg bw. Four major urinary metabolites were identified: phenylhydroquinone glucuronide, phenylhydroquinone sulfate, 2-phenylphenol sulfate, and 2-phenylphenol glucuronide, accounting for about 98% of the recovered dose in mice and 102% in rats. An additional

metabolite which accounted for about 2.7% of the recovered dose in rat urine was tentatively identified as the sulfate conjugate of 2,4'-dihydroxybiphenyl. No qualitative difference in metabolites was observed in male mice, but a dose-dependent, quantitative difference was noted in the extent of sulfation and glucuronidation of 2-phenylphenol. After a single dose of 25 mg/kg bw to mice, the sulfate was the major urinary metabolite, accounting for 56% of the recovered radiolabel, while the glucuronide accounted for 29%. After single or repeated doses of 1000 mg/kg bw, the glucuronide was the major metabolite, accounting for 48-60% of the urinary radiolabel, while the sulfate accounted for 20-27%. In rats given a single oral dose of 25 mg/kg bw, 2-phenylphenol sulfate was the major metabolite, accounting for 91% of the recovered radiolabel, while the glucuronide accounted for only 7%. Formation of phenylhydroquinone glucuronide and sulfate represented minor metabolic pathways, accounting for 11-23% and 2-7% of the radiolabel in mice and rats, respectively. The extent of conjugation was not dose-dependent in mice given a single oral dose of 25 or 1000 mg/kg bw of 2-phenylphenol. The authors concluded that 2phenylphenol is completely metabolized in mice and rapidly eliminated in the urine, predominantly as the sulfate and glucuronide conjugates. The extent of metabolism was qualitatively comparable in mice and rats, although quantitative differences were seen in the extent of conjugation (McNett et al., 1997).

The major metabolites identified in the urine of five male and five female Fischer 344 rats fed 20,000 ppm of sodium 2-phenylphenol (purity not given) in the diet for 136 days were glucuronide conjugates of 2-phenylphenol and 2,5-dihydroxybiphenyl. Trace amounts of phenyl-1,4-benzoquinone were also tentatively identified.

Unconjugated phenolic metabolites accounted for only 1% of the phenolic metabolites excreted; no other metabolites were found. By 24h after feeding, 55% of the dose had been recovered in males and 40% in females. A sex difference was found in the proportion of urinary metabolites, male rats excreting 1.8 times as much conjugated 2-phenylphenol and more than 7 times as much conjugated 2,5-dihydroxybiphenyl as female rats in 24-hour urine samples. No explanation was given for the inability to find the sulfate ester of 2-phenylphenol in urine in this study. As only 40-55% of the administered dose was recovered, it may have been present but not identified (Nakao et al., 1983).

Single oral doses of 5, 50, or 500 mg/kg bw of [^{14}C]2-phenylphenol (purity, 99.8%; specific activity, 19 mCi/mmol per L) or [^{14}C]sodium 2-phenylphenol (purity, 98.7%; specific activity, 19 mCi/mmol/L) were administered to groups of four male rats, and the urinary metabolites were identified and quantified. At the two lower doses, the major metabolites of both compounds were the glucuronide and sulfate ester conjugates of 2-phenylphenol, and unconjugated 2-phenylphenol and 2,5-dihydroxybiphenyl accounted for <2% of the total radiolabel recovered in urine at a limit of detection of 1-2%. Nearly identical high-performance liquid chromatograms were obtained for the two compounds. At 500 mg/kg bw, a further metabolite of both compounds was identified, which accounted for 20-30% of the urinary radiolabel and appeared to be a

conjugated dihydroxybiphenyl molecule, most likely with glucuronide and/or sulfate groups. The authors hypothesized that this metabolite is formed only at high doses as a result of saturation of normal glucuronide and sulfate ester conjugation pathways. Incubation of [^{14}C]2-phenylphenol with purified microsomes *in vitro* in the absence of conjugating substrates yielded large amounts of a material which co-chromatographed with 2,5-dihydroxybiphenyl. The semiquinone and quinone were not identified in these studies, but their formation was proposed on the basis of the results of similar studies on benzene (Reitz et al., 1983).

In conjunction with the data examining the mutagenic potential of OPP *in vitro* vs. *in vivo*, and the available data on biotransformation *in vivo*, it could be hypothesized that only minor amounts of free OPP metabolites are formed *in vivo* to act upon the urinary bladder epithelium through a predominantly non-genotoxic mechanism. The levels of oxidative metabolites formed *in vivo* may not be of sufficient concentration to act upon the target tissue through a genotoxic mechanism. Pharmacokinetic modeling could aid in determining an answer to this question.

