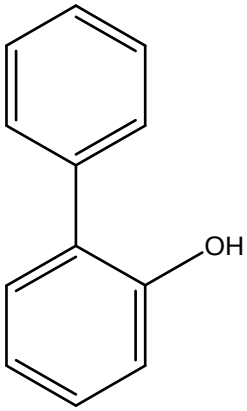
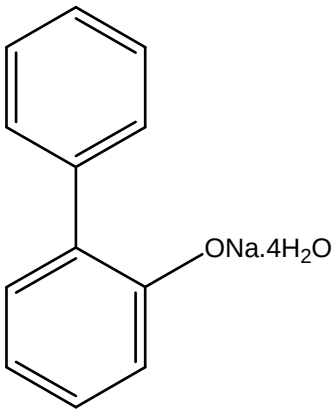
ORTHO-PHENYLPHENOL (OPP)

AND

SODIUM *ORTHO*-PHENYLPHENATE (SOPP)

RISK CHARACTERIZATION DOCUMENT DIETARY EXPOSURE





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LIST OF ABBREVIATIONS AND ACRONYMS

AB 2161	California Assembly Bill 2161
AB 2728	California Assembly Bill 2728
AIC	Akaike's Information Criterion
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase (formerly known as SGPT)
AST	Asparate Aminotransferase (formerly known as SPOT)
ARA	Arachidonic Acid
BBN	N-butyl-N-(4-hydroxybutyl)nitrosamine
BM	Basement Membrane
BMD	Benchmark Dose
BUN	Blood Urea Nitrogen
BrdU	Bromodeoxyuridine
CEC	Critical Exposure Commodity
CSFII	Continuing Survey of Food Intake by Individuals
Cu(II)	Divalent Copper Ion
Cyst	Cysteine
dGMP	Deoxyguanosine 3'-Phosphate Oligonucleotides
2,4'-DHB-S	Sulfate Conjugate of 2,4'-Dihydroxybiphenyl
DEEM	Dietary Exposure Evaluation Model
DNA	Deoxyribose Nucleic Acid
DMBA	7,12-Diemthyl-benz(a)anthracene
DPR	Department of Pesticide Regulation
ED_{10}	10% Effective Dose
ENEL	Estimated No-Observed-Effect Level
F0	Parental Generation
F1 & F2	First and Second Filial Generations, respectively
FANFT	N-[4-(5-nitro2-furyl)-2-thiazolyl]formamide
FDA	Food and Drug Administration
Fe(II)	Divalent Iron Ion (Ferrous Ion)
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
GD	Gestation Day
GLU	Glucose
γ-GPT	gamma-Glutamyl phosphotransferase
GSH	Reduced Glutathione
GSSG	Glutathione Disulfide
Hct	Hematocrit
HGPRT	Hypoxanthine-Guanine Phosphoribosyl Transferase
H_2O_2	Hydrogen Peroxide
J_{max}	Maximum Penetration Rate (Flux)
K_s	Ionization Constant of PSQ
K	Equilibrium constant

K_{oc}	Soil-Adsorption Coefficient
K_{ow}	Octanol-Water Partition Coefficient
K_p	Permeability Coefficient
LC_{50}	Median Lethal Concentration
LD_{50}	Median Lethal Dose
LDH	Lactate Dehydrogenase
LED_{10}	Lower Bound of the ED_{10}
LMS	Linearized Multistage Model
LOD	Limit of Detection
LOEL	Lowest-Observed-Effect-Level
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MOA	Mechanism of Action
MOE	Margin of Exposure
$NaHCO_3$	Sodium Bicarbonate
NH_4Cl	Ammonium Chloride
NO_3	Nitrate Radicals
NOEL	No-Observed-Effect-Level
NTP	National Toxicology Program
NZW	New Zealand White Rabbits
$O_2^{\cdot -}$	Superoxide Anion
O_3	Ozone
OH	Hydroxyl Radicals
OPP	*ortho*-Phenylphenol
OPP-G	Glucuronide conjugate of OPP
OPP-S	Sulfate conjugate of OPP
PAPS	Adenosine 3’-Phosphate-5’-Phosphosulfate
PBQ	Phenylbenzoquinone
PCT	Percent Crop Treated
PDP	Pesticide Data Program
PHQ	Phenylhydroquinone
PHQ^{2+}	Ionized PHQ
PHQ-GSH	Glutathione Conjugate of PBQ
PHS	Prostaglandin H Synthase
P/C	Protein-to-Creatinine
P/N	Papillary and (or) Nodular
POD	Point-of-Departure
PSQ	PHQ Semiquinone Radical
PSQ^-	Ionized PSQ
pKa	Ionization constant
RAC	Raw Agricultural Commodities
RBC	Red Blood Cells
RCD	Risk Characterization Document
RED	Reregistration Eligibility Decision
ROS	Reactive Oxygen Species

SB950	California Senate Bill 950
SCE	Sister Chromatid Exchange
SEM	Scanning Electron Microscopy
SLRL	Sex-linked Recessive Lethal
SOD	Superoxide Dismutase
SOPP	Sodium *ortho*-phenylphenate
TBZ	Thiabendazole
TK	Thymidine Kinase
TP	Total Protein
TPA	12-O-Tetradecanoylphorbol-13-acetate
TSCA	Toxic Substances Control Act
UDPGA	Uridine-5'-Diphosphoglucuronic Acid
UDS	Unscheduled DNA Synthesis
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
V_{max}	Maximum Velocity of an Enzyme Reaction

I. EXECUTIVE SUMMARY

Introduction *ortho*-Phenylphenol (OPP) and its sodium salt, sodium *ortho*-phenylphenate (SOPP), are fungicides for the post-harvest-treatments of fruits and vegetables, among other uses (e.g., disinfections and preservatives). The purpose of this dietary risk characterization document (RCD) is to evaluate the potential health hazards caused by OPP and (or) SOPP residues in food, because of their use for the post-harvest treatments, and any adverse effects identified in chronic, oncogenic, genetic, reproductive, and developmental studies in experimental animals (i.e., rats, mice, dogs, and [or] rabbits). An addendum to this document will address the health risk associated with exposures from other sources (e.g., ambient air, drinking water, occupational, and residential).

Toxicology Profile Available studies at low doses indicated that the pharmacokinetics of OPP in rats is similar to humans. In rats, absorption of OPP was almost complete and urine was the major excretion route of absorbed OPP followed oral exposure. Enzyme-mediated conjugation and oxidative reactions that resulted in biologically inactive products (e.g., sulfates and glucuronides) and reactive metabolites (e.g., phenylhydroquinone [PHQ] and phenylbenzoquinone [PBQ]) *in vitro* also occurred *in vivo*. Evidence indicates that nonenzymatic autoxidation of PHQ that produced biologically active metabolites *in vitro* may also occur *in vivo*. Under single and repeated dosing conditions, results in rats showed that conjugation reactions of OPP and PHQ shifted from mainly sulfation at the lower doses and glucuronidation at the higher doses. Unlike the conjugated form, unconjugated PHQ occurred only under the repeated dosing condition and the unconjugated PHQ formation followed a linear kinetics with its concentrations increased dose-dependently over the dose-range tested. Consistent with the sex-related difference in the xenobiotic enzyme expression *in vitro*, results from rats showed that males exhibited a greater ability to metabolize OPP than females *in vivo*; accordingly, the amount of unconjugated PHQ in urine was markedly higher in males than females.

Oral acute toxic effects of OPP in rats were ataxia and reduced body weight gain. At or near the median lethal dose (LD_{50}: 924-2700 mg/kg), OPP and (or) SOPP also produced the following clinical observations: rales, tussis, diuresis, depression, exophthalmos, lacrimation, ptosis, and abdominal inflation in rats, and a decrease of spontaneous movement, staggering gait, low respiratory rate, and depigmentation of hair in mice. Other effects that OPP induced in these species were damage in the liver, kidneys, lungs, and alimentary canal.

Under subchronic and chronic dosing protocols, OPP and SOPP affected the rat's kidneys and urinary bladder and SOPP induced tumors after exposure for as little as 13 weeks. The kidney effects included increased organ weight, reduced renal function, and elevated histologic-lesion incidences (nephritis, pelvis and [or] papilla hyperplasia, renal tubular proliferation, and renal tubular dilation). The bladder effects included increased organ weight and elevated histologic-lesion incidences (epithelial cell proliferation,

simple hyperplasia, papillary or nodular (P/N) hyperplasia [tumor precursor], papilloma [benign tumor], and carcinoma [malignant tumor]). SOPP favored the excretion of alkaline urine in rats and exhibited a higher tumorigenicity in the urinary bladder than OPP. Co-exposure to thiabendazole (TBZ), another fungicide used with SOPP for the post-harvest treatment, enhanced the tumorigenic effect of SOPP whereas co-exposure to $NaHCO_3$, an urine alkalinizing agent, enhanced the tumorigenic effect of OPP. In the chronically dosed rats, the kidney effects were greater in incidence and severity in females than males but the reverse was true for effects in the bladder. More than one strain of rats (i.e., F344 and Sprague-Dawley) exhibited these kidney and bladder effects of OPP. Other organs that OPP and (or) SOPP affected under chronic dosing protocols were eyes (including the optic nerves), spleen, heart, and pancreas (female only).

In chronically dosed mice, OPP and SOPP affected the kidneys and liver. The kidney effects included increased organ weight, reduced renal function, and elevated histologic-lesion incidences (tubular epithelium degeneration and necrosis, ductular epithelium degeneration and necrosis, tubular dilation, and papilla transitional cell necrosis and [or] pelvis cell debris). The liver effects included increased organ weight and elevated histologic-lesion incidences: focal necrosis, anisonucleosis, and pigment deposition in liver cells and phagocytes, foci of eosinophilic liver cells (tumor precursor), hepatocellular cancers (adenoma [benign tumor], carcinoma [malignant tumor], and hepatoblastoma [a variant of carcinoma]), and hemangioma (circulatory system tumor). The total numbers of liver tumors (i.e., liver-tumor multiplicity) also increased with dose. Other organs that OPP and (or) SOPP affected were spleen and heart (females only). Co-exposure to sodium bicarbonate or TBZ enhanced the renal and splenic toxicity of OPP.

Genotoxicity studies of OPP, SOPP, and their metabolites are available from Registrants and open literature. Many of the Registrant-submitted studies showed weak or negative genotoxicity. However, *in vivo* and *in vitro* studies published in the open literature supported the genotoxic potential of OPP and SOPP, and their metabolites. The evidence from these studies should not be ignored. Also, because different assays provided different information for the genotoxicity, it is not appropriate to dismiss positive studies simply by the relative number of negative *versus* positive reports. Collectively, the positive genotoxicity studies showed that OPP and SOPP induced gene mutations in eukaryotic systems *in vitro* without metabolite activation and caused more severe damage to chromosomes and DNA with than without metabolic activation *in vitro*. Regarding the *in vivo* studies, rats dosed orally and repeatedly with OPP or SOPP produced micronuclei (due to chromosomal breakage and loss), DNA breaks, and (or) DNA adducts in the urinary bladder (i.e., target organ). *In vitro* studies showed that PHQ and PBQ, metabolites of OPP and SOPP, possess clastogenic and aneugenic effects (i.e., breakage and loss) in chromosomes and abilities to induce covalent DNA adducts, single strand breaks, oxidation, and sister chromatid exchange irrespective of metabolic activation (i.e., they are biologically active). Also, *in vitro* evidence indicated that increased pH and metal ion concentrations enhanced the genotoxicity of PHQ, possibly *via* their promoting effects on PHQ autoxidation, with the resultant PBQ exhibited oncogenic potential in mammalian cells. Taken together, reactive species produced *via*

pH- and metal ion-dependent PHQ autoxidation may play an important role in the genotoxicity of PHQ. By corollary, a combination of the reactive species derived from different enzymatic and non-enzymatic pathways may be responsible for the enhanced genotoxic effects of OPP with metabolic activation *in vitro*, and the genotoxicity of OPP and SOPP *in vivo*. The overall data indicated that OPP and SOPP have genotoxic potential, and their metabolites may contribute to their genotoxicity *in vivo*.

Reproductive toxicity studies showed that while OPP did not negatively affect reproductive functions in rats, it produced effects in the pups: reduced pup weight on lactation days 14 and (or) 21 and stunting.

OPP and SOPP induced developmental toxicity in the rat, rabbit, and mouse. In these studies, maternal toxicity was either not observed (rabbits) or appeared to be minimal (mice) at the lowest dose whereat their fetuses exhibited some developmental effects: resorptions in OPP-treated rabbits and cleft palate in SOPP-treated mice.

Risk Assessment (1) Hazard Identification DPR uses two approaches to characterize the dietary risk associated with exposure to OPP and SOPP in humans: margin-of-exposure (MOE) for the acute and chronic exposures and excess cancer risk for the lifetime exposure. The MOE is a ratio of exposure-specific critical No-Observed-Effect-Level (NOEL) derived from an experimental toxicity study and an estimated human exposure using a computer program; i.e., Dietary Exposure Evaluation Model (DEEM™).

For evaluating the toxicity due to acute exposure, this assessment used two critical NOELs: 25 mg/kg/day for characterizing health risk in women in their childbearing years (i.e., effect that occurred only during pregnancy) and 150 mg/kg/day for characterizing health risk in the general population including infants, children, and adult males. The corresponding toxicological endpoints that DPR determined as the most critical (i.e., "sensitive") were resorption in rabbits (i.e., *in utero* effect) and ataxia and decreased body weight gain in rats (acute toxic effects). For the chronic exposure, this assessment also used two critical NOELs: 39 mg/kg/day for characterizing health risk in the males and 4.9 mg/kg/day for characterizing health risk in the females. The toxicological endpoints for the former were clinical pathology associated with the polydipsia and kidney effects and simple hyperplasia in the urinary bladder of rats whereas the later was cardiac degeneration and (or) fibrosis (i.e., cardiomyopathy). The use of two critical chronic NOELs is based on the effects in rats (i.e., kidney, bladder, and heart) and mice (i.e., heart) that showed different sensitive endpoints to OPP in males and females. The evidence to support the critical acute and chronic NOELs is consistent with different animal studies exhibiting similar effects within a comparable dose range.

For the cancer risk assessment, DPR determined that OPP and SOPP are "likely to be carcinogenic in humans" based the following weight of evidence evaluation: (1) occurrence of tumors in multiple animal species, strains, sexes, and sites, (2) genotoxic effects of OPP in mammalian cell systems, (3) abilities of OPP and its metabolites (e.g., PHQ) to interact with DNA *in vitro* and *in vivo*, (4) dose-dependent conversion of OPP to

PHQ, (5) potential relevance of the rat urinary bladder carcinogenic MOA to humans. The bases for applying low-dose extrapolation model for characterizing human health risk associated with OPP exposure are: (1) genotoxic mode of action (MOA) is more plausible than non-genotoxic MOA for bladder carcinogenicity of OPP in rats and (2) the MOA for mouse liver tumors is not known. Also, the low-dose extrapolation approach adopted by DPR is consistent with the USEPA Guidelines of Carcinogen Risk Assessment. That is, "*linear extrapolation is used as a default approach for characterizing the cancer risk when the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data*" (USEPA, 2005). DPR calculated a human equivalent potency slope factor at the BMD of 10% response (ED_{10}, LED_{10}) from modeling rat urinary bladder tumor incidence data. The best estimate of potency slope was 0.0017 (mg/kg/day OPP)$^{-1}$ and its 95th upper bound was 0.002 (mg/kg/day OPP)$^{-1}$. The use of rat tumor data for the low-dose extrapolation is justified based on the similarity of OPP pharmacokinetics in rats and in humans.

(2) Exposure Assessment The dietary exposure (and the subsequent risk estimates) covered the average U.S. population and 15 selected population subgroups. The criteria used for classifying each of these subgroups were geographic region, gender, ethnicity, or age. DEEM™ estimates the exposure using user-input residue data and food consumption pattern; the latter was based on data generated by the United States Department of Agriculture (USDA) during the 1994-1998 Continuing Survey of Food Intake by Individuals (CSFII). The residue data consisted of both estimated and measured values; the latter came from multi-year monitoring program by the U.S. Department of Agriculture's (USDA's) Pesticide Data Program (PDP). In this acute and chronic exposure assessment, DPR assume that OPP is the main chemical form for human exposure to OPP- and SOPP-treated RAC.

the high pKa value of OPP and the poorly buffered environment employed during the application of SOPP. For OPP, DPR presents the acute exposure as point estimate at the 95th, 97.5th, and 99th percentiles. At the 95th percentile, the estimated acute exposure range was 0.007-0.04 mg/kg/day whereas at the 97.5th and 99th percentiles, the exposure ranges were 0.013-0.07 mg/kg/day and 0.025-0.117 mg/kg/day, respectively; in all cases, the highest dietary exposure occurred in Children 1-2 yrs. The chronic exposures ranged from the lowest in Nursing Infants (0.051 μg/kg/day) to the highest in Children 1-2 yrs (0.417 μg/kg/day).

(3) Risk Characterization Both acute and chronic dietary exposures resulted in MOEs greater than 100, a value that is currently considered sufficient for protection for non-oncogenic effects. For the acute exposure, the MOEs ranged from ~10^3-10^4 at the 95th, 97.5th, and 99th percentiles. For the chronic exposure, MOEs for all population subgroups were >10^4. The excess cancer risk due to OPP exposure (i.e., 2.4×10^{-7}) was less than an upper bound value of 1×10^{-6} (i.e., less than one case in a population of a million), a risk value that is generally considered negligible.

Risk Appraisal The dietary risk assessment for OPP and SOPP contains uncertainties based on the limited toxicological and exposure data. For hazard identification, DPR selected acute NOELs from multiple-day oral developmental toxicity studies due to the lack of suitable single-day toxicity study. The acute dietary risk overestimation may occur if the NOELs selected from the developmental toxicity studies were based on endpoints that resulted from repeated rather than acute exposures for OPP. An additional area of uncertainty was the magnitude of the chronic NOELs for males and females. For the males, the NOEL was based, in part, on histopathology. The results of a subchronic study showed that more animals showed lesions by scanning electron microscopy (SEM) than by light microscopy. For the females, the NOEL was estimated (i.e., extrapolated) by applying a 10-fold uncertainty factor (UF) to the LOEL. The large response observed at the LOEL, however, may require a larger UF for the LOEL-to-NOEL extrapolation. Another issue for consideration is intraspecies variation in humans. Studies have shown that some humans exhibited sub-optimal Phase II detoxification and hence, potentially increased sensitivity to the effects of OPP.

For human dietary exposure assessment, the uncertainties included completeness of the food residue database, use of surrogate residue (i.e., estimated) data, and measurement errors (sampling and or reporting). Other areas of uncertainty are the assumptions of no exposure from commodities without legally established tolerance or co-exposure to other pesticides at concentrations that may modify the toxic effect of OPP or SOPP (e.g., thiabendazole); however, evaluation of their impact on the exposure estimates may require further investigation. Otherwise, there were no indications that any of the uncertainties substantially affected the exposure assessment of OPP.

Issues related to the Food Quality Protection Act The Food Quality Protection Act (FQPA) mandated the United States Environmental Protection Agency (USEPA) to address several issues in their risk assessment: potential increased sensitivity of infants and children, aggregate exposure from multiple routes, cumulative exposure from multiple sources, and potential for endocrine disruption.

USEPA recommended FQPA factor be reduced to 1x for OPP based on the findings that "the available developmental and reproductive toxicity studies showed no evidence of increased toxicity to offspring at the same or lower doses as those causing parental/systemic toxicity or evidence of more severe toxicity relative to parental/systemic toxicity." In contrast, the DPR analysis indicated that in a rabbit developmental toxicity study, the maternal toxicity was not observed at the lowest dose whereat fetal effects (resorption) were identified.

Of the three remaining issues, USEPA conducted detailed evaluations on only two: aggregate risk and endocrine disruption potential. Because OPP and SOPP have residential and occupational uses, aggregate exposure is possible, and DPR will evaluate data from these sources of exposure when they become available. Limited *in vitro* studies evaluated by USEPA and DPR demonstrated that OPP has some potential to act as an endocrine disruptor. For cumulative risk, USEPA assumed that OPP does not have a common mechanism of toxicity with other pesticides.

Tolerance Assessment (Acute Exposure Only) An acute-tolerance assessment for a single label-approved commodity evaluates the health protectiveness of the tolerance for that commodity. DPR conducted the tolerance assessment for foods with a significant impact on dietary exposure, a high consumption by young children, and major uses in California. The results indicated that MOEs at the 95th percentile were above the benchmark of 100 in different population subgroups for the analyzed foods.

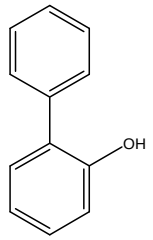
Conclusion This dietary health risk assessment indicates that no significant health concern exist (including cancer) due to the exposures to foods with legally allowed residues of OPP and SOPP under acute and chronic conditions.

II. INTRODUCTION

As required by AB2161 (Food and Agricultural Code Section 13134), the purpose of this dietary risk assessment document is to evaluate the potential health hazards caused by OPP and (or) SOPP residues in food, because of their use as fungicides for the post-harvest treatments. Because of the potential adverse effects identified in chronic, oncogenic, genetic, reproductive, and developmental studies in experimental animals, Department of Pesticide Regulation (DPR) of the California Environmental Protection Agency (Cal EPA) listed OPP as a high priority active ingredient for risk assessment. The state of California listed OPP and SOPP as chemicals known to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 ("Proposition 65") and added OPP to the state toxic air contaminants list under AB 2728. Also, the Carcinogenicity Peer Review Committee of the United States Environmental Protection Agency (USEPA) classified OPP and SOPP as a Group B2 carcinogen (i.e., probable human carcinogen) (Rinde and Dapson, 1994). Recently, the USEPA's Office of Pesticide Programs published a risk assessment for OPP for Reregistration Eligibility Decision (RED) assessment (USEPA 2006) wherein the Agent's Cancer Assessment Review Committee (CARC) (Kidwell, 2005) concluded that OPP and SOPP are "not likely to be carcinogenic to humans" below 200 mg/kg/day but "likely to be carcinogenic to humans" above 200 mg/kg/day.

II.A. PHYSICAL AND CHEMICAL PROPERTIES

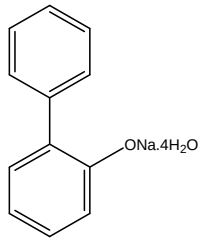
IUPAC	:	2-Biphenylol
Synonyms	:	*ortho*-phenylphenol; 2-hydroxybiphenyl
Trade Name	:	Dowicide 1; Dowicide 1E; Preventol O Extra
CAS Registry No	:	90-43-7
Structural Formula	:	



Empirical Formula	:	$C_{12}H_{10}O$	
Molecular Weight	:	170.21	
Melting Point	:	57^oC	(Dean, 1987)
pKa	:	9.55	(Dean, 1987)
Water Solubility	:	0.08 g/100g (25^oC)	(Dow, 2005)
Vapor Pressure	:	0.0017 mmHg (25^oC)	(Dow, 2005)
log K_{ow}	:	3.09-3.36	(Mackay *et al.*, 2000)
log K_{oc}		2.70-2.97	(Mackay *et al.*, 2000)

IUPAC	:	2-Biphenylol, Sodium Salt
Synonyms	:	*ortho*-phenylphenol, sodium salt

Trade Name	:	Dowicide A
CAS Registry No	:	132-27-4
Structural Formula	:	



Empirical Formula	:	$C_{12}H_{10}ONa.4H_2O$	
Molecular Weight	:	264.3	
Specific Gravity	:	1.3	(Dow, 2005)
Water Solubility	:	120 g/100g (25°C)	(Dow, 2005)
pH of Saturated H_2O	:	12.0-13.5 (25°C)	(Dow, 2005)
Vapor Pressure	:	N/A	
log K_{ow}	:	N/A	

Abbreviation: N/A, not available.

II.B. CHEMICAL IDENTIFICATION

OPP and SOPP are board-spectrum antimicrobial agents. The agricultural industry uses SOPP mainly to eradicate or inactivate pathogens on the surfaces of fruits and vegetables after harvest (i.e., post-harvest treatment) (IARC, 1983, 1999). The consumer-product industry uses OPP and (or) SOPP for formulating household- and institution-disinfectants and for preserving consumer products (Grossman, 1995). The pesticide manufacturing industry used SOPP as an inert ingredient in their product formulations (<2% in amount): turf-, garden-, and ornamental-insecticides and herbicides, insect repellent for pets, and indoor/outdoor crack and crevice insecticides (USEPA, 2006). Evidence for a clear understanding of the biocidic mechanism of OPP and SOPP is not available. However, experimental studies in mammalian cells showed that OPP induced a direct toxic effect (e.g., reduced viability) in rat hepatocytes *in vitro* (Nakagawa *et al.*, 1992a,b, 1993), and the investigators speculated that the effect was attributable to the disturbance of mitochondrial respiration and the depletion of protein and nonprotein thiols (e.g., enzymes and glutathione).

II.C. USE AND PRODUCT FORMULATION

USEPA established tolerances that allow the agricultural industry to use OPP and SOPP for the post-harvest treatment of 22 raw agricultural commodities (RAC) (40 CFR 180.129, 2005). The treatment methods are: dipping foam or solution (e.g., carrot, cucumber, cantaloupes, kiwi fruit), flooding (e.g., pineapple), and water spray or water-wax emulsions (e.g., citrus fruits) (Dezman *et al.*, 1986, Papadopoulou-Mourkidou,

1991). In California, the use of OPP and SOPP reported by DPR was 14-70 thousand pounds during 1991-2005, and the post-harvest application accounted for approximately 34% of the use in 2005 (the latest use data available) (DPR, 2006). Also, the DPR database indicates that there are 167 OPP- and SOPP-containing products available in California, with ~90% being disinfectants (DPR, 2006). The disinfectant-product forms include dust/powder, emulsifiable and flowable concentrates, granules/flake, impregnated materials, pressurized liquid/foggers/sprays, solution/liquid (ready-to-use), and aqueous concentrates.

II.D. ENVIRONMENTAL FATE

Because of their diverse applications, OPP and SOPP may potentially enter into the air, water, and (or) soil. For example, disposal of OPP- and SOPP-containing disinfectants would allow their release into aquatic environments from the wastewater storage and treatment facilities (e.g., residential septic tank and wastewater treatment plants, respectively) (Wick and Gschwend, 1988a,b; Ternes *et al.*, 1998). In the environment, the reactions in air, water, and soil and the transport among these environmental media determine the fate of OPP and SOPP.

In the air, OPP exists primarily in the gas phase because of its high vapor pressure (i.e., 0.0017 mmHg) (Bidleman, 1988); one of the potentially important degradation pathways for consideration is photolysis. However, analysis conducted by DPR indicated that the photolysis rate may be slow because OPP can only use a narrow range of the energetic sunlight for the reaction (Gore *et al.*, 1971**,** Sarakha *et al.*, 1993). Other potential atmospheric degradation pathways of OPP are hydroxyl (OH) radical reaction during the day, nitrate (NO_3) radical reaction during the night, and ozone (O_3) reaction throughout the day (Atkinson, 1991, 1994). In the absence of experimental data, DPR predicted that, using a structure-activity relationship developed by Atkinson (1987), the rate constants of OPP reactions with OH, NO_3, and O_3 were 5.8×10^{-11} cm^3 $molecule^{-1}$ s^{-1}, 1.37×10^{-11} cm^3 $molecule^{-1}$ s^{-1}, and 2.6×10^{-19} cm^3 $molecule^{-1}$ s^{-1}, respectively. Based on these estimated reaction rate constants and the concentrations of OH, NO_3, and O_3 in the urban atmosphere[1], DPR estimated the overall atmospheric lifetime[2] (τ) of OPP was 0.04 hr (or half-life = 0.03 hr)(Atkinson, 1986; 1994).

In the water, potentially important degradation pathways of OPP and SOPP for consideration include photolysis, hydrolysis, and biodegradation. Under the summer-sun of Woburn, MA, Wick and Gschwend (1998a) determined that the degradation half-life of OPP in pure oxygenated water was 44.3 hr. Similarly, based on the aqueous reaction rates of phenol with OH and RO_2 radicals, Howard *et al.* (1991) speculated that the half-lives of OPP in surface water would range from 66 to 3840 hr. Regarding the biodegradation, Wick and Gschwend (1998a) showed that the degradation rates of OPP ranged from 16.5 to 38.4 hr in the spring, summer, and fall but was negligible in winter.

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[1] OH radical = 1.6×10^6 molecule/cm^3, 12 hr average; NO_3 radical = 5.0×10^8 molecules/cm^3, 12 hr average; and O_3 = 2.4×10^8 molecule/cm^3, 24 hr average (Atkinson, 1986, 1994).

[2] Life-time is defined as time to decay to a concentration of 1/e (=0.368) of that initially present.

In the study by Gonsior *et al.* (1984), OPP in river water samples at 20±0.5^{o}C had half of it removed within 168 hr. In the same study, OPP incubated with acclimated and non-acclimated activated sludge had half-lives of 3 hr and 24 hr, respectively.

In the soil, Zbozinek (1984) reported microbial degradation of OPP. Using this information, Howard *et al.* (1991) estimated that half-life of OPP in the soil ranged from 24-168 hr.

Besides the physical and chemical loss processes, OPP can undergo intermedia transport through various diffusive and non-diffusive processes (e.g., deposition). Following the modeling approach of Mackay *et al.* (2000), Equilibrium Criterion Multimedia Environmental Model predicted that OPP would reside mainly in the medium to which it was discharged initially, based on the physical and chemical properties of OPP (i.e., estimated half-lives in the air, water, and soil, water solubility, vapor pressure, melting point, and log K_{ow}). The model also predicted that the rates of reaction in air, water, or soil were 2-3 orders of magnitude higher than that of inter-media transport. Taking these data altogether, OPP would not be persistent in the environment and its potential to undergo inter-media transport would not be of environmental significance.

III. TOXICOLOGY PROFILE

III.A. PHARMACOKINETICS

Summary: Pharmacokinetics data of OPP and SOPP are available in the rat, mouse, dog, cat, lactating goat, and human. Following the oral exposure, the amount of OPP absorbed was almost complete in rats (~85%), mice (~90%), and lactating goats (~80%); rats also absorbed SOPP to a similar extent. By contrast, the amount of OPP absorbed was ~50% in dogs and ~35%, in cats. Residual tissue radioactivity was low in rats and lactating goats after the single or repeated oral applications of radiolabeled OPP. The available data in [^{14}C]-OPP-treated cats and dogs were not sufficient to determine quantitatively the amount of radiolabel accumulated by tissues.

Multiple pathways transformed OPP into a large number of products as observed *in vitro*. The pathways mediated *via* rat liver microsomes or hepatocytes included oxidation, redox-recycling, sulfation, glucuronidation, and adduction with macromolecules; the products identified were phenylhydroquinone (PHQ), phenylbenzoquinone (PBQ), sulfate and glucuronide conjugates of OPP (OPP-S and OPP-G), glucuronide conjugate of PHQ (PHQ-G), PHQ glutathione (PHQ-GSH), and superoxide anions (i.e., reactive oxygen species [ROS]). PHQ *via* extra-hepatic metabolism (e.g., prostaglandin H synthase) and non-enzymatic autoxidation also produced PBQ. Human liver enzymes mediated the sulfation and oxidation of OPP *in vitro*; however, the enzymes showed no measurable activity for the glucuronidation of OPP.

In vivo, OPP-treated cats and dogs produced the same urinary metabolites found in rats and mice. However, in cats, the major urine constituent was unchanged OPP whereas in dogs, the major constituent was conjugated OPP (sulfate and glucuronide). Other metabolites also found in rats and mice were PHQ-G, PHQ-S, 2,4'-DHB-S (2,4'-dihydroxybiphenyl; rat only), unconjugated PHQ, and unconjugated PBQ. Enzyme-mediated conjugation and oxidative reactions that resulted in the formation of biologically inactive products (e.g., sulfates and glucuronides) and reactive metabolites (e.g., PHQ and PBQ) *in vitro* also occurred in OPP-treated rats *in vivo*. Evidence indicates that nonenzymatic autoxidation of PHQ that produced biologically active metabolites *in vitro* may also occur *in vivo*. Under single and repeated dosing conditions, results in rats and mice showed that conjugation reactions of OPP and PHQ shifted from mainly sulfation at the lower doses to glucuronidation at the higher doses, possibly after phenolsulfotransferase saturation. Unlike the conjugated form, unconjugated PHQ occurred only under the repeated dosing conditions and the unconjugated PHQ formation followed a linear kinetics with its concentrations increased dose-dependently over the dose-range tested. Consistent with the sex-related difference in the xenobiotic enzyme expression of rats *in vitro*, males showed a greater ability to metabolize OPP than females; accordingly, the amount of unconjugated PHQ in urine was markedly higher in males than females. Urine was the major excretion route of absorbed OPP in the species examined.

Dermal absorption of OPP also was studied in the rat and human. After a 4-hr dermal application of 4 μg/kg, rats absorbed ~43% of OPP. In humans, the percent of dermal absorption was ~43% at 6 μg/kg for 8 hr. The vehicle for the dose delivery had a modifying effect on OPP absorption by the skin. Only humans had metabolism data available from the dermal exposure. The metabolites identified were the same as in rats dosed orally with OPP. Also, evidence suggested that products from other reaction pathways (e.g., oxidation) in addition to OPP conjugates may have occurred at a higher dose, and sulfation was the dominant detoxification pathway of OPP at the low dose in humans.

III.A.1.a. Oral – Rat

Numerous *in vitro* and *in vivo* studies on the pharmacokinetics of OPP, SOPP, and their metabolites (e.g., phenylhydroquinone [PHQ] and phenylbenzoquinone [PBQ]) are available in the open literature. They are briefly described below.

Absorption

Nakao *et al.* (1983) reported that, in male F344 rats that received a single gavage dose of [^{14}C]-OPP (amount not specified), peak plasma radioactivity occurred within 1 hr. Reitz *et al.* (1983), Sato *et al.* (1988) and Bartels *et al.* (1998) treated male F344 rats with a single dose of radiolabeled OPP or SOPP (28-500 mg/kg and 250-500 mg/kg, respectively) and detected ~85% of the administered ^{14}C in urine at 24 hr. Bartels *et al.* (1998) also reported 86% urinary recovery of radiolabel in female F344 rats within 24 hours of receiving 27 mg/kg [^{14}C]-OPP. Viewing these data altogether, the absorption of OPP and SOPP from the gastrointestinal tract are rapid and almost complete.

Distribution

In the only available tissue distribution study in male F344 rats, Sato *et al.* (1988) found no difference in the radiolabel content in tissues between a single gavage dose of equimolar [^{14}C]-OPP and -SOPP. For both, the administered radiolabel amounted to <8% at 24 hr and <1% at 7 days in the tissues examined (i.e., adipose, intestine, liver, kidneys, blood, urinary bladder, stomach, and brain), indicating that the accumulation of OPP, SOPP, and (or) their metabolites by the rats was insignificant.

Metabolism

This section summarizes studies investigating the *in vitro* and *in vivo* metabolism of OPP and its metabolites.

In Vitro Studies Three types of *in vitro* metabolism study are available: hepatic, extra-hepatic, and non-enzymatic. Regarding the hepatic metabolism, incubation of OPP with rat liver microsomes produced phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) (Nakagawa and Tayama, 1989; Roy, 1990). Based on this

finding and chemical properties of quinoids, Roy (1990) proposed that OPP was sequentially oxidized, first to PHQ then *via* an intermediate PHQ-semiquinone radical (PSQ) to PBQ, with superoxide anion ($O_2^{\cdot-}$) as a co-product (Figure 1). The investigators also proposed a redox recycling between PHQ and PBQ in which PBQ was reduced by cytochrome P-450 reductase (with NADPH as a cofactor) back to PHQ, possibly, *via* PSQ (Roy, 1990). Other transformation pathways observed *in vitro* included glucuronidation, sulfation, and adduct formation (Wiebkin *et al.*, 1978; Nakagawa and Tayama, 1989; Nakagawa et al., 1992b). The metabolites identified in the presence of rat liver microsomes or hepatocytes were OPP-glucuronide (OPP-G), PHQ-glucuronide (PHQ-G), OPP-sulfate (OPP-S), and PHQ-GSH conjugate[3]. Wiebkin *et al.* (1978) found that sulfation was a more important Phase II reaction of OPP than glucuronidation at the low concentration (7 μM) whereas the reverse was true at the high concentration (50 μM). This dose-dependent shift in the Phase II metabolism may have been due to the saturation of phenolsulfotransferase rather than the depletion of substrate (Koster *et al.*, 1981).

For extra-hepatic metabolism of OPP and PHQ, two *in vitro* studies involving prostaglandin H synthase (PHS)[4] are available (Kolachana *et al.*, 1991; Freyberger and Degen, 1998). Kolachana *et al*, (1991) found that PHQ was co-oxidized with arachidonic acid (ARA) by PHS, with PBQ as a reaction product. In contrast, Freyberger and Degen (1998) reported that PHS only poorly oxidized OPP. Given that PHS is found at high levels in different rat tissues including urinary bladder, Kolachana *et al.* (1991) suggested that the PHS-mediated bioactivation of PHQ could effectively occur *in vivo*. However, Freyberger and Degen (1998) questioned this conclusion based on their finding that OPP, PHQ, and PBQ inhibited the activity of PHS-cyclooxygenase and therefore, the ARA-mediated PHQ-oxidation. The investigators further argued that considerable inhibition of PHS-cyclooxygenase could occur *in vivo*, because of the presence of high OPP and PHQ concentrations in the urine of rats exposed to OPP or SOPP (Hasegawa *et al.*, 1991).

In addition to the extra-hepatic metabolism, Kwok and Eastmond (1997) showed that PHQ could undergo nonenzymatic autoxidation *in vitro*. Based on the chemical properties of PHQ, the investigators proposed two different pathways over the pH range commonly found in rat urine (i.e., pH 6.3-7.6) (Figure 1). The first was an oxygen-dependent pathway wherein PHQ reacted sequentially with oxygen to form PSQ and then PBQ. In each of the reaction steps, $O_2^{\cdot-}$ was formed as a co-product. In the second pathway, ionized PSQ disproportionated to form PBQ and $O_2^{\cdot-}$. Accordingly, the formation of PBQ and $O_2^{\cdot-}$ increased with increasing pH. Because oxygen tension in body fluids is expected to be low, the investigators proposed that the second (i.e., pH-dependent) pathway may be the major mechanism of PHQ autoxidation *in vivo*.

<u>*In Vivo* Studies</u> Studies investigating the metabolism of OPP and SOPP in F344 rats (both sexes) found evidence of glucuronidation, sulfation, and oxidation in

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[3] PHQ-GSH conjugate was found to be a non-enzymatic reaction product between PBQ and GSH (Nakagawa and Tayama, 1989).

[4] PHS was found in the rat urinary bladder and kidney medulla; the enzyme also has both the activities of cyclooxygenase and peroxidase (Freyberger and Degen, 1998).

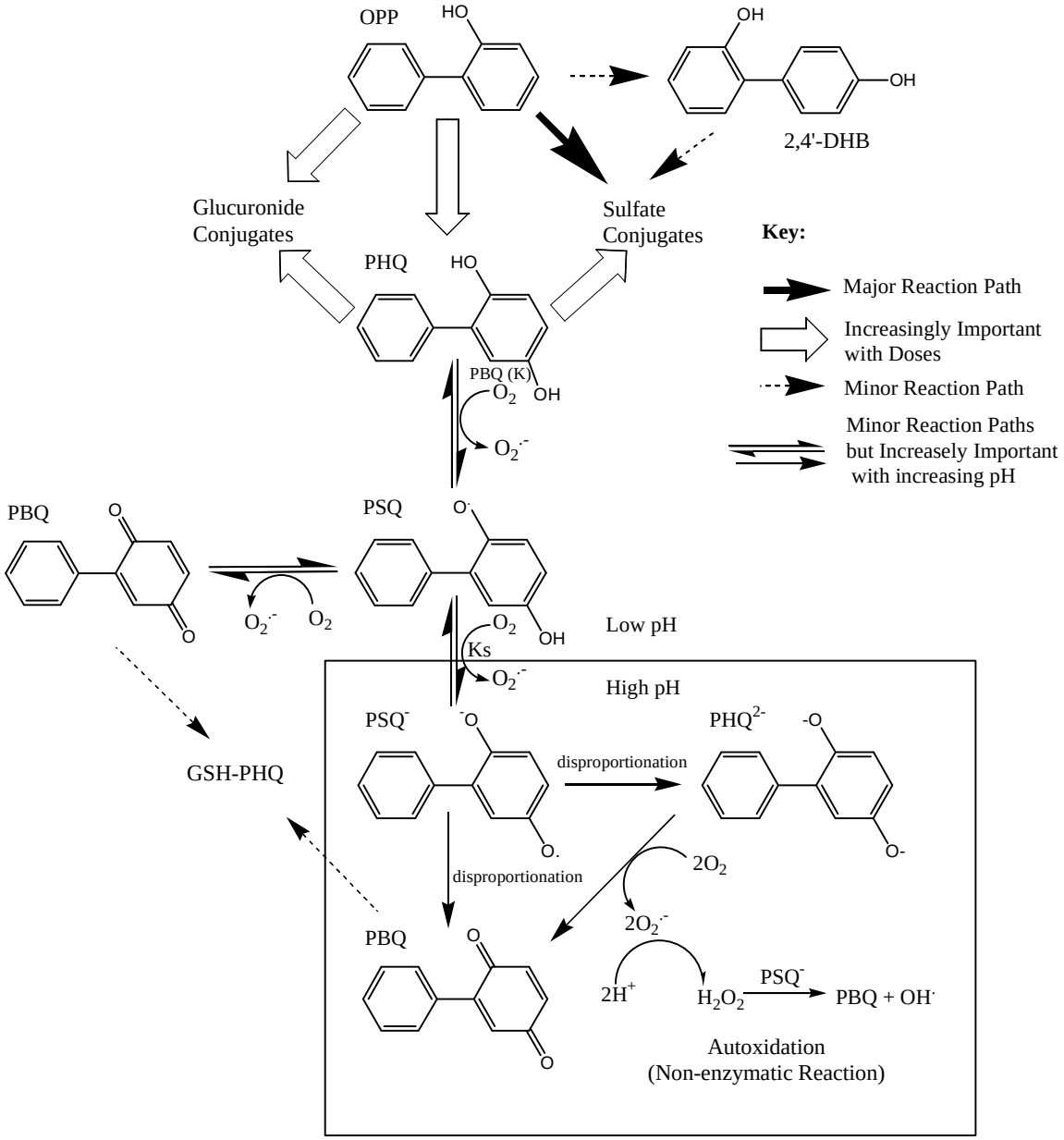

Figure 1 Transformation pathways of OPP[5]

[5] Abbreviations: OPP, *ortho*-phenylphenol; PHQ, phenylhydroquinone; 2,4'-DHB, 2,4'-dihydroxybiphenyl; PSQ, PHQ-semiquinone radical; PBQ, phenylbenzoquinone; PSQ^-, ionized PSQ; PHQ^{2+}, ionized PHQ; GSH-PHQ, glutathione conjugate of PBQ; $O_2^{\cdot-}$, superoxide anion; H_2O_2, hydrogen peroxide; OH, hydroxyl radical; K_s, ionization constant of PSQ; K, equilibrium constant.

male and female F344 rats. Bartels *et al.* (1998) and Smith *et al.* (1998) reported the occurrence of sulfate and glucuronide conjugates of OPP, PHQ, and 2,4'-dihyroxylbiphenyl (2,4'-DHB) in the urine of male rats after a single (27 mg/kg, gavage) or repeated dosing (56-294 mg/kg/day, dietary). In both cases, these conjugates amounted to ~95% of the total dose recovered. Smith *et al*, (1998) also reported a dose-dependent shift in the formation of conjugates in male rats. At the low dose (56 mg/kg/day), urinary sulfate and glucuronide accounted for 84% and 10%, respectively, of the recovered dose. At the high dose (924 mg/kg/day), sulfate and glucuronide accounted for 48% and 51%, respectively, of the recovered dose[6].