2. Mutagenicity

An analysis of the genetic toxicology data from over 130 studies with OPP was undertaken by Brusick (2005) who found that there was no indication of gene mutations in bacteria or in mammalian cells such as Chinese hamster ovary (CHO) cells and that positive results with mouse lymphoma (Tk$^{+/-}$) were generally associated with cytotoxicity. Similarly, clastogenicity, which was the most frequently observed genotoxic effect, was consistently linked with cytotoxicity. For OPP, the most common type of structural chromosome damage was chromosome breaks, an event that Brusick describes as typically resulting in cell death. Mixed results were found in studies assessing direct interaction with DNA damage. Based on the weight-of-the-evidence analysis, it was concluded that positive findings in genetic toxicology tests were related to 'excessive cytotoxicity, not direct DNA damage". Furthermore, Brusick (2005) states that agents that shift the normal cellular antioxidative balance and induce cytotoxicity are considered threshold-dependent because exposure levels that do not produce alterations in homeostasis do not produce DNA damage (i.e., genotoxicity). In other words, oxidative damage, eventually leading to cell lethality, only occurs at concentrations that have exceeded the levels that can be handled by normal homeostasis. This observation is supported by the analysis of the carcinogenic mechanism of o-phenylphenate (OPP) by Niho *et al.*(2002). From the dose- and time-response studies with OPP and urinary bladder carcinogenicity in rats, these investigators found that the tumor induction was a high-dose phenomenon, producing a steep dose response at 15,000 and 20,000 ppm but negative at < 10,000 ppm. Similarly, a steep time response curve was plotted with transitional cell carcinoma development only seen in 4% of the animals after 24 weeks of continuous oral exposure but increasing dramatically after 24 (53%) and 52 (71%) weeks. The non-linearity of this response suggested to the authors that the tumor response observed in these studies with OPP is consistent with a threshold effect.

3. Structure-Activity Relationship

Phenol and biphenyl may be considered structurally related chemicals in relation to ortho-phenylphenol but are not close analogs. Data available from the National Toxicology Program on the carcinogenicity of phenol show the compound to be negative for carcinogenicity in both rat and mouse (NIH PB# 80-1759). Biphenyl has been tested for genotoxicity by the National Toxicology Program and was found to be negative in Ames Salmonella assays.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

In a 90-day oral toxicity test (Iguchi S., et al., 1984) designed to determine the subchronic toxicity effects of repeated dietary exposure to o-phenylphenol (>98% purity) in F344/DuCrj rats. o-Phenylphenol (o-PP) was administered in feed to 10 rats/sex/dose at concentrations of 0.156, 0.313, 0.625, 1.25, or 2.5% (total intake: 182, 391, 761, 1669, or 2798 mg/kg/day for males; 202, 411, 803, 1650, or 3014 mg/kg/day, respectively) for 13 weeks. Animals were observed 2 times/day for changes in body weight and food and water consumption.

Mortality occurred in treated animals within 2 weeks of initiating the study, with death in 20% of males (4 days into study) and 10% of females (8 days into study) in the 2.5% dose group. Food consumption was slightly decreased in males and females of the 1.25% dose group. Males administered 2.5% o-PP exhibited significant decreases from control in food intake. The discrepancy in food intake was greatest in the first week but decreased as the study progressed. Females of this group also exhibited a reduction in food consumption that was significantly less than the control until week 8; however, the decreased food intake trend continued throughout the remainder of the study. Additionally, the 2.5% rats spilled an excessive amount of feed at the initial stage of the study and they tended to be thin throughout the study period.

There were no other effects on food consumption in animals of the other dose levels except for males treated with 0.313% o-PP. These rats showed significant increases in food consumption and food intake/body weight that appeared to be reflected in the body weight changes. Overall the feeding efficiency (increase in body weight over unit time in grams/feed intake in grams) was slightly lower in groups fed on feed containing high o-PP concentrations.

Water consumption was significantly decreased from controls in the first week of the study in the 1.25 and 2.5% dose groups. There were no significant changes from controls in body weight gain in animals treated with o-PP concentrations equal to or less than 0.625%. Weight gain was inhibited in males and females of the 1.25% dose group, with maximum inhibition ratios of 14 and 7%, respectively. The significant weight loss of 1.25% females occurred in the first 8 weeks

of the study. Body weight gain was significantly reduced from controls in both male and female rats in the 2.5% o-PP dose group.

The hemoglobin (Hgb) and mean red blood corpuscle hemoglobin concentration (MCHC) were significantly lower than controls in 1.25 and 2.5% females, while hematological analyses in the 2.5% males showed significant decreases from controls in red blood corpuscles (RBC), Hgb, and MCHC. There was a slight tendency for animals to be anemic in groups fed higher dosages of OPP. No treatment-related effects were observed in the serum analyses. Pathological and histological observations indicated treatment-related inflammation of the kidneys in both male and female rats (most pronounced in the 2.5% group) and abnormal growth in the mucous membrane of the male bladder (most pronounced in the 1.25% group).

The subchronic toxicity NOAEL is 0.625 % (761 mg/kg/day, males; 803 mg/kg/day, females). The subchronic toxicity LOAEL is 1.25% (1669 mg/kg/day, males; 1650 mg/kg/day, females), based on significant reductions in body weight gain and food and water consumption.