Both Reitz *et al.* (1983) and Smith *et al.* (1998) found evidence of OPP oxidation. Reitz *et al.* (1983) treated male rats with single gavage doses of OPP and found the amounts of conjugated PHQ in urine increased from non-detect (detection limit calculated as <1%) at 50 mg/kg to 25% at 500 mg/kg. Similarly, Smith *et al.* (1998) fed male rats with OPP-containing diets for 13 weeks and found a linear increase in the total amount of urinary PHQ-S and PHQ-G from 8% at 56 mg/kg/day to 35% at 924 mg/kg/day. Overall, these data indicated that, under both the single and repeated dosing protocols, OPP-to-PHQ conversion occurred in a dose-dependent manner.

Further evidence of the *in vivo* oxidation of OPP and SOPP was the occurrence of unconjugated PHQ and PBQ in urine of the treated rats (Morimoto *et al*., 1989, Hasegawa *et al.*, 1991, Bartels, *et al.* 1998, Smith *et al.*, 1998). Several patterns of these unconjugated metabolites were noticeable: (1) they were reported only in the repeated dosing studies (Hasegawa *et al.*, 1991; Bartels *et al.*, 1998; Smith *et al.*, 1998); (2) they amounted to up to 2% of total dose recovered in the urine (Smith *et al*., 1998); and (3) and their mode of formation appeared to follow a linear kinetics. That is, the relative concentration of OPP-to-PHQ appeared to be independent of dose (Levy, 1968). For example, in a 13-week OPP feeding study with the male rats, the increased concentrations of unconjugated OPP and PHQ (pooled sample from 11 animals) occurred dose-dependently from 56 to 924 mg/kg/day OPP whereas the calculated unconjugated OPP-to-PHQ concentration ratio within the dose range appeared to be constant (~1.3) (Smith *et al.*, 1998). Similarly, in a 20-week SOPP feeding study (5,000-20,000 ppm) with the same sex and strain of rats, there appeared to be a dose-dependent increase in the concentrations of unconjugated OPP and PHQ; the calculated unconjugated OPP-to-PHQ concentration ratios were ~1 at the mid and high doses (except at the lowest dose tested, which was ~0.2) (Morimoto *et al.*, 1989).

For PHQ, pH-dependent nonenzymatic autoxidation that produced reactive metabolites *in vitro* may also occur *in vivo*. Two studies that support the occurrence of PHQ autoxidation in the rat urinary bladder *in vivo* are Kwok and Eastmond (1997) and Kwok *et al.* (1999). In the first study, Kwok and Eastmond (1997) showed a striking linear correlation ($r^2 = 0.8$) between amount of reactive species generated through pH-dependent PHQ autoxidation and the preneoplastic and neoplastic lesion incidences

[6] The percent formations of OPP conjugate were calculated by DPR based on the amount of OPP-G, OPP-S, PHQ-G, and PHQ-S reported by Bartels *et al.* (1998).

of urinary bladder in OPP-treated rats. In the second study, Kwok *et al.* (1999) demonstrated a good correlation (r^2=0.94; $p<0.05$) of autoxidized PHQ to the radiolabels retained by urinary bladder protein in male F344 rats exposed to [^{14}C]-OPP.

Various short-term dietary studies demonstrated a sex-specific difference in the metabolism of OPP and SOPP (Nakao *et al.*, 1983, Morimoto *et al.*, 1989, Hasegawa *et al.*, 1991, Bartels *et al.*, 1998). Hasegawa *et al.* (1991) fed both sexes of rats with diets containing OPP or SOPP for 8 weeks and found ~6-7 times higher unconjugated PHQ concentration in the urine of males (296 and 225 μM, respectively) than females (40 and 37 μM, respectively). Using a similar dosing protocol, Morimoto *et al.* (1989) reported that after 20 weeks of dietary exposure at 20000 ppm SOPP, the males excreted ~24 times more unconjugated PHQ in the urine (1507 μM) than the females (62 μM). In another 20-week feeding study of SOPP, the amount of PHQ-G excreted in the urine was ~8 times higher in the males (157 μM) than the females (20 μM). Given that isoenzymes of the cytochrome P-450 including a male-specific isoform in 2C subfamilies (CYP2C11) metabolized OPP and that *in vitro* OPP-to-PHQ conversion by liver microsomes was ~3-6 times faster by the males than females (Ozawa *et al.*, 2000), the sex difference in OPP oxidation may have been associated with more extensive metabolism in the males.

Summary of *In Vitro* and *In Vivo* Studies Enzyme-mediated conjugation and oxidative reactions that resulted in the formation of biologically inactive products (e.g., sulfates and glucuronides) and reactive metabolites (e.g., PHQ and PBQ) *in vitro* also occurred in OPP-treated rats *in vivo*. Evidence indicates that nonenzymatic autoxidation of PHQ that produced reactive metabolites *in vitro* may also occur *in vivo*. Under repeated dosing conditions, results in rats showed that conjugation reactions of OPP and PHQ shifted from mainly sulfation at the lower doses to glucuronidation at the higher doses; however, unconjugated PHQ formation followed linear kinetics and the PHQ concentrations increased dose-dependently over the dose-range tested. Consistent with the sex-related difference in the xenobiotic enzyme expression of rats *in vitro*, males showed a greater ability to metabolize OPP than females; accordingly, the amount of unconjugated PHQ in urine was markedly higher in males than females.

Excretion

Excretion of the absorbed OPP occurred rapidly *via* urine. As mentioned previously, male rats excreted a majority (~85%) of the administered ^{14}C in urine at 24 hr after a single dose of radiolabeled OPP or SOPP (Reitz *et al.*, 1983; Sato *et al.*, 1988; Bartels *et al.*, 1998). The females also exhibited a similar result (Bartels *et al.*, 1998). Additional support for the rapid elimination was the similar recovery pattern after a single dose of radiolabeled OPP or SOPP *versus* after 14-day pretreatment of non-radiolabeled OPP or SOPP in diets.

Excretion of absorbed OPP also occurred in the bile. Sato *et al.* (1988) measured the amount of biliary excretion in male F344 rats after an oral dose of [^{14}C]-SOPP and found that a higher amount of radioactivity occurred in the bile (26%) than in feces (4%).

The investigators speculated that the intestine reabsorbed OPP and (or) its metabolites; i.e., the occurrence of enterohepatic recirculation.

III.A.1.b. Oral – Mouse

Three pharmacokinetic studies of OPP are available: one is in the open literature (Bajaj *et al.*, 1976) and the others were obtained from the Registrant (McNett *et al.*, 1997, Bartels *et al.*, 1998).

Absorption

Absorption of OPP occurred readily and was almost complete in the gastrointestinal tract of mice. Studies investigating the absorption of OPP *via* oral route (gavage) by male B6C3F1 mice indicated that recovery of radiolabel in the urine was ~90% within 24 hours after a single dose of [^{14}C]-OPP (15 or 800 mg/kg) (McNett *et al.*, 1997, Bartels *et al.*, 1998).

Metabolism

Two studies investigated the metabolism of OPP: the first was a single dosing study (15-800 mg/kg) with male B6C3F1 mice (Bartels *et al.*, 1998) and the second was a repeated dosing study (200 mg/kg/day for 9 days) with male Swiss-stain albino mice (Bajaj *et al.*, 1976). The conjugated urinary metabolites found in these studies were OPP-G, OPP-S, PHQ-G, and PHQ-S and they accounted for >98% of the total dose recovered in urine. Similar to the rats, mice also showed a dose-dependent shift in the sulfation-to-glucuronidation of OPP. The ratios of sulfates (i.e., OPP-S and PHQ-S) to glucuronides (OPP-G and PHQ-G) concentrations in the urine were 1.93 at the low dose (15 mg/kg) and 0.45 at the high dose (800 mg/kg) (Bartels *et al.*, 1998). Unchanged OPP and unconjugated metabolite also occurred in the repeated dosing study; the metabolite was dihydroxybiphenyl (presumably PHQ) (Bajaj *et al.*, 1976).

Excretion

Excretion of the absorbed OPP occurred rapidly *via* urine. As mentioned previously, recovery of radiolabel in the urine was ~90% at 24 hours after a single dose of [^{14}C]-OPP (15 or 800 mg/kg); the radiolabel recovered in the feces was ~9% within 48 hours (the only time point measured) (Bartels *et al.* 1998). These observations suggested that accumulation of OPP and its metabolites in tissues would be insignificant.

III.A.1.c. Oral – Dog

Four pharmacokinetic studies of OPP in beagle-type dogs are available in the open literature: Oehme (1971a), Oehme and Smith (1972), Rachofsky and Oehme (1975, 1976), and Savides and Oehme (1980).

Absorption

Two single-dose and one repeated dose studies investigated the oral absorption of OPP (Oehme, 1971a; Rachofsky and Oehme, 1975; Savides and Oehme, 1980). Oehme (1971a) reported that, after a single gavage dose of [^{14}C]-OPP (1 or 3 g/kg) to both sexes of dogs (number not specified), the administered radioactivity[7] in urine was ~57% within 72 hours; also, in each of the dose groups, the peak plasma radioactivity occurred at 1.5 hrs. Similarly, Rachofsky and Oehme (1975) reported a cumulative excretion of ~45% in five beagle dogs after a single gavage dose of [^{14}C]-OPP (amount and duration were not specified). In the repeated dosing study wherein the adults and puppies (3 animals/sex/age) received 0.27 mg/kg and 2.03 mg/kg [^{14}C]-OPP (in gelatin capsule), respectively, every other day for 7 weeks, the cumulative excretions of the administered dose in urine by adults and puppies were 54% and 45%, respectively (Savides and Oehme, 1980). Taken together, these data indicated that absorption of OPP by the dogs was rapid but incomplete.

Distribution

In the only tissue distribution study in beagle-type dogs (sex not specified), Rachofsky and Oehme (1975) found various amount of ^{14}C in tissues; i.e., in a decreasing order, liver, lung, kidneys, bile, brain, heart, and spleen after a gavage dose of [^{14}C]-OPP (time not specified). The amount of ^{14}C in the liver, brain, and lung remained the highest 120 hours after the dosing.

Metabolism

Two studies investigated the metabolism of OPP in beagle dogs (both sexes): one employed single dosing (Oehme and Smith 1972) and the other employed repeated dosing (Savides and Oehme, 1980). Unchanged OPP, OPP-S, OPP-G, and phenol occurred in the urine after a single gavage dose of 1 or 3 g/kg. The investigators stated that phenol was derived from cleavage of phenyl-phenol bond followed by the hydroxylation of phenyl ring. OPP, OPP-S, and OPP-G occurred in the urine after repeated dosing of 0.27 mg/kg to adult dogs and 2.03 mg/kg to puppies every other day for 7 weeks. The respective percentages of these metabolites were: 90%, 5%, and 5% for adult males, 87%, 8%, and 6% for adult females, 74%, 8%, and 19% for male puppies, and 68%, 9%, and 23% for female puppies. Based on these observations, the investigators concluded more OPP was metabolized to OPP-G in the puppies than adults and more to OPP-S in the adult females than males. It should be noted that there was no report on phenol formation under the repeated dosing.

Excretion

Available data indicated that the dogs excreted the absorbed OPP slowly. Rachofsky and Oehme (1975) reported a first order disappearance rate of 0.027 hr^{-1} of the

[7] Data on excretion of OPP in urine of dogs were presented as a graph.

plasma radioactivity (corresponding to an elimination half life of ~11 hr) after an oral dose (gavage) of [^{14}C]-OPP (amount not specified). In another single dose study, beagle dogs that received gavage doses of 1 or 3 g/kg [^{14}C]-OPP excreted most of the recovered OPP (~57%) in urine within the first 12 hr; the excretion continued at a progressively slower rate during the remaining experimental period (i.e., 72 hr) (Oehme, 1971a). In a repeated dosing study wherein the adults and puppies received 0.27 mg/kg and 2.03 mg/kg [^{14}C]-OPP, respectively, every other day for 7 weeks, the investigators stated that both age groups reached a plateau in the percent of administered dose excreted during their first week of dosing (Savides and Oehme, 1980). The investigators further stated that both had ~50% of administered radiolabel recovered in the urine at the end. None of these single and repeated dosing studies reported the percent of ^{14}C recovered in feces.

III.A.1.d. Oral – Cat

Five pharmacokinetic studies of OPP in domestic short-hair cats are available from the open literature: Oehme, (1971a), Oehme and Smith (1972), Rachofsky and Oehme (1976), and Savides and Oehme (1980).

Absorption

Two studies investigated the absorption of [^{14}C]-OPP: one employed single dosing (Oehme, 1971a) and the other employed repeated dosing (Savides and Oehme, 1980). Male and female domestic short-hair cats that received a single lethal dose of 1 or 3 g/kg [^{14}C]-OPP excreted <2% of the administered radioactivity in urine before death occurred at 24 and 6 hr, respectively (Oehme, 1971a). By contrast, in the repeated dosing study wherein the adults and kittens received 2.04 mg/kg and 1.16 mg/kg [^{14}C]-OPP in gelatin capsule, respectively, every other day with for 7 weeks, a steady state ^{14}C excretion occurred in the kittens after 3 weeks (31%) and the adults after 6 weeks (42%).

Distribution

In the only tissue distribution study, Oehme (1971a) found the highest ^{14}C in liver, kidneys, and spleen after an oral dose of radiolabeled OPP (sex and amount not specified). The investigators reported that the digestive tract ^{14}C content increased with proximity to the rectum[8]. The investigators speculated that the digestive tract ^{14}C distribution may have been due to unabsorbed OPP, biliary excretion, and (or) excretion of radiolabel by mucosal cells of the intestine.

Metabolism

Two studies investigated the metabolism of OPP in cats: one employed single dosing (Oehme and Smith 1972) and the other employed repeated dosing (Savides and Oehme, 1980). Unchanged OPP, OPP-S, OPP-G, and phenol occurred in the urine after a

[8] The investigator did not report any results regarding percent radiolabel recovered in different organs.

single dose of 1 or 3 g/kg. The investigators stated that phenol was derived from cleavage of phenyl-phenol bond followed by the hydroxylation of phenyl ring. OPP, OPP-S, and OPP-G occurred in the urine after repeated dosing of 1.16 mg/kg to adult cats and 2.04 mg/kg to kittens every other day for 7 weeks. The respective percentages of these metabolites were: 97%, 2%, and 0.3% for adult males, 96%, 3%, and 1.2% for adult females, 95%, 4%, and 1.2% for male kittens, and 98%, 2%, and 0.1% for female kittens. Based on these observations, the investigators concluded that more OPP was metabolized to OPP-G in the adult females than males. It should be noted that there was no report on phenol formation under the repeated dosing.

Excretion

Cats slowly excreted the absorbed OPP. In the repeated dosing study wherein the adults and kittens received 1.16 mg/kg and 2.04 mg/kg [^{14}C]-OPP, respectively, every other day for 7 weeks, a plateau in the percent of administered dose excreted occurred in adults after 6 weeks (42%) and kittens after 3 weeks (31%) (Savides and Oehme, 1980). The investigators did not report the amount of ^{14}C recovered in feces.

III.A.1.e. Oral – Lactating Goat

One pharmacokinetic study of OPP in lactating goats is available from the Registrant (Thalacker, 1997). In this study, the lactating goats excreted ~80% of administered ^{14}C in urine and up to ~10%, in feces after repeated dosing with 13.7 or 53.3 mg/kg/day [^{14}C]-OPP (encapsulated; 1 animal/dose) for 5 days. The investigators examined residue ^{14}C levels in the kidneys, liver, muscle (round), fat (omental and renal, mixed), and milk; none of the tissues examined had a ^{14}C level that exceeded 0.1% of the administered radioactivity. The only tissues examined for OPP, PHQ, and PBQ were milk, kidneys, and liver; none of these compounds exceeded the analytical method detection limits.

III.A.2.a. Dermal – Rat

One percutaneous absorption study of OPP in rats is available in the open literature (Cnubben *et al.*, 2002). The study employed two groups of male Wistar rats (4 animals/group), a dermal-exposure group that received 4.8 µg/kg [^{14}C]-OPP (in 60% aqueous ethanol) semi-occlusively for 4 hours[9] and an intravenous-exposure group that received a bolus dose of 50.4 µg/kg [^{14}C]-OPP[10] (in saline). The maximal plasma ^{14}C level was reached at 1 hr after the initiation of topical application. In both the dermal- and intravenous-exposure groups, the respective 48-hr cumulative excretions

[9] Based on the dosing concentration (12 µg OPP/µL), dosing volume (100 µL/0.25 kg), and surface area (10 cm^2), the topical dose calculated by DPR was 120 µg/cm^2 (i.e., [12 µg OPP/µL x 100 µL]/10 cm^2). The systemic dose was 4.8 µg/kg (i.e., 12 µg OPP/µL x 100 µL/0.25 kg x 1 kg/1000g).

[10] Based on the dosing concentration (25.2 µg/1000µL) and dosing volume (2000 µL/kg), the systemic dose calculated by DPR was 50.4 µg/kg (i.e., 25.2 µg/1000µL x 2000 µL/kg).

were 38% and 89% in urine and <1% and 2.2% in feces. Using these data, calculation showed that the dermal absorption of OPP was 43% (i.e., 38/89 x 100). It should be noted that at 48 hr, the amount of radioactivity remaining in the skin was 6.2% of the applied dose, as opposed to the 4-hr value of 24.3% that was determined in another experiment by the investigators. Hence, it would be reasonable to assume that given a long enough time, this ^{14}C remaining in the skin after 48 hours would still be available for absorption. Accordingly, the estimated 43% dermal absorption may underrepresent the total amount of OPP absorbed. Based on the plasma ^{14}C data, the investigators determined that the *in vivo* permeability coefficient (K_p) of OPP was 0.039±0.015 cm/hour. When viewing these data altogether, the rats absorbed OPP rapidly but incompletely *via* the skin.

III.A.2.b. Dermal – Human

One dermal absorption study of OPP in humans is on file at DPR (Selim, 1996)[11]. Based on this study, a separate report by Bartels *et al.* (1997; 1998) provided an in-depth evaluation of the results and another report by Timchalk *et al.* (1998) described the pharmacokinetic modeling of the data. Cnubben *et al.* (2002) also investigated the percutaneous absorption of OPP in human, and Hagadorn-Leweke and Lippold (1995) investigated the potential enhancing effect of vehicle on the dermal absorption. Pacific *et al.* (1991), Temellini *et al.* (1991), and Ozawa *et al.* (2000) investigated the metabolism of OPP *in vitro* using microsomes or cytosol derived from human tissues. Except for the study by Selim (1996), the others are reports in the open literature.

Absorption

This section summarizes studies investigating the dermal absorption of OPP. Selim (1996) measured the plasma and urine radioactivity of six male volunteers (19-27 years old) after a non-occlusive topical application of 6 μg/kg [^{14}C]-OPP (in 100% ethanol) to the forearm (volar aspect) for 8 hr. The maximum peak plasma ^{14}C occurred by 4 hr in the non-application arms after the dosing. The 5-day cumulative ^{14}C excretion in urine was 42.7% of the administered radioactivity. Timchalk *et al.* (1998) applied a one compartment pharmacokinetic model to the reported plasma radioactivity (Selim, 1996) and predicted that 44±16% of the applied dose would be absorbed and recovered in the urine, consistent with the observed amount of urinary radioactivity recovered. Based on the modeling results that the rate of absorption (0.104 hr^{-1}) was ~9 times slower than excretion (0.891 hr^{-1}), the investigators suggested that the skin may represent a rate-limiting step for the dermal penetration of OPP. Given that isolated epidermis was shown to be 11 times more permeable to OPP (permeability coefficient [K_p] = 0.0183 cm/hr) than the whole skin *in vitro* (K_p = 0.0016 cm/hr) (Cnubben *et al.*, 2002), the viable dermis may have been the cause of the slow absorption of OPP.

[11] This study was conducted in accordance with Good Clinical Practice Regulation.

In another *in vivo* percutaneous absorption study, Cnubben *et al.* (2002) applied ~153 μg/kg OPP[12] (in 60% aqueous ethanol) to the forearm of three male human volunteers (23-24 years old) and the treated forearm was placed into a humidified incubator (relative humidity 50±10%) for 4 hours. Each volunteer also received ~31 μg/kg OPP (in ethanol-saline)[13] intravenously for establishing the kinetics of absorbed OPP. Unlike other pharmacokinetic studies discussed previously in this risk characterization document (RCD), this study used non-radiolabeled OPP. The investigators expressed the amount of OPP absorbed following dermal exposure as the total amount of unchanged OPP plus its acid-labile conjugates (glucuronide and sulfate combined) excreted in the urine. Based on the total amount of OPP recovered, the 48-hr cumulative urinary excretions after the dermal and intravenous exposures were 15% and 61%, respectively, of the applied dose. It is noteworthy that in the dermal absorption study described previously, total OPP and its conjugates (i.e., OPP-G and OPP-S) accounted for 71% of the absorbed dose and 31% of the applied dose (i.e., 71% x 0.43) (Selim, 1996; Bartels *et al.*, 1998). Although Cnubben *et al.* (2002) used a higher topical dose, the total amount of absorbed dose recovered as OPP and its conjugates (15%) was lower than the 31% reported by Bartels *et al.* (1998) (31%), indicating that other metabolic pathway(s) (e.g., oxidation) may be important at higher doses. Using the concentration-time courses of OPP in plasma from the dermal and intravenous exposures, the investigators calculated that the amount of OPP penetrated per unit time (i.e., flux [J_{max}]) was 11.0±4.11 μg cm^{-2} hr^{-1} and K_p was 0.0158±0.0059 cm hr^{-1}.

Hagadorn-Leweke and Lippold (1995) investigated the potential enhancing effect of propylene glycol on the absorption of OPP in 14 healthy volunteers. The lateral side of each upper arm of these volunteers received a saturated solution of OPP (in 30% aqueous propylene glycol) for one hour. This dosing solution was replaced by a new one, and the total number of replacements was six. Based on the assumption that the amount of OPP absorbed by the skin was equal to the decreased concentration in the dosing solution, the investigators calculated the maximum penetration rate (i.e., $J_{max} = 125.6^{+51.99}_{-36.76}$ μg cm^{-2} hr^{-1} and $K_p = 0.028^{+0.012}_{-0.008}$ cm hr^{-1}). Using these values, the estimated amount of OPP absorbed after treating the whole-skin surface (1.8 m^2) with a saturated solution for 1 hr was 2226 mg, indicating that a considerable amount of OPP would have been absorbed under this experimental condition.

Distribution

There is no information on tissue distribution of OPP after its application to the human skin. Nevertheless, a modeling study showed that OPP had considerably lower

[12] Calculated value based on the information reported in Cnubben *et al.* (2002): average body weight of the human volunteers (78 kg; ranged from 68-88 kg), concentration of dosing solution (40 mg/ml), and the amount of solution applied (0.3 ml). That is (40 mg/ml x 0.3 ml)/78 kg = 0.153 mg/kg (or 153 μg/kg).

[13] Calculated value based on the information reported in Cnubben *et al.* (2002): average body weight of the human volunteers (78 kg; ranged from 68-88 kg), concentration of the dosing solution (2.5 mg/250 ml), infusion time (40 min), and infusion rate (6ml/min). That is (2.5 mg/250 ml x 40 min. x 6 ml/min)/78 kg = 0.03 mg/kg (or 31 μg/kg).

distribution volume than the total human blood volume (Timchalk *et al.*, 1998), indicating that the bioaccumulation potential of OPP in humans would be insignificant.

Metabolism

This section summarizes studies investigating metabolism of OPP *in vitro* and *in vivo*. Temellini *et al* (1991) incubated OPP with human mixed liver microsomes[14] *in vitro* and found evidence of sulfate formation in the presence of adenosine 3'-phosphate-5'-phosphosulfate (PAPS); however, with uridine-5'-diphosphoglucuronic acid (UDPGA), the corresponding glucuronide was not observed. Regarding the sulfation, Pacifici *et al.* (1991) assayed sulphotransferase activity in hepatic and extrahepatic sources and found that the maximum sulfation rate (V_{max}) of OPP in liver (852 pmol/min) was ~20 and ~250 times, respectively, faster than kidneys (40.4 pmol/min) and urinary bladder (3.3 pmol/min). Based on this observation, the investigators speculated that these organs may be more susceptible to the toxicity of OPP than liver. In another *in vitro* study with human liver microsomes, Ozawa *et al.* (2000) reported that the rate of OPP-to-PHQ conversion was as high as that by the male rat liver microsomes and that CYP1A2 was the most efficient human cytochrome P450 isozyme for transforming OPP to PHQ.

In vivo, there was evidence of glucuronidation, sulfation, and oxidation of OPP in humans. Bartels *et al.* (1997, 1998) reported that humans dosed topically with OPP excreted sulfates and glucuronides of OPP and its oxidative metabolite in urine (percent of absorbed dose in parentheses): OPP-S (68.3 %), 2,4'-DHB-S (12.4 %), PHQ-S (<0.6%)[15], OPP-G (3.5%), and PHQ-G (14.3 %). Other compounds identified in the urine included unchanged OPP (0.5 %) and 2 unknown compounds (0.6% each). Based on these observations, sulfation appeared to be the dominant detoxification pathway of OPP at the low dose, and hydroxylation of phenol or phenyl ring followed by conjugation (i.e., the formation of PHQ-S and 2,4'-DHB-S, respectively) appeared to be equally important.

Excretion

Excretion of the absorbed OPP occurred rapidly *via* urine. In a study by Selim (1996), five days following the administration of [^{14}C]-OPP in humans, the recovery of a topical dose of [^{14}C]-OPP in urine was 42.7% and in feces was 0.42%. The cumulative rate of excretion in the urine (percent of applied dose) was as follows: after 12 hours–30.9 %; 24 hours–38.9 %; 48 hours–41.5 %, indicating that elimination of the absorbed OPP occurred mainly within 24 hr after the dosing.

[14] PAPS and UDPGA are substrates for sulphotransferase and UDP-glucuronosyl transferase, respectively.
[15] PHQ-S was not detected and the detection limit was given in parentheses.

III.B. ACUTE TOXICITY

Summary: Table 1 summarizes the results of the oral and dermal median lethal dose (LD_{50}), inhalation median lethal concentration (LC_{50}), and eye and skin irritation potentials of OPP and SOPP. Among the experimental animals studied, oral acute lethality of OPP was the highest in cats, followed by mice, rats, and guinea pigs. It should be noted that, unlike rats and mice, pharmacokinetics of OPP in cats is considerably different from humans (see III.A. PHARMACOKINETICS). Based on the acute toxicity data in rats, technical OPP and SOPP are Toxicity Category III oral toxicants; however, SOPP appeared to be ~3 times more acutely toxic than OPP. Acute oral lethality of OPP and SOPP was similar in both sexes of rats and mice. At or near the median lethal dose, OPP and (or) SOPP also produced the following clinical observations: rales, tussis, diuresis, depression, exophthalmos, lacrimation, ptosis, and abdominal inflation in rats, and decrease of spontaneous movement, staggering gait, low respiratory rate, and depigmentation of hair in mice. Other effects that OPP induced in these species were damage in the liver (rats), kidneys (rats), lungs (mice), and alimentary canal (rats and mice). Metabolites of OPP (i.e., PHQ and PBQ) also were toxic to the rat liver and kidneys (the only species studied). PBQ was approximately two times more acutely toxic to the liver than PHQ, which in turn was two times more toxic than OPP. In humans, following an acute dermal exposure to phenylphenols, the organs affected were urinary bladder and, possibly, liver.

Studies with experimental animals indicated that SOPP and OPP are Toxicity Category I eye irritants and strong skin irritants; there was limited evidence that SOPP is a skin sensitizer. In humans, the skin effects reported *via* contact with OPP- or SOPP-containing consumer and industrial products included depigmentation and dermatitis; the latter also may have an immunologic involvement.

In the formulated products, although they contained much less OPP or SOPP, similar toxicity to the neat chemicals occurred in some cases. This observation suggests that other ingredients in the formulations also may contribute to the toxic effects noted.

III.B.1. Technical Materials

<u>III.B.1.a. Acute Oral Toxicity</u>

Twenty-one oral acute toxicity studies of OPP and (or) SOPP are available: 17 studies involved LD_{50} determination (Table 1) and 4 special toxicity studies involved target organ identification. The latter include three studies in rats (Robenek *et al.*, 1980; Fukumori and Sakai, 1985; Nakagawa and Tayama, 1988) and one study in mice (Tayama and Hiraga, 1984).

Of the 17 acute lethality studies, the majority are research reviews and summaries. Only two rat studies allow for an in-depth evaluation and contain sufficient information to support a Toxicity Category III hazard designation for OPP and SOPP by DPR (Hodge

et al., 1954; Norris, 1971a); however, none of them has information for establishing a No-Observed-Effect Level (NOEL). Clinical and (or) necropsy observations were available in some lethality studies with the rat, mouse, and cat. In the rats, the clinical observations included depression, lacrimation, rales, tussis, respiratory failure, diuresis, ptosis, exophthalmos, and abdominal inflation (Macintosh, 1945; Hodge *et al*., 1954; Norris *et al.*, 1971a; Tayama *et al.*, 1979); the necropsy observations included gastro- and duodeno-staxis[16] (Tayama *et al*., 1979). In the mice, the clinical observations included decreased spontaneous movement, staggering gait, and low respiratory rate (Tanguchi *et al.*, 1981a); the necropsy observations included bleeding from the digestive system (i.e., stomach and duodenum) and hemorrhage in the lungs (Ogata *et al*., 1979; Tanguchi *et al.*, 1981a). In the cats, the necropsy observations included hemorrhage in lungs, liver, alimentary canal, and myocardium (Oehme, 1971a).

III.B.1.a.1. Special Acute Oral Toxicity: Rat

Liver and kidneys were the targets of special toxicity studies in rats (Robenek *et al.*, 1980; Fukumori and Sasaki, 1985; Nakagawa and Tayama, 1988). One study also investigated the toxic effects of OPP metabolites (i.e., PHQ and PBQ) on these organs (Nakagawa and Tayama, 1988).

Robenek *et al.* (1980) found severe alterations in the nuclei and nucleoli, increased vacuole formation, and dilation of intercellular space in the liver of male Wistar rats 60 hr after the oral or subcutaneous applications of 2500 mg/kg OPP. It should be noted that all animals survived until sacrifice, and there were no changes in behavior or body weights in the treated rats.

Fukumori and Sasaki (1985) found histologic lesions in the kidneys of male Jcl:SD rats after the oral administration of 1500 mg/kg OPP for 3 day; the effects included disappearance or shortening of brush border in the renal tubular lumen and disruption of mitochondria in the proximal tubular cells. By contrast, the treated rats that were allowed to recover for 7 days showed no signs of OPP-induced degenerative changes in kidney tubules. Based on these observations, the investigators concluded that the kidney effect is reversible.

Male F344 rats that received an oral administration (*via* stomach tube) of 1400 mg/kg OPP exhibited dilation of renal cortical tubules and changes in centrilobular hepatocytes (nuclear pycnosis and eosinophilic cytoplasm) at 24 hr (Nakagawa and Tayama, 1988). At the same dose, elevated blood urea nitrogen (BUN) level (by 18%) and activities of alanine aminotransferase (ALT) and asparate aminotransferase (AST) (362% and 85%, respectively, both $p<0.05$) occurred at 24 hr. Another effect observed at 1400 mg/kg was the increase in relative kidney weight (27%, $p<0.05$). At the next lower dose (i.e., 700 mg/kg OPP), elevated ALT activity (13%, statistically not significant) and increased relative kidney weight occurred (10%, $p<0.05$). OPP treatment caused a dose-dependent decrease in renal and hepatic GSH levels; accordingly, pretreatment (*via*

[16] Gastro- and duodeno-staxis mean bleeding from the stomach and duodenum, respectively.

intraperitoneal injection) of the rats with an inhibitor for GSH synthesis, BSO[17], enhanced substantially the kidney and liver effects.

Increased ($p<0.05$) ALT and AST activities occurred in groups that received 1400 mg/kg PHQ (by 294% and 122%, respectively) or 700 mg/kg PBQ (by 318% and 82%, respectively) at 24 hr. In the latter group, there was also an increased ($p<0.05$) BUN level (by 165%). Compared to the 1400 mg/kg OPP-alone treatment, ALT activity was two times higher at 1400 mg/kg PHQ or 700 mg/kg PBQ; BUN level was two times higher at 700 mg/kg PBQ. Groups that received PHQ or PBQ had no effect on the mean relative liver weights; however, both 1400 mg/kg PHQ and 700 mg/kg PBQ caused a significant ($p<0.05$) increase in relative kidney weights (by 19% in both cases). Based on these observations, the investigators concluded the following: (1) high doses of OPP caused hepatic damage and, to a lesser extent, kidney damage; (2) GSH depletion *via* BSO pretreatment enhanced the hepatic and renal damage; and (3) PBQ induced hepatic and renal damage at a lower dose than PHQ and OPP.

III.B.1.a.2. Special Acute Oral Toxicity: Mouse

C57B1/6N male mice at 8-, 10- and 13-weeks old and females at 8-weeks old (10 animals/sex/age group) exhibited depigmentation of tactile hair two weeks after a single dose of 1000 mg/kg OPP (Tayama and Hiraga, 1984). Depigmentation of ordinary hairs[18] was also exhibited in eight 8-week old females (80% incidence), five 13-week old males (50% incidence), and ten 18-week old males (100% incidence) but not the 8-week old males. The investigators speculated that lack of effect in the 8-week-old males may be associated with the resting stage of hair growth.

III.B.1.b. Acute Dermal Toxicity

Three experimental dermal toxicity studies (Kahn, 1970; Carreon and New, 1981; Bomhard, 1991) and three human case reports are available (Gaches, 1975; Wysowski *et al.*, 1978; Needham *et al.*, 1980). Except for the study by Carreon and New (1981), the others are reports in the open literature.

Of the three experimental studies, two studies involved LD_{50} determination (Carreon and New, 1981; Bomhard, 1991) (Table 1) and one involved dermal toxicity identification (Kahn, 1970). Of the two acute lethality studies, only the study by Carreon and New (1981) in New Zealand White (NZW) rabbits is sufficient for an in-depth evaluation. DPR found that this study was unacceptable for establishing the LD_{50} and Toxicity Category because only a limited number of animals was tested. Also, the information did not allow the determination of NOEL. Nevertheless, the study contained clinical observations associated with the dermal toxicity of OPP: lethargy, erythema, edema, and necrosis; there were no treatment-related changes found at necropsy. In the

[17] L-buthionine-S,R-sulfoximime (BSO) is an inhibitor of the γ-glutamylcysteine synthetase.
[18] The investigators did not specify whether the ordinary hair is facial hairs, over hairs, and (or) under fur.

study by Kahn (1970), the pigmented guinea pig skin exhibited ulcers and scared-site depigmentation after an occlusive application of OPP (6% solution in 70% ethanol; exposure time not specified).

Among the three human case reports, one described the toxic effects in the urinary bladder (Gaches, 1975) and two described the effects in the liver (Wysowski *et al.* 1978; Needham *et al.*, 1980). Gaches (1975) reported that a 23-year-old male worker who was exposed repeatedly, *via* the dermal route, to a phenylphenol-containing wood preservative mixture exhibited purulent urethral discharge, frank hematuria, and passage of blood clot and tissue fragments. Endoscopic assessment indicated that the urinary bladder had a very small capacity; the bladder epithelium appeared to be "stripped off" and was replaced with granulation tissue. Given that other components of the wood preservatives have not been associated with urological effects, these observations suggest that urinary bladder is likely a target organ of phenylphenol in humans.

Two reports described the hospital outbreak of hyperbilirubinemia[19] in four states in the United States: New Jersey and Wyoming (Wysowski *et al.*, 1978) and California and Oregon (Needham *et al.*, 1980). In the former, the plausible cause of the effect in neonates was the increased use of phenolic disinfectants, which contained OPP. The route of entry could have been through the skin and (or) the respiratory tract. In the latter, the suspected cause of the effect in infants also was increased use of phenolic disinfectants. The disinfectant contained 9.8% OPP, 3.5% p-*tert*-amylphenol, and 3.5% 2-benzyl-4-chlorophenol and the serum concentrations in the affected infants were 0.5-1.9, 0.24-0.68, and 0.27-4.4 μg/ml, respectively.

III.B.1.c. Acute Inhalation Toxicity

Two acute inhalation toxicity studies (Lester and Bomhard, 1989; Landry *et al.*, 1992) and one human case report (Sesline *et al.*, 1994) are available. The study by Landry *et al.* (1992) is on file at DPR; others are reports in the open literature.

Of the two acute inhalation studies (Table 1), in-depth evaluation is possible only for the rat study by Landry *et al.* (1992). DPR determined that this study was unacceptable for establishing the LC_{50} and Toxicity Category because of various deficiencies including inadequate analysis of exposure chamber concentration. Also, the information contained did not allow for establishing an inhalation NOEL.

Regarding the human case report, Sesline *et al.* (1994) described an incident of the accidental human exposure to a disinfectant that contained OPP (0.21%). The local and systemic symptoms reported were consistent with the exposure to chemical irritants: headache, skin rash, upset stomach, malaise, and nausea. However, the establishment of a definite role of OPP in this poisoning episode is difficult, as the effect of other active ingredients (i.e., quaternary ammonium complex, 0.69% and bromine, 0.4%) cannot be ruled out.

[19] Hyperbilirubineia was defined by the investigators as one total bilirubin level ≥12.

III.B.1.d. Eye Irritation

Five eye irritation studies of OPP or SOPP in NZW rabbits are available (Norris, 1971a,b; Schreiber, 1981a; Pauluhn, 1983; Maertins, 1988) (Table 1). Two studies with information for an in-depth evaluation are on file at DPR (Norris, 1971a, b); both have sufficient information for supporting a Toxicity Category I hazard designation of OPP and SOPP. In these studies, the clinical observations were persistent corneal opacity, iritis, conjunctivitis, redness, chemosis, conjunctiva burning, necrosis, and exudates.

III.B.1.e. Dermal Irritation

Seven dermal irritation studies of OPP or SOPP in rabbits are available: Norris (1971b), Schreiber (1981b), Thyssen (1982), Pauluhn (1983), Suberg (1983), Maertins (1988), and Gilbert (1994). Except for the study by Norris (1971b) that is on file at DPR, the others are available in the open literature.

Results of all tests for skin irritation potentials of OPP and SOPP were positive (Table 1). In the study by Norris (1971b), clinical observations reported were erythema, edema, and necrosis after the 24-hr exposure period; at necropsy, there were no systemic treatment-related changes.

III.B.1.f. Dermal Sensitization

Two studies investigated the skin sensitization potentials of OPP and SOPP: one employed the guinea-pig maximization test (Andersen and Hamann, 1984) and the other used human skin patch test (Hodge *et al.*, 1954). Five human case reports also described effects associated with the skin sensitization potentials of OPP and SOPP: Ito *et al.* (1968), Kahn (1970), Adams (1981), Van Hecke (1986), and Tuer *et al.* (1986). The experimental study with guinea pigs and the human case reports are available in the open literature whereas the study by Hodge *et al.* (1954) is on file at DPR.

OPP and SOPP were negative and weakly positive, respectively, for the guinea-pig maximization test; the basis of the latter assessment was the one positive response noted (5% incidence) (Andersen and Hamann, 1984).

Hodge *et al.* (1954) measured the skin irritation potentials of OPP and SOPP after an occlusive application of OPP (5%) or SOPP solutions (0.1, 0.5, 1, or 5%) to the human skin (100 subjects/sex) for 5 days. For detecting the skin sensitization potentials of OPP and SOPP, the investigators performed a second occlusive application three weeks after the first application; this was administered essentially identically to the first except that the exposure duration was 2 days (instead of 5 days). The investigators stated (without data) that 5% OPP caused no sign of irritation in the skin examined after the patch removal whereas 5% and 1% solutions of SOPP were significantly irritating to the skin.

In addition, 0.5% SOPP caused very slight irritation in human skin but 0.1% SOPP solution caused no skin irritation. The investigators also stated (without data) that 5% OPP caused no sensitization reaction in human skin whereas the same result occurred at 0.1% solution of SOPP.