This study is classified as acceptable/guideline.

B) Chronic Toxicity/Carcinogenicity

Rat Study

In a combined chronic toxicity /carcinogenicity study (MRID 43954301) CDF rats from SASCO, Inc., Madison WI received ortho-phenylphenol, Technical Grade (99.5—100% a.i.; Batch # S-01-93, Mixture of Bayer AG, Leverkusen, Germany and Dow, Midland, Michigan) in the diet for 24 months at dose levels of 0, 800, 4000 and 8000 ppm in males, and 0, 800, 4000, and 10000 ppm in females (39, 200, and 402 mg/kg/day for males for the 800, 4000, and 8000 ppm dose groups and 49, 248, and 647 mg/kg/day for females fat: the 800, 4000, and 10000 ppm dose groups). An interim sacrifice group of twenty animals/sex for control and high dose groups and ten animals/sex for the low and mid dose groups were sacrificed at 12 months. Systemic toxicity was noted as decreased body weights ($p < 0.05$) and body weight gains in both males and females of the mid and high dose groups during the first 13 weeks of- the study (for the 2-year carcinogenicity group). At study termination, only the high dose groups had reduced body weights ($p < 0.05$) and body weight gains. Food consumption was slightly decreased in the 2-year carcinogenicity group in the high dose group at all time points measured and was decreased in the mid dose females at 13 weeks. Food efficiency determined for the first 13 weeks was slightly decreased in the mid dose group and greatly decreased in the high dose, group. There was an increase in observed masses in the urinary bladder of high dose males at 24 months. High dose females had an increased incidence of kidneys with pitted zones at 24 months. Mid and high dose females had an increase in wet/stained ventrum at 12 months and both high dose males and females had a similar observation at 24 months, this was attributed to the urine and red staining

in the perigenital area noted in the clinical observation data. Non-neoplastic observations noted an increase in incidence of calculus in the kidneys in high dose males at the 12 month sacrifice and the 24 month study termination. There was also increased hyperplasia of the urinary bladder at 12 and 24 months in high dose males (and high dose females at 24 months) along with an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in high dose males. High dose males and females also had an increase in cysts of the kidney at 24 months. High dose females had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney. **Based on the results of this study, the Systemic Toxicity NOEL is equal to 800 ppm (39 mg/kg/day for males and 49 mg/kg/day for females and the Systemic Toxicity LOEL is equal to 4000 ppm (200 mg/kg/day for males and 248 mg/kg/day for females) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity.**

This study is classified as Acceptable and satisfies guideline requirements (S83-5) for a combined chronic toxicity/carcinogenicity study in the rat.

Mouse Study

In a carcinogenicity study (MRID# 43545501) B6C3F1 albino mice (50/sex/dose group) from Charles River Laboratory, Portage, MI received ortho-phenylphenol (99.88% a.i.; Lot# 8800005-24, mixture of Dow Chemical Company and Miles, Inc. products) in the diet for 24 months at dose levels of 0 250, 500 and 1000 Mg/kg/day. A satellite group of ten animals/sex/dose group were sacrificed at 12 months.

Systemic toxicity was noted in treated females at 3 months as decreased body weight gain (10-12%), statistically significant but not dose related. At 12 and 24 months there was a 14-25% decrease in body weight gain in males and females of the mid dose and a 27-38% decrease in the high dose groups. Treated females had a slightly reduced food consumption during the first 90 days. Food efficiency for this period was slightly reduced for the male dose groups and variable for the female dosed groups (no dose response effect). At 1 year there was no treatment related effects on food consumption and at 2 years there was a slight increase in food consumption in all treated groups. There was an increase in absolute and relative liver weights at 12 and 24 months in all treated males and females; also, treated males had increased adrenal absolute and relative weights at 24 months. Spleen weights (absolute and relative) in the males and females were reduced in all treated groups. **The Systemic Toxicity LOEL is less than or equal to 250 mg/kg/day and the Systemic Toxicity NOEL lees than 250 mg/kg/day based on increased liver and reduced spleen weights and gross observations in the liver of all treated animals**

This study is classified as Core-Minimum data and satisfies the guideline requirement (83-2b) for a carcinogenicity study in the mouse.

5. Mode of Action

A. Urinary Bladder Tumors

The CARC followed the EPA's final guidelines for Carcinogen Risk Assessment (March 29, 2005) and the ILSI-RSI publication "A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action" (ILSI, 2003) for the evaluation of carcinogenic response in animal studies and in determination of the relevance to humans. The Hill criteria (Hill, 1965) for causation were used as a general guide.

1. Did the registrant provide sufficient evidence to establish a plausible mode of action for bladder tumors in the rat for OPP? The CARC concluded that the data submitted by the registrant was consistent with a non-linear mode of action for the bladder tumors observed in the rat. The CARC agreed with the registrant's statement on bladder tumors that "the oxidative damage to urothelial cells, including macromolecular binding to cellular proteins, caused by the quinone compounds elicits a hyperplastic response in the urinary bladder urothelium. In the continued presence of the quinone cytotoxicants, simple regenerative hyperplasia of the urothelium progresses to papillary and nodular hyperplasia, and then to papillomas and transitional cell carcinoma."

Key Events

A series of key events can be identified from the available data that support a non-linear mode of action for development of bladder tumors in rats from administration of OPP.

1) Shift in biotransformation of OPP at doses above 200 mg/kg/day

The biotransformation of OPP *in vivo* is of interest in that it has been postulated that a shift in the biotransformation of the chemical underlies the tumorigenic response observed in the urinary bladder of male rats from oral administration of the chemical in chronic studies. Biotransformation of OPP initially involves formation of phenolic metabolites (such as 2,4'-dihydroxyphenyl and phenylhydroquinone) in the liver through the action of cytochrome P-450 (demonstrated by Ozawa et al. [Xenobiotica 30(10), 1005-1017, 2000], by rat CYP2C11 and possibly CYP2E1, and human CYP1A2. OPP, phenylhydroquinone, and 2,4'-dihydroxybiphenyl can themselves undergo conjugation reactions through the action of either sulfotransferase or glucuronidation phase II reactions. Phenylhydroquinone can also be converted to phenyl-1,4-benzoquinone by a secondary peroxidase-mediated activation in the kidney and/or bladder involving the prostaglandin endoperoxide synthase (PHS) complex. The involvement of PHS has been suggested on the basis of data submitted to the Office of Pesticide Programs (D203250), where *in vitro* incubations were conducted with microsomal PHS from ram seminal vesicles using ortho-phenylphenol (OPP) or the metabolites phenylhydroquinone (PHQ) and

2-phenyl-1,4-benzoquinone (PBQ). This study demonstrated a role for PHS in conversion of PHQ to PBQ.

The presence of PHS in the bladder epithelium has been proposed by Kolachana et al. (Carcinogenesis 12(1): 145-149, 1991) as possibly responsible for the activation of phenylhydroquinone to reactive intermediates in the bladder and kidney. The generation of PBQ is considered dose-dependent, appearing in increased quantity only at higher (>200 mg/kg/day) doses of OPP. The shift in biotransformation products with increased dose of OPP has been postulated to be associated with the non-linear response observed in tumorigenicity of the urinary bladder, involving oxidative damage to cells and subsequent regenerative hyperplasia. With continued exposure, this process leads to development of tumors.

Support for this is observed from the study of Reitz et al (1983). In this study, single oral doses of 5, 50, or 500 mg/kg bw of [14C]2-phenylphenol (purity, 99.8%; specific activity, 19 mCi/mmol per L) or [14C]sodium 2-phenylphenol (purity, 98.7%; specific activity, 19 mCi/mmol/L) were administered to groups of four male rats, and the urinary metabolites were identified and quantified. At the two lower doses, the major metabolites of both compounds were the glucuronide and sulfate ester conjugates of 2-phenylphenol, and unconjugated 2-phenylphenol and 2,5-dihydroxybiphenyl accounted for < 2% of the total radiolabel recovered in urine at a limit of detection of 1-2%. Nearly identical high-performance liquid chromatograms were obtained for the two compounds. At 500 mg/kg bw, a further metabolite of both compounds was identified, which accounted for 20-30% of the urinary radiolabel and appeared to be a conjugated dihydroxybiphenyl molecule, most likely with glucuronide and/or sulfate groups. The authors hypothesized that this metabolite is formed only at high doses as a result of saturation of normal glucuronide and sulfate ester conjugation pathways. Incubation of [14C]2-phenylphenol with purified microsomes *in vitro* in the absence of conjugating substrates yielded large amounts of a material which co-chromatographed with 2,5-dihydroxybiphenyl. The semiquinone and quinone were not identified in these studies, but their formation was proposed on the basis of the results of similar studies on benzene.

2) Development of hyperplasia of urinary bladder epithelium

In a published study by Niho et al. (2002), rats receiving oral doses of sodium orthophenylphenol at 10,000 ppm and above for 13 weeks were observed with simple and papillary/nodular hyperplasia of the urinary bladder epithelium in increased incidence. Doses below 10,000 ppm (5000, 2500 ppm) produced no increase in this lesion. Administration of ortho-phenylphenol in the diet for 36 weeks to groups of 31 male F344 rats resulted in increased incidence of bladder hyperplasia (Fuji, Nakamura, and Hiraga, 1987).

In a second experiment also by Niho et al. (2002), groups of 50 rats were fed 20,000 ppm sodium ortho-phenylphenol for 12, 24, 52, or 104 weeks. At 12 weeks, no response was observed. At 24 weeks, only a slight increase in hyperplasia was observed in treated rats and a few bladder transitional cell carcinomas observed. At 52 weeks, significant increases in both hyperplasia and transitional cell carcinoma were observed in treated rats.