Of the five human case reports, two exposure scenarios can be classified: single (Adams, 1981; Tuer *et al.*, 1986) and repeated exposures (Ito *et al.*, 1968; Kahn, 1970; Adams, 1981; Van Hecke, 1986). In the former, dermatitis was the major effect reported whereas in the latter, depigmentation at the exposure site and (or) hyper-pigmentation in the surrounding areas also occurred (Ito *et al.*, 1968; Kahn, 1970). In addition, Tuer *et al.* (1986) found evidence for immunologic involvement of the skin effect induced by SOPP. It should be noted that all the skin reactions mentioned above were due to contacts with OPP- or SOPP-containing personal care products (e.g., hand cream) (Adams, 1981; Van Hecke, 1968), plastic cast with SOPP as preservative (Tuer *et al.*, 1986), hospital phenolic disinfectants that contained OPP (Kahn, 1970), and (or) industrial coolants formulated with OPP (Van Hecke, 1986).

III.B.1.g. Intraperitoneal - Mouse

Two studies by the intraperitoneal administration route are available: one is on file at DPR that involved LD_{50} determination (Macintosh, 1945; Table 1) and the other is an open literature study that investigated the mechanism of toxic action (Narayan and Roy, 1992). An in-depth evaluation of the study by Macintosh (1945) is not possible as it is available only as a summary report. The following briefly describes the study by Narayan and Roy (1992).

Narayan and Roy (1992) found reductions in nonprotein thiol (i.e., GSH) and protein thiol contents in urinary bladder and kidneys of BALB/C mice (sex not specified) after an intraperitoneal application of 600 mg/kg SOPP at 4 hr; the same thiol content reductions in these organs and the liver occurred at 100 mg/kg PBQ. Following the SOPP and PBQ treatments, the investigators also assayed nonprotein disulfide (GSSG) and protein disulfide contents (i.e., reduced nonprotein and protein thiols) in the urinary bladder; the treatments caused the bladder disulfide content to increase and thiol content to decrease. Given that nonprotein and protein disulfides are indicators of oxidative stress, the investigators concluded that oxidative stress induced by SOPP metabolism may be responsible for the reduction of thiol contents and, therefore, the SOPP-induced cellular toxicity.

III.B.2. Product Formulations

In California, there are 164 OPP- and SOPP-containing products available in the marketplace (DPR, 2006). To study the potential influence of other ingredient(s) on the toxicity of OPP and SOPP, DPR also evaluated results from acute toxicity tests with commercial products. Table 2 summarizes the LD_{50} (oral and dermal), LC_{50} (inhalation),

and dermal and eye irritation data of the representative products. As can be seen in Table 2, some formulations have toxicity similar to that of the neat materials (Table 1), suggesting that other ingredient(s) in the product may be toxic or have an enhancing effect on the toxicity of OPP and SOPP. In fact, Oehme (1971b) reported that a disinfectant solution that contained 0.125 % OPP was similarly toxic to cats when compared to the neat chemical.

III.B.3. Additional Acute Toxicity Studies

Other toxicological studies that may be useful for identifying acute NOELs are described in the III.G. DEVELOPMENTAL TOXICITY. The selection of the critical NOEL for characterizing the risk from acute exposure to OPP or SOPP is presented in IV. RISK CHARACTERIZATION.

Table 1 LD_{50}, LC_{50}, and Irritation Potentials (Eyes and Skin) of OPP and SOPP

Test/Species	Sex	OPP	Ref.	SOPP	Ref.
LD50		mg/kg (TC)		mg/kg (TC)	
Rat	M	2700 (III)	1	1049-1096	23
Rat	M	2850	2	924 (III)	17*
Rat	M	2600	3	1650	24
Rat	M	2980	4		
Rat	F	3600	2	731	17
Rat	F	2850	3	1550	24
Rat	M/F	2000-4000	5	591-846	25
Rat	M/F	2733	6		
Rat	NS	~ 3000	7		
Mouse	M	1200	8	683 – 1018	26
Mouse	M	3499	9		
Mouse	F	1050	8	812 – 1049	26
Mouse	F	3152	9		
Mouse	M/F	2000	10		
Guinea Pig	M/F	3500	11		
Cat	M/F	500	7		
Cat	M/F	<1000	12		
Intraperitoneal LD_{50}		mg/kg			
Rat	M/F	1500 (oily solution)	7		
Rat	M/F	500 (acacia-saline)	7		
Inhalation LC_{50}[a]		mg/m^3		mg/m^3	
Rat (1 hr)	M/F	> 949	13	> 1331	13
Rat (4 hr)	M/F	N/A	14		
Dermal LD_{50}		mg/kg			
Rat	M/F	>2000	15		
Rabbit	M/F	N/A	16		
Ocular Irritation					
Rabbit	NS	Irritant (I)	17*	Irritant (I)	27*
Rabbit	NS	Irritant	18	Corrosive	28, 29
Skin Irritation[a]					
Rabbit (4 hr)	NS	Irritant	19	Corrosive	29
Rabbit (4 hr)	NS	Irritant	20		
Rabbit (0.5 hr)	NS	Irritant	21		
Rabbit (4 hr)	NS	Irritant	22		
Rabbit (24 hr)	NS	Irritant	17	Irritant	28

Reference: 1-Hodge *et al.* (1954); 2-Hasegawa *et al.* (1989); 3-Tayama *et al.* (1980); 4-Loeser (1981); 5-Kaneda *et al.* (1978); 6-Gibert & Crissman, (1994); 7-Macintosh (1945); 8-Taniguchi *et al.* (1981a); 9-Tayama *et al.* (1983a); 10-Yanagisawa *et al.* (1978); 11-Dow (2005); 12-Oehme (1971a); 13-Lester and Bomhard (1989); 14-Landry *et al.* (1992); 15-Bomhard (1991); 16-Carreon and New (1981); 17- Norris (1971a); 18-Schreiber (1981a); 19-Schreiber (1981b); 20-Thyssen (1982); 21-Suberg (1983); 22- Gilbert (1994); 23-Tayama *et al.* (1979); 24-Taniguchi *et al.* (1981b); 25-Gilbert & Stebbins (1994); 26-Ogata *et al.* (1979); 27-Norris (1971b); 28-Pauluhn (1983); 29-Maertins (1988).

Abbreviations: NS: not specified, N/A: not available, M/F: males and females, TC, Toxicity Category.

[a] Exposure duration is given parentheses.

* Study was acceptable to DPR.

Table 2 LD_{50}, LC_{50}, and Irritation Potentials (Eyes and Skin) of OPP- and SOPP-Containing Products

Test (Species)	Sex	Formulation/Dose	Reference[a]
Oral LD_{50} (Rat)		**Liquid Concentrates**	
		(OPP : 1.5 – 7.92 %; SOPP : 14.5 – 23 %)	
OPP	M/F	1210 – >5000	1–3
SOPP	M/F	731 – 924	4,5
		Ready-To-Use Liquids	
		(0.78 – 12 %)	
OPP	M/F	3718 – 9226 mg/kg	6–10
		Pressurized Liquids /Sprays /Foggers	
		(0.08 – 0.21 %)	
OPP	M/F	> 5000 mg/kg	11–16
Inhalation LC_{50} (Rat)		**Pressurized Liquids /Sprays /Foggers**	
		(0.08 – 0.21 %)	
OPP	M/F	2.1 – 5.25 mg/l	11-17
Dermal LD_{50} (Rat)		**All Product Forms**	
OPP	M/F	> 2000 – 5000 mg/kg	1,6,8,11-16,18
Ocular Irritation (Rabbit)		**Liquid Concentrates**	
		(OPP : 1.5 – 7.92 %; SOPP : 14.5 – 23 %)	
OPP	M/F	Mild	2
SOPP	M/F	Severe	4–5
		Ready-To-Use Liquids	
		(0.78 – 12 %)	
OPP	M/F	Severe – Moderate	6, 9
		Pressurized Liquids /Sprays /Foggers	
		(0.08 – 0.21 %)	
OPP	M/F	Moderate – Mild	11,12, 14–16, 19
Dermal Irritation (Rabbit)		**Liquid Concentrates**	
		(OPP : 1.5 – 7.92 %; SOPP : 14.5 – 23 %)	
OPP	M/F	Mild – Negligible	1-3
SOPP	M/F	Severe	4, 5
		Ready-To-Use Liquids	
		(0.78 – 12 %)	
OPP	M/F	Severe – Negligible	6, 9, 20
		Pressurized Liquids /Sprays /Foggers	
		(0.08 – 0.21 %)	
OPP	M/F	Moderate – Negligible	12–16

Abbreviation: M/F, males and females.

[a] Reference : 1-Prince, (1987); 2-McElroy (1981); 3-Cantor (1991); 4-Norris (1971a); 5-Norris (1971b), 6-Shapiro (1987); 7-Rose (1990); 8-Robbins (1998a); 9-Schulz (1988); 10-American Biogenic Corporation (1990); 11-Cerven (1997a); 12-Kukulinski (1985); 13-Vaughn *et al.* (1975); 14-Robbins (1988b); 15-Doyle (1989); 16-Cerven (1997b); 17-Wnorowski (1996); 18-Deeniham (1987); 19-Prince (1996); 20-Hixon (1987).

III.C. SUBCHRONIC TOXICITY

Summary: Results of subchronic toxicity studies of OPP and SOPP are available in the rat, mouse, and dog. OPP affected kidneys and urinary bladder in rats. In males, the kidney effects included increased organ weight, reduced renal function, and induction of nephritis, papillary necrosis, pelvis and (or) papilla hyperplasia, renal tubular proliferation, and renal tubular dilation. The limited data indicated that OPP also affected kidneys in females (e.g., reduced urinary pH and nephritis). OPP affected the urinary bladder in males but not the females; the effects included increased organ weight, increased epithelial cell proliferation, and induction of simple hyperplasia, papillary or nodular (P/N) hyperplasia, and papilloma (but not carcinoma). SOPP also affected the kidneys and urinary bladder, as well as the liver, in rats. The liver effect of SOPP included significantly decreased alanine aminotransferase (ALT) activity in the males and females. Kidney effects induced by SOPP in both sexes included increased organ weight and pyelonephritis. Ingestion of SOPP (but not OPP) favored the excretion of alkaline urine in both sexes of rats. SOPP induced bladder tumors in males (papilloma and carcinoma) and females (papilloma only). In OPP- and SOPP-exposed males, the highest tumor incidences did not occur at the highest tested dose. The "umbrella-shape" bladder tumor dose-responses suggest an inverse relationship between bladder tumorigenesis and the nephritis, which occurred only at very high dose levels and in association with a decrease in urinary pH. Sodium bicarbonate (an urine-alkalizing agent) and thiabendazole (TBZ, a fungicide used with SOPP) enhanced the bladder tumorigenic effects of OPP and SOPP, respectively, whereas ammonium chloride (an urine-acidifying agent) suppressed the SOPP-induced bladder tumorigenicity. OPP and SOPP induced urinary bladder damage *via* a mechanism that did not involve precipitate formation.

Results of subchronic oral toxicity testing are available in mice for SOPP and dog for OPP. Except for body weight reduction, mice (both sexes) fed diets containing up to 40000 ppm SOPP for 13 weeks showed no treatment-related pathology in the liver, kidney, and bladder. In a 4-week dermal exposure study of OPP in mice (both sexes), the only effect reported was ulcerative skin lesions. In both sexes of dogs, OPP induced a dose-dependent increase in emetic activity.

Because this risk assessment addresses the potential acute and chronic dietary exposures, DPR uses the subchronic toxicity data for developing the toxic mode of action rather than selecting the dietary subchronic critical NOEL.

III.C.1. *ortho*-Phenylphenol

<u>III.C.1.a. Oral – Rat</u>

Four subchronic toxicity studies are on file at DPR: Macintosh (1945), Hodge *et al.* (1954), Nakamura *et al.* (1981), and Iguchi *et al.* (1984). DPR also obtained two studies on the mode of action of OPP from the Registrant (Christenson *et al.*, 1996a, b).

Macintosh (1945)

Since this study is available only as a summary report in the open literature, an in-depth review is not possible. Administration of OPP in feed to male white rats (strain not specified) at doses up to 200 mg/kg/day for 32 days caused no signs of illness, no effects on growth rate, and no change in hemoglobin or white blood cell levels (only endpoints investigated). The NOEL for this study was ≥200 mg/kg/day OPP.

Hodge *et al.* (1954)

Five groups of rats (12 animals/sex/dose; strain not specified) received diets containing 0, 1000, 3000, 10000 or 20000 ppm OPP (purity was not specified) for 12 weeks. Oral exposure to OPP caused no significant difference in survival between the treated and control groups. Hematology or blood urea nitrogen (BUN) analysis showed no effects in either sex at 20000 ppm (the only groups measured). The investigators reported a slightly decreased body weight gain at 20000 ppm[20] (both sexes). While 10000 ppm and 20000 ppm groups (both sexes) exhibited absolute weight increases in the liver, kidneys, and spleen, the investigators found no histologic lesions in these and other organs examined. Based on body weight decrease at 20000 ppm, the NOEL was 10000 ppm. Using DPR's default assumption that the daily food intake was ~5% of body weight, the estimated dose at this dietary level was 500 mg/kg/day.

Nakamura *et al.* (1981)

Four groups of male F344 rats (10 animals/dose) received diets containing 0, 6250, 12500, or 25000 ppm OPP (purity was not specified) for 13 weeks. The investigators reported that the time-weighted average doses were 0, 377, 763, and 1554 mg/kg/day, respectively. Except for a high-dose male sacrificed due to morbidity, OPP had no effect on survival. OPP had no effect on the BUN, total protein (TP), albumin (ALB), glucose (GLU), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). At 1554 mg/kg/day, two hematological parameters showed significant ($p<0.05$) decreases: hemoglobin (Hgb) concentration (3%) and red blood cell (RBC) counts (5%). Also, 763 and 1554 mg/kg/day groups had single cases of hematuria at week 12.

Throughout the study, reduced body weights occurred at 763 and 1554 mg/kg/day; however, these reductions (up to 12 and 22%, respectively) appeared to be related to reductions in feed consumption (g/animal/day) over the same periods (up to 12% and 23%, respectively). At 1554 mg/kg/day, increased water intake occurred starting at week 9; by week 13 (the last time point measured), water intake was 45% higher than the controls ($p<0.05$). Increased ($p<0.05$) relative kidney weights occurred in the low-, mid-, and high-dose groups (10-33%). The increase appeared to be independent of a general body weight reduction because the latter did not occur in the low-dose group.

[20] The investigators did not report any results regarding statistical analyses of the body weight data.

Decreases in urinary pH and protein concentration occurred at 763 and 1554 mg/kg/day (Table 3). Although the investigators did not measure the urine output, the decreased protein concentration is consistent with an increased urine output noted by Christenson *et al.* (1996) in the same rat strain. OPP caused an increase in the relative weights of urinary bladder in the low-, mid-, and high-dose groups by 10% (statistically not significant), 41% ($p<0.05$), and 47% ($p<0.05$), respectively. Although each of the exposed groups exhibited increase ($p<0.05$) in relative liver weights, the toxicological significance is uncertain without changes in serum liver enzymes (e.g., AST and ALT activities) and findings of historical lesions.

In conclusion, this study documented that OPP induced organ weight increases in the kidney and urinary bladder, higher urine output, and lower urinary pH. Based on increased relative kidney weights, the LOEL for this study was 377 mg/kg/day. DPR considered this study as supplemental information.

Iguchi *et al.* (1984)

Six groups of F344 rats (10-12 animals/sex/dose) received diets containing 0, 1560, 3130, 6250, 12500, and 25000 ppm OPP for 13 weeks. A separate report by Hiraga and Fujii (1984) described the histopathology data for kidneys and urinary bladder. Hiraga and Fujii (1984) reported that the respective time-weighted average doses were[21] 0, 98, 210, 410, 815, and 1493 mg/kg/day for the males and 0, 109, 221, 432, 888, and 1623 mg/kg/day for the females.

The only deaths were two males and one female in the 25000 ppm group. Serum chemistry showed no treatment-related effects. At 25000 ppm, hematological evaluation showed decreases ($p<0.05$) in the following parameters: RBC count (6%), Hgb concentration (7%), and mean corpuscular hemoglobin concentration (MCHC, 5%) in the males and Hgb concentration (6%), mean corpuscular hemoglobin (MCH) (4%), and mean corpuscular volume (MCV) (2%) in the females. At the next-lower dose (i.e., 12500 ppm), females also exhibited decreases ($p<0.05$) in Hgb concentration and MCH (4% and 3%, respectively). The 12500 and 25000 ppm groups had single cases of occult blood in the urine at week 13. Throughout most of the study, reduced body weights occurred in both sexes at 12500 and 25000 ppm. However, these reductions (up to 13% and 35% for the males; 9% and 32% for the females, respectively) appeared to be related to decreases in feed consumption (g/animal/day) over the same periods (up to 11% and 29%; 9% and 25%, respectively). In week 12, increased water intake (g/animal/day) occurred at 25000 ppm (13% for the males and 32% [$p<0.05$] for the females).

OPP affected kidneys and urinary bladder in the males (Table 4). In the kidneys, inductions of renal hyperplasia and nephritis (interstitial or pyelonephritis; $p<0.01$) occurred at 25000 ppm. Increased ($p<0.05$) relative kidney weights occurred in the 6250,

[21] The doses shown in the report by Iguchi *et al*. (1984) were approximately 2-fold higher. They appeared to be incorrect as they were also not consistent with the estimated dose reported by Nakamura *et al.* (1981), which appears to be the pilot study for Iguchi *et al.* (1984).

Table 3 Urinary Protein Concentration and pH Measurements made in Male F344 Rats in a 13-Week OPP Feeding Study (Nakamura *et al.*, 1981)

Parameters	Ppm			
	0	6250	12500	25000
Dose (mg/kg/day)	0	377	763	1554
No. of Animals[a]	10	7	9	6
Protein Conc. (mg/dL)[b]	90	109 (121)	84 (93)	27 (30)*
pH[b]	6.7	7.0	6.4*	6.2*

[a] Samples that contained sperm were omitted. One death occurred at the high dose.
[b] Mean value calculated by DPR based on categorical data measured in week 12 using test tape. Percent of the negative controls is given in parentheses. Mean pH = -log (Σ (number of animals x 10^{-pH})/total number of animals).
[*] Non-parametric Shirley's test (Shirley, 1977), as calculated by DPR; significant at $p<0.05$.

Table 4 Nonneoplastic and Neoplastic Lesions in the Kidneys and Urinary Bladder of Male and Female F344 Rats in a 13-Week OPP Feeding Study (Iguchi *et al.*, 1984; Hiraga and Fujii, 1984)

Dose Ppm (mg/kg/day)	Kidneys		Urinary Bladder	
	Transitional Cell Hyperplasia	Interstitial Nephritis or Pyelonephritis	Hyperplasia[a]	Papilloma
Males				
0	0/11 (0%)[+++]	0/11 (0%)[+++]	0/12 (0%)	0/12 (0%)
1560 (98)	0/11 (0%)	0/11 (0%)	0/12 (0%)	0/12 (0%)
3130 (210)	0/11 (0%)	0/11 (0%)	0/12 (0%)	0/12 (0%)
6250 (410)	0/11 (0%)	0/11 (0%)	0/12 (0%)	0/12 (0%)
12500 (815)	0/11 (0%)	0/11 (0%)	6/12 (50%)**[,b]	6/12 (50%)**[,b]
25000 (1493)	3/11 (27%)	7/11 (64%)**	0/12 (0%)	0/12 (0%)
Females				
0	0/12 (0%)	0/12 (0%)[+++]	0/12 (0%)	0/12 (0%)
1560 (109)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
3130 (221)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
6250 (432)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
12500 (888)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)
25000 (1623)	0/12 (0%)	6/12 (50%)**	0/12 (0%)	0/12 (0%)

[a] No distinction between nonneoplastic simple hyperplasia and preneoplastic papillary and (or) nodular (P/N) hyperplasia.
[b] All males at this dose level had lesions; either transitional cell hyperplasia or papillomas.
** Fisher exact test, as calculated by DPR; significant at $p<0.01$.
+++ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.001$.

12500, and 25000 ppm groups (5-25%). The increased relative organ weights appeared to be independent of a general body weight reduction because the latter did not occur in the 6250 ppm group. Decreased ($p<0.05$) urinary protein concentration occurred at 25000 ppm (Table 5). This is consistent with an increased urine output noted in the same rat strain by Christenson *et al.* (1996a). In the bladder, increased ($p<0.01$) incidences of hyperplasia and papilloma occurred at 12500 ppm. However, none of these lesions occurred in the 25000 ppm group. The reduction in survival at the high dose was too slight to account for the absence of bladder tumors in this group. Hence, other factor(s) may have contributed to the reduction in the bladder tumorigenicity of OPP (see IV.C. RISK CHARACTERIZATION for additional discussion). Also, increases ($p<0.05$) in absolute bladder weight occurred at 12500 ppm (40%) and the relative weights at 12500 and 25000 ppm (49% and 60%, respectively).

OPP also induced toxicity in the kidneys of females. Increased ($p<0.05$) incidence of nephritis occurred at 25000 ppm (Table 4) and, in this same group, there was a decrease ($p<0.001$) in urinary pH (Table 5). Unlike the males, OPP had little effect on the urinary bladder of females.

Another organ that OPP affected was liver. Increased ($p<0.05$) relative liver weights occurred in the low-, mid-, and high-dose groups of males and females (6250 ppm, 9% males only, 12500 ppm, 12% and 13%, respectively, 25000 ppm, 21% and 31%, respectively). However, the toxicological significance of liver weight changes is uncertain without changes in serum liver enzymes (e.g., AST and ALT activities) and findings of histological lesions.

In conclusion, this study documented that OPP induced nonneoplastic lesions, together with organ weight increases, in kidneys (nephritis) and urinary bladder (hyperplasia). Also, the detection of tumors in urinary bladder of the males occurred as early as 13 weeks of exposure at a relatively high dose level. The NOEL of this study was 3130 ppm (210 mg/kg/day), based on increased relative kidney weight in the males at the LOEL of 6250 ppm (410 mg/kg/day). DPR considered this report as supplemental information. The USEPA determined a NOEL of 6250 ppm for the study and noted the significant reductions in body weight gain and food and water consumption at 12500 ppm (USEPA, 2006).

Christenson *et al.* (1996a)

This study is the first of a two-part investigation on the mode of action of OPP in kidneys and urinary bladder; the second part will be discussed next. Four groups of male F344 rats received diets containing 0, 1000, 4000, or 12500 ppm OPP (>99% pure) for 13 weeks. The investigators reported that the time-weighted average doses were 0, 54, 224, and 684 mg/kg/day. Initially, the control and 684 mg/kg/day groups each consisted of 30 animals whereas the other two groups each had 20 animals. After the 13-week exposure, 10 animals in each of the control and 684 mg/kg/day groups were given OPP-free diets for an additional 4 weeks, referred to as the 4-week recovery in the discussion below.

Table 5 Urinary Protein Concentration Measurement made in Male and Female F344 Rats in a 13-Week OPP Feeding Study (Iguchi *et al.*, 1984)

Parameters	ppm					
	0	1560	3130	6250	12500	25000
Male						
Dose (mg/kg/day)	0	98	210	410	815	1493
No. of Animals	8	7	10	9	8	8
Protein Conc. (mg/dL)[a]	74	109 (147)	65 (88)	99 (133)	56 (77)	23 (31)**
pH[a]	6.4	6.3	6.3	6.6	6.5	6.2
Female						
Dose (mg/kg/day)	0	109	221	432	888	1623
No. of Animals	10	10	10	10	10	9
Protein Conc (mg/dL)[a]	69	65 (94)	34 (49)*	31 (45)*	35 (51)*	54 (78)
pH[a]	6.4	6.3	6.3	6.5	6.3	6.0***

[a] Mean value calculated by DPR based on categorical data measured in week 13 using test tape. Percent of the negative controls is given in parentheses. Mean pH = -log (Σ (number of animals x 10^{-pH})/total number of animals).

*,**,*** Non-parametric Shirley's test (Shirley, 1977), as calculated by DPR; significant at $p<0.05$, $p<0.1$, and $p<0.01$, respectively.

From each of the treatment groups, serial sacrifice occurred at weeks 4 and 13 for 10 rats; for the control and 684 mg/kg/day groups, an additional sacrifice occurred at week 17. The only clinical sign reported was urine stain in the 224 and 684 mg/kg/day groups. No unscheduled deaths occurred in any of the test groups. The investigators indicated (without data) that reduced ($p<0.05$) body weight occurred at 648 mg/kg/day, with no concurrent reduction in feed consumption.

In the kidneys, after 13 week-treatment, three 684 mg/kg/day males exhibited renal tubular proliferation. Of the males exhibiting this lesion, one also had renal tubular dilation. Increased urinary volume occurred in the 224 mg/kg/day (statistically not significant) and 684 mg/kg/day ($p<0.05$) groups (Table 6). Correspondingly, there were reductions ($p<0.05$) in electrolytes (e.g., sodium, calcium, and chloride) and protein concentrations in these groups. There also was a slight reduction (statistically not significant) in the specific gravity at the high dose. Because protein-to-creatinine (P/C) ratio[22] appeared to be constant (~1) across all treatment groups, the decreased urinary protein concentration may have been due to increase in urine volume (i.e., dilution) instead of reduced protein excretion. OPP caused no significant difference in urinary pH between the treated and the control groups.

In the bladder, induction ($p<0.05$) of simple hyperplasia (i.e., nonneoplastic lesion) occurred at 648 mg/kg/day after 4 weeks of exposure (Table 7); by 13 weeks, albeit the effect did not achieve statistical significance, one animal exhibited papillary or nodular [P/N] hyperplasia (i.e., preneoplastic lesion). Although bromodeoxyuridine (BrdU)-labeling index, an indicator of cell proliferation, appeared to decrease after 4 weeks, the index increased with doses (54-648 mg/kg/day) and achieved statistical significance ($p<0.05$) at 648 mg/kg/day in both weeks 4 and 13 (Table 8). Lesion-severity scores based on SEM analyses of the bladder appeared to increase with dose and duration of exposure (Table 9). Also, more animals had lesions in the bladder as indicated by SEM than by histopathology (Table 7 *vs.* Table 9).

At the end of the 4-week recovery period, 684 mg/kg/day group still exhibited some urinary-tract effects: one animal exhibited renal tubular proliferation, two animals had renal tubular dilation, and one animal had mild hyperplasia in the bladder (see also Table 7). Although at 684 mg/kg/day, the bladder BrdU labeling index was similar to the controls, bladder lesion-severity scores based on SEM still remained increased ($p<0.05$) for this group (Table 9). Because of these observations, the investigators speculated that OPP-induced simple hyperplasia was reversible. Based on the increased SEM lesion-severity scoring observed in the bladder, the LOEL was 54 mg/kg/day.

Christenson *et al.* (1996b)

In this second of the two-part mode of action studies, five groups of male F344

[22] Expressing protein excretion in terms of the ratio of protein to creatinine (P/C ratio) allows the protein values to be adjusted for differences in urine volume (Finco, 1997).

Table 6 Selected Urinalysis Measurements for Male F344 Rats in a 13-Week OPP Feeding Study (Christenson *et al*., 1996a)

Parameters[a]	mg/kg/day (ppm)			
	0 (0)	54 (1000)	224 (4000)	684 (12500)
Volume (ml)	6.6±0.5	6.5±0.4 (98)	7.5±0.7 (114)	9.3±0.8 (141)*
Protein (mg/dL)[b]	160±5	149±7 (93)	124±6 (77)*	93±7 (58)*
Creatinine (mg/dL)	131±6	145±8 (110)	137±9 (105)	91±6 (69)*
P/C Ratio[c]	1.2	1.0	0.9	1.0
Chloride (mg/dL)	419±18	415±16 (99)	358±15 (85)*	256±12 (61)*
Calcium (mg/dL)	22.6±1.4	19.9±1.1 (88)	18.7±0.8 (83)*	12.4±0.8 (55)*
Potassium (mg/dL)	383±16	375±19 (98)	374±24 (98)	255±15 (66)*
Sodium (mg/dL)	178±6	141±9 (79)*	138±8 (77)*	115±8 (65)*
Specific Gravity	1.066±0.004	1.072±0.003	1.069±0.003	1.058±0.003
PH	7.3±0.1	7.1±0.0	7.2±0.1	7.2±0.1

[a] Mean ± standard error for groups of 9-10 animals. Percent of the negative controls is given parentheses. All measurements took place in week 13.

[b] Regression analysis performed by DPR indicated a negative association between the urine volumes and protein concentrations ($r^2 \geq 0.94$).

[c] Calculated by DPR based on the protein and creatinine concentrations in the previous two rows.

* Significantly different from the negative controls at $p \leq 0.05$, as reported by the investigators.

Table 7 Nonneoplastic and Preneoplastic Lesions in the Urinary Bladder of Male F344 Rats in a 13-Week OPP Feeding Study Followed by a 4-Week Recovery Period (Christenson *et al*., 1996a)

Lesion Types	Sacrifice Time	mg/kg/day (ppm)			
		0 (0)	54 (1000)	224 (4000)	684 (12500)
13-Week Feeding					
Simple Hyperplasia	Week 4	0/10	0/10	0/10	5/10*
	Week 13	0/10	0/10	0/10	3/10
P/N Hyperplasia	Week 4	0/10	0/10	0/10	0/10
	Week 13	0/10	0/10	0/10	1/10
4-Week Recovery					
Mild Hyperplasia[a]	Week 17	0/10	ND	ND	1/10

Abbreviation: ND, not done.
[a] The investigators did not state whether the lesion was simple hyperplasia or papillary or nodular hyperplasia.
* Fisher exact test, as calculated by DPR; significant at $p<0.05$.

Table 8 BrdU-Labeling Index of Epithelium in the Urinary Bladder of Male F344 Rats in a 13-Week OPP Feeding Study Followed by a 4-Week Recovery Period (Christenson *et al*., 1996a)[a]

Sacrifice Time	mg/kg/day (ppm)			
	0 (0)	54 (1000)	224 (4000)	684 (12500)
13-Week Feeding[b]				
Week 4	0.05±0.01	0.10±0.02	0.23±0.08	2.52±0.53*
Week 13	0.17±0.03	0.09±0.02	0.13±0.03	1.29±0.38*
4-Week Recovery[b]				
Week 17	0.07±0.02	ND	ND	0.05±0.02

Abbreviation: ND, not done.
[a] The administration of BrdU occurred 5 minutes prior to sacrifice during weeks, 5, 14, and 18 of the study.
[b] Mean ± standard error for groups of 9-10 animals. Labeling index is the percentage of cells counted that were labeled.
* Significantly different from the controls at $p<0.05$, as reported by the investigators.

Table 9 Epithelial Lesions in the Urinary Bladder of Male F344 Rats as seen by Scanning Electron Microscopy in a 13-Week OPP Feeding Study Followed by a 4-Week Recovery Period (Christenson *et al*., 1996a)

Severity Score Classification[a]	Sacrifice Time	mg/kg/day (ppm)			
		0 (0)	54 (1000)	224 (4000)	684 (12500)
13-Week feeding		Numbers of Animals			
Class 1	Week 4	9	9	5	
Class 2		1	1	3	4
Class 3				1	1
Class 4				1	3
Class 5					2
Total		10	10	10*	10*
Class 1	Week 13	8	4	1	
Class 2		1	2	7	
Class 3		1	3	2	1
Class 4			1		2
Class 5					7
Total		10	10*	10**	10**
4-Week Recovery		Numbers of Animals			
Class 1	Week 17	2	ND	ND	
Class 2		6	ND	ND	3
Class 3		1	ND	ND	3
Class 4		1	ND	ND	1
Class 5			ND	ND	3
Total		10			10*

Abbreviation: ND, not done.

[a] Class 1, a normal urinary bladder. Class 2, occasional foci of one to a few necrotic or exfoliated cells. Class 3, cobblestone appearance and (or) more extensive and larger foci of necrosis and (or) exfoliation. Class 4, extensive necrosis and the appearance of rounded cells in addition to polygonal cells. Class 5, obvious pilling of round cells (hyperplasia), the cells usually having uniform and (or) pleomorphic microvilli rather than microridges.

*,** Non-parametric multiple comparison test (Shirley's test) between the treated groups and the controls (Shirley, 1977), as calculated by DPR; significant at $p<0.05$ and $p<0.01$, respectively.

rats (22 animals/dose) received diets containing 0, 800, 4000, 8000, or 12500 ppm OPP for 13 weeks. The investigators reported that time-weighted average doses were 0, 56, 282, 556, and 924 mg/kg/day, respectively. At the end of the study, from each of these groups, the investigators used 10 animals for histopathology and 12 animals for identification of DNA-adducts. The following describes the results of histopathology and III.E. GENOTOXICITY details the results of DNA adduct formation.

Reduced ($p<0.05$) body weights occurred in the 556 and 924 mg/kg/day groups (by 6% and 12%, respectively). Increases ($p<0.05$) in the absolute kidney weight occurred at 556 mg/kg/day (5%) and the relative weights at 556 and 924 mg/kg/day (12% and 18%, respectively). Also, increased urine volumes occurred at 556 mg/kg/day (by16%) and 924 mg/kg/day (by 18%) (Table 10); correspondingly, there were reductions in urinary osmolality and creatinine concentrations. The negative associations between urinary volume and these urinalysis parameters were statistically significant at $p<0.05$ (Table 10).

In the bladder, simple hyperplasia occurred at 556 and 924 mg/kg/day ($p<0.05$) (Table 11); increased ($p<0.05$) BrdU-labeling index also occurred in these same groups. SEM analyses showed an increase ($p<0.05$) in lesion severity scores of the bladder at 556 mg/kg/day (the only dose group examined). At this same dose, more animals had lesions in the bladder as indicated by SEM than by histopathology (Table 11). Also, increases ($p<0.05$) in the absolute bladder weight occurred at 924 mg/kg/day (20%) and the relative weights at 556 and 924 mg/kg/day (18% and 36%, respectively). Based on body weight reduction, increased relative bladder weight, increased absolute weight of the kidney, and increased labeling index, the LOEL was 556 mg/kg/day. The NOEL was 282 mg/kg/day.

In conclusion, the results of the two-part mode of action investigation indicated that OPP-induced bladder lesions were visible by both light microscope and SEM at a relatively high dose level but only by SEM at lower doses. Also, these studies documented that OPP induced polyuria in rats.

III.C.1.b. Oral – Mouse

One 12-week oral study in the mice is available in the open literature (Ito *et al.*, 1968). The investigators dosed two groups of C-57 type mice (5 animals/treatment; gender not specified) daily *via* the oral route with 0.2 ml OPP or *para*-phenylphenol (PPP) in olive oil for 12 weeks. Two OPP-treated mice exhibited depigmentation of hair whereas all PPP-treated mice exhibited the depigmentation effect.

III.C.1.c. Oral – Dog

One 4-week palatability/probe oral dog study is on file at DPR (Cosses *et al.*, 1990). The investigators dosed four groups of beagle dogs (2 animals/sex/dose) by gavage at 0, 100, 200, or 300 mg/kg/day OPP (100% purity) for 4 weeks (5 days per

Table 10 Selected Urinalysis Measurements for Male F344 rats in a 13-Week OPP Feeding Study (Christenson *et al*., 1996b)

Parameters[a,b]	mg/kg/day (ppm)				
	0 (0)	56 (800)	282 (4000)	556 (8000)	924 (12500)
Volume (ml)	8.3	8.6 (104)	8.6 (104)	9.6 (116)	9.8 (118)
Osmolality (mOs/kg H_2O)	2222	2085 (94)	2050 (92)	1948 (88)	1779 (80)
Creatinine (mg/dL)	114	111 (97)	107 (94)	94 (82)	86 (75)

[a] Means for groups of 12 animals. The values as a percent of the controls are given in parentheses.
[b] Regression analysis performed by DPR indicated that urinary volume was negatively associated with creatinine concentration and osmolality ($r^2 \geq 0.94$ for both parameters).

Table 11 Nonneoplastic Lesion, BrdU-Labeling Index, and Epithelial Lesions as seen by Scanning Electron Microscopy in the Urinary Bladder of Male F344 Rats in a 13-Week OPP Feeding Study (Christenson *et al*., 1996b)

Parameters	mg/kg/day (ppm)				
	0 (0)	56 (800)	282 (4000)	556 (8000)	924 (12500)
Simple Hyperplasia	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/10 (20%)	7/10 (70%)*
BrdU-Labeling Index[a]	0.10±0.02	0.12±0.02	0.17±0.05	0.45±0.14*	0.57±0.12*
Severity Score Classification[b]			Number of Animals		
Class 1	6	ND	ND		ND
Class 2	4	ND	ND	2	ND
Class 3		ND	ND		ND
Class 4		ND	ND	2	ND
Class 5		ND	ND	6	ND
Total	10			10*	

Abbreviation: ND, not done.
[a] Mean ± standard error for groups of 9-10 animals. The administration of BrdU occurred 5 minutes prior to sacrifice during week 13 of the study.
[b] See Table 13.
* Significantly different from the negative controls at $p<0.05$, as reported by the investigators.

week). Administration of OPP up to 300 mg/kg/day had no effects on body weight, feed consumption, ophthalmology, clinical chemistry, hematology, urinalysis, and pathology. The only clinical sign was vomiting; the frequency and volume of vomitus increased with the amount of OPP received. Because of the absence of adverse toxicological effects, the NOEL was ≥300 mg/kg/day.

III.C.1.d. Dermal – Mice

A dermal toxicity study of OPP is on file at DPR (NTP, 1986). Six groups of Swiss CD-1 mice (10 animals/sex/dose) received topical applications of acetone solutions (0.1 ml/application) containing 0, 6.0, 11.4, 20.8, 35.7, or 55.5 mg OPP (>99% pure) for 3 days per week for 3 weeks (hours not specified). All mice survived to the end of the study, and the treatments had no effect on body weights. The only effect reported was ulcerative skin lesions at the application site. All mice dosed at 20.8, 35.7, or 55.5 mg exhibited the skin lesions. Six males and nine females at 11.4 mg and seven females at 6.0 mg also exhibited the skin effect.

III.C.2. Sodium *ortho*-Phenylphenate

III.C.2.a. Oral – Rat

One 13-week oral rat study is on file at DPR (Iguchi *et al.*, 1979). In this study, seven groups of F344 rats (10 animals/sex/dose) received diets containing 0, 1250, 2500, 5000, 10000, 20000 or 40000 ppm SOPP for 13 weeks. The investigators reported that the time-weighted average doses were 0, 85, 177, 353, 706, 1384, and 2487 mg/kg/day for the males and 0, 87, 177, 352, 694, 1338, and 2431 mg/kg/day for the females. A separate report by Hiraga and Fujii (1981) described the histopathology data for kidneys and urinary bladder[23].

All animals survived to the end of the study. Hematological evaluation showed decreases in RBC count, Hgb concentration, and hematocrit (Hct) in the males at 40000 ppm. At this same dose, females also showed decreases in Hgb concentration and Hct, together with MCV and MCH. Similar hematological changes occurred in female groups at 5000, 10000, and 20000 ppm. The percent decrease of each of these effects was ≤ 6% but, except for the decreased MCH in 10000 ppm females, the decreases were statistically significant at $p<0.05$. For the duration of the study, reduced ($p<0.05$) body weights occurred in both sexes at 40000 ppm. However, these reductions (up to 22% for the males and 19% for the females) appeared to be related to decreases in feed consumption over the same periods (up to 17% and 21%, respectively). At 20000 ppm, males exhibited reduced ($p<0.05$) body weight intermittently for the first two weeks whereas

[23] Although Iguchi *et al.* (1979) did not specify where the histopathology data were published, it was assumed that the data reported in Hiraga and Fujii (1981) were generated in the study by Iguchi *et al.* (1979) because of the identical experimental design and the same investigator (Hiraga) in both reports.

the females exhibited reduced ($p<0.05$) body weight for the entire study duration; in the females, a relationship between reduced body weight (up to 17%) and reduced feed consumption (up to 19%) was evident. Increased water intake (g/animal/day) occurred in the males at 20000 ppm (12-32%, $p<0.05$) and 40000 ppm (9-26%; not statistically significant) for the duration of the study. There was no increase in water intake in any of the treated female groups.

SOPP affected the kidneys and urinary bladder. In the kidneys, both sexes exhibited pyelonephritis, which occurred exclusively at the high dose (Table 12). Increased ($p<0.05$) relative kidney weights occurred in 40000 ppm males (32%) and females (20%); the same increase ($p<0.05$) occurred in the 20000 ppm male and female groups (14% for both sexes) and 10000 ppm male group (6%). The 20000 pm males also exhibited increased ($p<0.05$) absolute kidney weight (13%). Urinalysis in both sexes indicated that decreased ($p<0.05$) urinary protein concentration occurred at 40000 ppm and increased urinary pH occurred at 20000 ppm ($p<0.01$) and 40000 ppm (statistically not significant in the males) (Table 13). In the females, another effect identified at 40000 ppm was elevated BUN (12%; $p<0.05$).

In the bladder, SOPP induced papillomas and carcinomas in males and only papillomas in females (Table 12). In the males, while almost all animals in the 20000 ppm group had bladder tumors (i.e., papilloma or carcinoma), only 10% had carcinoma at 40000 ppm. The observation that the high-dose males exhibited mainly nephritis and the maximal bladder tumor incidence occurred at the next lower dose whereat the animals exhibited no nephritis also was seen in 13-week feeding study of OPP (Hiraga and Fujii, 1984) (see IV.C. RISK CHARACTERIZATION for additional discussion). In the females, the increased papilloma incidence was biologically significant (albeit statistically not significant) given the rarity of this tumor type at this age for this strain of rats (Haseman *et al.*, 1990)

Another organ that SOPP affected was liver (both sexes). In males, increases ($p<0.05$) in the absolute liver weight occurred at 20000 ppm (18%) and the relative weights at 20000 ppm (17%) and 40000 ppm (28%). Although the investigators found no histologic lesions in the liver of males, serum chemistry showed decreases ($p<0.05$) in ALT activities at 5000, 10000, 20000, and 40000 ppm (17%, 27%, 31%, and 39%, respectively) and AST activities at 10000, 20000, and 40000 ppm (19%, 21%, and 27%, respectively). There were increases ($p<0.05$) in serum albumin (ALB, 6%) and total serum protein (TP, 6%) in the males at 40000 ppm. In females, increased absolute liver weights occurred at 20000 ppm (8%) and 40000 ppm (10%) (both were not statistically significant). Serum chemistry showed decreased ($p<0.05$) ALT activities in females at 20000 and 40000 ppm (13% and 30%, respectively).

In conclusion, this study documented the following: (1) SOPP affected the kidneys and urinary bladder, as well as the liver; (2) more males exhibited tumors in urinary bladder than females after the exposure for 13 weeks; and (3) an inverse relationship existed between bladder tumorigenesis and nephritis. Based on increased relative kidney weight and decreased AST and ALT activities in males at 10000 ppm, the

Table 12 Nonneoplastic Lesion in the Kidneys and Neoplastic Lesions in the Urinary Bladder of Male F344 Rats in a 13-week SOPP Feeding Study (Iguchi *et al.*, 1979, Hiraga and Fujii, 1981)[a]

Dose ppm (mg/kg/day)	Kidney	Urinary Bladder	
	Pyelonephritis	Transitional Papilloma	Transitional Carcinoma
Male			
0	0/10 (0%)	0/10 (0%)	0/10 (0%)
1250 (85)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2500 (177)	0/10 (0%)	0/10 (0%)	0/10 (0%)
5000 (353)	0/9 (0%)	0/9 (0%)	0/9 (0%)
10000 (706)	0/10 (0%)	1/10 (10%)	0/10 (0%)
20000 (1384)	0/9 (0%)	4/9 (44%)*	5/9 (56%)**
40000 (2487)	6/10 (60%)**	0/10 (0%)	1/10 (10%)
Female			
0	0/10 (0%)	0/10 (0%)	0/10 (0%)
1250 (87)	0/9 (0%)	0/9 (0%)	0/9 (0%)
2500 (177)	0/9 (0%)	0/9 (0%)	0/9 (0%)
5000 (352)	0/9 (0%)	0/9 (0%)	0/9 (0%)
10000 (694)	0/10 (0%)	0/10 (0%)	0/10 (0%)
20000 (1338)	0/10 (0%)	0/10 (0%)	0/10 (0%)
40000 (2431)	1/10 (10%)	2/10 (20%)	0/10 (0%)

[a] Summarized incidences of papilloma and carcinoma, as reported by the investigators. Sacrifice of all animals occurred at the end of the study.

*,** Fisher exact test, as calculated by DPR; significant at $p<0.05$ and $p\leq0.01$, respectively.

Table 13 Urinary Protein Concentration and pH Measurements made in Male and Female F344 Rats in a 13-Week SOPP Feeding Study (Iguchi *et al.*, 1979, Hiraga and Fujii, 1981)

Parameters	ppm						
	0	1250	2500	5000	10000	20000	40000
Males							
Dose (mg/kg/day)	0	85	177	352	706	1384	2487
Protein Conc. (mg/dL)[a]	72	72 (100)	58 (81)	65 (90)	72 (100)	51 (71)	34 (47)*
pH[a]	6.4	6.4	6.6	6.6	6.5	7.2**	6.8
Females							
Dose (mg/kg/day)	0	87	177	352	694	1338	2431
Protein Conc. (mg/dL)[a]	34	31 (91)	41 (121)	21 (62)	21 (62)	27 (79)	15 (44)*
pH[a]	6.1	6.2	6.3	6.3	6.2	6.6**	6.6**

[a] Mean value calculated by DPR based on categorical data measured at week 13 in 10 animals using test tape. Percent of the negative controls is given in parentheses. Mean pH = -log (Σ (number of animals x 10^{-pH})/total number of animals).

*,** Non-parametric Shirley's test (Shirley, 1977), as calculated by DPR; significant at $p<0.05$ and $p<0.01$, respectively.

NOEL was 5000 ppm (i.e., 353 mg/kg/day). DPR considered this study as supplemental information.

III.C.2.b. Oral – Mouse

One oral mouse study is available from the open literature (Shibata *et al.*, 1985). In this study, six groups of $B6C3F_1$ mice (10 animals/sex/dose) received diets containing 0, 2500, 5000, 10000, 20000, 40000 ppm SOPP for 13 weeks. The investigators reported that the time-weighted average doses were 0, 414, 730, 1581, 3529, and 5375 mg/kg/day for the males and 0, 558, 1021, 1926, 4294, and 6349 mg/kg/day for the females. The only death was a male in the 2500 ppm group. Without presenting any data, the investigators stated that SOPP had no significant effects on hematology (no specification of endpoints) and the following urinalysis parameters: excretion of protein, glucose, ketones, bilirubin, occult blood, or urobilinogen. However, both sexes at 40000 ppm and females at 20000 ppm had decreased ($p<0.05$) urinary specific gravity. In addition, both sexes at 40000 ppm excreted slightly alkaline urine (pH 7.5-7.6), which was in contrast to the other groups whose urine was slightly acidic (pH 6.1-7.0).

Reduced body weights occurred in both males and females at 40000 ppm, starting at week 1; the final weights were 21% and 18% lower, respectively, than the controls ($p<0.05$). However, in these groups, the body weight reduction paralleled a ~40% reduction in average feed consumption. Reduced ($p<0.05$) body weight also occurred in males at 10000 and 20000 ppm; their final body weights were 5% and 8% lower than the controls, respectively. The investigators stated (without data) that significantly increased absolute liver weights occurred in females at 10000 ppm and both sexes at 20000 ppm. Increased ($p<0.05$) relative liver weights occurred in females at 5000, 10000, 20000, and 40000 ppm (9%, 15%, 21%, and 25%, respectively) and males at 10000, 20000, and 40000 ppm (7%, 19%, and 17%, respectively). Despite the liver weight effects, the investigators reported no gross or histologic lesions in the liver of any SOPP-exposed animals. The investigators also conducted scanning electron microscopy (SEM) analyses in the urinary bladder, using one animal/sex at weeks 4, 8, and 13 from the control and 20000 ppm groups. The bladder surface epithelium of the animals from the 20000 ppm group appeared by SEM to be comparable to that seen in the controls. Urinary bladder of the 40000 ppm animals showed no detectable lesions using light microscopy; however, the investigators did not report results from SEM analysis (not explained). Based on decreased body weight in males at 10000 ppm, the NOEL was 5000 ppm (i.e., 730 mg/kg/day SOPP).

III.C.3. Special Subchronic Toxicity Studies

Four special subchronic toxicity studies in rats are available in the open literature. Three studies involved investigating the mode of action of OPP and SOPP in the urinary bladder and kidneys (Fujii *et al.*, 1987; Shibata *et al.*, 1989; and St. John *et al.*, 2001) and one study involved

determining the interaction of thiabendazole (TBZ), another fungicide used with SOPP for post-harvest treatments, on bladder tumorigenicity of SOPP (Fujii *et al.*, 1986).

III.C.3.a. Mode of Action

Fujii *et al.* (1987)

This special toxicity study was to evaluate the modifying effects of co-exposure to sodium bicarbonate ($NaHCO_3$) and ammonium chloride (NH_4Cl) (urine-alkalizing and -acidifying agents, respectively) on the OPP- and SOPP-induced bladder oncogenicity. Five groups of male F344 rats (30-31 animals/treatment) were involved: three groups received diets containing 0, 12500 ppm OPP, or 20000 ppm SOPP and the other two groups receiving 12500 ppm OPP or 25000 ppm SOPP also received $NaHCO_3$ (4000 ppm) and NH_4Cl (10000 ppm), respectively, in drinking water. Urinalysis indicated that the drinking water additives acted as expected: animals exposed to OPP alone and SOPP alone exhibited urinary pH of 6.4 and 7.0, respectively, whereas animals exposed to OPP plus $NaHCO_3$ and SOPP plus NH_4Cl exhibited urinary pH of 7.0 (i.e., alkalized) and 5.9 (i.e., acidified), respectively. After consuming diets containing 12500 ppm OPP or 20000 ppm SOPP for 26 weeks, the rats developed preneoplastic and neoplastic lesions in the urinary bladder (Table 14). When OPP-treated rats were co-exposed to $NaHCO_3$, papilloma incidences in the bladder were comparable to SOPP-alone treatment and were significantly ($p<0.05$) higher than the incidence in the OPP-alone group. By contrast, SOPP-treated rats exposed to NH_4Cl had a bladder papilloma incidence that was significantly ($p<0.05$) less than the incidence of the SOPP-alone group.

In kidneys, all four treated-groups had nephritis (slight-grade and moderate-to-severe grade) (Table 14). However, SOPP plus NH_4Cl group had an increased ($p<0.05$) incidence of slight-grade nephritis compared to the incidences in the other treatment groups, including the group exposed to SOPP-alone. Given that an inverse relationship between nephritis and bladder tumors also had been reported in 13-week feeding studies of OPP and SOPP (Hiraga and Fujii, 1981, 1984), the investigators speculated that the decreased carcinogenic effect in the urinary bladder in the SOPP plus NH_4Cl group may be due to the nephritis. It was hypothesized that as the nephritis progressed, the urine became more acidic, thereby inhibiting both transformation and proliferation of the bladder epithelium. Based on this observation, the investigators concluded the following: (1) SOPP was a more potent rat bladder carcinogen than OPP; (2) $NaHCO_3$ enhanced the carcinogenic effect of OPP whereas NH_4Cl suppressed the tumorigenic potency of SOPP; and (3) the difference in potency between OPP and SOPP as bladder carcinogens may be related to the difference in urinary pH.

Shibata *et al.* (1989)

This special toxicity study was to investigate the occurrence of nephrotoxic effects of OPP and SOPP in relation to their urinary bladder effects. Three groups of male F344 rats (20

Table 14 Urinary Bladder Lesions and Kidney Nephritis of Male F344 Rats in a 26-Week Feeding Study with OPP and SOPP Supplemented with $NaHCO_3$ and NH_4Cl in Drinking Water (Fujii *et al.*, 1987)[a]

Lesions	Controls	12500 ppm OPP		20000 ppm SOPP	
		alone	plus $NaHCO_3$	Alone	plus NH_4Cl
Bladder					
Simple Hyperplasia	1/30 (3%)	4/31 (13%)	2/31 (6%)	2/31 (6%)	3/31 (10%)
P/N Hyperplasia	0/30 (0%)	13/31 (42%)[#]	9/31 (29%)[#]	7/31 (23%)[#]	1/31 (3%)
Papilloma	0/30 (0%)	12/31 (39%)[#]	20/31 (65%)[#,$]	21/31 (68%)[#,$]	3/31 (10%)[@]
Carcinoma	0/30 (0%)	0/31 (0%)	0/31 (0%)	1/31 (3%)	0/31 (0%)
Kidney Nephritis					
Slight	NA	3/31 (10%)	5/31 (16%)	6/31 (19%)	16/31 (52%)[**]
Moderate-Severe	NA	2/31 (6%)	2/31 (6%)	1/31 (3%)	3/31 (10%)

[a] $NaHCO_3$: 4000 ppm; NH_4Cl: 10000 ppm; NA: data not reported.
[#, $, @] Significantly ($p<0.05$) different from the controls, OPP-alone, and SOPP-alone groups, respectively, as reported by the investigators.
[**] Fisher exact test, as calculated by DPR, significantly different from other dose groups at $p<0.01$.

animals/group) received diets containing 0, 20000 ppm OPP or 20000 ppm SOPP, respectively, for up to 24 weeks with 5/group sacrifice at 4, 8, 16, and 24 weeks. Unlike the previous study by Fujii *et al.* (1987), the OPP- and SOPP-treated groups did not receive equimolar dosing; i.e., the former would have consumed ~36% more moles of OPP than the latter based on an assumption of a similar daily feed consumption. After 4 weeks of exposure, OPP and SOPP groups had increased ($p<0.05$) BrdU-labeling index in renal papilla and (or) pelvis; SEM analysis showed cell surface alternations in the renal pelvis and papilla in both groups, but only the OPP group exhibited lesions visible by light microscopy (renal papillary necrosis). Histological examination of the kidneys (by microscope only) at week 8, 16, and 24 found renal papillary necrosis and papillary hyperplasia in the OPP and SOPP groups, indicating that epithelial necrosis was first induced and later regeneration and hyperplasia followed. In contrast to the papilla, SOPP affected only the renal pelvis: hyperplasia seen at weeks 16 and 24 was not preceded by necrotic events. Other histological findings included interstitial nephritis at weeks 16 and 24 in the OPP groups, simple hyperplasia in the urinary bladder starting at week 8 in the SOPP group, and P/N hyperplasia in the bladder at weeks 16 and 24 in the SOPP group. In conclusion, this study documented that OPP and SOPP affected the kidneys early in the exposure and at the same time that the urinary bladder was being affected.

St. John *et al.* (2001)

This special toxicity study investigated the role of precipitate formation in OPP- and SOPP-induced bladder carcinogenesis. Three groups of male F344 rats (10 animals/dose) received diets containing 0, 12500 ppm OPP or 20000 ppm SOPP for 10 weeks. Both OPP and SOPP groups had increased ($p<0.05$) incidences of simple hyperplasia (70% and 100%, respectively). Of the animals exhibiting simple hyperplasia, one of the SOPP-treated and three of the OPP-treated also exhibited P/N hyperplasia. In the OPP- and SOPP-groups, urinary bladder also had increases ($p<0.05$) in BrdU-labeling index and SEM lesion severity scoring. The latter indicated that all animals in the SOPP and OPP groups exhibited hyperplasia (Class 5 score; see Table 9 for the severity score description). Since urinalysis showed no formation of abnormal crystals or increase in number of crystals and calculi for any groups, the investigators concluded that OPP and SOPP induced urinary bladder damage *via* a mechanism that did not involve precipitate formation.

III.C.3.b. Interaction with Other Fungicides

Fujii *et al.* (1986)

This special study was to evaluate the interaction of TBZ on SOPP-induced bladder carcinogenesis. In this 13-week study, six groups of F344 rats (10 animals/sex/dose) were involved: four groups received diets containing 0, 2000 ppm TBZ, 10000 ppm SOPP or 20000 ppm SOPP and the other two SOPP groups (at 10000 and 20000 ppm, respectively) received diets containing 2000 ppm TBZ. SOPP-treated males at 10000 ppm had a single case of

hyperplasia in the urinary bladder (Table 15). When increased to 20000 ppm, SOPP induced a marked increase in the incidences of papilloma and carcinoma in the male urinary bladder. At 10000 ppm SOPP, TBZ appeared to increase papillomas and carcinomas in the males whereas at 20000 ppm SOPP, TBZ appeared to enhance the progression of papillomas to carcinomas, without changing the total incidence of combined papilloma and carcinoma. Females at 20000 ppm SOPP had four cases of hyperplasia. No other females in this study exhibited this hyperplasia and none of the female groups developed bladder tumors. Based on these observations, the investigators concluded that co-exposure to TBZ enhanced SOPP-induced urinary bladder carcinogenesis in the males after as little as 13 weeks.

Table 15 Nonneoplastic and Neoplastic Lesions in the Urinary Bladder of Male and Female F344 Rats in a 13-Week TBZ and (or) SOPP Feeding Study (Fujii *et al.*, 1986)[a]

Treatment	Hyperplasia[b]	Papilloma	Carcinoma	Papilloma and Carcinoma
Males				
0	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2000 ppm TBZ	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
10000 ppm SOPP	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
20000 ppm SOPP	2/10 (20%)	5/10 (50%)*	3/10 (30%)	8/10 (80%)[#]
10000 ppm SOPP + 2000 ppm TBZ	1/10 (10%)	6/10 (60%)*	2/10 (20%)	8/10 (80%)[#]
20000 ppm SOPP + 2000 ppm TBZ	1/10 (10%)	0/10 (0%)	8/10 (80%)*	8/10 (80)[#]
Females				
0	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2000 ppm TBZ	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
10000 ppm SOPP	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
20000 ppm SOPP	4/10 (40%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
10000 ppm SOPP + 2000 ppm TBZ	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
20000 ppm SOPP + 2000 ppm TBZ	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)

[a] Summarized incidences of papilloma and carcinoma, as reported by the investigators.
[b] The investigators made no distinction between simple hyperplasia and P/N hyperplasia.
[*] Fisher exact test, as calculated by DPR; significant at $p<0.05$.
[#] Significantly different from the controls at $p<0.05$, as reported by the investigators.

III.D. CHRONIC TOXICITY/ONCOGENICITY

Summary: Results of chronic toxicity and oncogenicity studies of OPP and SOPP are available in the rat, mouse, and dog; the result of a SOPP tumorigenicity study in urinary bladder is also available in the guinea pig and hamster. Toxicity of OPP and SOPP exhibited sex- and species differences. In rats, the effects of OPP occurred mainly in kidneys and urinary bladder; available data indicated that OPP also affected the eyes, optic nerves, spleen, and heart. The kidney effects in males included increased organ weight, reduced renal function, and increased incidences of several nonneoplastic lesions (pyelonephritis, interstitial nephritis, and pelvis/papilla hyperplasia). In comparison to the males, the incidence and severity of the kidney effects were greater in the females. The bladder effects in males included increased organ weight, increased incidences of nonneoplastic lesions (simple hyperplasia), preneoplastic lesions (papillary or nodular [P/N] hyperplasia), and neoplastic lesions (papilloma and carcinoma). In addition, co-exposure to sodium bicarbonate enhanced the bladder tumorigenicity of OPP. In comparison to the males, dietary exposure to OPP had little effect on the urinary bladder in females including the lack of tumor induction.

SOPP affected the urinary tract and pancreas (acinar cell focal atrophy) in rats. The kidney effects in both sexes included increased interstitial nephritis and pyelonephritis, with the effects being more severe in the females. SOPP induced tumors in the kidneys and urinary bladder of males but only bladder tumors in females. Ingestion of SOPP favored the excretion of alkaline urine in the males. Co-exposure to thiabendazole (TBZ), another fungicide used with SOPP for post-harvest treatments, enhanced the urinary bladder tumorigenicity of SOPP. In OPP- and SOPP-exposed males, there were instances wherein the highest tumor incidences in the urinary bladder did not occur at the highest dose tested. The “umbrella-shape” bladder tumor dose-responses suggested an inverse relationship between incidences of bladder neoplasm and nephritis, the latter occurring only at very high dose levels. Using an initiation-promotion study design, OPP and SOPP promoted the carcinogenesis in urinary bladder initiated by a known rat bladder carcinogen, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), *in vivo*.

In mice, OPP affected the liver, kidneys, and spleen. The liver effects in males and females included increased organ weight, increased incidences of nonneoplastic lesions (focal necrosis, anisonucleosis, and pigment deposition in liver cells and phagocytes), preneoplastic lesions (foci of eosinophilic cells), neoplastic lesions (adenoma, hepatoblastoma, and carcinoma), and increased liver-tumor multiplicity. The kidney effects in males included increased organ weight, reduced renal function, and increased incidences of nonneoplastic lesions (tubular epithelium degeneration and necrosis, ductular epithelium degeneration and necrosis, tubular dilation, and papilla transitional cell necrosis and [or] pelvis cell debris). The spleen effect that OPP induced in the male was organ atrophy. Co-exposure to sodium bicarbonate or TBZ enhanced the renal and splenic toxicity of OPP.

SOPP also affected liver and kidneys, together with heart (females only), in mice. Unlike the rats, urinary pH of mice (both sexes) was slightly acidic (ranging from pH = 5.8 to 6.8) and ingestion of SOPP or $NaHCO_3$ did not favor the sustained excretion of alkaline urine in males

(only sex studied). Mice exposed to OPP or SOPP exhibited an increased incidence of circulatory-system tumors (hemangioma and [or] hemangiosarcoma). Similar to the results of oral toxicity studies, liver and kidneys also appeared to be the targets of OPP absorbed *via* the skin (both sexes).

Dogs dosed orally with OPP for 1 year (both sexes) exhibited no adverse toxicological effects. Male mouse, guinea pigs, or hamsters developed no neoplastic and nonneoplastic lesions in the urinary bladder after exposure to SOPP for 4 to 48 weeks.

III.D.1. *ortho*-Phenylphenol

III.D.1.a. Oral – Rat

Chronic toxicity and (or) oncogenicity studies with OPP by Hodge *et al.*, (1954), Hiraga and Fujii (1984), and Wahle and Christenson (1996) are on file at DPR. Two reproductive toxicity studies that are on file at DPR also have information on the urinary tract effects of OPP (Eigenberg, 1989b, Eigenberg and Lake, 1995).

Hodge *et al.* (1954)

Four groups of Wistar rats (25 animals/sex/dose) received diets containing 0, 200, 2000, and 20000 ppm OPP (purity was not specified) for 2 years. In the exposed as well as the control groups, only 22-32% of the animals were alive at the end of 24 months. Although the investigators examined the body weight, organ weight, hematology, urinalysis, pharmacokinetics (tissue distribution), and histopathology, the report presented only selected data. Decreased ($p<0.01$) body weights occurred in males (10%) and females (6%) at 20000 ppm. Another effect observed at this dose level was increased relative testis weight (46%). The investigators stated (without data) that there was an increase in incidences of renal tubular cystic dilation and pyelonephritis. Because of insufficient reporting, DPR considered this study unacceptable for filling SB950 chronic toxicity data requirements.

Hiraga and Fujii (1984)

This report consisted of two studies. The first was a 13-week study, which was discussed in III.C. SUBCHRONIC TOXICITY. In the second study, four groups of male F344 rats (20-24 animals/dose) received diets containing 0, 6250, 12500, or 25000 ppm OPP (>98% pure) for 91 weeks. The investigators reported that the time-weighted average doses were 0, 269, 531, and 1140 mg/kg/day. A separate report by Nakamura *et al.* (1982) described results of organ weight analysis, hematology, serum chemistry, and urinalysis. In the treated groups, survival showed an inverse relationship with dose (269 mg/kg/day, 100%, 531 mg/kg/day, 71% [$p<0.05$], and 1140 mg/kg/day, 65% [$p<0.05$]). Increased ($p<0.05$) white blood cell count occurred at 1140 mg/kg/day (by 20%). Hematuria occurred at 531 and 1140 mg/kg/day; the respective incidences

were 60% and 90% at week 30, and 82% and 50% at week 90. Both the control and the treated groups excreted slightly acidic urines (pH 6.0 to 6.5) at week 90 (the only time those measurements were made) (Table 16).

For the duration of the study, reduced body weight occurred at 531 and 1140 mg/kg/day. These reductions (up to 8% and 21%, respectively), however, appeared to be related to the reductions in feed consumption over the same periods (up to 10% and 23%, respectively). Increased ($p<0.05$) water intake occurred at 1140 mg/kg/day, starting at week 9; by week 89 (the last measurement), the water intake was 102% higher than the controls. Increased ($p<0.05$) water intake also occurred at 531 mg/kg/day, starting at week 17; by week 89, water intake was 29% higher than the controls. The dose-dependent increase in water intake and decrease in the onset time suggest that the treatment may have affected the water metabolism in the 531 and 1140 mg/kg/day groups.

The principle treatment-related effects were nonneoplastic lesions in the kidneys and neoplastic changes in the urinary bladder (Table 17). In kidneys, the incidence of renal pelvis/papilla hyperplasia showed an increase ($p<0.001$) at 1140 mg/kg/day whereas the combined incidences of nephritis showed a dose-related increase starting at 531 mg/kg/day and becoming statistically significant ($p<0.001$) at 1140 mg/kg/day. Also, increases ($p<0.05$) in the absolute kidney weight occurred at 1140 mg/kg/day (48%) and in the relative weights at 531 and 1140 mg/kg/day (9% and 95%, respectively). Serum chemistry and urinalysis showed that elevated ($p<0.05$) blood urea nitrogen (BUN) occurred at 1140 mg/kg/day (34%) and decreased ($p<0.05$) urinary protein concentration in each of the OPP-exposed groups (Table 16). Although the investigators reported polydipsia instead of urine volume, the decreased protein concentration would be consistent with an increased urine volume, a relationship that Christenson *et al.* (1996a) noted in the same rat strain.

Urinary bladder exhibited both nonneoplastic (simple hyperplasia) and neoplastic lesions (papilloma and carcinoma) at 1140 mg/kg/day (Table 17). Paradoxically, while almost all animals in 531 mg/kg/day group had bladder tumors, only 17% had these tumors at 1140 mg/kg/day. It is possible that the reduced survival at the high dose caused the decreased tumor incidence; i.e., the high dose animals did not live long enough for tumors to appear (Bailer and Portier, 1988). However, the treatment-induced mortality in the 1140 mg/kg/day group may not be the sole cause of the tumor reduction based on a subchronic dietary study of OPP involving male F344 rats (Hiraga and Fujii, 1984). In that subchronic study, dose-response for papillomas exhibited a downturn at the high dose (1493 mg/kg/day) even though the treatment did not significantly affect the survival of that group. The decreased bladder tumor incidence may be due to nephrotoxicity (i.e., nephritis) that was also induced at the high dose (Fujii *et al.*, 1987) (see III.C. SUBCHRONIC TOXICITY). Increased ($p<0.05$) absolute and relative bladder weights occurred at 1140 mg/kg/day (46% and 82%, respectively); the 531 mg/kg/day group showed the same effects but at much higher levels (310% and 345%, respectively).

Another organ that OPP affected was the spleen. Each of the treated groups had decreased absolute spleen weight (16-24%). The decrease was considered to have occurred

Table 16 Urinary Protein Concentration and pH Measurements made in Male F344 Rats in a 91-Week OPP Feeding Study (Hiraga and Fujii, 1984)

Parameters	ppm			
	0	6250	12500	25000
Dose (mg/kg/day)	0	269	531	1140
Total Animal Tested[a]	17	16	6	8
Protein Conc. (mg/dL)[b]	959	248 (26)**	98 (10)**	74 (8)**
pH[b]	6.02	6.04	6.05	6.04

[a] Differences in sample size among dose groups were not explained.
[b] Mean value calculated by DPR based on categorical data measured in week 90 using test tapes. Percent of the negative controls is given in parentheses. Mean pH = -log (Σ (number of animals x 10^{-pH})/total number of animals).
** Non-parametric Shirley's test (Shirley, 1977), as calculated by DPR; significant at $p<0.01$.

Table 17 Nonneoplastic, Preneoplastic, and Neoplastic Lesions in the Kidneys and Urinary Bladder of Male F344 Rats in a 91-Week OPP Feeding Study (Hiraga and Fujii, 1984)

Organ/Lesions[a]	ppm			
	0	6250	12500	25000
Dose (mg/kg/day)	0	269	531	1140
Kidneys				
Hyperplasia (Pelvis/Papilla)	0/24 (0%)$^{+++}$	0/20 (0%)	0/24 (0%)	12/23 (52%)***
Pyelonephritis	0/24 (0%)$^{+++}$	0/20 (0%)	1/24 (4%)	15/23 (65%)***
Interstitial Nephritis	0/24 (0%)$^{+++}$	0/20 (0%)	2/24 (8%)	8/23 (35%)**
Combined Nephritic Lesions[b]	0/24 (0%)$^{+++}$	0/20 (0%)	3/24 (13%)	23/23 (100%)***
Bladder				
Simple Hyperplasia	0/24 (0%)$^{+++}$	2/20 (10%)	0/24 (0%)	7/23 (30%)**
Papilloma	0/24 (0%)	0/20 (0%)	3/24 (13%)	2/23 (8%)
Carcinoma	0/24 (0%)	0/20 (0%)	20/24 (83%)***	2/23 (8%)
Combined Tumor[c]	0/24 (0%)$^{+}$	0/20 (0%)	23/24 (96%)***	4/23 (17%)*

[a] Summarized lesion incidences, as reported by the investigators. Time of death was not reported, and no corrections were made for instances of early death.
[b] Combined incidences of pyelonephritis and interstitial nephritis, as reported by the investigators.
[c] Combined papilloma and carcinoma incidences, as reported by the investigators.
*, **, *** Fisher exact test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.
+, ++, +++ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

independently of a general body weight reduction because the latter did not occur in the low-dose group. At 269 mg/kg/day, serum chemistry showed that there were increases ($p<0.05$) in activities of serum aspartate aminotransferase (AST, 37%), alanine aminotransferase (ALT, 17%), and alkaline phosphatase (ALP, 24%). Because the 531 and 1140 mg/kg/day groups had no elevated enzyme activities, it is doubtful whether these effects at the low dose represent liver injury attributable to OPP.

In conclusion, the results of this study indicated the following: (1) OPP affected the kidneys and urinary bladder, as well as, the spleen; (2) OPP-induced nephrotoxicity (i.e., nephritis) may affect the tumorigenesis in urinary bladder; and (3) OPP induced polydipsia. Given that the test employed only one sex and the histopathology information was missing for major organs besides the kidneys and bladder, DPR determined that this study was unacceptable for filling the SB950 chronic toxicity and oncogenicity data requirements.

Wahle and Christenson (1996)

Four groups of F344 rats (46-50 animals/sex/dose) received diets containing 0, 800, 4000, or 8000 ppm (males)/10000 ppm (females) OPP (≥ 99.5% pure) for 2 years. The investigator reported that the time-weighted average doses were 0, 39, 200, and 402 mg/kg/day for the males and 0, 49, 248, and 647 mg/kg/day for the females. For an interim sacrifice at 1 year, the control and high-dose groups involved 20 rats per sex whereas the low- and mid-dose groups involved 10 rats per sex. In addition, 80 animals receiving treatments (10/sex/dose) served as a pool of replacement animals until their sacrifice occurred at about one month after the replacement took place. The replacement involved 12 animals, 6 of which were in the high-dose female groups (three for the interim sacrifice group and three for the terminal sacrifice group). Three of the 6 high-dose females that were replaced had perigenital urine staining and the other three, red ocular discharge. The former constituted a treatment-related finding.

The survival of male and female groups exposed to OPP was not statistically different from the controls (Wahle and Christenson, 1999). Reductions ($p<0.05$) in body weights followed a dose-response in both sexes at ≥4000 ppm OPP. The body weight reductions were evident by week 3 and persisted for the rest of the study. At the high dose, the reduction was ~10% for both sexes and paralleled significant ($p<0.05$) decreases in feed consumption (g/animal/day) of 4-8%.

Urine pH values were similar between the control and treated groups for the duration of the study, generally ranging between 7.5-8.2 for the males and 7.0-7.9 for the females (i.e., slightly alkaline). At the high and mid doses, decreased ($p<0.05$) urinary protein concentrations occurred in both sexes for almost the entire study (Table 18). Decreased ($p<0.05$) urine specific gravity occurred in the high-dose males for the first year and high-dose females for most of the study. At the mid dose, decreased ($p<0.05$) urine specific gravity also occurred at week 26 with the males and at weeks 13 and 26 with the females. Although the investigators did not measure water intake, other studies indicated that chronic oral exposure of OPP caused polydipsia in rats

Table 18 Selected Urinalysis Parameters for Male and Female F344 Rats in a 2-Year OPP Feeding Study (Wahle and Christenson, 1996)

Sex/ Parameters[a]/ Study Week	ppm			
	0	800	4000	8000/10000[b]
Males				
Dose (mg/kg/day)	0	39	200	402
Protein Conc. (mg/dL)[c]				
13	53±36	36±23 (68)	34±24 (64)	32±17 (60)*,#
26	137±85	90±26 (66)	50±34 (36)*,#	40±26 (29)*,#
51	270±73	280±62 (104)	120±62 (44)*,#	61±37 (23)*,#
79	290±45	300±0 (103)	167±102 (58)*,#	86±29 (30)*,#
103	300±0	300±0 (100)	230±98 (77)*,#	76±34 (25)#
Specific Gravity				
13	1.050±0013	1.053±0.009	1.044±0.01	1.044±0.005*,#
26	1.059±0.013	1.055±.007	1.049±0.008*,#	1.042±0.003*,#
51	1.050±0.008	1.049±0.01	1.048±0.01	1.038±0.008*,#
79	1.037±0.011	1.041±0.007	1.037±0.006	1.034±0.006
103	1.035±0.006	1.038±0.005	1.036±0.008	1.031±0.008
Females				
Dose (mg/kg/day)	0	49	248	647
Protein Conc. (mg/dL)[c]				
13	25±21	35±34 (140)	13±9#,* (52)	11±8#,* (44)
26	28±18	22±9 (79)	10±10#,* (36)	11±8#,* (39)
51	65±85	65±64 (100)	20±9#,* (31)	17±8#,* (26)
79	180±114	166±115 (92)	37±33#,* (21)	18±23#,* (10)
103	266±85	254±92 (95)	132±117#,* (52)	38±65#,* (14)
Specific Gravity				
13	1.052±0.018	1.058±0.016	1.044±0.011*	1.044±0.009*
26	1.055±0.016	1.052±0.015	1.044±0.007#,*	1.043±0.007#,*
51	1.044±0.009	1.048±0.007	1.042±0.007	1.040±0.006
79	1.038±0.007	1.038±0.007	1.035±0.005	1.032±0.007#,*
103	1.035±0.005	1.038±0.006	1.034±0.011	1.030±0.007*

Abbreviation: N: negative trend.
[a] Mean ± standard error for groups of 20 animals.
[b] Males and females received diets containing 8000 ppm and 10000 ppm OPP, respectively.
[c] Categorical data. Percent of the negative controls is given in parentheses.
[#] Parametric Dunnett's test, as reported by the investigators; significant at $p<0.05$.
[*] Non-parametric Shirley's test (Shirley, 1977), as calculated by DPR; significant at $p<0.05$.

(e.g., Hiraga and Fujii, 1984). The decreased urinary protein concentration and urine specific gravity observed in this study would be consistent with an increased urine output related to polydipsia, a relationship that Christenson *et al.* (1996a) noted in the same rat strain.

Animals exposed to OPP exhibited increases in two types of perigenital findings (i.e., clinical observations): dark red (hence blood) discharge from the penis and urine stain. Dark red discharge from the penis occurred only in the terminal sacrificed males at the high dose (36%; $p<0.05$). In the interim and terminal sacrificed males at 8000 ppm, the incidence of urine stain was 20% ($p=0.053$) and 36% ($p<0.05$), respectively. In the interim and terminal sacrificed females at 10000 ppm, the incidence of urine stain was 70% ($p<0.05$) and 64% ($p<0.05$), respectively. Unlike the males, the mid- and low-dose females also had increased incidence of urine staining. In the terminal sacrifice females, the incidences of urine stain at 0, 800, and 4000 ppm were 24%, 42% ($p<0.05$), and 54% ($p<0.05$), respectively. Similarly, in a 4-week range-finding study that was conducted prior to the main study (reported in Wahle and Christenson, 1996), 59% the female rats fed a diet containing ~12500 ppm OPP (~625 mg/kg/day OPP[24]) exhibited urine strain ($p<0.05$); also, in this dose group, there was a significant ($p<0.05$) increase in urine volume (40%)[25].

Several serum chemistry changes repeatedly achieved statistical significance ($p<0.05$). Those seen at three or more of the five times that measurements were taken and included the following. Decreased creatine kinase and lactate dehydrogenase activities occurred in males at 8000 ppm on days 89, 179, and 551 and in females at 10000 ppm on days 179, 354, and 551. Increased serum albumin occurred in males at 4000 and 8000 ppm on days 179, 354, 718 and in females at 10000 ppm on days 89, 179, and 551; often the changes were accompanied by decreases in globulin, without changes in total protein. Increased blood urea nitrogen (BUN) occurred in males on days 179 (mid and high doses), 354 (mid and high doses) and 551 (high dose) and in females on days 89 (low, mid, and high dose), 179 (mid and high doses), 551 (high dose), and 718 (high dose). Decreased triglycerides levels occurred in males at 8000 ppm on days 179, 551, and 718 and at 4000 ppm on days 179, 354, and 718; often the changes in males were accompanied by a decrease in cholesterol. In females, decreased triglycerides levels occurred on days 551 (mid and high doses) and 718 (high dose); these were not accompanied by significant changes in cholesterol.

Statistically significant hematological changes sometimes occurred in the mid- and (or) high-dose groups (both sexes) but the magnitude of these changes made it doubtful whether these changes were treatment related. The most consistent hematological changes were decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) observed in the high-dose female group. However, for both indices, these changes amounted to decreases of ≤3%.

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[24] DPR performed the calculation using a default assumption of ~5% daily feed consumption.

[25] The investigators found no significant difference in urine volumes among the treated groups using parametric statistical techniques (Wahle and Christenson, 1999). Because urinalysis data have skewed distributions (NTP, 2004), DPR applied non-parametric multiple comparison methods as recommended by National Toxicology Program (NTP, 2004) to re-evaluate the data.

OPP affected the kidneys (both sexes) and urinary bladder (mainly in males). In the terminal sacrifice males, the main kidney effect was cystic tubular dilation/degeneration: this lesion had 34% incidence at 8000 ppm ($p<0.05$). By contrast, at this same dose, the bladder exhibited multiple effects (Table 19): simple hyperplasia, papillary nodular and (or) nodular hyperplasia, papilloma, and carcinoma in both the interim and terminal sacrifice groups. Consistent with the proposal that P/N hyperplasia, papilloma, and carcinoma constitute a morphological continuum of urinary bladder neoplasia (Jokinen, 1990), the terminal sacrifice males at 8000 ppm exhibited a markedly increased incidence of carcinoma ($p<0.01$) in association with decreased incidences of P/N hyperplasia and papilloma. At 4000 ppm, the terminal sacrifice male group also exhibited a dose-related increase in simple hyperplasia (12% incidences) and carcinoma (4% incidence). While the tumor incidence was not statistically significant, DPR considered it a probable treatment-related effect because of the rare spontaneous occurrence of this tumor (<0.5%) in this strain of rats (Wahle and Christenson, 1996). Other effects ($p<0.01$) observed in the bladder at 8000 ppm included the following (incidence in parentheses): congestion (32%), hemorrhage (18%), mineralization (36%), necrosis (40%), and calculus (42%).

In females, OPP induced toxicity in the kidneys with little effect on the urinary bladder. The kidney effects occurred almost exclusively in the terminal sacrifice females and only at the high dose. At necropsy, kidneys of 15 females (30% incidence) were pitted or had an abnormal texture ($p<0.05$). At histology, increased ($p<0.05$) incidence of kidney lesions included the following (incidences in parentheses): papilla mineralization (24%), cystic tubular dilation/degeneration (74%), cortical infarct (58%), tubular hyperplasia (60%), and acute inflammation (22%). In the case of cystic tubular dilation/degeneration, the incidence in the interim sacrifice females at the high dose was 25% ($p<0.05$). By contrast, urinary bladder findings were limited to 12% incidence of simple hyperplasia ($p<0.05$) and a single case of P/N hyperplasia; these occurred only in the terminal sacrifice females in the high dose group. No urinary bladder tumors were observed in females.

Other organs that OPP affected were the eyes and heart. At ophthalmology, increased ($p<0.05$) incidence of cataract occurred in the terminal sacrifice males at 8000 ppm (61% incidence *vs.* 36% in the controls) and increased ($p<0.05$) incidences of cataract, uveitis, and corneal vascularization occurred in the females at 4000 ppm (incidences of 27%, 22%, and 22%, respectively; the respective incidence in the controls were 7%, 4%, and 2%). The female histological data for the 4000 ppm group exhibited increased ($p<0.05$) incidences of retinal degeneration (27% incidence *vs.* 7% in the controls) and optic nerve atrophy (29% incidence *vs.* 13% in the controls). The incidences of these eye effects did not similarly increase in the 10000 ppm female group. Supplemental data submitted by the Registrant indicated that there were problems in the reporting of the various evaluations that pertain to the eyes and optic nerves (ophthalmology, clinical observations, necropsy, and histology) for this study. Since OPP and its metabolites are chemically similar to the metabolites of naphthalene, which are responsible for its cataractogenic activity (Gehring, 1971, Wells *et al.*, 1989), potential injury to the vision system attributable to OPP administration in both sexes should not be dismissed without further investigation.

Table 19 Nonneoplastic, Preneoplastic, and Neoplastic Lesions in the Urinary Bladder of Male F344 Rats in a 2-Year OPP Feeding Study (Wahle and Christenson, 1996)[a]

Lesions	mg/kg/day[b] (ppm)			
	0 (0)	39 (800)	200 (4000)	402 (8000)
Simple Hyperplasia				
Interim sacrifice	0/20 (0%)$^{+++}$	0/10 (0%)	0/10 (0%)	20/20 (100%)***
Terminal sacrifice	2/50 (4%)$^{+++}$	2/50 (4%)	6/50 (12%)	42/50 (84%)***
Papillary/ Nodular Hyperplasia				
Interim sacrifice	0/20 (0%)$^{+++}$	0/10 (0%)	0/10 (0%)	20/20 (100%)***
Terminal sacrifice	1/50 (1%)$^{+++}$	0/50 (0%)	0/50 (0%)	43/50 (86%)***
Papilloma				
Interim sacrifice	0/20 (0%)$^{+++}$	0/10 (0%)	0/10 (0%)	6/20 (30%)*
Terminal sacrifice	0/50 (0%)$^{+++}$	1/50 (2%)	0/50 (0%)	6/50 (12%)*
Carcinoma				
Interim sacrifice	0/20 (0%)$^{++}$	0/10 (0%)	0/10 (0%)	3/20 (15%)[b]
Terminal sacrifice	0/50 (0%)$^{+++}$	0/50 (0%)	2/50 (4%)[b]	34/50 (68%)***
Combined Tumors[d]				
Interim sacrifice	0/20 (0%)$^{+++}$	0/10 (0%)	0/10 (0%)	9/20 (45%)***
Terminal sacrifice	0/50 (0%)$^{+++}$	1/50 (2%)	2/50 (4%)[c]	40/50 (80%)***

[a] Summarized lesion incidences, as reported by the investigators.
[b] Time-weighted average doses, as reported by the investigators.
[c] Incidence exceeded the incidence of urinary bladder carcinoma in historical control males at the conducting laboratory (0.49% [2/409]) (Wahle and Christenson, 1996).
[d] Combined incidence of papilloma and carcinoma, as performed by DPR.
*,**,*** Fisher Exact test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.
+,++,+++ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.