Christenson et al. (Dow Chemical Corporation, 1996), administered OPP in the diet to groups of 22 male F344 rats/dose at doses of 0, 800, 4000, 8000, or 12,500 ppm (equivalent to 0, 56, 280, 560, and 920 mg/kg/day) for 13 weeks. Urine was collected during weeks 12-13 and 13-14 for analysis of metabolites, and bladders were collected from 12 animals/dose group for analysis of the urothelium by 32P-postlabelling. Simple hyperplasia was observed at 8000 and 12,500 ppm OPP. The glucuronide and sulfate conjugates of OPP and the hydroxylated metabolite phenylhydroquinone were the major urinary metabolites, the major conjugate at all doses being the sulfate conjugate. An increase in labeling index of the bladder epithelium was observed at 8000 and 12,500 ppm OPP, but there was no evidence of formation of DNA adducts by OPP. The conclusions of this study were that the carcinogenicity of OPP in male rats is due to mild cytotoxicity with consequent regenerative hyperplasia mediated by an indirect, dose-dependent cytotoxic effect on the bladder epithelium leading to regenerative hyperplasia and subsequent tumorigenesis of epigenetic origin rather than direct metabolic activation of OPP to reactive metabolites capable of forming DNA adducts.

In a study by Smith et al. (1998), changes in urinary composition, urinary metabolites, cytotoxicity and regenerative hyperplasia and DNA adducts in male rats given OPP in the diet were examined. Groups of rats fed OPP in the diet at 0, 1000, 4000, and 12,500 ppm for 13 weeks showed evidence of cytotoxicity and hyperplasia at the high dose only after 13 weeks. By week 17, there was no evidence of hyperplasia in this group. In a second experiment, doses of 0, 800, 4000, and 12,500 ppm OPP were administered, and 32-P postlabeling experiments as well as metabolite determinations and urinalysis were added to the parameters measured. As in the first experiment, a significant increase in hyperplasia of the urinary bladder was observed in the 12,500 ppm dose group. An increased labeling index was observed in the urothelium of the 8000 and 12,500 ppm dose groups but not at lower doses.

3) Progression of simple hyperplasia to nodular hyperplasia, papilloma, and transitional cell carcinoma with continued exposure to high doses of OPP

Long-term studies of oral administration of ortho-phenylphenol have demonstrated the development of urinary bladder tumors in rats. As mentioned previously, studies by Niho et al. (2002) have demonstrated the progression of the hyperplastic lesion to the tumorigenic response from continued administration of high doses of ortho-phenylphenol. These studies have also consistently demonstrated a threshold response, in that doses below a certain level produce no significant increases in either hyperplasia or neoplasia.

Reversibility of Effects

In a study by Smith et al. (1998), changes in urinary composition, urinary metabolites, cytotoxicity and regenerative hyperplasia and DNA adducts in male rats given OPP in the diet were examined. Groups of rats fed OPP in the diet at 0, 1000, 4000, and 12,500 ppm for 13 weeks showed evidence of cytotoxicity and hyperplasia at the high dose only after 13 weeks. By week 17, there was no evidence of hyperplasia in this group.

B. Liver Tumors

With regard to the mouse liver tumors observed following administration of OPP, the CARC concluded that the positive tumorigenic response was based primarily on the increase in liver adenomas, which were found to be significant at the 500 and 1000 mg/kg/day dose levels. Although there was an increase in incidence of carcinoma at the 500 and 1000 mg/kg/day dose levels, the increase was within historical control range for the strain of mouse, and statistical significance was not identified at these dose levels for the carcinoma incidence. Available data on the effect of OPP on the liver also suggest a non-linear mode involving depletion of glutathione in the development of liver tumors. OPP was shown to cause acute hepatocellular damage after i.p injection of 900 mg/kg to male Fischer rats, and hepatic and renal glutathione were shown to be depleted by OPP by 6 hours post-dose. Pre-treatment with buthionine sulfoximine (gamma-glutamylcysteine synthetase inhibitor) potentiated the renal and hepatic toxicity of OPP (Nakagawa and Tayama, 1989). Using rat hepatocytes, Nakagawa et al. (1992) showed enhancement of cytotoxicity of OPP after addition of diethylmeleate, a glutathione depletor, and protection against cytotoxicity using dithiothreitol, cysteine, N-acetytl-L-cysteine, or ascorbic acid. The CARC considered this information to be limited in support of a non-linear mode of action for development of the liver tumors and could not conclude without additional data that this indeed is the mode of action behind development of the liver tumors.