In the terminal sacrifice females, the incidences of cardiac degeneration and (or) fibrosis in both the controls and high-dose groups were comparable (54% and 46%, respectively) [26]. By contrast, the mid and low-dose groups exhibited increased ($p<0.05$) incidences (84% and 75%, respectively). Although the elevated incidence did not occur at the high dose females, this group showed reductions in feed consumption and body weight gain and feed restriction/body weight reduction is known to reduce the incidence and severity of cardiac fibrosis in both sexes of F344 rats (Imai *et al.*, 1991). In the 2-year terminal sacrifice males, the incidences of vascular mineralization involving the wall of the heart-base vessels in the 0, 800, 4000, and 8000 ppm groups were 2%, 8%, 22% ($p<0.05$) and 11%, respectively.

In conclusion, this study documented the following: (1) OPP affected the kidneys and urinary bladder, as well as the eyes (including the optic nerves) and heart; (2) tumors occurred only in the urinary bladder of males; and (3) females exhibited greater severity and incidence of kidney lesions than males. Based on increased simple hyperplasia incidence in the urinary bladder of males and decreased urine protein concentration, decreased urinary specific gravity, and increased BUN levels in both sexes, the NOEL was 800 ppm. The potential eye effects in females (retinal degeneration and optic nerve atrophy) also achieved the same NOEL. The NOEL was 39 mg/kg/day OPP based on males. With respect to the heart effect, based on increase in cardiac degeneration and (or) fibrosis incidence in females, the LOEL was 800 ppm (i.e., 49 mg/kg/day). The USEPA established a NOEL of 800 ppm for this study (30 mg/kg/day for males and 49 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 (Dapson, 1996). Because in some areas this study was not conducted in accordance with the FIFRA guidelines, the study was classified as unacceptable. However, since this study clearly demonstrated toxic effects in the kidneys and bladder (including tumorigenicity), DPR required no other long-term study for satisfying the data need under the California Birth Defects Prevention Act of 1984 (SB 950). DPR uses this study for selecting the critical chronic NOEL for assessing the risk associated chronic exposure to OPP (see IV. RISK CHARACTERIZATION for details).

Eigenberg (1989b) and Eigenberg and Lake (1995)

These reports were reproductive-toxicity studies (up to 43 weeks in duration) using Sprague-Dawley rats. Kidneys in the males exhibited nonneoplastic lesions and urinary bladder in both sexes exhibited nonneoplastic, preneoplastic, and neoplastic effects (see III.F. REPRODUCTIVE TOXICITY for details).

III.D.1.b. Oral – Mouse

[26] Cardiomyopathy was evaluated based on the original data submitted to DPR by Wahle and Christenson (1996) because there was no indication in a rebuttal submitted by the Registrant that re-evaluation of the heart effect was done in a blind fashion and otherwise was done in compliance with guidelines (e.g., Fenner-Crisp [1994]).

Mikuriya *et al.* (1989a) and Quast and McGuirk (1995) studied the chronic toxicity and oncogenicity of OPP. The former is available from the open literature while the latter is on file at DPR.

Mikuriya *et al.* (1989a)

Four groups of male B6C3F1 mice (20 animals/dose) received diets containing 0, 6500, 13000, or 26000 ppm OPP (≥99.7% pure) for 52 weeks. The investigators reported that respective time-weighted average doses were 0, 92, 198, and 447 mg/kg/day[27]. The only death occurred in 13000 ppm group at week 40. OPP had no effects on ALT, AST, γ-GPT, and LDH activities. Urine pH values were similar between the control and treated groups for the duration of the study, generally ranging between 5.8 and 6.8 (i.e., slightly acidic).

Exposure to OPP for 52 weeks resulted in reductions ($p<0.05$) in body weights at 13000 and 26000 ppm (25% and 43%, respectively). Feed consumption of the treated groups was not significantly different from the controls, except the 26000 ppm group, which showed a decrease ($p<0.05$) over the first 10 weeks. Water intake at 26000 ppm was lower ($p<0.05$) than the controls over the first 6 weeks, then it started to increase ($p<0.05$) at week 30; by week 51 (last time point measured), water intake was 69% higher than the controls. Increased ($p<0.05$) water intake also occurred at 6500 and 13000 ppm, starting at weeks 38 and 10, respectively; and by week 51, water intakes were 38% and 100%, respectively, higher than the controls. Although the effect on water intake seemed to be maximal in the mid-dose group, overall, the increased water intake and decreased onset time suggest that all OPP-exposed groups may have affected water metabolism.

OPP affected the urinary bladder and kidneys. In urinary bladder, increases ($p<0.05$) in the absolute weight occurred at 13000 ppm (37%) and the relative weights at 13000 ppm (70%) and 26000 ppm (59%). OPP induced nonneoplastic lesions in the kidneys ($p<0.05$ when all severity grades are combined) at the low, mid, and high doses (Table 20). In addition to the increased incidence, the lesion severity (e.g., tubular epithelium degeneration) also appeared to increase with dose. Reduced ($p<0.05$) absolute kidney weights occurred in each of the OPP-treated groups (7-24%) but the dose-response seemed consistent with the general body weight reductions noted in these groups (12-43%).

Other organs that OPP affected were the spleen and liver. The main effect in the spleen was atrophy; this occurred in each of the exposed groups (mainly grade of very slight, $p\leq0.05$ in each case) (Table 20). In the liver, although only OPP-treated groups had tumors (type not stated), the tumor incidences did not represent a dose response: the respective incidences at 0, 6500, 13000, and 20000 ppm OPP were 0%, 5%, 10%, and 5%. Multiple nonneoplastic lesions

[27] Time-weight average doses were not reported in this study; however, the investigators reported the information in another 52-week OPP dietary study (Mikuriya *et al*., 1992).

Table 20 Nonneoplastic Lesions in the Kidneys and Liver of Male B6C3F1 in a 52-Week OPP Feeding Study (Mikuriya *et al*., 1989a)

Organ/Lesions/ Severity Score	mg/kg/day[a] (ppm)			
	0 (0)	92 (6500)	198 (13000)	447 (26000)
Kidney				
Degeneration of Tubular Epithelium				
Very Slight	0/20 (0%)	7/20 (35%)**	11/20 (55%)**	4/20 (20%)
Slight	0/20 (0%)	6/20 (30%)*	9/20 (45%)**	15/20 (75%)***
Moderate	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)
Combined[b]	0/20 (0%)	13/20 (65%)***	20/20 (100%)***	20/20 (100%)***
Necrosis of Tubular Epithelium				
Very Slight	0/20 (0%)	0/20 (0%)	4/20 (20%)	6/20 (30%)*
Slight	0/20 (0%)	0/20 (0%)	1/20 (5%)	2/20 (10%)
Combined[b]	0/20 (0%)	0/20 (0%)	5/20 (25%)*	8/20 (40%)**
Dilation of Tubules				
Very Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)
Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	3/20 (15%)
Moderate	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)
Combined[b]	0/20 (0%)	0/20 (0%)	0/20 (0%)	5/20 (20%)*
Degeneration of Ductular Epithelium				
Very Slight	0/20 (0%)	0/20 (0%)	1/20 (5%)	3/20 (15%)
Necrosis of Ductular Epithelium				
Very Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	4/20 (20%)
Necrosis of Transitional Cells in Papilla and (or) Cell Debris in Pelvis				
Very Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)
Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	8/20 (40%)**
Moderate	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)
Combined[b]	0/20 (0%)	0/20 (0%)	0/20 (0%)	10/20 (50%)***
Spleen				
Atrophy				
Very Slight	0/20 (0%)	4/20 (20%)*	8/20 (40%)**	9/20 (45%)**

(continued)

Table 20 Nonneoplastic Lesions in the Kidneys and Liver of Male B6C3F1 in a 52-Week OPP Feeding Study (Mikuriya *et al*., 1989a)(continued)

Organ/Lesions/ Severity Score	mg/kg/day[a] (ppm)			
	0 (0)	92 (6500)	198 (13000)	447 (26000)
Liver				
Focal Necrosis of Liver Cells				
Very Slight	0/20 (0%)	0/20 (0%)	2/20 (10%)	5/20 (25%)*
Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	8/20 (40%)**
Combined[b]	0/20 (0%)	0/20 (0%)	2/20 (10%)	13/20 (65%)***
Anisonucleosis of Liver Cells with Enlargement				
Very Slight	0/20 (0%)	0/20 (0%)	20/20 (100%)***	17/20 (85%)***
Slight	0/20 (0%)	0/20 (0%)	0/20 (0%)	3/20 (15%)
Combined[b]	0/20 (0%)	0/20 (0%)	20/20 (100%)***	20/20 (100%)***
Deposit of Pigment in Liver Cells and Phagocytes				
Very Slight	0/20 (0%)	0/20 (0%)	7/20 (35%)**	10/20 (50%)***
Slight	0/20 (0%)	0/20 (0%)	1/20 (5%)	7/20 (35%)**
Combined[b]	0/20 (0%)	0/20 (0%)	8/20 (40%)**	17/20 (85%)***

[a] These values are the time-weighted average doses as reported by the investigators; the values were reported in a later study (Mikuriya *et al*., 1992).
[b] Combined incidences of lesions having any severity score, as performed by DPR.
*,**,*** Fisher exact test, as calculated by DPR; significant at $p \leq 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

occurred in the 13000 and 26000 ppm groups: focal necrosis of liver cells, anisonucleosis[28], and pigment deposition in liver cells and phagocytes (Table 20). Except for the focal necrosis, each of these effects at both doses was a statistically ($p<0.05$) significant finding. Increases ($p<0.05$) in absolute liver weight occurred at 13000 ppm (11%) and the relative weights at 6500 ppm (18%), 13000 ppm (39%), and 26000 ppm (41%).

In conclusion, the result of this study indicated that OPP affected the liver, kidneys, urinary bladder, and spleen. Also, this study documented that OPP induced polydipsia in mice. Based on the induction of renal tubular epithelium degeneration, spleen atrophy, increased water intake, and increased relative liver weight, the LOEL was 6500 ppm (i.e., 92 mg/kg/day). This report was not a FIFRA guideline study (e.g., only one sex was tested).

Quast and McGuirk (1995)

Four groups of B6C3F1 mice (48-50 animals/sex/dose) received diets containing OPP (≥99.7% pure) for 2 years to achieve constant doses of 0, 250, 500, or 1000 mg/kg/day. In addition, each dose group included 9-10 mice per sex for an interim sacrifice at 1 year. OPP did not affect survival of the interim sacrifice animals (both sexes) and the terminal sacrifice males. Survival in the terminal sacrifice females was unusual in that the low- and mid-dose groups had the largest numbers of animals not surviving to terminal sacrifice, 20 and 22 ($p<0.05$)[29], respectively, compared to 12 in the control group and 14 in the high-dose group. Clinical observations and hematology did not identify any treatment-related effect. In addition, investigators observed no feed wastage in the daily animal inspections. At the interim sacrifice, increases ($p<0.05$) in serum alkaline phosphatase (ALP) activities occurred at the low-, mid-, and high-dose male groups (by 40%, 85%, and 90%, respectively) and in the mid- and high-dose female groups (by 14%[30] and 32%, respectively). At the terminal sacrifice, increased ALP activities occurred in the mid- and high-dose male groups (by 14% and 83%, respectively; both at $p<0.05$); ALP in the high-dose female group was increased (by 34%) but the change did not achieve statistical significance. Urine pH values were similar between the control and treated groups (8-10 animals/sex) for the duration of the study. With few exceptions, urine pH was ≤7 at the interim sacrifice and ≤6.5 at the terminal sacrifice (i.e., slightly acidic). Decreased ($p<0.05$) urine specific gravity occurred in the mid- and high-dose females at the interim and terminal sacrifices. In the males, only the decrease at the interim sacrifice in the high-dose group was statistically significant ($p<0.05$).

Body weights among the test groups (by sex) were comparable after the first 13 weeks of treatment. At week 21, body weight reduction ($p<0.05$) occurred at the high dose (both sexes) (3-5%). By 1 year, the mid- and high-dose groups (both sexes) exhibited body weight reductions ($p<0.05$): 7% reduction at the mid-dose and 13-16% at the high dose. The degree of reduction

[28] Anisonucleosis is irregularity in the size of the nuclei.
[29] Fisher exact test (12/48 *vs.* 22/50), as calculated by DPR.
[30] Non-parametric Shirley's test (Shirley, 1977) calculated by DPR; significant at $p<0.05$.

continued to increase with time with the females, but not with the males. At 2 years, the mid- and high-dose females had body weights reduced by 13% ($p<0.05$) and 20% ($p<0.05$), respectively, whereas the mid and high-dose males had body weights reduced by 7% (statistically not significant) and 13% ($p<0.05$), respectively. The body weight reductions occurred with no concurrent reductions in feed consumption; rather, increases in cumulative feed consumption (g/animal) occurred at the low, mid, and high doses: by 4%, 2%, and 9% for the males and by 3%, 8%, and 5% for the females, respectively[31].

OPP-treated females exhibited kidney hypertrophy. Increased absolute kidney weights occurred in the low-, mid-, and high-dose females groups at the interim sacrifice (by 2-4%, statistically not significant); the same occurred at the terminal sacrifice except that the high-dose group showed a decrease by 1%. Consistent with the changes in absolute kidney weights in the mid- and high-dose female groups in the presence of reduced body weights, increased relative kidney weights ($p<0.05$) occurred in each of the OPP-exposed groups[32] at the interim as well as terminal sacrifices. In males, the mid- and high-dose groups exhibited decreased ($p<0.05$) absolute kidney weights at the interim (by 14% and 16%, respectively) and terminal sacrifices (by 7% and 14%, respectively). However, given that non-significant effects occurred in the relative kidney weights at these times, the decreased absolute weights appeared to be a reflection of the decreased body weights occurring with these groups. Renal tubular epithelial cells in sexually mature males (but not the females) have lipid vacuoles. In the interim and terminal sacrificed groups, every male exposed to OPP had decreased vacuolation in renal tubular epithelial cells, starting with the low dose ($p<0.001$ based on all grades combined). In addition, in the terminal sacrifice groups (both sexes), the severity grading for degeneration/regeneration of the renal tubules decreased in each of the OPP-treated groups, starting with the low dose ($p<0.05$).

Another organ in which OPP induced hypertrophy and nonneoplastic changes was the liver. At interim sacrifice, increases in the absolute liver weights occurred in the low-, mid-, and high-dose male groups (by 17%, 7%, and 25% [$p<0.05$], respectively) and the corresponding female groups (by 9%, 17%, and 27%, respectively, $p<0.05$ each). In addition, increased ($p<0.05$) relative liver weights occurred in each of the OPP-treated male and female groups at the interim sacrifice. At terminal sacrifice, increased ($p<0.05$) absolute liver weights still occurred in both mid- and high-dose groups: in the males by 15% and 11%, respectively, and in the females by 37% and 23%, respectively. However, the high incidences of liver tumors in the terminal sacrifice groups may have complicated the determination that the liver weight data constitute evidence of sustained liver hypertrophy (Tables 21 and 22). All groups (both sexes) exposed to OPP for 1 year as well as 2 years exhibited an increase ($p<0.05$) in the incidence of an accentuated lobular pattern (an area wherein cells were larger and their cytoplasm showed increased eosinophilia). In the terminal sacrifice groups, hepatocellular vacuolation consistent with fatty change occurred at a 20% incidence in the control males but the high-dose males had no such hepatocellular vacuolation ($p<0.001$); the corresponding incidences in the mid- and low-

[31] The investigators did not report the individual feed consumption data nor any statistical analyses of these data.
[32] William's t-test (William, 1972) calculated by DPR; significant at $p\leq0.05$.

dose males were 14% (statistically not significant) and 6% ($p<0.05$). In females, neither the control nor OPP-exposed groups had this hepatocellular-vacuolation finding.

Liver in the OPP-treated groups also exhibited preneoplastic and neoplastic lesions (Tables 21 and 22). Increased incidence of foci of eosinophilic cells occurred in the mid- and high-dose terminal sacrifice groups (both sexes); the effect in male groups also achieved statistically significance ($p\leq0.01$). Increased ($p<0.01$) incidences of adenoma occurred in the terminal sacrifice males at the mid and high doses. Although the treatments did not affect the incidence of carcinoma in the males, the terminal sacrifice groups at the low, mid, and high doses had a rarely observed variant of hepatocellular carcinoma, hepatoblastoma. Only the incidence of hepatoblastoma at the mid dose was statistically significant ($p<0.05$). However, DPR considered that the induction of this tumor was a treatment-related effect at each dose level because of its rare spontaneous occurrence (<0.1%) in this strain of mice (Haseman *et al*., 1999). In addition to the effect on tumor incidences, OPP affected the average number of liver tumors per mouse (i.e., tumor multiplicity). In the terminal sacrifice males, the total numbers of liver tumors (all types combined) per group were 57, 83, 111, and 108 for the control, low-, mid-, and high- dose groups, respectively. The respective numbers of terminal sacrifice males bearing two or more liver tumors were 14, 23, 30, and 30. Five was the maximum number of liver tumors observed: one male in the negative-control group exhibited this maximum whereas four occurred in each of the OPP-exposed male groups. In females, except for a significantly ($p<0.05$) increased carcinoma incidence at the low dose for the terminal sacrifice animals, the statistical comparisons between the treated groups with the controls did not identify any significantly increased liver-tumor incidences (Table 22). However, as with the males, OPP affected the average number of liver tumors per mouse in the females. In the terminal sacrifice females, the total numbers of liver tumors (all types combined) per group were 20, 29, 42, and 39 for the control, low-, mid-, and high-dose groups, respectively. The respective numbers of terminal sacrifice females bearing two or more liver tumors were 5, 7, 12, and 12; three was the maximum number of liver tumors observed in the negative-control group whereas the maximums in the low- mid- and high-dose groups were 3, 7, and 5, respectively.

Another neoplasm observed in OPP-treated animals was vascular tumors (hemangiomas and hemangiosarcomas). There were two indications that elevated incidence of vascular tumors may have occurred in the low-dose male group. First, the low-dose males had four animals with liver hemangioma (8% incidence), compared to only three liver hemangiomas occurring among 1350 males (0.2% incidence) in the NTP database (Haseman *et al.*, 1999); the respective incidences in the control, mid- and high-dose male groups were 2%, 0%, and 4%. Second, the low dose males had 6 animals with hemangiosarcoma in the spleen, compared to one in the control group ($p=0.053$)[33]. However, 12% (6/49) as the low-dose incidence may not be an estimation of the full incidence and may be an underestimate because the investigators only examined 15 of 34 spleens microscopically. Of the spleens examined, 40% (6/15) exhibited a

[33] Fisher exact test (1/50 *vs.* 6/49), calculated by DPR.

Table 21 Preneoplastic and Neoplastic Lesions in the Liver of Male B6C3F1 Mice in a 2-Year OPP Feeding Study (Quast and McGuirk, 1995)

Lesions[a]/Sacrifice	mg/kg/day[b]			
	0	250	500	1000
Foci of Eosinophilic Cells				
Interim Sacrifice	0/10 (0%)	0/9 (0%)	0/10 (0%)	1/10 (10%)
Terminal Sacrifice	3/50 (6%)$^{+++}$	6/49 (12%)	12/49 (24%)*	16/50 (32%)***
Adenoma				
Interim Sacrifice	2/10 (20%)$^{+}$	1/9 (11%)	1/10 (10%)	5/10 (50%)
Terminal Sacrifice	27/50 (54%)$^{++}$	33/49 (67%)	40/49 (82%)**	41/50 (82%)**
Carcinoma				
Interim Sacrifice	0/10 (0%)	0/9 (0%)	0/10 (0%)	0/10 (0%)
Terminal Sacrifice	11/50 (22%)	5/49 (10%)	14/49 (29%)	12/50 (24%)
Hepatoblastoma[c]				
Interim Sacrifice	0/10 (0%)	0/9 (0%)	0/10 (0%)	0/10 (0%)
Terminal Sacrifice	0/50 (0%)	2/49 (4%)	6/49 (12%)*	3/50 (6%)
Combined[d]				
Interim Sacrifice	2/10 (20%)$^{+}$	1/9 (11%)	1/10 (10%)	5/10 (50%)
Terminal Sacrifice	32/50 (64%)$^{++}$	36/49(73%)	45/49 (92%)***	43/50 (86%)**

[a] Incidence given per animals at risk (excluding animals dead before 52-week or the appearance of first tumor) (Gart *et al.*, 1979).
[b] By design, the animals were exposed to a constant dose throughout the entire study.
[c] A rarely observed variant of hepatocellular carcinoma (Haseman *et al.*, 1999).
[d] Combined tumor incidences of adenoma, carcinoma, and hepatoblastoma, as reported by the investigators.
*,**,*** Fisher exact test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$ respectively.
+,++,+++ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.

Table 22 Preneoplastic and Neoplastic Lesions in the Liver of Female B6C3F1 Mice in a 2-Year OPP Feeding Study (Quast and McGuirk, 1995)

Lesions[a]/Sacrifice	mg/kg/day[b]			
	0	250	500	1000
Foci of Eosinophilic Cells				
Interim Sacrifice	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2-Year Sacrifice	2/48 (4%)[+]	1/49 (2%)	5/48 (10%)	6/50 (12%)
Adenoma				
Interim Sacrifice	0/10 (0%)	2/10 (20%)	0/10 (0%)	1/10 (10%)
2-Year Sacrifice	13/48 (27%)	14/49 (29%)	17/48 (35%)	19/50 (38%)
Carcinoma				
Interim Sacrifice	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2-Year Sacrifice	2/48 (4%)	8/49 (16%)*	6/48 (13%)	5/50 (10%)
Hepatoblastoma				
Interim Sacrifice	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
Terminal Sacrifice	0/48 (0%)	0/49 (0%)	0/48 (0%)	0/50 (0%)
Combined[c]				
Interim Sacrifice	0/10 (0%)	2/10 (20%)	0/10 (0%)	1/10 (10%)
2-Year Sacrifice	14/48 (29%)	21/49 (43%)	18/48 (38%)	21/50 (42%)

[a] Incidence given per animals at risk (excluding animals dead before 52-week or the appearance of first tumor) (Gart *et al.*, 1979).
[b] By design, the animals were exposed to a constant dose throughout the entire study.
[c] Combined tumor incidences of adenoma and carcinoma, as reported by the investigators.
[*] Fisher exact test, as calculated by DPR; significant at $p \leq 0.05$.
[+] Cochran-Armitage trend test, as calculated by DPR; significant at $p < 0.05$

vascular tumor, compared to 2% in the control males[34]. An increased incidence of hemangiosarcomas in the spleen also occurred in the low-dose female group. Four of the 23 spleens examined microscopically (17%) from the low-dose females exhibited hemangiosarcoma, compared to one among the 48 spleens examined in the controls (2% incidence). The main evidence used by the investigators to argue that OPP did not induce vascular tumors was the observation that the incidences in the high-dose groups (wherein all major organs were examined) were not significantly increased. In males, the high-dose group observed to be bearing a vascular tumor (all sites combined) was 10%, compared to 10% in the control group. In the females, the corresponding incidences were 10% and 6% for the high-dose and control groups, respectively. However, there is a significant correlation between reduction in body weight and hemangioma/hemangiosarcoma incidence (Sheldon *et al.*, 1995, Seilkop, 1995) and, as discussed earlier, reduced body weights were observed in the second year of the study at the mid and high doses (both sexes).

In conclusion, this study documented that OPP-treated mice exhibited toxicity in the liver, kidneys, and spleen. The investigators established no systemic NOEL due to the toxicity observed at the lowest dose tested. This conclusion was in agreement with the DPR's conclusion that at the low dose, there were increased relative liver weight, increased serum alkaline phosphatase activities, induction of hepatoblastoma, and increased tumor multiplicity in the liver of males. DPR determined that this study was acceptable for filling the SB950 data requirements of oncogenicity in mice. The USEPA established a systemic LOEL of less than 250 mg/kg/day for the study based on increased liver and reduced spleen weights and gross observations in the liver of all treated animals (Dapson, 1995).

III.D.1.c. Oral – Dog

Two 1-year oral dog studies are on file at DPR (Hodges *et al.*, 1954 and Cosse *et al.*, 1990).

Hodge *et al.* (1954)

Since this study is available only as a summary report in the open literature, an in-depth review is not possible. Four groups of mongrel dogs (2 animals/sex/dose) received doses of 0, 0.02, 0.2, or 0.5 g/kg/day OPP (purity not specified) *via* capsule for one year. The only findings were illness and a rapid body-weight decline in one male at 0.5 g/kg/day at 4 months. However, the investigators stated that the moribund conditions were likely attributable to a distemper-type of illness, rather than a direct effect of OPP. At necropsy, dogs at 0.5 g/kg/day had slightly increased kidney weights. DPR considered this study as supplemental information.

[34] By experimental design, all major organs (including spleen) were examined microscopically for each animal in the control and high dose groups. However, for the low- and mid-dose groups, the spleen was examined only if the animals had died on test or an observation had been made about the site during the necropsy conducted at terminal sacrifice.

Cosse *et al.* (1990)

This report consisted of two studies. The first was a 4-week study, which was discussed in III.C. SUBCHRONIC TOXICITY. In the second study, four groups of beagle dogs (4 animals/sex/dose) were dosed by gavage at 0, 30, 100, or 300 mg/kg/day OPP (100% purity) for one year (5 days per week). Administration of OPP up to 300 mg/kg/day had no effects on body weight, feed consumption, ophthalmology, hematology, urinalysis, and pathology. At week 18, two males at 300 mg/kg/day died due to gavaging errors. The only clinical sign was vomiting: the frequency and volume of vomitus increased with the amount of OPP received. The NOEL for this study was ≥300 mg/kg/day, based on the absence of adverse toxicological effects in the OPP-exposed dogs. DPR considered this study acceptable for filling SB950 data requirements.

III.D.1.d. Dermal – Mouse

A dermal oncogenicity study of OPP is on file at DPR (NTP, 1986). This report consisted of two studies. The first was a 4-week study, which was discussed in the III.C. SUBCHRONIC TOXICITY. The second study employed an initiation-promotion experimental design involving five groups of Swiss CD-1 mice (50 animals/sex/group): an acetone vehicle control, a positive control that received an initiator (7,12-dimethyl- benz(a)anthracene [DMBA]) once followed by repeated applications of a promoter (12-O-tetradecanoylphorbol-13-acetate [TPA]), an initiator control that received DMBA once followed by repeated applications of acetone, a group that received repeated applications of OPP (>99% pure), and a promotion group that received DMBA once followed by repeated applications of OPP. The investigators applied topically the following doses (in 0.1 ml acetone solution): 0.05 mg DMBA (one time), 55 mg OPP, or 0.005 mg TPA (3 days per week for OPP and TPA). The exposure duration for all treatments was up to 102 weeks. Except for the positive controls, treatments did not affect survival and body weights. Skin neoplasms occurred in the following groups (respective male and female incidences in parentheses): DMBA/TPA (38% and 64%), DMBA/acetone (12% and 18%), and DMBA/OPP (18% and 16%). Since the tumor incidences were not significantly different between the DMBA/acetone and DMBA/OPP groups, the investigators concluded that OPP was not a promoter. No skin neoplasms occurred in mice (both sexes) dosed with OPP alone or acetone alone.

Mice receiving repeated applications of OPP exhibited nonneoplastic lesions at the application sites as well as local and remote locations (i.e., systemic effects). The local lesions were ulcers, active chronic inflammation, hyperkeratosis, and acanthosis. The systemic effects included increased ($p \leq 0.05$[35]) incidences of liver focal necrosis in the males (60% incidence) and kidney tubule dilation in both sexes (males, 13% incidence; females, 21% incidence).

[35] Fisher exact test, as calculated by DPR.

In conclusion, this study documented that OPP *via* dermal absorption induced systemic effects in the liver and kidneys. DPR considered this study unacceptable for filling SB950 data requirements because the study was not a FIFRA guideline type study.

III.D.2. Sodium *ortho*-Phenylphenate

III.D.2.a. Oral – Rat

Hiraga and Fujii (1981), Hiraga (1983), and Niho *et al.* (2002) studied the oncogenicity of SOPP. A separate report by Fujii (1980) discussed the data regarding time to death, tumor-onset time, and urinary-tract histopathology in the report by Hiraga and Fujii (1981). Except for the study by Niho *et al.* (2002), these studies are on file at DPR.

Hiraga and Fujii (1981)

This report consisted of two studies. The first was a 13-week study, which was discussed in III.C. SUBCHRONIC TOXICITY. In the second study, seven groups of male F344 rats (20-21 animals/dose) received diets containing 0, 1250, 2500, 5000, 10000, 20000 or 40000 ppm SOPP (≥ 95% pure) for 91 weeks. The investigators reported that the time-weighted average doses were 0, 62, 125, 250, 500, 1000, and 2000 mg/kg/day. Chronic exposure to SOPP caused a decrease ($p<0.01$) in survival at 1000 and 2000 mg/kg/day. However, survival was more affected at 1000 mg/kg/day (62% survival) compared to 80% survival at 2000 mg/kg/day. Hematuria was the only clinical sign reported; each of the SOPP-exposed groups exhibited this effect starting at week 45. The investigators presented no other information on clinical chemistry, hematology, or urinalysis.

SOPP affected the kidneys and urinary bladder (Table 23). Kidneys exhibited both nonneoplastic (pyelonephritis) and neoplastic (carcinoma) changes. In addition to the increased ($p<0.01$) carcinoma incidence in renal papilla at 2000 mg/kg/day, low incidences of the kidney tumor also occurred in the renal pelvis at 250-2000 mg/kg/day and the papilla at 250-1000 mg/kg/day. While these tumors were not statistically significant in terms of incidence, DPR considered that these were treatment-related findings because transitional cell carcinomas of the kidneys are rare spontaneous neoplasms in male F344 rats (0.2% incidence in the historical controls of NTP bioassay [Haseman *et al*., 1990]). Urinary bladder also exhibited carcinomas (Table 23). While almost all animals in the 1000 mg/kg/day group had the bladder tumor, only 40% had the tumor at 2000 mg/kg/day. In addition, at 2000 mg/kg/day, the time when the carcinoma first appeared was week 74 whereas the first carcinoma appeared in week 55 at 1000 mg/kg/day. It is possible that a reduction in survival due to SOPP toxicity at the high dose influenced the decreased tumor incidence; i.e., the high dose animals did not live long enough for tumors to appear (Bailer and Portier, 1988). However, DPR adjusted the tumor incidences for intercurrent mortality and found that the downturn in the tumor dose response remained (last row in Table 23). Further evidence that SOPP-induced mortality in the 2000 mg/kg/day group may not be the sole cause of the tumor reduction comes from a subchronic dietary study of SOPP.

Table 23 Nonneoplastic and Neoplastic Lesions in the Kidneys and Urinary Bladder of Male F344 Rats in a 91-Week SOPP Feeding Study (Hiraga and Fujii, 1981)

Lesion type	ppm						
	0	1250	2500	5000	10000	20000	40000
Dose (mg/kg/day)	0	62	125	250	500	1000	2000
Kidneys							
Pyelonephritis	0/20 (0%)$^{+++}$	0/20 (0%)	0/20 (0%)	0/21 (0%)	0/21 (0%)	1/21 (5%)	19/20 (95%)***
Renal Papilla Carcinoma	0/20 (0%)$^{+++}$	0/20 (0%)	0/20 (0%)	1/21 (5%)	0/21 (0%)	1/21 (5%)	10/20(50%)***
Renal Pelvis Carcinoma	0/20 (0%)$^{++}$	0/20 (0%)	0/20 (0%)	1/21 (5%)	1/21 (5%)	0/21 (0%)	3/20 (15%)
Bladder							
Carcinoma	0/20 (0%)$^{+++}$	0/20 (0%)	0/20 (0%)	0/21 (0%)	6/21 (29%)*	19/21 (90%)^{a,***}	8/20 (40%)**
Carcinoma (Adjusted)^{b}	0%	0%	0%	0%	29%	93%	41%

[a] A carcinosarcoma was found also in one rat in this group.
[b] Tumor incidences after adjusting for intercurrent mortality using the individual animal data reported by Fujii (1980) and a computation method (Poly-3 test) utilized by the National Toxicology Program (NTP, 2000).
*,**,*** Fisher exact test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.
$^{++,+++}$ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.01$ and $p<0.001$, respectively.

involving male F344 rats (Iguchi *et al.*, 1979; Hiraga and Fujii, 1981). In that subchronic study, there was a marked downturn in the dose response for the carcinomas at the highest dose tested (2487 mg/kg/day) even though there was 100% survival in all groups in that study (Table 12). As explained in III.C. SUBCHRONIC TOXICITY, the reduced tumor incidence at the high dose coincided with an increased incidence of nephrotoxicity (Fujii *et al.*, 1987). It was speculated that the decreased bladder carcinoma incidences at the high dose in Table 23 may be due to the 95% incidence of pyelonephritis that also was induced in that group.

In conclusion, the result that the highest tumor incidence in the urinary bladder did not occur at the highest dose tested may have been due to an inverse relationship that existed between bladder tumorigenesis and nephrotoxicity (i.e., nephritis) rather than a reduction in survival. DPR considered this study unacceptable because it was not a FIFRA guideline study (e.g., only one sex was tested).

Hiraga (1983)

This report consisted of two studies. In the first study, three groups of F344 rats (50 animals/sex/dose) received diets containing 0, 7000, or 20000 ppm SOPP (95.5% pure) for the males and 0, 5000, or 10000 ppm SOPP for the females. The investigators reported that the time-weighted average doses were 0, 270, and 770 mg/kg/day for the males and 0, 224, and 466 mg/kg/day for the females. After 104 weeks, the surviving animals received SOPP-free diets for an additional 2 weeks. Hence, the overall study duration was 106 weeks. DPR referred to this extension as the 2-week recovery study in the discussion below. To detect possible delayed effects of SOPP on urinary bladder tumor development, the investigators conducted a second study. This was similar to the first study except for the following. The second study had an additional dose group: these animals received a diet containing 2500 ppm SOPP, resulting in time-weighted average doses of 95 mg/kg/day for the males and 113 mg/kg/day for the females. Also, the second study had only 25 animals/sex/dose and after the 104 weeks of dietary exposure to SOPP, the animals received SOPP-free diets until their natural death. Hence, the overall duration of the second study was approximately 160 weeks. DPR referred to this as the 56-week recovery study in the discussion below.

In the 2-week recovery study, survivals of males in the control, low-dose, and high-dose groups at 104 weeks were 70%, 88%, and 20% ($p<0.01$), respectively[36]. The corresponding female survivals were 84%, 82%, and 86%. In the 56-week recovery study, the investigators did not discuss or report the results of survival at 104 weeks. Instead, the report indicated that the mean survival times for males in the control and low-, mid- and high-dose groups were 111, 121, 114, and 82 weeks ($p<0.001$), respectively. The corresponding survival times for the females were 108, 111, 117, and 123 weeks.

[36] Data on survival and body weight for the negative-control and treated groups were presented as graphs and in a summary table. For the 2-week and 56-week recovery studies, no individual data were reported.

Chronic exposure to 10000 ppm resulted in reduced body weights in females, reaching a maximum reduction of 9% at week 74 during the 2-week recovery study and a maximum of 5% at week 95 during the 56-week recovery study (Fujii and Hiraga, 1985)[37]. Body weight reductions also occurred in the males at 20000 ppm; the maximum reductions, 12% and 18% in the 2-week and 56-week recovery studies, respectively, occurred at week 90. In both studies, the only clinical sign reported was hematuria in the 20000 ppm males starting at week 40. A separate report by Tada *et al.* (1985) described the hematological measurements in the 2-week recovery study (both sexes); the only effect was a decreased MCV in the males at 20000 ppm.

In the 2-week recovery study in males, SOPP affected urinary bladder and kidneys, with the former being the more severely affected (Table 24). Dose-dependent increase in carcinoma occurred in the bladder starting at 7000 ppm and culminating with a 92% incidence at 20000 ppm ($p<0.001$). DPR considered that the carcinoma induced at 7000 ppm, albeit not statistically significant, was a treatment-related finding because of the rare spontaneous occurrence of this tumor (<0.2%) in this strain of rats (Haseman *et al.*, 1990). In the kidneys, both nonneoplastic changes (interstitial nephritis and pyelonephritis) and neoplastic changes (transitional cell papilloma and carcinoma) occurred at low incidences in the high-dose males. Like the tumor types found in the bladder, these tumors in the kidneys are rare spontaneous neoplasms in male F344 rats (Haseman *et al.*, 1990). As a result, DPR considered that the kidney tumor incidences at 20000 ppm were treatment-related effects despite their not achieving statistical significance. In the 56-week recovery study, essentially the same urinary-tract effects were found in males, except that no dose groups had neoplasms in the kidneys (Table 24).

SOPP also induced toxicity in the urinary bladder and kidneys of females (Table 25). However, the kidney appeared to be more severely affected in females than males. In the 2-week recovery study, the incidences of interstitial nephritis exhibited a dose-related increase starting at 5000 ppm and reaching statistical significance ($p<0.001$) at 10000 ppm. Also, increased ($p<0.01$) incidence of pyelonephritis occurred at 10000 ppm. In the bladder, incidence of neoplastic changes (papilloma and carcinoma combined), albeit not statistically significant, appeared to increase with dose; the incidence of nonneoplastic change (simple hyperplasia) also exhibited the same dose-dependent increase. In the 56-week recovery study, kidney and bladder lesions occurred mainly in females at 10000 ppm (Table 25). In the kidney, interstitial nephritis and pyelonephritis were still evident. However, the incidences of these kidney lesions were lower than the 2-week recovery study. The investigators did not discuss this observation. It is plausible that some of the kidney lesions may have regressed after the SOPP exposure ceased, resulting in the decreased incidences noted. It should be noted that for the purposes of chronic dietary risk assessment, DPR assumes that a lesion that does not persist after the termination of chemical exposure has the same toxicological significance as a lesion that does persist. In the bladder, the combined tumor incidence (papilloma and carcinoma) was identical to the 2-week recovery study.

[37] The maximal reductions in body weight were reported in Fujii and Hiraga (1985) but not in Hiraga (1983). However, it was assumed that the data reported in Hiraga (1983) were published by Fujii and Hiraga (1985) because of the essentially identical experimental design and results in both reports. Also, in both reports, the investigators did not report any results regarding statistical analyses of the body weight data.

Table 24 Neoplastic and Nonneoplastic Lesions in the Urinary Bladder, Kidneys, and Pancreas of Male F344 Rats in 2-Week and 56-Week Recovery Studies After Exposure to SOPP for 104 Weeks (Hiraga, 1983)

Study/Organ/Lesions	ppm			
	0	2500	7000	20000
Dose (mg/kg/day)	0	95	270	770
2-Week Recovery Study				
Bladder				
Simple Hyperplasia	0/50 (0%)	ND	0/50 (0%)	1/50 (2%)
Papilloma	0/50 (0%)	ND	0/50 (0%)	1/50 (2%)
Carcinoma	0/50 (0%)$^{+++}$	ND	2/50 (4%)	46/50 (92%)***
Combined Tumors[a]	0/50 (0%)$^{+++}$	ND	2/50 (4%)	47/50 (94%)***
Kidneys				
Interstitial Nephritis	0/50 (0%)	ND	1/50 (2%)	2/50 (4%)
Pyelonephritis	0/50 (0%)$^{++}$	ND	0/50 (0%)	3/50 (6%)
Papilloma/Carcinoma[a]	0/50 (0%)$^{++}$	ND	0/50 (0%)	3/50[b] (6%)
Pancreas				
Focal Atrophy (Acinar Cells)[c]	14/50 (28%)	ND	21/50 (42%)	14/50 (28%)
56-Week Recovery Study				
Bladder				
Simple Hyperplasia	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)
Papilloma	0/25 (0%)	0/25 (0%)	2/25 (8%)	2/25 (8%)
Carcinoma	0/25 (0%)$^{+++}$	0/25 (0%)	1/25 (4%)	21/25 (84%)***
Combined Tumors[a]	0/25 (0%)$^{+++}$	0/25 (0%)	3/25 (12%)	23/25 (92%)***
Kidneys				
Interstitial Nephritis	0/25 (0%)$^{++}$	0/25 (0%)	1/25 (4%)	3/25 (12%)
Pyelonephritis	0/25 (0%)$^{++}$	0/25 (0%)	0/25 (0%)	3/25 (12%)
Papilloma/Carcinoma[a]	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)

Abbreviation: ND, not done.

[a] Combined incidences of papilloma and carcinoma, as reported by the investigators.

[b] One animal with transitional cell papilloma and two with transitional cell carcinoma in the renal pelvis. These rats also had carcinoma in the urinary bladder.

[c] Focal atrophy of acinar cells was reported in the 2-week recovery study but not in the 56-week recovery study for reasons that were not explained.

*,*** Fisher Exact test, as calculated by DPR; significant at $p<0.05$ and $p<0.001$, respectively.

$^{++,+++}$ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.01$ and $p<0.001$, respectively.

Table 25 Neoplastic and Nonneoplastic Lesions in the Urinary Bladder, Kidneys, and Pancreas of Female F344 Rats in 2-Week and 56-Week Recovery Studies After Exposure to SOPP for 104 Weeks (Hiraga, 1983)

Study/Organ/Lesions	ppm			
	0	2500	5000	10000
Dose (mg/kg/day)	0	113	224	466
2-Week Recovery Study				
Bladder				
Simple Hyperplasia	0/50 (0%)	ND	1/50 (2%)	4/50 (8%)
Papilloma	0/50 (0%)	ND	1/50 (2%)	3/50 (6%)
Carcinoma	0/50 (0%)	ND	0/50 (0%)	1/50 (2%)
Combined Tumors[a]	0/50 (0%)[+]	ND	1/50 (2%)	4/50 (8%)
Kidneys (Pelvis)				
Interstitial Nephritis	0/50 (0%)[+++]	ND	3/50 (6%)	11/50 (22%)***
Pyelonephritis	0/50 (0%)[+]	ND	0/50 (0%)	9/50 (18%)**
Papilloma/Carcinoma[a]	0/50 (0%)[++]	ND	0/50 (0%)	0/50 (0%)
Pancreas				
Focal Atrophy (Acinar Cells)[b]	2/50 (4%)[++]	ND	8/50 (16%)*	11/50 (22%)**
56-Week Recovery Study				
Bladder				
Simple Hyperplasia	0/25 (0%)	0/25 (0%)	0/24 (0%)	0/25 (0%)
Papilloma	0/25 (0%)	0/24 (0%)	0/24 (0%)	1/25 (4%)
Carcinoma	0/25 (0%)	0/24 (0%)	0/24 (0%)	1/25 (4%)
Combined Tumors[a]	0/25 (0%)[+]	0/24 (0%)	0/24 (0%)	2/25 (8%)
Kidneys (Pelvis)				
Interstitial Nephritis	0/25 (0%)[++]	0/25 (0%)	0/24 (0%)	3/25 (12%)
Pyelonephritis	0/25 (0%)[++]	0/25 (0%)	1/24 (4%)	3/25 (12%)
Papilloma/Carcinoma[a]	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)

Abbreviation: ND, not determined.
[a] Combined incidence of papilloma and carcinoma, as reported by the investigators.
[b] Focal atrophy of acinar cells was reported in the 2-week recovery study but not in the 56-week recovery study for reasons that were not explained.
[*,**,***] Fisher Exact test, as calculated by DPR: significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.
[+,++,+++] Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Another organ that SOPP affected was the pancreas. In the 2-week recovery study, both of the SOPP-exposed female groups had increased ($p<0.05$) incidences of focal atrophy of pancreatic acinar cells (Table 25). In males, increased incidence of the pancreatic lesion occurred in the mid-dose group (statistically not significant) but not in the high-dose group (Table 24). Whether the decreased survival in males at the high dose affected the incidence of this pancreatic lesion at that dose could not be assessed due to the lack of individual data in the reporting. In the 56-week recovery study, the investigators did not report data for nonneoplastic lesions in the pancreas (and other organs, except for the kidneys and bladder). Nevertheless, as a health protective consideration, DPR assumes the nonneoplastic effect identified in the pancreas in the 2-week recovery study to be a valid finding.