Summary of Mode of Action

Urinary Bladder Tumors

In summary, the data are sufficient to support a mode of action for development of urinary bladder tumors in male rats from administration of OPP only at high doses. The key events are as follows:

1) High doses of OPP leads to saturation of phase II detoxification enzyme pathways, resulting in increased oxidative metabolites PHQ and/or PBQ. The amount of these oxidative metabolites in the urine of male rats is sufficient to cause cytotoxicity but not direct genotoxicity. Cytotoxicity occurs through oxidative damage to cells.

2) Oxidative damage to the urinary bladder epithelium (including macromolecular binding to cellular proteins) caused by the quinone metabolites elicits a hyperplastic regenerative response in urinary bladder. Continued presence of these quinone metabolites results in the progression of the hyperplastic response to papillary and nodular hyperplasia, and eventually to the urinary papillomas and carcinomas observed in long-term feeding studies. Evidence suggests that there are not sufficient oxidative metabolites generated *in vivo* to result in a genotoxic mode of action, but that a non-genotoxic mode of action is operative.

Liver Tumors

The evidence supporting a non-linear mode of action for liver tumors observed in mice after administration of high doses of OPP is limited, and additional information would be required to establish a mode of action for development of these tumors.

V. WEIGHT-OF-THE-EVIDENCE CONSIDERATIONS

1. Carcinogenicity

Rat

- In male rats, the incidences of urinary bladder papillomas, transitional cell carcinomas, and/or combined papillomas and/or transitional cell carcinomas for the control, 800, 4000, and 8000 ppm dose groups (0, 39, 200, and 402 mg/kg/day), respectively, were as follows:

  Papillomas: 0/69 (0), 1/60 (2%), 0/60 (0), 11/68 (16%)
  Transitional cell carcinomas: 0/70 (0), 0/60 (0), 2/60 (3%), 37/70 (53%)
  Combined: 0/70 (0), 1/60 (2%), 2/60 (3%), 48/70 (69%)

  Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls, for urinary bladder papillomas, transitional cell carcinomas, and papillomas and/or transitional cell carcinomas combined, all at $p < 0.01$. Although historical control data from the testing laboratory were not provided, urinary bladder tumors are considered to be a rare tumor. There is a marginal tumor response for transitional cell carcinomas (3%, not statistically significant, vs 0%, controls) at the mid-dose of 4000 ppm (200 mg/kg/day) which is supported by hyperplasia at this dose as well as the tumors at the high dose. The CARC considered the urinary bladder tumors at the high dose to be treatment-related.

- There were no compound-related increases in tumors in female rats.

- Dosing at the high dose of 8000 ppm was considered to be adequate and not excessive in both sexes. This was based on significantly decreased body weight gains (males, 15% and females, 22%) during the first 13 weeks of the study at the high dose; decreased body weight of 11% and 15% in males and females at week 104 at the high dose respectively; increased incidence of urinary bladder masses in high dose males at 24 months, and an increased incidence of several non-neoplastic lesions in high dose males at 24 months, including bladder calculus, congestion, hemorrhage, nodular hyperplasia, mineralization, and necrosis.

Mouse

- In male mice, the incidences of liver adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 250, 500, and 1000 mg/kg/day dose groups, respectively, were as follows:

  Adenomas: 29/60 (48%), 34/58 (59%), 41/59 (69%), 46/60 (77%)
  Carcinomas: 11/60 (18%), 5/58 (9%), 14/59 (24%), 12/60 (20%)
  Combined: 34/60 (57%), 36/58 (62%), 46/59 (78%), 48/60 (80%)

  Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the controls, for liver adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 500 mg/kg/day dose group with the controls for liver adenomas and adenomas and/or carcinomas combined, both at $p < 0.05$. The incidence of liver adenomas (77%) and combined adenomas and/or carcinomas (80%) at the 500 and 1000 mg/kg/day exceeded the historical control average (30%, adenomas, 42%, combined) and range (4-60%, adenomas; 10-68%, combined) from NTP. Historical control data from the testing laboratory were not provided. The CARC considered the male mouse liver tumors, adenoma driven, at 500 and 1000 mg/kg/day to be treatment-related.

- Female mice had a significant difference in the pair-wise comparison of the 250 mg/kg/day dose group with the controls for liver carcinomas at $p < 0.05$. There were no other statistically significant findings for female mice. The CARC did not consider the liver tumors in female mice to be treatment-related.

- The CARC considered the mid-dose of 500 mg/kg/day dose to be adequate, and not excessive, in both sexes for assessing the carcinogenic potential of OPP based on decreases in body weight gain. At 12 and 24 months, a decrease of 14-25% in body weight gain in males and females at the mid dose of 500 mg/kg/day was observed, and a decrease of 27-38% in body weight gain was observed at the high dose of 1000 mg/kg/day. Accentuated lobular pattern of the liver was observed in male mice at the 500 and 1000 mg/kg/day dose levels at 12 months, and in male and female mice at 24 months. The 1000 mg/kg/day dose, a limit dose, was considered to be excessive by the CARC due to the decreases in body weight gain.

2. Mutagenicity

Based on the available data regarding the mutagenicity of OPP, there is no clear evidence of mutagenicity. Positive results generally seen in cytogenetic assays were associated with excessive cytotoxicity and not related to direct damage to DNA. The proposed mechanism for severe cytotoxicity is oxidative damage which is supported by the

evidence showing the non-linearity of the response for urinary bladder tumors observed in rats. Thus the tumor response observed in the rat studies with OPP is consistent with a threshold effect involving oxidative damage leading to cytotoxicity and not a direct DNA damaging effect. An MOA for the liver tumors seen in mice was not determined at this time.

3. Mode of Action

- In summary, the mode of action supported for development of **urinary bladder tumors** in male rats from administration of OPP only at high doses is as follows:

  ·High doses of OPP leads to saturation of phase II detoxification enzyme pathways, resulting in increased oxidative metabolites PHQ and/or PBQ. The amount of these oxidative metabolites in the urine of male rats is sufficient to cause cytotoxicity but not direct genotoxicity. Cytotoxicity occurs through oxidative damage to cells.

  ·Oxidative damage to the urinary bladder epithelium (including macromolecular binding to cellular proteins) caused by the quinone metabolites elicits a hyperplastic regenerative response in urinary bladder. Continued presence of these quinone metabolites results in the progression of the hyperplastic response to papillary and nodular hyperplasia, and eventually to the urinary papillomas and carcinomas observed in long-term feeding studies. Evidence suggests that a non-genotoxic mode of action is operative.

Liver tumors observed in mice after administration of high doses of OPP have been postulated to be the result of a threshold effect involving the depletion of cellular glutathione and oxidative damage; Bomhard et al. (2002) have postulated a non-genotoxic mechanism of action for development of the liver tumors. However, convincing evidence in support of a mode of action is limited and no conclusion can be reached regarding a mode of action for the development of liver tumors.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC used multiple descriptors for the classification of ortho-Phenylphenol and sodium ortho-phenylphenol.

OPP and SOPP were classified as "Not Likely to be Carcinogenic to Humans" based on convincing evidence that carcinogenic effects are not likely below a defined dose range (i.e, below 200 mg/kg/day). This classification is based on the following: convincing evidence that a non-linear mode of action for bladder tumors was established in rats. High doses of OPP lead to saturation of phase II detoxification enzyme pathways, resulting in increased amounts of the oxidative metabolites PHQ and/or PBQ. The generation of PBQ is considered dose-dependent, appearing in increased quantity only at higher doses of OPP (>200 mg/kg/day). The shift in biotransformation products with increased dose of OPP has been postulated to be associated with the non-linear response observed in tumorigenicity of the urinary bladder, involving oxidative damage to cells and subsequent regenerative hyperplasia. With continued exposure, this process leads to development of tumors. Evidence suggests that a non-genotoxic mode of action is operative.

OPP and SOPP were also classified as "Likely to be Carcinogenic to Humans," based on the presence of urinary bladder tumors in rats and the presence of liver tumors in mice at doses above 200 mg/kg/day. This classification is based on the fact that insufficient data were provided to support a mode of action for the mouse liver tumors. Although the tumors were benign and observed only in one sex at high doses, more data are required for any conclusion to be drawn regarding the mode of action for these tumors.

[*Note:* Although the majority of the CARC voted for the "Not Likely..." classification, several CARC members voted for "Suggestive Evidence of Carcinogenicity." This classification was based on the presence of bladder tumors in male rats with an established mode of action and benign liver tumors in mice in 1 sex (males) seen at the limit dose and one-half the limit dose in a susceptible strain of mice. After additional comments, the CARC concluded that multiple descriptors were appropriate for the classification. Thus, as noted above, OPP is classified "not likely" below the 200 mg/kg/day dose, but "likely to be carcinogenic" above this dose.]

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The CARC noted that although both chemicals are classified as "Likely to be Carcinogenic to Humans" above a defined dose range, quantification of cancer risk is not required since the NOAEL selected for the chronic Reference Dose would address the concerns for the precursor events leading to development of bladder and liver tumors. The non-cancer assessment for OPP established a chronic Reference Dose using a NOAEL of 39 mg/kg/day from

the combined chronic toxicity/carcinogenicity study in rats (MRIDs 43954301, 44852701, 44832201) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity at the LOAEL of 200 mg/kg/day. The NOAEL of 39 mg/kg/day selected for the chronic RfD is sufficiently protective of the key events involved in the carcinogenic mode of action, which are not present at doses below 200 mg/kg/day in the rat and is also protective of liver adenomas occurring at ≥500 mg/kg/day in the mouse. Thus, the precursor events leading to development of bladder and liver tumors are not likely to occur using the NOAEL selected for the chronic RfD value and this value is thus protective against development of tumors.