In conclusion, the results of this study indicated the following: (1) SOPP affected the kidneys and urinary bladder, as well as the pancreas; and (2) the urinary bladder effects (both nonneoplastic neoplastic lesions) were more severe in the males than females but the reverse is true for the kidney effects (nonneoplastic lesions only). Based on increased incidences of focal atrophy of pancreatic acinar cells and interstitial nephritis in the females in the 2-week recovery study, the LOEL was 5000 ppm (i.e., 224 mg/kg/day). Because of insufficient data in the areas of hematology and ophthalmology, DPR found this study unacceptable for filling the SB950 chronic toxicity requirements, but acceptable as a rat oncogenicity study based on FIFRA guidelines.

Niho *et al.* (2002)[38]

This report consisted of two studies. The first study involved evaluating the bladder carcinogenic effect of SOPP in response to increasing doses; the second study involved determining the carcinogenic effect as a function of increasing times of treatment and correspondingly decreasing times for recovery. In the first study, six groups of male F344 rats (50 animals/dose) received diets containing 0, 2500, 5000, 10000, 15000, or 20000 ppm SOPP (97% pure). After 104 weeks, the surviving animals received SOPP-free diets for an additional 8 weeks. Hence, the overall study duration was 112 weeks. In the second study, three groups of rats (50 animals/exposure duration) received diets containing 20000 ppm SOPP for 12, 24, or 52 weeks. At the end of these exposure periods, the animals received SOPP-free diets until their scheduled sacrifices at week 112. Hence, the corresponding recovery periods were 100, 88, and 62 weeks. The investigators used the data from the animals that received 0 or 20000 ppm in the first study as the data for the control and 104-week exposure groups, respectively, for the second study. SOPP exerted equivocal effects on survival and body weight in these studies. The lowest survival rate occurred in the 15000 ppm group (first study). At study week 112, ~40% were alive, compared to 60% survival in the controls. The test groups showed only small differences in body weight. However, in terms of body weight gain, the 20000 ppm and 15000 ppm groups from the first study did exhibit reductions starting week 4 that generally were statistically

[38] The study was completed in 1985 but was not reported until 2002 by the investigators (personal communication with M. Shibutani [2004]).

significant ($p<0.05$). Sustained hematuria and anemia occurred in the first study at 15000 and 20000 ppm, starting from week 58. The investigators reported no other data regarding serum chemistry measurements, hematology, or urinalysis, except for the urine pH.

SOPP affected the urinary bladder (Table 26). In the first study, the bladder exhibited dose-related increases ($p<0.01$) in the incidences of simple hyperplasia, P/N hyperplasia, and neoplastic changes (papilloma and carcinoma), with statistically significant ($p<0.05$) effects being observed at 10000, 15000, and 20000 ppm. In addition, there was a close correspondence between the developments of simple hyperplasia, P/N hyperplasia, papilloma, and carcinoma in relation to doses.

In the second study, rats that received diets containing 20000 ppm SOPP for 12 weeks followed by 100 weeks of recovery (i.e., SOPP-free diets) did not exhibit any lesions in the urinary bladder (Table 26). This is an unexpected finding based on the results of subchronic dietary studies of SOPP involving male F344 rats (Hiraga and Fujii, 1981; St John *et al*., 2001). In these subchronic studies, male rats that were given SOPP at 20000 ppm for ~13 weeks and sacrificed (i.e., no recovery period) exhibited a significant incidence of urinary bladder lesions, including neoplastic ones in some studies (e.g., Hiraga and Fujii, 1981) (see III.C. SUBCHRONIC TOXICITY for details). Hence, in this second study, it may be that after 12 weeks of dietary exposure to SOPP, these rats also had urinary bladder lesions (apparently not any neoplastic ones) that regressed during the 100-week recovery period. If this is true, the occurrence of simple hyperplasia in the rats whose dietary exposure to 20000 ppm SOPP lasted 24 weeks (followed by 88 weeks of recovery) raises the following possibilities. First, simple hyperplasia that is considered nonneoplastic in nature never fully regressed after 24 weeks of 20000 ppm. Second, studies with genotoxic rat urinary bladder carcinogens, e.g. N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), have documented that short-term dosing (e.g., 4 weeks) produced a diffuse, simple hyperplasia that regressed and then reappeared as foci of simple hyperplasia, P/N hyperplasia, and tumors (Fukushima *et al*., 1982). Hence, by analogy, simple hyperplasia induced during the 24-week exposure in this second study may have regressed but subsequently the lesion reappeared, presumably progressing to P/N hyperplasia (and possibly papilloma) and eventually reaching the carcinoma stage in at least two animals (Study 2 in Table 26). The fact that there was a close correspondence between the development of simple hyperplasia, P/N hyperplasia, and carcinoma in relation to exposure times in this second study further supported that the nonneoplastic lesion progressed to preneoplastic lesion, which then eventually became tumors in the bladder. Whether the lesions in the 24-week-exposed group that were present at the end of the OPP-exposed period completely regressed is important. If indeed there were complete regressions, the simple hyperplasia that reappeared, even though there was no apparent stimulus provoking the response, would need to be considered effectively as a preneoplastic lesion. Also, it would argue that a genotoxic mechanism altered the DNA during the 24 weeks of exposure and this DNA change allowed the tissues to undergo the new growth that gave rise to the lesions, which included carcinoma.

SOPP also affected the kidneys. Compared to the bladder, the treatments had lesser effect on the kidneys (Table 27). In the first study, the major effects noted were nonneoplastic lesions:

Table 26 Nonneoplastic, Preneoplastic, and Neoplastic Lesions of the Urinary Bladder in Male F344 Rats in Two 112-Week Duration SOPP Feeding Studies (Niho *et al.*, 2002)

Parameters	Hyperplasia		Papilloma	Carcinoma	Tumors Combined[a]
	Simple	Papillary/Nodular			
Study 1: 104 Weeks of Diets Containing up to 20000 ppm SOPP, Followed by an 8-Week Recovery					
Dose (ppm)					
0	0/47 (0%)$^{+++}$	0/47 (0%)$^{++}$	0/47 (0%)$^{+}$	0/47 (0%)$^{+++}$	0/47 (0%)$^{+++}$
2500	1/44 (2%)	0/44 (0%)	0/44 (0%)	1/44 (2%)	1/44 (2%)
5000	1/43 (2%)	0/43 (0%)	0/43 (0%)	1/43 (2%)	1/43 (2%)
10000	19/44 (43%)**	5/44 (11%)*	1/44 (2%)	3/44 (7%)	4/44 (9%)$^{\#}$
15000	35/49 (71%)**	29/49 (59%)**	2/49 (4%)	29/49 (59%)**	30/49 (61%)**
20000	47/48 (98%)**	42/48 (88%)**	3/48 (6%)	34/48 (71%)**	37/48 (77%)**
Study 2: 0 to 104 Weeks of a Diet Containing 20000 ppm SOPP, Followed by a Recovery Period Lasting up to 100 Weeks					
Dosing Time/Recovery Time (weeks)					
0/NA[b]	0/47 (0%)$^{+++}$	0/47 (0%)$^{+++}$	0/47 (0%)	0/47 (0%)$^{+++}$	0/47 (0%)$^{+++}$
12/100	0/43 (0%)	0/43 (0%)	0/43 (0%)	0/43 (0%)	0/43 (0%)
24/88	3/45 (7%)	2/45 (4%)	0/45 (0%)	2/45 (4%)	2/45 (4%)
52/60	29/45 (64%)**	24/45 (53%)**	0/45 (0%)	24/45 (53%)**	24[c]/45 (53%)**
104/8[b]	47/48 (98%)**	42/48 (88%)**	3/48 (6%)	34/48 (71%)**	37/48 (77%)**

Abbreviation: N/A, not applicable.
[a] Combined papilloma and carcinoma incidences, as reported by the investigators.
[b] These are the data for these groups reported for the first study.
[c] One of the 24 animals with carcinoma had a metastasis to the lung.
[#] Fisher exact test, as calculated by DPR; significant at p=0.051.
[*,**] Statistically different from the negative controls at p<0.05 and p<0.01, respectively, as reported by the investigators.
[+,++,+++] Cochran-Armitage trend test, as calculated by DPR; significant at p<0.05, p<0.01 and p<0.001, respectively.

Table 27 Nonneoplastic and Neoplastic Lesions of Kidneys in Male F344 Rats in Two 112-Week Duration SOPP Feeding Studies (Niho *et al.*, 2002)

Parameters	Papillary Mineralization	Pelvic Hyperplasia	Transitional Cell Carcinoma	Renal Cell Carcinoma
Study 1: 104 Weeks of Diets Containing up to 20000 ppm SOPP, Followed by an 8-Week Recovery				
Dose (ppm)				
0	0/47 (0%)$^{+++}$	1/47 (2%)$^{++}$	0/47 (0%)	0/47 (0%)
2500	3/44 (7%)	4/44 (9%)	0/44 (0%)	0/44 (0%)
5000	3/43 (7%)	3/43 (7%)	0/43 (0%)	0/43 (0%)
10000	4/44 (9%)	6/44 (14%)	0/44 (0%)	0/44 (0%)
15000	4/49 (8%)	11/49 (22%)**	0/49 (0%)	0/49 (0%)
20000	10/48 (21%)**	10/48 (21%)**	0/48 (0%)	0/48 (0%)
Study 2: 0 to 104 Weeks of a Diet Containing 20000 ppm SOPP, Followed by a Recovery Period Lasting up to 100 Weeks				
Dosing Time/Recovery Time (weeks)				
0/NA[a]	0/47 (0%)$^{+++}$	1/47 (2%)$^{++}$	0/47 (0%)	0/47 (0%)
12/100	0/43 (0%)	0/43 (0%)	0/43 (0%)	0/43 (0%)
24/88	2/45 (4%)	2/45 (4%)	0/45 (0%)	0/45 (0%)
52/60	4/45 (9%)	9/45 (20%)**	1/45 (2%)	1/45 (2%)
104/8[a]	10/48 (21%)**	10/48 (21%)**	0/48 (0%)	0/48 (0%)

Abbreviation: N/A, not applicable.
[a] These are the data for these groups reported for the first study.
** Significantly different from the negative controls at $p<0.01$, as reported by the investigators.
$^{++,+++}$ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.01$ and $p<0.001$, respectively.

renal papillary mineralization and pelvic hyperplasia. All exposed groups exhibited these lesions, with the maximum incidence of pelvic hyperplasia reaching ~20% in the two highest dose groups ($p<0.01$). In the second study, after exposure for 24 weeks followed by recovery for 88 weeks, two animals had renal pelvic hyperplasia. For the animals whose exposure was extended to 52 weeks (therefore, a recovery period of 60 weeks), the incidence of pelvic hyperplasia increased to 20% ($p<0.01$). Also, in the 52-week exposed group, there was one animal with renal pelvis carcinoma and one with renal cell carcinoma.

In conclusion, the results of this study are consistent with the supposition that SOPP-induced nonneoplastic lesion in urinary bladder may have progressed to preneoplastic lesion, which then eventually became tumors. Also, the lesion progression may involve a genetic mechanism.

III.D.2.b. Oral – Mouse

One two-year oral mouse study is on file at DPR (Ito, 1983). Four groups of B6C3F1 mice (50 animals/sex/dose) received diets containing 0, 5000, 10000, and 20000 ppm SOPP (97% pure) for 96 weeks. After 96 weeks, the surviving animals received SOPP-free diets for an additional 8 weeks. The investigators reported that the time-weighted average doses were 0, 591, 1451, and 3009 mg/kg/day for the males and 0, 480, 1464, and 3081 mg/kg/day for the females. SOPP had no effects on survival and feed consumption (both sexes). There was a slight increase in water intake (g/mouse/day) at 5000 ppm (males, 4%; females, 9%), followed by greater increases at 10000 ppm (males, 12%; females, 22%) and 20000 ppm (males, 28%; females, 47%)[39]. The apparent dose-dependent increase in water intake suggests that SOPP may have affected water metabolism. Both the controls as well as the treated groups at week 104 excreted slightly acidic urines (majority at pH 6.0). Decreased ($p<0.01$) urine specific gravity occurred in the mid- and high-dose males and each of the SOPP-exposed female groups at week 104. Increased ($p<0.01$) serum alkaline phosphatase (ALP) activity occurred in the low-, mid-, and high-dose female groups (by 33%, 36%, and 86%, respectively). The increased ALP activity did not occur in any SOPP-exposed male group. Each of the SOPP-exposed female groups and the high-dose male group exhibited reductions in body weights. The body weights at week 96 for the low-, mid-, high-dose females, and the high-dose males were lower ($p<0.05$) than the controls by 7%, 12%, 21%, and 9%, respectively.

SOPP affected the liver more severely in the males than females. In males, there was a dose-dependent increase in carcinoma, with statistically significant ($p<0.05$) effects observed at the mid and high doses (Table 28). Increased incidences of hemangiosarcoma and (or) hemangioma occurred also in the liver in each of the SOPP-exposed male groups. Only the incidence of hemangiosarcoma at the mid dose was statistically significant. However, DPR considered that the occurrence of hepatic hemangiomas in each of the SOPP-exposed male

[39] Water intakes were averaged over the entire study by the investigators. Statistical analyses of the water-intake data were not reported by the investigators.

Table 28 Neoplastic Lesions in the Liver of Male B6C3F1 Mice in a 96-Week SOPP Feeding Study (Ito, 1983)

Lesions	ppm			
	0	5000	10000	20000
Dose (mg/kg/day)	0	591	1451	3009
Carcinoma	4/49 (8%)	9/50 (18%)	13/50 (26%)[#]	14/50 (28%)[#]
Hemangiosarcoma	0/49 (0%)	3/50 (6%)	5/50 (10%)[#]	3/50 (6%)
Hemangioma	1/49 (2%)	3/50 6%)	2/50 (4%)	1/50 (2%)
Combined Tumor[a]	1/49 (2%)	6/50 (12%)	7/50 (14%)*	4/50 (8%)

[a] Combined incidences of hemangiosarcoma and hemangioma, as performed by DPR. Because the lack of individual histology data, DPR assumed that each of these circulatory-system tumors occurred in different animals.
[#] Significantly different from the negative controls at $p<0.05$, as reported by the investigator.
* Fisher exact test, as calculated by DPR; significant at $p<0.05$.

Table 29 Neoplastic Lesions in the Liver of Female B6C3F1 Mice in a 96-Week SOPP Feeding Study (Ito, 1983)

Lesions	ppm			
	0	5000	10000	20000
Dose (mg/kg/day)	0	480	1464	3081
Carcinoma	4/49 (8%)	5/50 (10%)	7/50 (14%)	0/50 (0%)
Hemangiosarcoma.	0/49 (0%)	0/49 (0%)	0/49 (0%)	0/49 (0%)
Hemangioma	5/49 (10%)	6/49 (12%)	9/49 (18%)	3/49 (6%)

groups was treatment-related because of their rare spontaneous occurrence in this strain of mice. For example, Haseman *et al.* (1999) reported that in the NTP database of feeding studies, only 3 hemangiomas occurred among 1350 livers from untreated male B6C3F1 mice (0.2% incidence). In the females, treatment did not significantly affect the incidences of carcinoma (Table 29). While no hepatic hemangiosarcomas occurred in any female group, all groups exhibited hepatic hemangiomas, including the controls. According to the NTP data (Haseman *et al.*, 1999), only 5 hepatic hemangiomas occurred among 1350 livers from untreated female B6C3F1 mice (0.4% incidence). Therefore, although the occurrence of 6-18% incidences of hepatic hemangiomas in the SOPP-exposed female group would seem to represent a treatment-related effect based on the rarity of the tumor, such a conclusion has to be withheld given that the control group exhibited a tumor incidence of 10%.

SOPP exhibited contrasting effects on kidney and heart weights in the two sexes. In males, decreased absolute kidney weights occurred in the low-dose (7%, $p<0.05$), mid-dose (4%, not statistically significant), and high-dose groups (12%, $p<0.01$). The decreases at the low and mid doses appeared to be independent of a general body weight reduction because the latter did not occur in these male groups. In females, increased ($p<0.05$) absolute kidney weight occurred in the low-, mid-, and high-dose groups (by 9%, 6%, and 2%, respectively). The smaller absolute kidney weight increases at the mid and high doses paralleled the reduction in body weights (12% and 21%, respectively) at these dose levels. In males, SOPP had no effect on the absolute heart weight in each of SOPP-exposed groups. In females, the 5000, 10000, and 20000 ppm groups had absolute heart weights increased by 19% ($p<0.05$), 13% ($p<0.05$), and 0%, respectively. The lack of an increased absolute heart weight in the high-dose females presumably was due to the 21% reduction in body weight in this group.

In conclusion, this study documented that SOPP-treated mice exhibited toxicity in the liver (including tumors), kidneys, and heart. Also, SOPP induced polydipsia in mice. Based on decreased body weight, increased absolute weights of the kidney and heart, and increased ALP activity in the females, the LOEL was 5000 ppm (i.e., 480 mg/kg/day SOPP). DPR considered this study unacceptable for filling SB950 data requirements because of histological data inconsistencies.

III.D.3. Special Chronic Toxicity and Oncogenicity Studies

III.D.3.a. Oral – Rat

Five special toxicity studies in rats are available in the open literature. Four studies involved investigating the mode of action of OPP and SOPP in the urinary bladder (Fukushima *et al.*, 1983, 1985, 1989; Inoue, 1993) and one study involved determining the modifying effect of thiabendazole (TBZ), another fungicide used with SOPP for post-harvest treatments, on bladder tumorigenicity of SOPP (Fujii *et al.*, 1986).

III.D.3.a.1. Mode of Action

Fukushima *et al.* (1983)

This special toxicity study was to evaluate the carcinogenic activity of SOPP and OPP in rat urinary bladder using an initiation and promotion experimental design. Male F344 rats received drinking water that contained N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) for 4 weeks (i.e., initiation) followed by OPP- (or) SOPP-containing diets for 32 weeks (i.e., promotion); therefore, the total study duration was 36 weeks. Results showed that 20000 ppm SOPP greatly enhanced the P/N hyperplasia, papilloma, and carcinoma induced by 0.05% BBN (97%, 100%, and 100%, respectively, in the BBN/SOPP group *vs.* 34%, 23%, and 7%, respectively, in the BBN-alone group). Because SOPP alone induced preneoplastic (86% incidence) and neoplastic (17% incidence) lesions in the bladder, the investigators concluded that SOPP possessed promoting as well as initiating activities (i.e., SOPP was a complete carcinogen). By contrast, while 20000 ppm OPP treatment following BBN exposure (0.05%) tended to increase the individual incidences of P/N hyperplasia (57%), papilloma (37%), and carcinoma (20%), these increases were small in magnitude and, individually, were not statistically increased over the values observed in the BBN-alone group. In addition, OPP-alone group had no histologic lesions in the bladder. Hence, OPP was not like SOPP in terms of initiating and (or) promoting urinary bladder cancer.

Fukushima *et al.* (1985)

This special toxicity study was to evaluate the carcinogenic activity of SOPP and OPP in rat urinary bladder using two study designs: initiation-promotion and serial sacrifice. In the former, male F344 rats received drinking water that contained 0.01% BBN for 4 weeks (i.e., initiation) followed by 20000 ppm OPP- (or) SOPP-containing diets for 64 weeks (i.e., promotion); therefore, the total study duration was 68 weeks. In the latter study, the animals received 2500-20000 ppm SOPP-containing diets for up to 104 weeks (sacrifice at weeks 4, 8, 12, 24, 36, and 104 weeks) or OPP-containing diets for 12 weeks (sacrifice at 4, 8, and 12 weeks).

Compared to the BBN-only group, SOPP increased the numbers of P/N hyperplasia sites per 10 cm of basement membrane (BM)[40] ($p<0.05$). In addition, BBN/SOPP group had an increased incidence of papilloma over the BBN-only group (72% and 40% incidences, respectively). It should be noted that SOPP alone induced P/N hyperplasia, papilloma, and carcinoma; the respective incidences were 68%, 18%, and 21%. OPP treatment (20000 ppm) following BBN exposure (0.01%) tended to increase the individual incidences of P/N hyperplasia and papilloma (54% and 35% incidences, respectively). However, these increases were small in magnitude and, individually, were not statistically increased over the values observed in the BBN-alone group (35% and 24% incidences, respectively). In addition, except for one case of P/N hyperplasia, OPP-alone group had no histologic lesions in the bladder.

[40] Number/10 cm BM is an indicator for tumor multiplicity.

Hence, akin to what was observed in the study by Fukushima *et al.* (1983), OPP was not like SOPP in terms of initiating and (or) promoting urinary bladder cancer.

With the serial sacrifices, the investigators stated that 20000 ppm SOPP group exhibited simple hyperplasia at week 4 (no data), P/N hyperplasia at week 36 (40% incidence), and tumors (papilloma and carcinoma, 40% incidence each) at week 104. In addition, scanning electron microscopy (SEM) analysis on the luminal surface from the 20000 ppm SOPP group revealed surface changes starting at week 4. In the 10000 ppm SOPP group, the animals exhibited simple hyperplasia at weeks 36 and 104 (10% and 67% incidences); however, the investigators found no other bladder lesions. Lower dose groups had no histologic lesions at week 36 or 104. In the study with OPP, the only finding was surface changes of a slight degree (by SEM analysis) at 20000 ppm in weeks, 4, 8, and 12.

Fukushima *et al.* (1989)

This special toxicity study was to evaluate the modifying effects of co-exposure to sodium bicarbonate ($NaHCO_3$, urine-alkalizing agent) on the OPP-induced bladder oncogenicity. Seven groups of male F344 rats (30-31 animals/dose) received diets containing 0, 6400 ppm $NaHCO_3$, 20000 ppm SOPP, 12500 ppm OPP, or 12500 ppm OPP supplemented with 1600, 3200, or 6400 ppm $NaHCO_3$ for 104 weeks. Urinary bladders of all dose groups exhibited histologic lesions after 104 weeks (Table 30). At 12500 ppm OPP, P/N hyperplasia was the only lesion identified. By contrast, rats dosed with OPP plus 1600 or 3200 ppm $NaHCO_3$ had both P/N hyperplasia and carcinomas; the latter also had a higher incidence of P/N hyperplasia. At OPP plus 6400 ppm, the animals exhibited P/N hyperplasia, papilloma, and carcinoma; these lesion incidences were comparable to the SOPP-alone group. One carcinoma occurred in the $NaHCO_3$-alone group; however, this may not be treatment-related because other studies indicated that tumor induction in the rat urinary bladder by carbonate required much higher dietary concentration (e.g., 3%, [Lina *et al*., 1994]). Based on these observations, the investigators concluded that SOPP was a more potent rat bladder carcinogen than OPP. In addition, the carcinogenic effect of OPP was enhanced by the $NaHCO_3$ supplementation of the diet.

In addition to the bladder tumors, the investigators monitored urinary pH[41] and electrolytes, including sodium, in the 7 groups. Throughout the study, urinary pH values ranged from slightly acidic (pH 6-7) in the control and the OPP-alone groups to slightly alkaline (pH 7-8) in the SOPP-alone and OPP plus 6400 ppm $NaHCO_3$ groups; the increase in urine pH appeared to follow a dose response based on the feed concentrations of $NaHCO_3$. Mean urinary sodium concentrations measured in the groups dosed with 20000 ppm SOPP and OPP plus 6400 ppm, 3200 ppm, or 1600 ppm $NaHCO_3$ were 109%, 89%, 52%, and 59% higher, respectively, than in the controls; each increase was statistically significant ($p<0.05$). As described previously, there was also a corresponding dose-dependent increase in the bladder tumor

[41] The investigators did not report statistical analyses of the urinary pH data.

Table 30 Preneoplastic and Neoplastic Lesions in the Urinary Bladder of Male F344 Rats in a 104-Week Feeding Study (Fukushima *et al*., 1989)

Treatments	P/N Hyperplasia	Papilloma	Carcinoma
20000 ppm SOPP	8/29 (28%)**	3/29 (10%)	12/29 (41%)***
12500 ppm OPP + 6400 ppm $NaHCO_3$	7/29 (24%)*	2/29 (7%)	9/29 (31%)**
12500 ppm OPP + 3200 ppm $NaHCO_3$	8/29 (28%)**	0/29 (0%)	4/29 (14%)*
12500 ppm OPP + 1600 ppm $NaHCO_3$	4/26 (15%)*	0/26 (0%)	4/26 (15%)*
12500 ppm OPP	3/27 (11%)	0/27 (0%)	0/27 (0%)
6400 ppm $NaHCO_3$	0/28 (0%)	0/28 (0%)	1/28 (4%)
0	0/27 (0%)	0/27 (0%)	0/27 (0%)

*,**,*** Significantly different from the negative controls at $p<0.05$, $p<0.01$, $p<0.001$, as reported by the investigators.

incidences with increasing amount of $NaHCO_3$ co-administered with OPP in the diets. Based on these observations, the investigators concluded that increased urinary pH plus increased urinary sodium ion concentration due to the $NaHCO_3$ supplementation enhanced the carcinogenic effect of OPP.

III.D.3.a.2. Interaction with Other Fungicides

Fujii *et al.* (1986)

This special toxicity study evaluated the modifying effect of TBZ on SOPP-induced bladder carcinogenesis. This was a 65-week study and involved six groups of F344 rats (15 animals/sex/dose): four groups received diets containing 0, 2000 ppm TBZ, 10000 ppm SOPP or 20000 ppm SOPP and the other two SOPP groups (at 10000 and 20000 ppm, respectively) received diets containing 2000 ppm TBZ. In the males, increasing SOPP dose from 10000 ppm to 20000 ppm resulted in a marked progression of hyperplasia to papilloma and carcinoma in the urinary bladder (Table 31). At 10000 ppm SOPP, co-exposure to TBZ caused an increase in carcinomas compared to the corresponding SOPP-only group. At 20000 ppm SOPP, however, co-exposure to TBZ did not have any enhancing effect. The investigators speculated that the lack of enhancement was the result of optimal tumor induction achieved already by the high-dose group. Unlike the males, increasing SOPP dose from 10000 ppm to 20000 ppm did not cause a significant increase in tumor incidence in the females. While co-exposure of 10000 ppm SOPP with TBZ did not have an enhancing effect, the co-exposure of 20000 ppm caused a synergistic increase in papillomas and carcinomas in the females. The investigators concluded that co-exposure to TBZ enhanced the SOPP-induced bladder carcinogenesis in both sexes.

III.D.3.b. Oral – Mouse

Four special toxicity studies in mice are available in the open literature. One study involved investigating the modifying effect of $NaHCO_3$ on the toxicity of OPP (Fujii *et al.*, 1989a) and the other three involved determining the effect of thiabendazole (TBZ) in the presence or absence of $NaHCO_3$ on the OPP toxicity (Mikuriya *et al*., 1989b, 1992, Fujii *et al.*, 1989b). Because the same control data appeared in these four studies and Mikuriya *et al.* (1989a) (described previously in III.D.1.b. Oral-Mouse) and the same OPP-alone data appeared in the studies by Mikuriya *et al*., (1992) and Mikuriya *et al.* (1989a), DPR assumed that the same research group conducted these five studies concurrently even though the results appeared in 1989 and 1992.

Fujii *et al.* (1989a)

This 52-week feeding study was a companion study to Mikuriya *et al.* (1989a) with the intent of determining whether exposure to sodium bicarbonate ($NaHCO_3$; an urine-alkalizing agent) would enhance the effects caused by OPP in mice. This study involved five groups of

Table 31 Nonneoplastic and Neoplastic Lesions of the Urinary Bladder in Male and Female F344 Rats in a 65-Week TBZ and (or) SOPP Feeding Study (Fujii *et al*., 1986)

Treatment	Hyperplasia[a]	Papilloma	Carcinoma	Combined Tumors[b]
Males				
0	0/14 (0%)	0/14 (0%)	0/14 (0%)	0/14 (0%)
2000 ppm TBZ	0/15 (0%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
10000 ppm SOPP	6/15 (40%)*	0/15 (0%)	0/15 (0%)	0/15 (0%)
20000 ppm SOPP[c]	0/15 (0%)	5/15 (33%)*	10/15 (67%)***	15/15 (100%)[#]
10000 ppm SOPP + 2000 ppm TBZ	2/15 (13%)	1/15 (7%)	11/15 (73%)***	12/15 (80%)[#]
20000 ppm SOPP + 2000 ppm TBZ	1/15 (7%)	4/15 (27%)	10/15 (67%)***	14/15 (93)[#]
Females				
0	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
2000 ppm TBZ	0/14 (0%)	0/14 (0%)	0/14 (0%)	0/14 (0%)
10000 ppm SOPP	3/15 (20%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
20000 ppm SOPP[c]	3/15 (20%)	1/15 (7%)	1/15 (7%)	2/15 (13%)
10000 ppm SOPP + 2000 ppm TBZ	2/15 (13%)	1/15 (7%)	0/15 (0%)	1/15 (7%)
20000 ppm SOPP + 2000 ppm TBZ	0/15 (0%)	6/15 (40%)**	6/15 (40%)**	12/15 (80%)[#]

[a] The investigators made no distinction between simple hyperplasia and P/N hyperplasia.
[b] Combined incidences of papilloma and carcinoma, as reported by the investigators.
[c] Two males (13% incidence) and three females (20% incidence) also had transitional cell hyperplasia in the renal pelvis.
*,**,*** Fisher exact test, as calculated by DPR; significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.
[#] Significantly different from the negative controls at $p<0.05$, as reported by the investigators.

male B6C3F1 mice (20 animals/dose): one group received basal diet and additive-free drinking water (i.e., controls) and other four groups received diets containing 0, 6500, 13000, or 26000 ppm OPP and their drinking water contained 8000 ppm $NaHCO_3$. The $NaHCO_3$-alone treatment resulted in one death whereas $NaHCO_3$ plus OPP at 13000 or 26000 ppm resulted in three and four deaths, respectively. Because OPP-alone treatment at these same levels had no effect on survival (Mikuriya *et al.*, 1989a), these observations suggest that the combined treatment of $NaHCO_3$ and OPP may be more toxic than either treatment alone.

Except for the liver, no neoplastic lesions occurred in any dosed groups in this study. For the most part, the effects caused by dual exposure to OPP and $NaHCO_3$ were comparable to those observed in mice exposed to OPP alone (Mikuriya *et al.*, 1989a). The most significant difference between the studies was the following. Exposure to $NaHCO_3$ alone was slightly toxic to the kidneys. The incidence of degeneration of tubular epithelium (grades slight and very slight combined) was 37% ($p<0.01$) in the $NaHCO_3$-alone group. The inherent renal toxicity of $NaHCO_3$ may explain why dual exposure to OPP and $NaHCO_3$ resulted in LOELs for some renal lesions that were lower than those observed in the OPP-alone testing. In Mikuriya *et al.* (1989a), the incidence of necrosis of renal tubular epithelium in the 0, 6500, 13000, and 26000 ppm OPP groups were 0%, 0%, 25%, and 40%, respectively (Table 20) whereas in this study, the incidences in the corresponding OPP groups co-exposed to $NaHCO_3$ were 5%, 16%, 83%, and 50%. In addition, the LOEL in Mikuriya *et al.* (1989a) for necrosis of renal ductular epithelium was 26000 ppm OPP (Table 20) whereas the LOEL became 13000 ppm OPP (based on 28% incidence) when animals were co-exposed to $NaHCO_3$. The investigators also monitored urine pH in the five groups. The animals whose drinking water did not contain additives excreted slightly acidic urine (pH between 5.8 and 6.8) with few exceptions during the entire study. The four groups exposed to $NaHCO_3$ initially excreted urine that was slightly alkaline: at week 5, 50-70% of the animals in each of these groups excreted urine with pH between 7.0 and 7.4. However, by week 13, only 10-30% of the animals in each of the $NaHCO_3$-exposed groups excreted slightly alkaline urine. At weeks 39 and 52, 80-100% of the animals in each of the groups exposed to $NaHCO_3$ excreted urine with pH between 5.8 and 6.8. The failure of oral exposure to $NaHCO_3$ to induce a sustained state of alkalinuria in mice was in contrast to the success that was achieved in similar studies in rats (Fukushima *et al*., 1989).

In conclusion, this study documented that co-exposure to $NaHCO_3$ enhanced the kidney effects induced by OPP. Also, as opposed to the rats, $NaHCO_3$ did not induce sustained state of alkalinuria in mice.

Mikuriya *et al.* (1989b, 1992)

The intent of this 52-week feeding study was to determine whether co-exposure to TBZ would have the same effect in mice as it had in rats (Fujii *et al.*, 1986) in enhancing the toxicity of OPP. This study involved five groups of male B6C3F1 mice (20 animals/dose): one group received basal diet (i.e., controls) and other four groups received diets containing 2000 ppm TBZ plus either 0, 6500, 13000, or 26000 ppm OPP. Urine pH values were similar between the control

and treated groups for the duration of the study, generally ranging between pH 5.8 and 6.8 (i.e., slightly acidic). The TBZ-alone treatment resulted in two deaths whereas TBZ plus OPP at 13000 or 26000 ppm resulted in five and six deaths, respectively. Because OPP-alone treatment at these same levels had no effect on survival (Mikuriya *et al.*, 1989a), these observations suggest that the combined treatment of TBZ and OPP may be more toxic than either treatment alone.

Except for the liver, no neoplastic lesions occurred in any dosed groups and the neoplastic lesions in the liver were few in number and did not appear to be related to treatment. For the most part, incidences of nonneoplastic lesions in the groups with dual exposure to OPP and TBZ were consistent with an additive effect between the incidences observed in the OPP-alone testing reported by Mikuriya *et al.* (1989a) and those observed in the animals exposed to TBZ alone. Examples of possible synergistic effects were the following. First, exposure to TBZ alone resulted in toxicity in the spleen: the incidence of hemosiderosis in the spleen (grade of very slight) was 45% ($p<0.001$). This spleen lesion did not occur in the groups exposed to OPP only. By contrast, the hemosiderosis incidences increased to 63%, 100%, and 100% in the groups exposed to TBZ along with 6500, 13000, and 26000 ppm OPP, respectively. Second, exposure to TBZ alone was slightly toxic to the kidneys: the incidence of necrosis of ductular epithelium in the kidneys was 5%. The incidences of necrosis of ductular epithelium in the 6500, 13000, and 26000 ppm OPP-alone groups were 0%, 0%, and 20%, respectively (Table 21). By contrast, the incidences in the corresponding OPP groups co-exposed to TBZ increased to 11%, 25%, and 64%.

Fujii *et al.* (1989b)

The intent of this 52-week feeding study was to determine whether co-exposure to both thiabendazole (TBZ) and $NaHCO_3$ would enhance the effects caused by OPP in mice. This study involved four groups of male B6C3F1 mice (20 animals/dose): one group received basal diet and additive-free water (i.e., controls) and other three groups received drinking water containing 8000 ppm $NaHCO_3$ and diets containing 2000 ppm TBZ plus 6500, 13000, or 26000 ppm OPP.

Except for the liver and lung, no neoplastic lesions occurred in any dosed groups in this study. Liver tumors (type not stated) occurred in two animals exposed to 6500 ppm OPP plus $NaHCO_3$ and a lung tumor in one animal exposed to 26000 ppm plus $NaHCO_3$ plus TBZ. For the most part, each of the incidences of nonneoplastic lesions in the groups with co-exposure to OPP, TBZ, and $NaHCO_3$ were consistent with an additive effect of the incidences observed in animals exposed to OPP-alone, $NaHCO_3$-alone, or TBZ-alone in the studies by Mikuriya *et al.* (1989a), Fujii *et al.* (1989a), and Mikuriya *et al.* (1989b), respectively.

III.D.3.c. Oral – Miscellaneous Species

One study involved investigating the effect of SOPP on the urinary bladder of hamsters and guinea pigs (male only), together with rats and mice, is available in the open literature (Hasegawa *et al.*, 1990). Groups of F344 rats, B6C3F1 mice, Syrian golden hamsters, and Hartley guinea pigs (40 animals/species) received diets containing 0 or 20000 ppm SOPP for up to 48 weeks. From each of the SOPP-exposed groups, serial sacrifice occurred at weeks 4, 8, 12, 24, 36, and 48; for the controls, however, serial sacrifice occurred only at weeks 12 and 48. Urinalysis indicated that ingestion of SOPP favored the excretion of slightly alkaline urine in rats: pH values measured at weeks 12 and 48 were 7.2 and 7.1, respectively, compared to 6.7 and 6.9 in the controls. By contrast, untreated mice excreted slightly alkaline urine at 12 weeks (pH 7.8) and slightly acidic urine at 48 weeks (pH 6.5); at neither time did exposure to SOPP result in a significant increase in urine pH. Untreated hamsters and guinea pigs excreted alkaline urine at 12 weeks (pH 8.7 and 8.3, respectively) and at 48 weeks (pH 7.6 and 7.8, respectively); at neither time did exposure to SOPP result in a significant increase in urine pH.

Only the urinary bladder of rats examined by microscopic and SEM analyses exhibited changes. At histology, the incidences of simple hyperplasia and P/N hyperplasia in rats increased with duration of exposure. The incidences of the former were 20%, 40%, and 100% at weeks 4, 8, and 12-48, respectively. The incidences of P/N hyperplasia were 20%, 60%, and 80% at weeks 24, 36, and 48, respectively. SEM detected cell surface alternations in rats, starting at week 4 (first sacrifice time); also, the number of affected bladders appeared to increase with the duration of exposure to SOPP. Based on these observations, the investigators concluded that rat was the most sensitive species for SOPP-induced bladder carcinogenicity. Since the rat also was the most sensitive species (compared to mice, hamster, and guinea pigs) for the urinary bladder carcinogenesis induced by BBN as well as by two other chemicals structurally similar to BBN, N-ethyl-N-(4-hydroxyl-butyl)nitrosamine and N-butyl-N-(3-carboxypropyl)nitrosamine, the investigators further concluded that rats may be the most sensitive species for bladder carcinogens in general.

III.D.3.d. Dermal – Mouse

Two special toxicity studies are available in the open literature. One study involved investigating the oncogenic effect of SOPP (Takahashi *et al.*, 1989) and the other investigating the effect of its metabolites i.e., phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) (Sato *et al.*, 1990).

Takahashi *et al.* (1989)

This study employed an initiation-promotion experimental design, which involved 8 treatment groups of female CD-1 mice (20 animals/treatment). For the initiation treatments, the investigators applied the following doses (in 0.1 ml DMSO solution) topically twice weekly for 5 weeks: SOPP (10μg), 7,12-dimethyl-benz(a)anthracene (DMBA, 10μg), or DMSO. For the promotion treatment, starting one week after the last initiation treatment, the investigators

applied 0.1 ml acetone solution of SOPP (5 mg), 12-O-tetradecanoylphorbol-13-acetate (TPA, 10μg), or acetone (vehicle) to the skin twice weekly for 47 weeks.

Except for the group given DMBA/TPA (i.e., the positive-control group that was initiated with DMBA and promoted with TPA), survival was not affected by the treatments. Mice dosed with DMSO/acetone, SOPP/acetone, or DMSO/SOPP exhibited no skin tumors. The incidences of skin tumors in the other five treatment groups were as follows: DMBA/TPA, 100%; DMBA/acetone, 25%; SOPP/TPA, 5%; DMBA/SOPP, 75%; and DMSO/TPA, 10%. There was no significant difference in the skin tumor incidences between mice dosed with SOPP/TPA or DMSO/TPA. However, the skin tumor incidence in mice dosed with DMBA/SOPP was higher ($p<0.01$) than the DMBA/acetone group. Based on these observations, the investigators concluded that SOPP was a promoter rather than an initiator in the two-stage mouse skin carcinogenesis model.

Sato *et al.* (1990)

This study employed an initiation-promotion experimental design involving ten groups of female CD-1 mice (25 animals/treatment). For the initiation treatment, the investigators applied the following doses (in 0.1 ml DMSO solution) topically twice weekly for the first 5 weeks: PHQ (20 mg), PBQ (2 mg), DMBA (10μg), or acetone (vehicle) only. For the promotion treatment, starting one week after the last initiation treatment, the investigators applied PHQ (10 mg in DMSO), PHQ (1 mg in DMSO), TPA (10μg in acetone), or acetone to the skin twice weekly for 34 weeks.

Except for the DMBA/TPA group, treatments did not significantly affect survival. Mice exposed continuously to DMSO, PHQ, or PBQ exhibited no skin tumors. The incidences of skin tumors in the other 7 groups were as follows: DMBA/TPA, 96%; DMBA/acetone, 36%; PHQ/TPA, 8%; PBQ/TPA, 11%; DMBA/PHQ, 42%; DMBA/PBQ, 52%; and DMSO/TPA, 8%. There were no significant differences in the tumor incidences between mice dosed with DMBA/PHQ or DMBA/PBQ compared to the DMBA/acetone group. In addition, the skin tumor incidences in mice dosed with PHQ/TPA or PBQ/TPA were not significantly different from the DMSO/TPA group. Based on these observations, the investigators concluded that neither PHQ nor PBQ possessed initiating and (or) promoting activities.

III.E. GENOTOXICITY

Summary: Genotoxicity studies of OPP, SOPP, and their metabolites are available from Registrants and the open literature. Many of the Registrant-submitted studies showed weak or negative genotoxicity. However, *in vivo* and *in vitro* studies published in the open literature supported the genotoxic potential of OPP and SOPP and their metabolites. The evidence from these studies should not be ignored. Because different assays provide different information for the genotoxicity, it is not appropriate to dismiss the positive studies simply by the relative number of negative *versus* positive reports. Indications of gene mutation and damage to chromosomes and DNA by OPP, SOPP and their metabolites are available in numerous *in vitro* and *in vivo* bioassays and are summarized below.

Gene mutation studies collectively contained sufficient data to determine that OPP has genotoxic potential in mammalian cells *in vitro* in the absence or presence of metabolic activation. Similarly, limited data available in the gene mutation studies also indicate that SOPP with no metabolic activation has genotoxic potential in eukaryotic systems *in vitro*. OPP exhibited potential for inducing chromosomal damage (i.e., clastogenicity and endoreduplication) in mammalian cells *in vitro* and metabolic activation enhanced the effect. Evidence indicated that OPP was capable of interacting non-covalently with DNA *via* intercalation and complex formation *in vitro*. Among the *in vitro* tests for DNA damage, positive results occurred in microbial tests for non-specific DNA damage and mammalian cell tests for induction of SCE in the absence or presence of metabolic activation. Under metabolic activation, OPP-DNA reactions *in vitro* resulted in DNA-adduct formation and strand breakage, indicating that reactive OPP metabolite(s) formed could cause damage to biomacromolecules.

In vivo micronucleus test for clastogenicity (chromosomal break) and aneugenicity (chromosomal loss) yielded positive responses in the urinary bladder (but not bone marrow) of rats exposed to OPP or SOPP. This finding is consistent with the tissue-specific tumorigenic potential and the possible mechanism through reactive metabolites of OPP and SOPP. Rats dosed orally and repeatedly with SOPP had DNA adduct formation in the urinary bladder. Although other DNA binding studies in the rat urinary bladder *in vivo* yielded negative results, the study design may have limited the detection of DNA adducts. DNA breakage was another form of genetic damage observed in the urinary bladder of rats exposed to OPP or SOPP. Another effect identified in the urinary bladder of rats treated with OPP or SOPP was agglutination of the epithelial cells with concanavalin A, an effect that has been generally correlated with the carcinogenicity of the tested substances.

PHQ and PBQ were the only two metabolites of OPP and SOPP with genotoxicity data *in vitro* and (or) *in vivo*. Although none of these studies with genotoxicity findings complied with the protocols under the Toxic Substances Control Act (TSCA) guidelines, collectively they contain sufficient data to indicate that PHQ and PBQ possess the potential to damage chromosomes (clastogenicity, aneugenicity, and endoreduplication) and the ability to induce covalent DNA adducts, single strand breakage, oxidation, and sister chromatid exchange irrespective of metabolic activation (i.e., they are biologically active). *In vitro* studies without

metabolic activation showed that sulfhydryl compounds and scavengers of reactive oxygen species (ROS) inhibited the chromosomal and DNA effects of PHQ and that PHQ autoxidation resulted in the formation of reactive quinoid (e.g., PBQ) and ROS. Also, *in vitro* evidence indicates that increased pH and metal ion concentrations enhanced the genotoxicity of PHQ, possibly *via* their promoting effects on PHQ autoxidation, with the resultant PBQ exhibiting oncogenic potential in mammalian cells. Taken together, reactive species produced *via* pH- and metal ion-dependent PHQ autoxidation may play an important role in the genotoxicity of PHQ. By corollary, a combination of the reactive species derived from different enzymatic and non-enzymatic pathways may be responsible for the enhanced genotoxicity of OPP with metabolic activation *in vitro* and the genotoxicity of OPP and SOPP *in vivo*. The overall data indicated that OPP and SOPP have genotoxic potential, and their metabolites may contribute to their genotoxicity *in vivo*.

Seven genotoxicity studies with OPP and (or) SOPP submitted by the Registrant are on file at DPR (Reitz *et al.* 1983; Brusick, 1976; Cline and McMahon, 1977; Kaneda *et al.*, 1978; Shirasu *et al.*, 1978; Suzuki *et al.*, 1985; and NTP, 1986). Although none is a guideline-type study (e.g., Toxic Substances Control Act [TSCA] guidelines [Federal Register, 1985]), DPR considered that these studies collectively contain information to determine the genotoxic potentials of OPP and SOPP and, therefore, fulfill the data requirements under the California Birth Defects Prevention Act of 1984 (SB950). In addition to these Registrant-submitted studies, there are numerous open literature reports on the mutagenic potentials of OPP, SOPP, and their metabolites (e.g., phenylhydroquinone [PHQ] and phenylbenzoquinone [PBQ]). The following describes these open literature studies and those on file at DPR.

III.E.1. *ortho*-Phenylphenol

III.E.1.a. Gene Mutation

Table 32 summarizes the results of studies for gene mutation. Thorough evaluation for some of the available 14 reports on *Salmonella typhimurium* tests is not possible due to reporting only in the abstract form (Nishioka and Ogasawara, 1979; Shirasu *et al*., 1978; Takahashi, 1978), missing information on dose (Hanada, 1977; Shirasu *et al*., 1978; Nishioka and Ogasawara, 1979; Pagano *et al*., 1988), and lack of individual data (Cline and McMahon, 1977; Moriya *et al.*, 1983). Also, one study did not appear to achieve cytotoxicity at the highest tested dose (Brusick, 1976). Of the six remaining studies, all the tests with metabolic activation were negative (Ishidate *et al.*, 1984; Fujita *et al*., 1985; NTP, 1986; Kojima and Hiraga, 1978; Kojima *et al.*, 1983; Hirayama *et al*., 1981). Among the five studies without metabolic activation, OPP was weakly mutagenic in TA1535 in the study by NTP (1986) while results of all other studies that employed a comparable dose range were negative (Fujita *et al*., 1985; Kojima and Hiraga, 1978; Kojima *et al.*, 1983; Hirayama *et al*., 1981). In the only available report of *Escherichia coli* test, OPP produced a negative result in the absence of metabolic activation (Kojima and Hiraga, 1978).

Studies investigating gene mutation in different mammalian cell systems yielded different results: negative response in Chinese Hamster Ovary (CHO-WB1 HPGRT) cells (Brendler, 1992), but positive response in $TK^{+/-}$ mouse lymphoma cells (NTP, 1986) and ouabain-resistant human RSa cells (Suzuki *et al.*, 1985) in the absence and (or) presence of metabolic activation. All three mammalian cell studies indicated that challenge to the test systems was sufficient, as suggested by the occurrence of cytotoxicity. An *in vivo* test with *Drosophilia melanogaster* was negative for sex-linked recessive mutation (NTP, 1986). Results from gene mutation indicate that OPP did not show mutagenic effects in bacteria but showed genotoxicity in mammalian cells *in vitro.*

III.E.1.b. Chromosomal Damage

Table 33 summarizes the results of studies for chromosomal damage *in vitro* and *in vivo*. In the absence of metabolic activation, the *in vitro* chromosomal aberration tests of OPP were negative in Chinese Hamster Lung (CHL) fibroblasts and Chinese Hamster Ovary (CHO) cells (Ishidate *et al.*, 1984; NTP, 1986). OPP also tested negative for chromosomal aberrations in CHO cells in the presence of metabolic activation (NTP, 1986). However, at a dose range that showed minimal cytotoxicity (as indicated by cell progression delay that was measured concurrently in the test), the OPP-treated CHO-K1 cells exhibited chromosomal aberrations and endoreduplication (ERD)[42] in the absence of metabolic activation; both the genotoxic and cytotoxic effects occurred at a lower dose with than without metabolic activation (Tayama-Nawai *et al.* 1984; Tayama *et al.* 1989). In an abstract report, OPP with no metabolic activation was positive for chromosomal aberrations in human diploid fibroblasts (Takahashi, 1978). Taken together, results of chromosomal aberration studies indicated that OPP damages chromosomes in mammalian cells *in vitro*.

Regarding the genotoxic and cytotoxic effects observed in CHO-K1 cells, cysteine (Cyst) and reduced glutathione (GSH) suppressed the effects induced by OPP only in the presence of metabolic activation (S9 mix) (Tayama and Nakagawa, 1991). The suppression of effects by Cyst and GSH (i.e., radical scavengers) suggested that electrophilic metabolites of OPP also contributed to the cytotoxic and genotoxic effects noted. Chromatographic analyses of the cell mixtures with OPP-S9-Cyst/GSH detected sulfhydryl-compound adducts of PHQ. Based on these observations, the investigators concluded that two different processes may have caused the toxic effects in CHO-K1 cells: the direct effect of OPP in the absence of metabolic activation and electrophilic reaction of OPP metabolite(s) (e.g., PHQ) in the presence of metabolic activation.

The abstract report by Shirasu *et al.*, (1978) and the comprehensive study by Balakrishnan and Eastmond (2006) found no chromosomal aberrations in the bone borrow (i.e., a non-target organ) of rats treated with single or repeated doses of OPP. Kaneda *et al*. (1978), using a similar dose range, found that OPP did not induce dominant lethal effects in male mice. By contrast, Balakrishnan *et al.* (2002a) studied the genotoxicity of OPP in the rat urinary bladder at a dose level whereat repeated application in the diet for 2 years caused tumor induction in the organ and found increased cell proliferation and micronuclei formation after 2 weeks. The latter effect also appeared to be enhanced by the high concentration of sodium ions that the investigators also examined in the study. In a follow-up study, Balakrishnan and Eastmond (2006) reported that increased formation of micronuclei in the urinary bladder of OPP-treated rats was due to chromosome break and loss. Also, in that follow-up study, while the investigators included three OPP doses with observed tumors after dosing for 2 years, only the two higher doses caused the increased micronuclei formation and cell proliferation after 2 weeks.

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[42] Chromosomal aberrations and endoreduplication (ERD) were detected in OPP-treated CHO-K1 cells. The chromosomal aberrations observed were chromatid exchanges, chromatid breaks, dicentrics and ring chromosomes whereas the ERD consisted of microscopically non-visible duplication of the chromosomes within the cell nucleus leading to the formation of diplochromosomes in the following mitosis. The mechanism of ERD formation was suggested as the result of genome amplification (replications) without a preceding cell division, which may have been associated with specific chromosomal damage and concomitant cell-cycle arrest (Tayama-Nawai *et al.*, 1984).

This result suggested that the micronuclei bioassay designed to detect large-scale chromosomal events may not be sensitive enough for other genetic changes that may contribute to the OPP-induced tumorigenicity. Using 2 chromosomal probes, fluorescence *in situ* hybridization (FISH) studies showed that 2-week treatment of OPP in the diet caused no increase in the number of targeted chromosomes (i.e., hyperdiploidy) of the rat urinary bladder (Balakrishnan *et al,*. 2002b; Balakrishnan and Eastmond, 2003). Based on the micronuclei bioassay and FISH results, Balakrishnan and Eastmond (2006) concluded that OPP-induced aneuploidy may involve a unique mechanism that favored chromosomal loss in the urinary bladder. A known genotoxic chemical carcinogen that exhibited higher chromosomal loss than gain is benzene (Eastmond *et al.*, 2001).

III.E.1.c. DNA Damage

Table 34 summarizes the results of studies on the effects of OPP on DNA *in vitro* and *in vivo*; these DNA effects included binding, nonspecific damage, breakage, oxidation, sister chromatid exchange (SCE), and cell transformation.

(a) DNA Binding

Results of covalent binding of OPP to DNA are available in calf thymus DNA (Ushiyama *et al.*, 1992) and rat liver DNA *in vitro* (Pathak and Roy, 1992, 1993). All of these studies showed DNA-adduct formation only in the presence of metabolic activation. This suggested that reactive OPP metabolite(s) have potential for damage to biomacromolecules. Also, in the studies with rat liver DNA, the detection of four major ^{32}P-postlabeled DNA adducts was independent of the activation system employed (i.e., cytochrome P-450 or prostaglandin H synthase [PHS]), (Pathak and Roy, 1992, 1993). These DNA adducts had chromatographic mobility which matched closely with adducts from the reaction between PBQ and purified DNA and the reaction between PBQ and deoxyguanosine 3'-phosphate oligonucleotides (dGMP), indicating that PBQ was one of the DNA-binding metabolites.

Evidence suggests that OPP may have a direct effect on DNA. Gottesfeld *et al*. (1971) found evidence for non-covalent interaction between OPP and DNA and speculated that OPP was capable of interacting non-covalently with nucleic acid *via* (1) intercalation between nucleic bases and (2) hydrogen bond formation with purine-ring nitrogens (i.e., complex formation). Chemicals that intercalate between base pairs (e.g., 9-aminoacridine) may lead to mutation *via* physical distortion of DNA during replication (Casarett *et al.*, 2001).

The three available i*n vivo* studies investigating DNA adduct formation in the rat urinary bladder yielded negative results (Reitz *et al.*, 1983; Smith *et al.*, 1998; Kwok *et al.*, 1999). However, the study design may have limited the detection of DNA adducts. In the studies by Reitz *et al.* (1983) and Kwok *et al.* (1999), although both groups of investigators used radiolabeled OPP, the detection was measured only after a single dose. Given that repeated dosing favored the formation of OPP reactive metabolites *in vivo* (see III.A. PHARMACOKINETICS), the single dose protocol would limit the DNA-adduct formation. The

study by Smith *et al.* (1998) has several limitations. Although the investigation of DNA adduct formation occurred after repeated OPP dosing, the amount of sample available was relatively small for using only isolated bladder epithelium, and the detection of adduct was through ^{32}P postlabeling instead of using radiolabeled OPP. In addition, the investigators did not apply methods for enhancing the ^{32}P-postlabelled-adduct detection and appropriate controls for evaluating the fate of DNA adducts during sample processing (i.e., extraction and enrichment procedures). Thus, the study lacks sensitivity to detect adducts formation associated with OPP. Also, there are examples of carcinogen that detection of DNA adducts occurred only under repeated but not single dosing protocol (e.g., pentachlorophenol [Lin *et al.*, 2002]) and of genotoxic rodent bladder carcinogens (e.g., *o*-anisidine [Ashby *et al.*, 1994]) that induced mutations in DNA *via* a mechanism other than covalent adduct formation.

(b) Nonspecific DNA Damage

Five *in vitro* studies investigating DNA damage are available: three studies have sufficient details for an in-depth evaluation (Hanada, 1977; Kojima & Hiraga, 1978; Hirayama *et al.*, 1981) and two studies are available in an abstract form only (Nishioka and Ogasawara, 1979; Shirasu *et al.*, 1978). Over a similar dose range, results of all the three tests were positive regardless of the microbial system used (i.e., *Escherichia coli* or *Bacillus subtilis*) (Hanada, 1977; Kojima & Hiraga, 1978; Hirayama *et al.*, 1981).

(c) DNA Breakage and Oxidation

Results of DNA breaks and (or) oxidation are available in purified DNA and mammalian cells *in vitro*. With no metabolic activation, each of the three available studies produced a negative result (Nagai *et al.*, 1990, 1995; Henschke *et al.*, 2000). In one study that also tested DNA breakage in the presence of metabolic activation, the result was positive (Nagai *et al.*, 1990). Therefore, akin to what was concluded for the DNA binding, DNA breaks in the presence of metabolic activation may be due to reactive OPP metabolite(s), which have the potential for damage to biomacromolecules molecules *in vivo*.

Results of *in vivo* DNA breaks are available in rat urinary bladder (Morimoto *et al.*, 1987) and two studies in different mouse organs (Sasaki *et al.*, 1997; Brendler-Schwaab, 2000). In the rat study, OPP did not induce DNA breaks when it was injected intravescially into the urinary bladder, bypassing the liver metabolism (Morimoto *et al.*, 1987). In one of the two mouse studies, liver and kidneys exhibited DNA breaks, among other organs examined (Sasaki *et al.*, 1997); however, another study did not show the DNA breakage in these organs (Brendler-Schwaab, 2000). Although the dose ranges were similar, these mouse studies employed different detection methods (Table 34) and data evaluation criteria. That is, in the study by Sasaki *et al.* (1997), criterion of positive result was based on a statistical method whereas in the study by Brendler-Schwaab (2000), it was based on the observation that the COMET tail length (indicator of DNA fragmentation) exceeded 25% of the negative controls (rationale not explained). Because Brendler-Schwaab (2000) presented no statistical test results, any further comparison of these data are not possible. Hence, DPR considered the results from

these two studies on DNA breakage as “mixed" instead of “in contrast” as suggested by Brendler-Schwaab (2000).

(d) Sister Chromatid Exchange (SCE)

In the absence of metabolic activation, results of all available *in vitro* tests for SCE were positive in CHO cells (NTP, 1986) and CHO-K1 cells (Tayama *et al.*, 1983b; Tayama-Nawai *et al,* 1984). Also, CHO-K1 cells exhibited evidence of SCE induction in the presence of metabolic activation, with the effect occurring at a lower dose with than without metabolic activation (Tayama *et al.*, 1983b).

Previously, Tayama and Nakagawa (1991) described the OPP genotoxic mechanism of action for chromosomal damage in CHO-K1 cells (see III.E.1.b). Using the same test system, the investigators observed similar results regarding SCE. That is, Cyst and GSH suppressed the effects of OPP only in the presence of metabolic activation (S9 mix) (Tayama and Nakagawa, 1991). With the same test system, the investigators subsequently (Tayama and Nakagawa, 1994) reported that ascorbate (i.e., antioxidant) inhibited the SCE induction in the presence of metabolic activation, while catalase, mannitol, or superoxide dismutase (SOD)[43] caused no inhibition. These results support the notion that two different mechanisms may be involved in the SCE induction: a direct effect of OPP in the absence of metabolic activation and an electrophilic reaction of OPP metabolite(s) in the presence of metabolic activation (Tayama and Nakagawa, 1991). Also, the investigators concluded that the involvement of reactive oxygen radicals including H_2O_2 and $O_2^{\cdot -}$ in the latter process was minor (Tayama and Nakagawa, 1994).

(e) Mitotic Gene Conversion and Cell Transformation

OPP was negative in one study on mitotic gene conversion with *Saccharomyces cerevisiae* irrespective of metabolic activation (Brusick, 1976). In an *in vivo* study, Honma *et al.* (1983) reported positive concanavalin A agglutination[44] in rat bladder epithelial cells after 1 week of OPP dietary exposure at the dose range that resulted in tumor induction after a longer dosing period (e.g., 13 weeks [Hiraga and Fujii, 1984]).

Summary of OPP Genotoxicity Studies Results from gene mutation studies indicate that OPP did not show mutagenic effects in bacteria but showed genotoxicity in mammalian cells *in vitro.* OPP, at doses with minimal cytotoxicity, caused chromosomal aberrations and endoreduplication (i.e., chromosomal damage) in mammalian cells *in vitro*. Evidence indicated that OPP was capable of interacting non-covalently (i.e., intercalation and complex formation) with DNA *in vitro*. Among the *in vitro* tests for DNA damage, positive results occurred in microbial tests for non-specific DNA damage and mammalian cell tests for induction of SCE in the absence or presence of metabolic activation. Results of these studies of gene mutation,

[43] Catalase, mannitol, and superoxide dismutase are scavengers of H_2O_2, OH radicals, and superoxide anions, respectively.
[44] An effect correlated with the carcinogenicity of the tested substance (Honma *et al.*, 1983).

chromosomal effects, and DNA damage showed that metabolic activation enhanced the effects of OPP *in vitro*. Under metabolic activation, OPP-DNA reactions *in vitro* resulted in DNA-adduct formation and strand breakage. *In vivo* micronucleus tests for clastogenicity (chromosomal break) and aneugenicity (chromosomal loss) yielded positive responses in the urinary bladder (target organ) but not bone marrow (non-target organ) of rats exposed to OPP. Although DNA binding studies in the rat urinary bladder *in vivo* yielded negative results, the study design may have limited the detection of DNA adducts. DNA breakage was another form of genetic damage observed in the urinary bladder of rats exposed to OPP. Another effect identified in the urinary bladder of rats treated with OPP was agglutination of the epithelial cells with concanavalin A, an effect that has been generally correlated with the carcinogenicity of the tested substances. Taken together, evidence of gene mutation, chromosomal aberrations, and DNA damage suggest that OPP has genotoxic potential and its metabolites may have contributed to its genotoxic effects *in vivo*.

III.E.2. Sodium *ortho*-Phenylphenate

III.E.2.a. Gene Mutation

Five *in vitro* studies for gene mutation in bacterial and eukaryotic systems are available (Table 35). Negative results occurred in the three reports of *S. typhimurium* test and one report of *E. coli* testing in the absence and (or) presence of metabolic activation (Kojima and Hiraga, 1978, Reitz *et al.*, 1983, Mortelmans *et al*., 1986). SOPP with no metabolic activation caused a dose-related increase in segregation indexes in *Aspergillus nidulans* (Georgopoulos *et al*., 1976). All these studies indicated that challenge to the test systems was sufficient, as suggested by the occurrence of cytotoxicity.

III.E.2.b. Chromosomal Damage

Table 35 summarizes the results of studies for chromosomal damage *in vitro* and *in vivo*. In the absence of metabolic activation, the two *in vitro* chromosomal aberration tests of SOPP were negative in CHL fibroblasts and CHO-K1 cells (Yoshida *et al.*, 1979; Ishidate, 1988).

The *in vivo* systems used for testing the clastogenicity of SOPP were a dominant lethality assay (rats and mice) (Ogata *et al*., 1978a, 1980) and bone marrow chromosomal aberration test (Yoshida *et al.*, 1979). Both tests produced negative results in each of the species tested. By contrast, the rat urinary bladder was positive for cell proliferation and micronucleus induction at a SOPP concentration whereat repeated application caused tumor induction in the organ (Tadi-Uppala *et al.*, 1996; Balakrishnan and Eastmond, 2006).

III.E.2.c. DNA Damage

Table 36 summarizes the results of studies for the effects of SOPP on DNA *in vitro* and *in vivo*; these DNA effects included nonspecific damage, binding, breakage, oxidation, and cell transformation.

(a) Nonspecific DNA Damage

SOPP with no metabolic activation was negative for the *Rec* assay with *B. subtilis* (Kojima and Hiraga, 1978). SOPP was negative for unscheduled DNA synthesis (UDS) in the rat primary hepatocytes *in vivo* (Reitz *et al*., 1983).

(b) DNA Binding

Results of covalent binding of SOPP to DNA *in vivo* are available in the mouse skin (Pathak and Roy, 1993) and the rat urinary bladder (Reitz *et al.*, 1983; Ushiyama *et al.*, 1992). After a topical application of SOPP to mouse skin, Pathak and Roy (1993) found four major adducts by ^{32}P postlabeling. These adducts were identical to those obtained by reacting OPP with purified DNA *in vitro* (Pathak and Roy, 1993). Regarding the rat urinary bladder, Reitz *et al.* (1983) did not find evidence of DNA adduct formation after a single dose. However, given that repeated dosing favored the formation of OPP reactive metabolites *in vivo* (see III.A. PHARMACOKINETICS), the single dosing protocol would likely be insufficient for evaluating DNA-adduct formation. Indeed, Ushiyama *et al.* (1992) detected a single predominant adduct of DNA by ^{32}P postlabeling after the repeated dosing for 13 weeks. This DNA adduct had the chromatographic mobility which matched with adducts from PHQ-DNA and PBQ-dGMP reactions, indicating that PBQ was one of the DNA-binding metabolites. Taken together, these results indicated that reactive metabolites (e.g., PBQ) of SOPP are capable of binding to DNA *in vivo*.

(c) DNA Breaks and Cell Transformation

Results of *in vivo* DNA break formation are available in two single dosing studies in different organs in the mouse (Sasaki *et al.*, 2002) and the rat (Sekihashi *et al.*, 2002) and a repeated dosing study in the rat urinary bladder (Morimoto *et al.*, 1989). The results of both single dosing studies showed that DNA breaks occurred in the stomach, colon, liver, kidneys, and urinary bladder, among other organs examined (Sasaki *et al.*, 2002; Sekihashi *et al.*, 2002). Over a similar dose range, repeated application of SOPP induced DNA breaks in the rat urinary bladder in a dose dependent manner (Morimoto *et al.*, 1989).

In an *in vivo* study, Honma *et al.* (1983) reported positive concanavalin A agglutination[45] in rat bladder epithelial cells after 1 week of SOPP dietary exposure at the dose range that resulted in tumor induction after a longer dosing period (e.g., 13 weeks [Hiraga and Fujii, 1981]).

[45] An effect correlated with the carcinogenicity of the tested substance (Honma *et al.*, 1983).

Summary of SOPP Genotoxicity Studies Gene mutation studies indicate that SOPP with no metabolic activation has genotoxic potential in eukaryotic systems *in vitro*. While limited data suggest that SOPP was not like OPP in terms of the effects on chromosomal and DNA damage *in vitro*, studies showed that SOPP caused micronuclei, DNA adducts, and DNA breakage in the rat urinary bladder (i.e., target organ) *in vivo*. Another effect identified in the urinary bladder of rats treated with SOPP was agglutination of the epithelial cells with concanavalin A, an effect that has been generally correlated with the carcinogenicity of the tested substances. Taken together, evidence of gene mutation, chromosomal aberrations, and DNA damage suggest that SOPP has genotoxic potential and that enhancement of its genotoxic effects occurred *in vivo*.

III.E.3. Phenylhydroquinone (PHQ)

Table 37 summarizes the results of available studies for gene mutation, chromosomal damage, and DNA damage.

III.E.3.a. Gene Mutation

PHQ was negative in the only available study on gene mutation using Chinese hamster V79 cells (HGPRT) supplemented with arachidonic acid (Lambert, 1992).

III.E.3.b. Chromosomal Damage

In the absence of metabolic activation, PHQ-treated CHO-K1 cells exhibited cytotoxicity but not chromosomal aberrations (Tayama *et al.*, 1989; Tayama and Nakagawa, 1991). However, in the presence of metabolic activation (S9 mix), PHQ caused chromosomal damage (i.e., chromosomal aberrations and ERD) in the CHO-K1 cells with observed cytotoxicity (Tayama *et al.*, 1989); the presence of sulfhydryl compounds and S9 mix caused further suppression of cytotoxicity and clearer revelation of chromosomal damage in the PHQ-treated cells (Tayama and Nakagawa, 1991). With no metabolic activation, chromatographic analyses of the PHQ-treated cell mixtures indicated the presence of PBQ (a cytotoxic metabolite [Nakagawa *et al.*, 1992a,b, 1993]) whereas in the presence of a sulfhydryl compound, the analyses found PHQ-GSH conjugate[46]. Also, the result of a supplemental study indicated that PHQ autoxidized to PBQ in a stoichiometric manner. Taken altogether, these observations suggest that the negative result in the chromosomal aberration test with no metabolic activation may have been due, in part, to the cytotoxic effect of PBQ. That is, in the presence of PBQ, the cells may not survive long enough for PHQ-induced cytogenetic effect to appear (Tayama and Nakagawa, 1991). By corollary, because chromatographic analyses of the cell mixtures with OPP-S9-Cyst/GSH detected sulfhydryl-compound adducts of PHQ (Tayama and Nakagawa, 1991), PHQ and PBQ may be responsible for the enhanced genotoxic and cytotoxic effects of OPP with metabolic activation *in vitro*.

[46] PHQ-GSH is a non-enzymatic reaction product of PBQ and GSH (Nakagawa and Tayama, 1989).

Studies investigating micronuclei formation in different mammalian cell systems yielded mixed results: a positive result in Chinese hamster V79 cells (Lambert and Eastmond, 1994) and a negative result in ovine seminal vesicle (OVS) (Freyberger and Degen, 1998). All these mammalian cell studies indicated that challenge to the test systems was sufficient, as suggested by the occurrence of cytotoxicity.

III.E.3.c. DNA Damage

(a) DNA Binding

Two test systems used for studying covalent binding of OPP to DNA *in vitro* were purified DNA (Grether *et al*., 1989; Ushiyama *et al.*, 1992; Pathak and Roy, 1992, 1993) and HL-60 cells (Horvath *et al.*, 1992). All of these studies yielded positive results in DNA binding irrespective of metabolic activation. Although the investigators did not measure PHQ autoxidation, other studies indicated the PHQ autoxidation may have occurred under the experimental protocol employed (i.e., at pH ≥7.0 and 37°C) (Tayama *et al.*, 1989; Kwok and Eastmond, 1997). Taken altogether, these observations suggest that the DNA binding may be due to reactive PHQ metabolite(s) formed *via* non-enzymatic autoxidation. In addition to covalent binding, there was evidence for non-covalent interaction between PHQ and DNA (Gottesfeld *et al*., 1971).

In an *in vivo* study, Pathak and Roy (1993) measured DNA adducts after topical application of PHQ to mouse skin and found four major adducts by ^{32}P postlabeling. These adducts were identical to those found in the mouse skin treated with SOPP *in vivo* (Pathak and Roy, 1993).

(b) DNA Breakage

Results of DNA break formation in the absence of metabolic activation are available in two different test systems: purified DNA (Inoue *et al.*, 1990; Nagai *et al.*, 1990, 1995; Murata *et al.*, 1999; Okubo *et al.*, 2000) and mammalian cells (Murata *et al.*, 1999; Henschke *et al*., 2000). In some of these studies, the investigators also conducted the DNA-breakage test in the presence of metal ions (e.g., Cu [II] or Fe [II]) (Inoue *et al.*, 1990; Nagai *et al.*, 1995; Murata *et al.*, 1999) and (or) radical scavengers (Inoue *et al.*, 1990; Nagai *et al.*, 1990; Okubo *et al.*, 2000). Under the experimental protocols employed (Table 38), results of all studies were positive. Also, catalase and methionine[47] inhibited the DNA breaks (Inoue *et al.*, 1990; Nagai *et al.*, 1990) and there was evidence of PHQ autoxidation (Inoue *et al.*, 1990; Murata *et al.*, 1999; Okubo *et al.*, 2000). Regarding the effect of metal ions, several results were noticeable: (1) the DNA breaks occurred at a lower dose with than without the presence of Cu(II) (Inoue *et al.*, 1990; Nagai *et al.*, 1995; Murata *et al.*, 1999); (2) enhancement of PHQ autoxidation occurred the presence of Cu(II) ions (Inoue *et al*., 1990; Murata *et al*., 1999); and (3) superoxide dismutase and *tert*-butyl

[47] Methionine is a scavenger of OH radicals (Inoue *et al.*, 1990)

alcohol did not exhibit the suppressing effect on DNA breaks in the presence of Cu(II) (Inoue *et al.*, 1990). Overall, these observations suggest that DNA breaks may have been caused by reactive oxygen species (ROS) and that two autoxidation pathways of PHQ may have involved: one is metal-dependent and the other is metal-independent.

One *in vivo* study of DNA breaks in the rat urinary bladder is available (Morimoto *et al.*, 1987). The results showed that intravescially injected PHQ did not induce breaks in the DNA.

(c) DNA Oxidation

Six *in vitro* studies investigating DNA oxidation in the absence of metabolic activation are available. In some of these studies, the investigators also conducted the DNA-oxidation test in the presence of metal ions (Nagai *et al.*, 1995; Murata *et al.*, 1999), radical scavengers (Nagai *et al.*, 1995), and (or) inhibitor of ROS detoxifying enzyme (Nakagawa and Tayama, 1996). Except for one study available as an abstract only (Cai and Roy, 1999), an in-depth evaluation is possible for the two studies with purified DNA (Nagai *et al.*, 1995; Murata *et al.*, 1999) and the three studies with mammalian cells (Nakagawa and Tayama, 1996; Murata *et al.*, 1999; Henschke *et al.*, 20000). Under the experimental protocols employed (Table 38), results of the five studies were positive. Enhancement of the oxidative effect occurred in the presence of metal ions (copper or iron [Nagai *et al.*, 1995]) and an inhibitor of catalase (Nakagawa and Tayama, 1996) whereas suppression of the effect occurred with the scavengers of ROS (e.g., catalase, sodium benzoate, and sodium azide[48]) (Nagai *et al.*, 1995) and ion chelating agents (e.g., EDTA and bathocuproine[49]). Therefore, akin to what was concluded for the DNA breakage, DNA oxidation in the absence of metabolic activation may have been due to reactive oxygen species that were formed *via* PHQ autoxidation. Also, the copper ions present in animal tissues could facilitate the oxidative DNA damage *in vivo* (Nagai *et al.*, 1995)

(d) Sister Chromatid Exchange (SCE)

Results of investigating the effects of metabolic activation, radical scavengers, and (or) incubation pH on SCE induction are available in CHO-K1 cells *in vitro* (Tayama *et al.*, 1989; Tayama and Nakagawa, 1991, 1994). Tayama *et al.* (1989) reported a positive result in the SCE test irrespective of metabolic activation. Regarding the effects of radical scavengers, Tayama and Nakagawa (1991, 1994) found that, in the absence of metabolic activation, Cyst, GSH, catalase, or ascorbate inhibited the SCE induction whereas SOD plus catalase, SOD, or 3-amino-1,2,4-triazole (i.e., catalase inhibitor) intensified the effect. Also, the amount of unchanged PHQ in the incubation mixture was higher in the radical-scavenger treatments with than without the cytogenetic effect suppressed. Results of a supplemental study showed that SOD accelerated but catalase and GSH suppressed the rate of PHQ autoxidation. Taken together, inhibition of SCE by catalase, Cyst, GSH, and ascorbate indicated that these scavengers suppress PHQ autoxidation.

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[48] Sodium azide is a typical scavenger of OH radicals (Nagai *et al*., 1995).
[49] This is a specific chelator for monovalent copper ions (Nagai *et al*., 1995).

For investigating the effect of pH, Tayama and Nakagawa (1994) conducted the study in PHQ-treated CHO-K1 cells in the absence of metabolic activation. At a given dose of PHQ, an increase in pH caused an increase in the frequencies of SCE; also, there was a corresponding reduction in unchanged PHQ in the incubation mixtures. Catalase and SOD had suppressing and enhancing effects, respectively, on the SCE induction and PHQ loss. These observations support the notion that autoxidation is important for the cytogenetic effect of PHQ. Moreover, this study documented that the PHQ-induced cytogenetic effect is a pH-dependent process.

Summary of PHQ Genotoxicity Studies Evidence indicated that PHQ causes chromosomal aberrations, endoreduplication, micronuclei formation, and DNA damage (i.e., covalent adduct formation, breakage, oxidation, and sister chromatid exchange) irrespective of metabolic activation *in vitro*. Also, studies without metabolic activation showed that sulfhydryl compounds and scavengers of reactive oxygen species (ROS) inhibited the chromosomal and DNA effects of PHQ and that the formation of reactive quinoid and reactive oxygen species *via* PHQ autoxidation may have occurred. In the absence of metabolic activation, increasing pH or metal ion concentrations caused an increase in the genotoxic effects of PHQ. Taken together, these results suggest that reactive species produced *via* pH- and metal ion-dependent PHQ autoxidation may play an important role in the genotoxicity of PHQ *in vitro* and potentially the genotoxicity of OPP *in vivo*.

III.E.4. Phenylbenzoquinone (PBQ)

Table 38 summarizes the results of available studies for gene mutation, chromosomal damage, and DNA damage.

III.E.4.a. Gene Mutation

PBQ was negative for gene mutation in the two available studies with mammalian cells: V79 cells (Lambert, 1992) and AHH-1 human lymphoblastoid cells (Reid *et al.*, 1998).

III.E.4.b. Chromosomal Damage

Studies investigating chromosomal damage yielded different results in different mammalian cell systems (Table 38): negative response in V79 cells (Lambert and Eastmond, 1994) and CHL cells (Ishidate, 1988), but positive response in CHO-K1 cells (Tayama and Nakagawa, 1991).

Regarding the positive responses observed in CHO-K1 cells, sulfhydryl compounds (e.g., GSH) suppressed the chromosomal aberrations and ERD independent of metabolic activation (Tayama and Nakagawa, 1991). Also, chromatographic analyses of the cell mixtures detected sulfhydryl-compound adducts of PBQ (e.g., PHQ-GSH). Based on these observations, the

investigators concluded that PBQ itself could act directly as an electrophile for inducing the genetic damage.

II.E.4.c. DNA Damage

(a) DNA Binding

Results of covalent binding of PBQ to DNA *in vitro* are available in purified DNA (Horvath *et al.*, 1992; Ushiyama *et al.*, 1992; Pathak and Roy, 1992; Zhao *et al*., 2002) and mammalian cells (Horvath *et al.*, 1992; Zhao *et al.*, 2002) in the absence of metabolic activation. All of these studies reported positive results in the DNA binding.

(b) DNA Breakage and Oxidation

Results of DNA breaks and (or) oxidation are available in the absence of metabolic activation in purified DNA and mammalian cells *in vitro* (Inou *et al*., 1990; Nagai *et al*., 1990, 1995; Murata *et al.*, 1999; Henschke *et al*., 2000). In some of these studies with purified DNA, the investigators also examined the breakage and oxidative effects in the presence of Cu(II), NADP/NADPH, and (or) H_2O_2 (Ionu *et al.*, 1990, Nagai *et al.*, 1990, Murata *et al.*, 1999). Under the experimental protocols employed (Table 38), the results from all available studies were positive. Also, in the study by Henschke et al. (2000), PBQ induced both DNA breakage and oxidation at concentrations whereat the cytotoxicity was minimal (Table 38).

Two separate studies examined DNA breaks in the rat urinary bladder (Morimoto *et al.*, 1989) and forestomach *in vivo* (Morimoto *et al.*, 1991), respectively. PBQ injected intravescially or administered perorally produced DNA breaks in the urinary bladder and the stomach, respectively. However, at the same concentration, direct contact of OPP or PHQ in the rat urinary bladder produced no increased DNA breakage (Morimoto *et al.*, 1989). These results indicate that PBQ was a reactive metabolite to urinary bladder and stomach epithelium.

(c) Sister Chromatid Exchange (SCE)

PBQ was positive in the induction of SCE in CHO-K1 cells irrespective of metabolic activation (Tayama and Nakagawa, 1991). Also, sulfhydryl compounds (e.g., GSH) suppressed the SCE induction. Therefore, akin to what was concluded for the chromosomal aberrations, the SCE induction may be due to PBQ, which acted directly as an electrophile for inducing the genetic damage.

(d) Cell Transformation

PBQ was positive in one study with BALB/3T3 cells for cell transformation using an initiation-promotion study design (Sakai *et al.*, 1995). It is noteworthy that the test design of cell transformation *in vitro* is to simulate the events that occur during oncogenic transformation processes *in vivo* (Brusick, 1987).

Summary of PBQ Genotoxicity Studies Studies showed that PBQ causes damage to chromosome (i.e., clastogenicity and endoreduplication) and DNA (i.e., covalent adduct formation, breakage, oxidation, and sister chromatid exchange) irrespective of metabolic activation *in vitro*. Also, *in vitro* evidence suggests that PBQ acts directly as an electrophile for inducing genetic damage and has oncogenic potential. The higher intrinsic reactivity of PBQ compared to OPP and PHQ is indicated by the *in vivo* results that only PBQ produced DNA breakage when in direct contact with different rat tissues including the urinary bladder.

Table 32 Results of Tests for the Induction of Point Mutation by OPP

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
Gene Mutation	*S. typhimurium* TA1535, TA1536, TA1537, TA1538	No details given	-	Pos. TA1536	Hanada, 1977	No details were given in the report.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA98, TA 100	0, 3.3, 10, 33, 40, 60, 80, 100, 120, 140, 150, 200 µg/plate (99.9% pure)	±; r,h	Pos.; - TA1535	NTP, 1986*	Weakly positive responses occurred at 80 µg/plate and higher. Results also were reported in Haworth *et al.* (1983).
Gene Mutation	*S. typhimurium* TA98, TA 100	No details given	±;r	Pos.; ± TA98	Nishioka & Ogasawara, 1979	The study was published as an abstract.
Gene Mutation	*S. typhimurium* TA97a, TA102	0, 1, 5, 10, 50, 100 µg/plate (purity: ns)	±;r	Neg.	Fujita *et al*., 1985	Cytotoxicity was noted at ≥50 µg/plate.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA 100	0, 0.025, 0.25, 2.5, 25 µg/plate (purity: ns)	±;r	Neg.	Brusick, 1976*	No evidence of cytotoxicity was achieved at the highest dose tested.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA 100	0.1-1000 µg/plate (purity: ns)	±;r	Neg.	Cline & McMahon, 1977*	No individual data were reported.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA 100	No details given	±; ns	Neg.	Shirasu *et al*., 1978	Study was published as an abstract; no individual data were reported.
Gene Mutation	*S. typhimurium* TA98, TA 100	0, 1, 10, 100, 1000 µg/plate (purity: ns)	±;r	Neg.	Kojima & Hiraga, 1978	Cytotoxicity was observed at 1000 µg/plate.

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; h, hamster liver microsomal S9 fraction; Pos., positive result; Neg., negative result.
[a]*Salmonella typhimurium*
* Study evaluated by DPR was found to be unacceptable.