VIII BIBLIOGRAPHY

Bomhard, E.M., et al. (2002): O-Phenylphenol and its sodium and Potassium Salts: A toxicological assessment. Critical Reviews in Toxicology 32(6): 551-626.

Brunsman, L. 2005. Orthophenylphenol: Qualitative Risk Assessment Based on CDF(F-344)/BR Rat and B6C3F1 Albino Mouse Dietary Studies. May 19, 2005, TXR No. 0053394.

Brusick, D. (2005). Analysis of genotoxicity and the carcinogenic mode of action for orthophenylphenol. Environ and Mol Mutagen 45:460-471.

Christenson et al. (1996): Technical Grade Ortho-phenylphenol: A 32-P Postlabeling study to Examine the Potential for the Formation of DNA Adducts in the Urinary Bladder of the Male Rat. Bayer Ag., Report No. 94-972-AV.

Fukushima, Shoji et al. (1983): Promoting Effect of Sodium o-phenylphenate and o-phenylphenol on two-stage urinary bladder carcinogenesis in Rats. Gann 74: 625-632.

Fukushima, Shoji et al. (1985): Pathological Analysis of the Carcinogenicity of Sodium o-phenylphenate and o-phenylphenol. Oncology 42: 304-311.

Haseman, J.K., Hailey, J.R., and Morris, R.W. (1998): Spontaneous neoplasm incidence in Fischer 344 rats and B6C3F1 mice in two year carcinogenicity studies: a National Toxicology Program update. Toxicol. Pathol. 26(3): 428-441.

Iguchi, S., Takahashi, H., Fujii, T., Fukumori, N., Mikuriya, H., Tada, Y., Yuzawa, K., and Hiraga, K. (1984). Subchronic Toxicity of o-Phenylphenol (OPP) by Food Administration to Rats (Study ID: 88-2-26). Annual Report of Tokyo Metropolitan Research Laboratory. No. 35, pp. 407-415.

Kolachana, P. et al. (1991): Metabolism of phenylhydroquinone by prostaglandin (H) synthase: possible implications in o-phenylphenol carcinogenesis. Carcinogenesis 12(1): 145-149.

McNett, D.A., Timchalk, C., Mendrala, A.L. (1997): Ortho-phenylphenol (OPP): Metabolism of 14C-labelled OPP in B6C3F1 mice and Fischer 344 rats. Unpublished report No. HET K-001024-060 from Toxicological Research Laboratory, Health and Environmental Sciences, Dow Chemical Co., Midland, Michigan, USA.

Murata, M. et al. (1999): Oxidative damage to cellular and isolated DNA by metabolites of a fungicide ortho-phenylphenol. Carcinogenesis 20(5): 851-857.

Nagai, F. et al. (1995): Formation of 8-hydroxydeoxyguanosine in calf thymus DNA treated *in vitro* with phenylhydroquinone, the major metabolite of O-phenylphenol. Carcinogenesis 16(4): 837-840.

Nakagawa, Y. and Tayama, S. (1996): Induction of 8-hydroxy-2'-deoxyguanosine in CHO-K1 cells exposed to phenyl-hydroquinone, a metabolite of ortho-phenylphenol. Cancer Letters 101: 227-232.

Niho, N; Shibutani, M; Toyoda, K; Sato, H; Hirose, A; Imaida, K et al. (2002): Dose- and time-response studies of sodium o-phenylphenate urinary bladder carcinogenicity in rats. Food and Chemical Toxicology 40: 715-722.

Ozawa, S. et al. (2000): Metabolic activation of o-phenylphenol to a major cytotoxic metabolite, phenylhydroquinone: role of human CYP1A2 and rat CYP2C11/CYP2E1. Xenobiotica 30(10): 1005-1017.

Pathak, D.N. and Roy, D. (192): Examination of microsomal cytochrome P450-catalyzed *in vitro* activation of o-phenylphenol to DNA binding metabolite(s) by 32-P postlabeling technique. Carcinogenesis 13(9): 1593-1597.

Quast, J.F., McGuirk, R.J. (1995): Ortho-Phenylphenol: Two Year Dietary Chronic Toxicity/Oncogenicity Study in B6C3F1 Mice. Dow Chemical Company, Midland, Michigan. Submitted under **MRID 43545501**.

Reitz, R.H. et al. (1983): Molecular mechanisms involved in the Toxicity of Orthophenylphenol and its Sodium salt. Chem.-Biol. Interactions 43: 99-119.

Rinde, E. and Dapson, S. 1994. Carcinogenicity Peer Review of Orthophenylphenol (OPP) and Sodium Orthophenylphenol (SOPP). Memo, August 24, 1994. HED Document No. 011196.

Wahle, B.F. and Christenson, W.R. (1996): Technical Grade ortho-Phenylphenol: A combined Chronic Toxicity/Oncogenicity Study in the Rat. Bayer Corporation, Pittsburgh, PA and Dow Chemical Company, Midland, Michigan. Submitted under **MRID 43954301.**