Table 32 Results of Tests for Induction of Gene Mutation by OPP (Continued)

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
Gene Mutation	S. typhimurium TA98, TA 100	0, 1, 10, 100 µg/plate (purity: ns)	±;r	Neg.	Hirayama et al., 1981	Number of plates/dose was not specified in the report. Cytotoxicity was observed at 100 µg/plate.
Gene Mutation	*S. typhimurium* TA1535, A1537, TA1538, TA98, TA 100	Tested dose up to 5000 µg/plate (99.9% pure)	±;r	Neg.	Moriya *et al.* 1983	Individual data were not reported
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	0, 50, 100, 200, 300 µg/plate (purity: ns)	±;r, m	Neg.	Kojima *et al.*, 1983	Cytotoxicity was observed at the highest dose tested with no metabolic activation. With mouse S9, only TA100 and TA 98 were tested.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA92, TA94, TA98, TA100	6 doses used (not given); the highest dose tested was reported to be 500 µg/plate (purity: ns)	+; r	Neg.	Ishidate *et al.*, 1984	There was no indication that the maximum dose tested also was cytotoxic.
Gene Mutation	*S. typhimurium* TA100, TA98, TA97, TA102	No details given	±;r	Neg.	Pagano *et al.*, 1988	
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA 100; Host-Mediated, Male JCL:ICR mice	0, 200, or 600 mg/kg (purity: ns); oral for 5 days	N/A	Pos. TA98	Takahashi, 1978	Takahaski (1978) re-evaluated the study by Shirasu *et al.* (1978). Both studies were published in abstract form.
Gene Mutation	*E coli WP2*	0, 1, 10, 100, 1000 µg/ml (purity: ns)	-	Neg.	Kojima & Hiraga, 1978	Cytotoxicity was observed at the highest dose tested.

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; m, mouse liver microsomal S9 fraction; Pos., positive result; Neg., negative result; N/A, not applicable.
[a]*Salmonella typhimurium, Escherichia coli*

Table 32 Results of Tests for Induction of Gene Mutation by OPP (Continued)

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
Gene Mutation	CHO-WB1cells (HGPRT)	0, 37, 74, 147, 294, 441, 588 μM (>99% pure)	±;r	Neg.	Brendler, 1992	Cytotoxicity was observed at ≥294 μM without metabolic activation and ≥441 μM, with metabolic activation.
Gene Mutation	Mouse lymphoma (L5178Y/TK$^-$)	Study 1: 0, 118, 176, 235, 294, 353 μM Study 2: 0, 1.9, 3.7, 7.4, 14.7, 29.4 μM	±;r	Pos.	NTP, 1986*	Positive response was observed at ≥235 μM without metabolic activation and 29.4 μM, with metabolic activation. Relative cell growth also was reduced at these doses. Data were presented in a summary table. A second trial without S9 was performed but no data were presented. OPP purity: ns.
Gene Mutation	Human Rsa cells ($Na^+/K^=$ ATPase locus)	0, 88, 118, 147, 176 μM (purity: ns)	-	Pos.	Suzuki *et al.*, 1985*	Mutation frequency appeared to be 100 times greater than the controls at 176 μM with a linear increase with concentration. Cytotoxicity was observed at ≥147 μM (≤40% survival).
Gene Mutation	SLRL *D. melanogaster*	Fed at 250 ppm or received injections of 500 ppm (purity: ns)	N/A	Neg.	NTP, 1986*	The assay was conducted with three broods of 3, 2, 2 days. Results also were reported in Woodruff *et al.* (1985).

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; microsomal S9 fraction; Pos., positive result; Neg., negative result; N/A, not applicable; SLRL, sex-linked recessive lethal test.
[a]*Drosophilia melanogaster*
* Study evaluated by DPR was found to be unacceptable.

Table 33 Results of Tests for the Induction of Chromosomal Damage by OPP

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Chrom. Aberration	CHL fibroblasts	0, 74, 147, 294 μM (purity: ns)	-	Neg.	Ishidate *et al.*,1984	No evidence of cytotoxicity was achieved at the highest dose tested. Results also were reported in Ishidate (1988).
Chrom. Aberration	CHO cells	Study 1: 0, 353, 413, 471 μM (-r) Study 2: 0, 413, 471, 529 μM (+r)	±;r	Neg.	NTP, 1986*	No information on cytotoxicity was available. Data were presented in a summary table. OPP purity: ns.
Chrom. Aberration	CHO-K1 cells	0, 294, 441, 588, 735, 882, 1029 μM (>99% pure)	-	Pos.	Tayama-Nawai *et al.*, 1984	Positive response was observed at ≥558 μM ($p<0.05$). Increased cell cycle delay was observed at ≥735 μM; cell division was inhibited at 1029 μM.
Chrom. Aberration	CHO-K1 cells	Study 1: 0, 147, 294, 441, 588, 735, 882, 1029 μM plus 15% r Study 2: 588 μM plus 5-50% r	+; r	Pos	Tayama *et al.*, 1989	Positive response was observed at ≥147 μM ($p<0.05$); elevated ERD and cell cycle delay were induced dose-dependently. OPP was >99% pure)
Chrom. Aberration	CHO-K1 cells	Study 1: 0, 588, 735, 882 μM (-r) plus 10 mM Cyst/GSH Study 2: 588 μM (+r) plus 0.3-30 mM Cyst/GSH	±;r	Pos	Tayama & Nakagawa, 1991	Unchanged OPP, PHQ, PHQ-Cyst/GSH adducts were identified in the cell culture media with metabolic activation added. OPP was >99% pure.
Chrom. Aberration	Human Fibroblasts	0.6-5.9 μM (purity: ns)	-	Pos.	Takahashi, 1978	The report was published as an abstract.
Chrom. Aberration	Wistar Rats (Males); Bone Marrow	0, 50, 100, 200, 400, 800 mg/kg for 5 days or single doses of 250, 500, 1000, 2000, 4000 mg/kg (purity: ns)	N/A	Neg.	Shirasu *et al.*, 1978*	The report was published as an abstract.

Abbreviations: ns, purity not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Cyst, cysteine; GSH, reduced glutathione; Pos., positive result; Neg., negative result; chrom., chromosomal; ERD, endoreduplication.
* Study evaluated by DPR was found to be unacceptable.

Table 33 Results of Tests for the Induction of Chromosomal Damage by OPP (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Dominant Lethal	C3H Mice (Males)	0,100, 500 mg/kg (99.7% pure) by gavage for 5 days; 15 animals/dose.	N/A	Neg.	Kaneda *et al.*, 1978*	Each male was mated to 2 untreated females weekly for 6 weeks. Results also were reported in Shirasu *et al* .(1978).
Hyperdiploidy	F344 Rats (Males); Urinary Bladder	0, 20000 ppm OPP, 20000 ppm (OPP plus NaCl) or 20000 ppm NaCl in diet for 2 weeks (purity: ns); 5-8 animals/treatment.	N/A	Neg.	Balakrishnan *et al.*, 2002b	Hyperdiploidy was examined by FISH, which detected gain in a targeted chromosome. The investigators concluded that polyploid cells that commonly found in the urinary bladder complicated the analysis.
Hyperdiploidy	F344 Rats (Males); Urinary Bladder	0, 80, 800, 2000, 4000, 12500 ppm OPP in diet for 2 weeks (purity: ns); 4 animals/treatment.	N/A	Neg.	Balakrishnan & Eastmond 2003	Hyperdiploidy was examined by FISH, which detected gain in two targeted chromosomes.
Micronuclei Formation	F344 Rats (Males); Bone Marrow	0 or 8000 ppm OPP in diet for 15 days (purity: ns); 3-4 animals/treatment.	N/A	Neg.	Balakrishnan & Eastmond 2006	
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm OPP, 20000 ppm (OPP plus NaCl) or 20000 ppm NaCl in diet for 2 weeks (purity: ns); 9 animals/treatment.	N/A	Pos.	Balakrishnan *et al.*, 2002a	Positive responses were observed in all treated groups, as with the cell proliferation. The results also were published in Tadi-Uppala *et al.*(1996) and Balakrishnan *et al.* (1999).
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 2000, 4000, 8000, 12500 ppm OPP in diet for 15 days (purity: ns); 3-4 animals/treatment.	N/A	Pos.	Balakrishnan & Eastmond 2006	Increased ($p<0.05$) micronuclei formations were observed in dose groups at 8000 and 12500 ppm but not at 4000 ppm, as with the cell proliferation. OPP-induced micronuclei resulted from both chromosomal loss (CREST-positive) and breakage (CREST-negative).

Abbreviations: ns, purity not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; FISH, fluorescence *in situ* hybridization; CREST, centromeric antinuclear antibody.
* Study evaluated by DPR was found to be unacceptable.

Table 34 Results of Tests for the Induction of DNA Damage by OPP

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
DNA Binding	Rat Liver DNA	1000 μM (purity: ns) plus cofactor COH cofactor.	±;r	Pos.; +	Pathak & Roy, 1992	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Calf Thymus DNA	Study 1: 40mM (-r) Study 2: 25 nM [U-^{14}C] OPP (+r)	±;r	Pos.; +	Ushiyama *et al.*, 1992	Radioactivity was measured by liquid scintillation counting. OPP purity: ns.
DNA Binding	Herring Sperm DNA	0-50 μM (purity: ns)	-	Pos.	Gottesfeld *et al*, 1971	OPP inhibited deoxyribonuclease I activity. The number of moles OPP *vs.* nucleotide needed for the inhibition was 1.46.
DNA Binding	Rat Liver DNA	1000 μM plus cofactors COH or ARA	+;s	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	F344 Rats (Males); Urinary Bladder	0 or 500 mg/kg [^{14}C]-OPP by gavage (98% pure); 8 animals/dose.	N/A	Neg.	Reitz *et al.*, 1983*	DNA purified pooled urinary bladder; radioactivity was detected by liquid scintillation counting.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 15, 50, 125, 250, 500, 1000 mg/kg [^{14}C]-OPP by gavage (>99% pure); 4 animals/dose.	N/A	Neg.	Kwok *et al.*, 1999	Radioactivity was detected by accelerator mass spectrometry.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 800, 4000, 8000, 12500 ppm in diet for 13 weeks (≥ 99.5% pure); 12 animals/dose.	N/A	Neg.	Smith *et al.*, 1998	The respective time-weighted average doses were 0, 56, 282, 556, and 924 mg/kg/day. Results also were reported in Christenson *et al.*, (1996b)
DNA Damage	*E. coli* WP2/CN571, WP2uvrA/WP100	No details given	-	Pos.	Nishioka & Ogasawara, 1979	The report was available in an abstract form.
DNA Damage	*E. coli* WP/CM571, WpuvrA/WP100	1, 2, 4 mg/disc (purity: ns)	-	Pos.	Hirayama *et al.*, 1981	Cytotoxic dose was not indicated in the report.

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; s, skin homogenate; Pos., positive result; Neg., negative result; N/A, not applicable; COH, cumene hydroxide; ARA, arachidonic acid.
[a] *Escherichia coli;*
* Study evaluated by DPR was found to be unacceptable.

Table 34 Results of Tests for the Induction of DNA Damage by OPP (Continued)

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
DNA Damage	*B. Subtilis* H17/M45	0.1-1 mg/plate	-	Pos.	Hanada, 1977	Cytotoxic dose was not indicated in the report.
DNA Damage	*B. Subtilis* H17/M45	No details given	-	Neg.	Shirasu *et al.*, 1978	The report was available in an abstract form.
DNA Damage	*B. Subtilis* H17A/M45T	0.01, 0.1, 1, 10 mg/disc	-	Pos.	Kojima & Hiraga, 1978	Cytotoxic dose was not indicated in the report.
DNA Break	pUC18 DNA (*E. Coli* JM83)	1mM (purity: ns)	±;r	Pos.:+	Nagai *et al.*, 1990	Reaction time was 30 min. With metabolic activation, PHQ and PBQ were detected.
Oxidative Damage	Calf thymus DNA	0, 1, 1000, 10000 μM (purity: ns)	-	Neg.	Nagai *et al.*, 1995	8-OHdG was the endpoint for oxidative damage. Reaction time was 30 min.
DNA Break & Oxidative Damage	V79 cells	50, 200, 300, 400 μM (purity: ns)	-	Neg.	Henschke *et al.*, 2000	8-OHdG and SSB were the endpoints for oxidation and breakage. Reaction time was 30 min.
DNA Break	F344 Rats (Males); Urinary Bladder	0.05% OPP (purity: ns) in saline; intravesical injection.	N/A	Neg.	Morimoto *et al.*, 1987	DNA breaks were detected by alkaline elution assay. The exposure time was 10 min. Results also were reported in Morimoto *et al.* (1989).
DNA Break	CD-1 Mice (Males)	0, 2000 mg/kg by gavage (purity: ns); 4 animals/dose/sacrifice time at 3, 8, or 24 hr.	N/A	Pos.	Sasaki *et al.*, 1997	Modified COMET was used to detect DNA breaks in isolated nuclei from homogenized tissues (liver, kidneys, lung, and brain) and scraped mucosa (stomach and urinary bladder).
DNA Break	CD-1 Mice (Males)	0, 250, 2000 mg/kg by gavage (purity: ns); 4 animals/dose/sacrifice time at 3, 8, or 24 hr	N/A	Neg.	Brendler-Schwaab, 2000	Conventional COMET was used to detect DNA breaks in cells from perfused liver and kidneys. Two deaths occurred at 2000 mg/kg.

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; 8-OHdG, 8-hydroxyguanosine; SSB, single strand break; COMET, single cell gel electrophoresis.

[a] *Bacillus subtilis; Escherichia coli;*

Table 34 Results of Tests for the Induction of DNA Damage by OPP (Continued)

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
SCE	CHO cells	Study 1: 0, 87.6, 118, 176 μM (-r) Study 2: 0, 147, 294, 444 μM (+r)	±; r	Pos.;-	NTP, 1986*	Positive response was observed at 176 μM (11.4/cell vs. 8.9/cell). Data were presented in a summary table.
SCE	CHO-K1 cells	0, 147, 294, 588, 882 μM (> 99% pure)	±; r	Pos.	Tayama *et al.*, 1983b	Positive responses were observed at 882 μM (8.5/cell vs. 5.5/cell) and ≥588 μM ($p<0.05$) in the absence and presence of metabolic activation, respectively.
SCE	CHO-K1 cells	0, 294, 441, 588, 735, 882, 1029 μM (>99% pure)	-	Pos.	Tayama-Nawai *et al.*, 1984	Positive response was observed at ≥588 μM ($p<0.05$). Increased cell cycle delay was observed at ≥735 μM; cell division was inhibited at 1029 μM.
SCE	CHO-K1 cells	Study 1: 0, 588, 735, 882 μM (-r) plus 10 mM Cyst/GSH Study 2: 588 μM (+r) plus 0.3-30 mM Cyst/GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Unchanged OPP, PHQ, PHQ-Cyst/GSH adducts were identified in the cell culture media with metabolic activation added. OPP was >99% pure.
SCE	CHO-K1 cells	Study 1: 0, 147, 294, 441, 588, 735, 882, 1029 μM plus 15% r Study 2: 588 μM plus 5-50% S9 mix	+; r	Pos.	Tayama *et al.*, 1989	Positive response was observed at ≥147 μM ($p<0.05$). Elevated ERD and cell cycle delay were induced dose-dependently. OPP was >99% pure.
SCE	CHO-K1 cells	294 μM (>99% pure) plus SOD, catalase, mannitol, or ascorbate	+,r	Pos.	Tayama & Nakagawa, 1994	SCE, ERD, and cell cycle delay were the endpoints studied.
MGC	*Sac. Cerevisiae* D4	0.15, 1.47, 14.7, 147 μM (purity: ns)	±;r	Neg.	Brusick, 1976	
Cell Transform	F344 Rats (Males); Urinary Bladder	1000, 5000, 10000, 20000 ppm OPP in diet for 1 week (purity: ns); 5 animals/dose.	N/A	Pos.	Honma *et al*, 1983	Positive response was observed at ≥10000 ppm ($p<0.05$).

Abbreviations: ns, not stated; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; N/A, not applicable; Cyst, cysteine; GSH, reduced glutathione; MGC, mitotic gene conversion; ERD, endoreduplication.
* Study evaluated by DPR was found to be unacceptable.

Table 35 Results of Tests for the Induction of Gene Mutation and Chromosome Damage by SOPP

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Gene Mutation	*S. typhimurium* TA100, TA98	0,1,10,100,1000 μg/plate (purity ns)	±; r	Neg.	Kojima & Hiraga, 1978	Cytotoxicity was observed at the highest dose tested.
Gene Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	0.25, 2.5, 25, 125, 250 μg/plate (purity: 72%)	±; r	Neg.	Reitz *et al.*, 1983*	Cytotoxicity was observed at 125 and 250 μg/plate.
Gene Mutation	*S. typhimurium* TA100, TA1535, TA1537, TA98	0.3, 1.0, 3.3, 10, 33, 100, 333 μg/plate (purity ns)	-	Neg.	Mortelmans *et al.*, 1986	Cytotoxicity was observed at the highest dose tested.
Gene Mutation	*E. coli* WP2	0.01, 0.1, 1, 10 mg/plate (purity ns)	-	Neg.	Kojima & Hiraga, 1978	Cytotoxicity was observed at the highest dose tested.
Gene Mutation	*A. nidulans*	0, 11, 19, 38 μM (purity ns)	-	Pos.	Georgopoulos *et al.*, 1976	Growth inhibition at the low, mid-, and high-doses were 30%, 48%, and 69%., respectively The results also were reported in Kappas & Georgopoulos (1975)
Chrom. Aberration	CHO-K1 cells	0, 47, 95, 189, 378 μM (purified)	-	Neg.	Yoshida *et al.*, 1979	Cytotoxicity was observed at ≥189 μM.
Chrom. Aberration	CHL cells	0, 114, 227, 454 μM (95% pure)	-	Neg.	Ishidate, 1988	Cytotoxicity was observed at the highest test dose.
Chrom. Aberration	JCL:ICR Mice (Males) or F344 Rats (Males); Bone Marrow	Mouse Study: 0, 300, 600, 1200 mg/kg by gavage; Rat Study: 0, 10000, 2000, 4000 ppm in diet for 13 weeks	N/A	Neg.	Yoshida *et al.*, 1979	OPP purity: ns; number of animals used in each of the experiments were not specified.

Abbreviations: ns, not stated; N/A, not applicable; Act., activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result

[a] *Salmonella typhimurium, Escherichia coli, Aspergillus nidulans*

Table 35 Results of Tests for the Induction of Gene Mutation and Chromosome Damage by SOPP (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Dominant Lethal	CD-1 Mice (Males)	0, 1250, 25000, 10000, 20000, 40000 ppm in diet for 8 weeks (purity: ns); 30 animals/dose, except for the controls in which 50 animals were used	N/A	Neg.	Ogata *et al.*, 1978a	The respective time-weighted average doses were 0, 119, 222, 446, 2125, and 4008 mg/kg/day. Each male was mated to 2 untreated females for 4 days.
Dominant Lethal	F344 Rat (Males)	0, 10000, 20000, 40000 ppm in diet for 3 months (purity: ns); 20 animals/dose, except for the controls in which 25 animals were used	N/A	Neg.	Ogata *et al.*, 1980	The respective time-weighted average doses were 0, 706, 1384, and 2487 mg/kg/day. Each male was mated to 1 untreated female for 4 days.
Micronucleus Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm in diet for 2 weeks (purity: ns); 9 animals/treatment. Other groups tested included 20000 ppm OPP plus NaCl or 20000 ppm NaCl.	N/A	Pos.	Tadi-Uppala *et al.*, 1996	Positive response was observed in all treated groups, as with the cell proliferation. Study was published as an abstract. Individual data were obtained from the investigators.
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm SOPP in diet for 15 days (purity: ns).	N/A	Pos.	Balakrishnan & Eastmond 2006	Both micronuclei formation and cell proliferation increased ($p<0.05$) over the controls.

Abbreviations: ns, not stated; N/A, not applicable; Act., activation; Pos., positive result; Neg., negative result.

Table 36 Results of Tests for the Induction of DNA damage by SOPP

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
DNA Damage	*B. Subtilis* H17A/ M45T	0.01, 0.1, 1, 10 mg/disc (purity: ns)	-	Neg.	Kojima & Hiraga, 1978	Growth inhibition was observed at ≥1 mg/disc.
UDS	F344 rats Primary Hepatocytes	0.1, 1, 10, 100, 1000, 10000 μM (70% pure)	-	Neg.	Reitz *et al.*, 1983	Cytotoxicity was observed at ≥1000 μM.
DNA Binding	CD-1 Mice (Females); Skin	0, 10, 20 mg (97% pure) topical dosing for 4 hours; 6 animals/dose.	N/A	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 500 mg/kg [^{14}C]-SOPP (72% pure) by gavage; 8 animals/dose.	N/A	Neg.	Reitz *et al.*, 1983	DNA was purified from pooled urinary bladder samples; radiolabel was detected by liquid scintillation counting.
DNA Binding	F344 Rats (Males); Urinary Bladder	20000 ppm in diet for 13 weeks (purity: ns); 6 animals/dose.	N/A	Pos.	Ushiyama *et al.*, 1992	DNA adducts were detected by ^{32}P postlabeling.
DNA Break	F344 Rats (Males); Urinary Bladder	0, 2500, 5000, 10000, 20000 ppm in diet for 3-5 months (purity: ns); 2 animals/dose.	N/A	Pos.	Morimoto *et al.*, 1989	Increased DNA breaks occurred at 10000 and 20000 ppm by alkaline elution assay.
DNA Break	ddY Mice (Males)	0, 10, 100, 1000, 2000 mg/kg (purity: ns); 4 animals/dose and sacrificed at 3 hr. An additional 2000 mg/kg/day group that was sacrificed at 24.	N/A	Pos.	Sasaki *et al*,2002	Modified COMET assay was used to detect DNA breaks in isolated nuclei from homogenized tissues (liver, kidneys, lung, and brain) and scraped mucosa (stomach, colon, and bladder).
DNA Break	F344 Rats (Males)	0 or 2000 mg/kg (>98%); gavage; 4 animals/dose/sacrifice time.	N/A	Pos.	Sekihashi *et al*, 2002	See the description above. Animals were sacrificed at 3, 8, or 24 hr after dosing.
Cell Transform	F344 Rats (Males); Urinary Bladder	1000, 5000, 10000, 20000 ppm in diet for 1 week (purity: ns); 5 animals/dose	N/A	Pos.	Honma *et al*, 1983	Positive response was observed at ≥10000 ppm ($p<0.05$).

Abbreviations: N/A, not applicable; Act., activation; -, without microsomal S9 fraction; Pos., positive result; Neg., negative result; UDS, unscheduled DNA synthesis; COMET, single cell gel electrophoresis.
[a] *Bacillus subtilis*

Table 37 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PHQ

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Gene Mutation	V79 Cells (HGPRT)	31- 250 μM (purity: ns) plus ARA	-	Neg.	Lambert, 1992	The results also were reported in Lambert and Eastmond (1994).
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 27, 54, 134 μM (-r) Study 2: 0, 27, 54, 134, 269, 537, 672, 806 μM (+r) PHQ >99% pure.	±,r	Pos.; +	Tayama *et al.*, 1989	Without metabolic activation, cell cycle delay was observed at ≥27 μM; cell division was inhibited at 134μM. With metabolic activation, positive response was observed at ≥269 μM; elevated ERD and cell cycle delay were induced dose-dependently. Cell division was inhibited at 806 μM. No PBQ was found in the incubation mixture in the presence of metabolic activation.
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 54, 134, 269 μM (-r) Study 2: 0, 54, 134, 269, 538, 1075, 2150, 3226 μM (-r) plus 10 mM Cyst/GSH Study 3: 538 μM PHQ (+r) plus 0.3-30 mM Cyst or GSH	±,r	Pos.; +	Tayama & Nakagawa, 1991	Without metabolic activation, cell cycle delay was observed at ≥54 μM; cell division was inhibited at 269μM. With GSH/Cyst added, cell division was observed at up to 2150μM. PHQ-GSH/Cyst adducts were identified in both the absence and presence of metabolic activation. PHQ >98% pure.
Micronuclei Formation	V79 cells	0, 31. 62, 93, 108, 125, 140, 156, 187 μM (+ARA) (purity: ns)	-	Pos.	Lambert, 1992	Positive response occurred at 31 μM and 125-187 μM. PHQ-induced micronuclei resulted from chromosomal loss (CREST-positive). Cell growth was reduced in a dose-dependent manner.
Micronuclei Formation	OSV cells	0, 27, 81, 269 μM	-	Neg.	Freyberger & Degen, 1998	Moderate cytotoxicity was observed at 269 μM
DNA Binding	Calf Thymus DNA	0, 100, 1000, 10000 μM (purity: ns)	-	Pos.	Grether *et al.*, 1989	Reaction was carried out at pH 7.4 and 37°C; the reaction time was 90 min. Positive response was observed at 10000 μM.

Abbreviations: ns, purity not stated; Act, activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; Cyst, cysteine; GSH, reduced glutathione; ARA, arachidonic acid; ERD, endoreduplication CREST, centromeric antinuclear antibody.

Table 37 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PHQ (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
DNA Binding	Calf Thymus DNA	40 μM (purity: ns)	-	Pos.	Ushiyama *et al.*, 1992	Reaction was carried out at pH 7.0 and 37°C; reaction time was 60 min.
DNA Binding	HL-60 cells	0-500 μM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	Reaction was carried out at 37°C; reaction time was 8 hr.
DNA Binding	Rat Liver DNA	100 μM plus COH or NADPH	+; r	Pos.	Pathak & Roy, 1992	Reaction was carried out at pH 7.5 and 37°C; reaction time was 120 min. Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Rat Liver DNA	1000 μM plus COH, ARA, or hemin plus H_2O_2	+;s	Pos.	Pathak & Roy, 1993	Reaction was carried out at pH 7.5 and 37°C; reaction time was up to 240 min. Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Herring Sperm DNA	0-50 μM (purity: ns)	-	Pos.	Gottesfeld *et al.*, 1989	PHQ inhibited deoxyribonuclease I activity. The number of moles OPP *vs.* nucleotide needed for the inhibition was 1.
DNA Binding	CD-1 Mice (Females)	5 mg (97% pure); 4 hr topical dosing	N/A	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Break	Purified DNA	Study 1: 1-200000 μM Study 2: 1000 μM plus catalase, SOD, and other scavengers including methionine and *tert*-butyl alcohol Study 3: 4000-20000 μM	-	Pos.	Nagai *et al.*, 1990	Both reactions were carried out at pH 8 and 37°C; reaction time was 30 min. pUC18 DNA was used in studies 1 and 2 whereas and ^{32}P-5'-end-labeled DNA fragment was used in study 3. PHQ > 98% pure.
DNA Break	pUC18 DNA	0, 1000, 3000μM (purity: ns) plus radical scavengers	-	Pos.	Okubo *et al.*, 2000	Reaction was carried out at pH 8 and 37°C; ; reaction time was 60 min. Moutan Cortex and Paeoniae Radix were the radical scavengers used. ESR detected PSQ and OH radicals in the incubation mixtures.

Abbreviations: ns, purity not stated; Act, activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; ESR, electron spin resonance; PSQ, PHQ-semiquinone.

Table 37 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PHQ (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
DNA Break	PUC18 DNA	0, 1000 μM (purity: ns)	-	Pos.	Nagai *et al.*, 1995	Reaction were carried out at pH 8 and 37°C; reaction time was 30 min. DNA Breaks were enhanced by Cu(II) or Fe (II)
DNA Break	DNA fragments	2, 5, 10 μM plus 20 μM Cu(II) (purity: ns)	-	Pos.	Murata *et al*,.1999	Reaction was carried out at pH 7.9 and 37°C; reaction time was 60 min. ESR detected signal of PSQ, which was enhanced by Cu(II).
DNA Break	Purified DNA	Study 1: 50-500 μM plus Cu(II) Study 2: 0.1 mM plus 10 μM Cu(II) plus catalase, SOD, and other radical scavengers including methionine and *tert*-butyl alcohol. PHQ purity: ns.	-	Pos.	Inoue *et al.*, 1990	Reaction was carried out at pH 7.9 and 37°C; reaction time was 10 min. ^{32}P-5'-end-labeled DNA fragment and 10 μM Cu(II) were used in each studies. ESR and UV detected PSQ and PBQ, respectively; these signals were enhanced by Cu(II).
DNA Break	HL-60 cells	0, 5, 10, 15, 20 μM (purity: ns)	-	Pos.	Murata *et al.*, 1999	Positive response was observed at >10 μM.
DNA Break	V79 cells	0, 25, 30, 35, 45 μM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	Positive response was observed at ≥35 μM ($p<0.05$). Reduction in cell survival was 20% at 35 μM.
DNA Break	F344 Rats (Males); Urinary Bladder	0.05% PHQ (purity: ns) in saline; intravesical injection.	N/A	Neg.	Morimoto *et al.*, 1987	DNA breaks were detected by alkaline elution assay. The exposure time was 10 min. Results also were reported in Morimoto *et al.* (1989).
Oxidative Damage	Calf Thymus DNA	Study 1: 0, 1, 10, 100, 1000, 10000 μM Study 2: 1000 μM plus radical scavengers Study 3: 100 μM plus 0.01-100 μM Cu(I) or Cu(II) and chelating agents PHQ purity: ns	-	Pos.	Nagai *et al.*, 1995	Reaction was carried out at pH 7.8 and 37°C; reaction time was 30 min. 8OHdG levels showed a dose-related increase at ≥10 μM. The radical scavengers used were catalase, sodium benzoate, sodium azide, *tert*-butyl alcohol, or mannitol. The chelating agents used were bathocupreine, o-phananthroline, and EDTA.

Abbreviations: ns, purity not stated; Act, activation; ±, with and without microsomal; Pos., positive result; Neg., negative result; N/a, not applicable; 8-OHdG, 8-hydroxydeoxyqguanine; ESR, electron spin resonance; PSQ, PHQ-semiquinone.

Table 37 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PHQ (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Oxidative Damage	Calf Thymus DNA	PHQ (purity: ns) plus Cu(II)	-	Pos.	Cai & Roy, 1999	8OHdG was the endpoint. Report was published as an abstract.
Oxidative Damage	Calf Thymus DNA	0, 5, 10, 15, 20 μM plus Cu(II) (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG formation increased ($p<0.05$) at 20μM.
Oxidative Damage	CHO-K1 cells	Study 1: 50 μM (purity: ns) Study 2: cell pretreated with AT (inhibitor of catalase) and (or) DeFe (chelating agent)	-	Pos.	Nakagawa & Tayama, 1996	Reaction was carried out at pH 7.4 and 37°C; reaction time was 30-120 min.
Oxidative Damage	HL-60 cells	0, 5, 10, 15, 20 μM (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG increased ($p<0.05$) at 20μM.
Oxidative Damage	V79 cells	0, 5, 20 μM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	8-OHdG increased ($p<0.05$) at 20μM.
SCE	CHO-K1 cells	Study 1: 0, 27, 54, 134 μM (-r) Study 2: 0, 27, 54, 134, 269, 403, 538, 672, 806 μM (+r) PHQ >99% pure	±; r	Pos.	Tayama *et al.*, 1989	In the absence and presence of metabolic activation, the respective doses for the positive response were ≥27 and ≥134 μM (both at $p<0.05$); the respective doses for the inhibition of cell division were 134 and 806 μM.
SCE	CHO-K1 cells	Study 1: 0, 27, 54, 134 μM (-r) Study 2: 0, 54, 134, 269, 538, 1075, 2150, 3226 μM (-r) plus 10 mM Cyst/GSH Study 3: 538 μM (+r) plus Cyst or GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Unchanged PHQ and PHQ-Cyst/GSH adducts were identified in the cell culture media without and with metabolic activation. PHQ >99% pure
SCE	CHO-K1 cells	Study 1: 54 μM plus radical scavengers Study 2: 14 μM at pH 7.3, 7.6, or 8.0 plus SOD, catalase, or both.	-	Pos.	Tayama & Nakagawa, 1994	Radical scavengers used were catalase, ascorbate, GSH, SOD, Mannitol, SOD and catalase, or AT. PHQ >98% pure.

Abbreviations: ns, purity not stated; Act, activation; ±, with and without microsomal; Pos., positive result; Cyst, cysteine; GSH, reduced glutathione; SOD, superoxide dismutase; 8-OHdG, 8-hydroxydeoxyqguanine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyquanosine; AT, 3-amino-1,2,4-triazole.

Table 38 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PBQ

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Gene Mutation	V-79 cells (HGPRT)	6, 12, 25 μM (purity: ns)	-	Neg.	Lambert, 1992	Cell survival was 40% at 6 μM.
Gene Mutation	AHH-1 cells (HGPRT)	0, 2.5, 5, 10, 25 μM (purity: ns)	-	Neg.	Reid *et al.*, 1998	Cell survival was 30% at 10 μM.
Micronuclei Formation	V-79 cells	0, 6, 12, 25, 37, 50 μM (purity: ns)	-	Neg.	Lambert & Eastmond, 1994	Cell survival reduced dose-dependently at ≥6 μM.
Chrom. Aberrations	CHL cells	0, 59, 118, 236 μM (purity: ns)	-	Neg.	Ishidate, 1988	Information on cytotoxicity was not given.
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 7, 14, 27, 54 μM (-r); Study 2: 27-543 μM (-r) plus Cyst Study 3: 27-2174 μM (-r) plus GSH Study 4: 0, 27, 54, 136, 272 μM (+r) Study 5: 272 μM (+r) plus Cyst/GSH PBQ was >98% pure.	±; r	Pos.	Tayama & Nakagawa, 1991	Positive responses were observed at 27 μM (-r) and at 54 μM (+r) (both at $p<0.05$); ERD increased at 14 μM (-r) and 136 μM (+r). Cell cycle delay increased at 27 μM (-r) and 136 (+r) μM. Cyst and GSH were 10 mM each.
DNA Binding	Liver DNA (Rats)	3.4 μM (purity: ns)	-	Pos.	Pathak & Roy, 1992 a	Four major and other minor adducts of DNA were detected by ^{32}P-postlabaling.
DNA Binding	Calf Thymus DNA	2470 μM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	
DNA Binding	Calf Thymus DNA	40000 μM (purity: ns)	-	Pos.	Ushiyama *et al.*, 1992	
DNA Binding	Calf Thymus DNA	16300 μM (purity: ns)	-	Pos.	Zhao *et al.*, 2002	PBQ-^{2}N-dG was the major adduct.
DNA Binding	HL-60 cells	25-250 μM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	DNA adducts increased at ≥25 μM.
DNA Binding	HepG2 Hepatoma	0, 6.25, 12.5, 25, 50 μM (purity: ns)	-	Pos.	Zhao *et al.*, 2002	Positive response was observed at 50 μM.

Abbreviations: Act, activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction; Pos., positive result; Neg., negative result; GSH, reduced glutathione; Cyst, cysteine.

Table 38 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PBQ (Continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
DNA Break	Plasmid pbcNI DNA	100 μM (purity: ns) plus Cu(II) and H_2O_2	-	Pos.	Inoue *et al.*, 1990	Reaction time was 10 min. DNA breaks were detected by gel electrophoresis.
DNA Break	E. coli pUC18 DNA	0, 4, 40 μM (> 99% pure) plus NADPH & NADH	-	Pos.	Nagai *et al.*, 1990	Reaction time was 30 min. DNA breaks were detected by gel electrophoresis.
DNA Break	DNA Fragments	2, 5, 10 μM (purity: ns) plus Cu(II) and NADH	-	Pos.	Murata *et al.*, 1999	Reaction time was 60 min. DNA breaks were detected by gel electrophoresis.
DNA Break	HL-60 cells	0, 5, 10, 15, 20 μM (purity: ns)	-	Pos.	Murata *et al.*, 1999	DNA breaks were detected by pulse gel electrophoresis. Positive response was observed at ≥10 μM.
DNA Break	V79 cells	0, 20, 25, 30 μM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	DNA breaks were detected by alkaline elution assay. Reductions in cell survival were 10%, 25%, and 40% at the low, mid, and high doses.
DNA Break	F344 Rats Urinary Bladder	Males: 0.0005%, 0.005%, 0.05%, or 0.1% PBQ Females: 0.05% or 0.1% PBQ solutions (in saline) Intravesical injection.	N/A	Pos.	Morimoto *et al.*, 1989	Exposure time was 10 min. DNA breaks were detected by alkaline elution assay. Results also were reported in Morimoto *et al.* (1987).
DNA Break	F344 Rats (Males) Forestomach	0, 0.001%, 0.1% solution (> 99% pure); gavage (corn oil)	N/A	Pos.	Morimoto *et al.*, 1991	Exposure time was 3 hr. DNA breaks were detected by alkaline elution assay.
Oxidative Damage	Calf thymus DNA	0, 1, 1000, 10000 μM (purity: ns)	-	Pos.	Nagai *et al.*, 1995	The reaction time was 30 min. 8OHdG increased at ≥1000 μM.

Abbreviations: Act, activation; ±, with and without microsomal S9 fraction; N/A, not applicable; Pos., positive result; 8-OHdG, 8-hydroxydeoxyqguanine.

Table 38 Results of Tests for the Induction of Gene Mutation, Chromosome Damage, and DNA Damage by PBQ (continued)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Oxidative Damage	Calf Thymus DNA	0, 5, 10, 15, 20 μM plus NADH	-	Pos.	Murata *et al.*, 1999	The reaction time was 60 min. 8-oxodG was induced dose-dependently.
Oxidative Damage	Calf Thymus DNA	Ns	-	Pos.	Cai & Roy, 1999	Article was published as an abstract.
Oxidative Damage	HL-60 cells	0, 5, 10, 15, 20 μM (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG increased at 20 μM ($p<0.05$).
Oxidative Damage	V79 cells	0, 5, 20 μM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	8OHdG increased at 20 μM ($p<0.05$). Cell viability was reduced by 10% at this dose.
SCE	CHO-K1 cells	Study 1: 0, 7, 14, 27, 54 μM (-r) Study 2: 27-543 μM (-r) plus Cyst Study 3: 27-2174 μM (-r) plus GSH Study 4: 0, 27, 54, 136, 272 μM (+r) Study 5: 272 μM (+r) plus Cyst/GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Positive responses were observed at 27 μM (-r) and at 54 μM (+r) (both at $p<0.05$). Cell cycle delay increased at 27 μM (-r) and 136 (+r) μM. Cyst and GSH were 10 mM. PBQ was >98% pure.
Cell Transform	BALB/3T3 cells	2.2, 2.7, 3.3,3.8 μM (>99% pure)	-	Pos.	Sakai *et al.*, 1995	Positive response observed at ≥3.3 μM ($p<0.05$).

Abbreviations: Act, activation; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction ; Pos., positive result; GSH, reduced glutathione; Cyst, cysteine; 8-OHdG, 8-hydroxydeoxyqguanine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyquanosine.

III.F. REPRODUCTIVE TOXICITY

Summary: Results of two reproductive toxicity studies involving two generations with two litters per generation are available for OPP. One study did not have adequate information for assessing possible effects on fertility and mating. In the second study, OPP did not negatively affect reproductive functions. In both studies, the same effect occurred in pups: reduced body weight on lactation days 14 and (or) 21.

Parental effects included the following: reduced body weight; nonneoplastic lesions in the kidneys (chronic active inflammation and renal pelvis debris) and ureters (dilatation and hyperplasia); nonneoplastic, preneoplastic, and neoplastic lesions in the urinary bladder (simple hyperplasia, P/N hyperplasia, and carcinoma); and ovarian cysts.

Oral – Rat

Two reproductive-toxicity studies are on file at DPR. The Registrant submitted the first study to DPR twice as Eigenberg (1989a) and as Eigenberg (1989b). For reasons of brevity, this document discusses mainly the second report. Despite its revised reporting, DPR considered that the study was not in compliance with the FIFRA guidelines. As a result, the Registrant conducted a second study (Eigenberg and Lake, 1995).

Eigenberg (1989b)

Four groups of Sprague-Dawley (SD) rats (35 animals/sex/dose) received diets containing OPP at nominal doses of 0, 40, 140, or 490 mg/kg/day for two generations. The exposure to OPP in F0 rats occurred for 15 and ~31 weeks before their 1st and 2nd matings, respectively, and for a total of ~43 weeks before sacrifice. The exposure to OPP in F1 rats (F1b offspring of F0 parents) occurred for 10 and ~22 weeks before their 1st and 2nd matings, respectively; they were 34-40 weeks old when sacrificed.

Number of parental animals dead or sacrificed due to their moribund state was nineteen (12 F0 animals [6/sex] and 7 F1 animals [5 males, 2 females]). The causes of eleven male unscheduled deaths were incisors and lower jaw problems (6 animals), weight loss (1 animal), problems related to severe nephropathy and hemorrhaging into the abdominal cavity from an endothelioma (1 animal), disseminated lymphoma (1 animal), and undetermined reason (2 animals). Among the 8 female unscheduled deaths, four occurred on their respective gestation days (GD) 22 or 24 and, therefore, appeared to be related to birthing problems. Other causes for female unscheduled departures were incisor problems, a front-leg mass (fibrous histiocytoma), problems related to bloody inguinal discharge (urinary bladder and kidney lesions), and undetermined reason. Regarding the female unscheduled departures occurring on GD 22 or 24, two of them pertained to control dams during the F1a mating trial. Other findings with the F0 control dams included the following. In the F1a trial, 7% (23/330) of the pups were dead when delivered and 6% (20/330) died within the first four days after delivery. One control dam had an

F1a litter consisting of a single stillbirth. In the F1b mating trial, only a third of the control dams proved to be fertile and one of the 8 that did get pregnant delivered only "placental buttons." Consistent with the former, the control dams exhibited a reduced number of estrus cycles before the F1b mating trial: a group of 10 control dams had a combined total of only 10 cycles over a two-week period instead of the expected 23-28 cycles. Collectively, the loss of 19 parental animals in what amounted to a subchronic study design and the observations made in the F0 control dams would suggest that there were problems with the test animals used in this study.

Both sexes of F0 animals dosed at 490 mg/kg/day showed reduced ($p<0.05$) body weight, starting at test weeks 7-8 and continuing until the end of the F0 portion of the study. At the end of their first premating period (week 15), the body weight reductions ($p<0.05$) of males and females at 490 mg/kg/day were 8% and 7%, respectively; and at the F0 terminal sacrifice (weeks 43-44), their reductions were 6% and 12%, respectively. At the start of their first premating period (week 43), F1 males in the 40 and 490 mg/kg/day groups exhibited body weight reductions (by 13% and 12%, respectively, both at $p<0.05$) whereas the four F1 female groups showed comparable body weights. In the case of the high-dose F1 males, their body weights stayed reduced by 11-13% ($p<0.05$) throughout the F1 portion of the study, including at the F1 terminal sacrifice. Therefore, outside of the body-weight reduction evident at the start of the F1 portion of the study, consumption of up to 490 mg/kg/day in the F1 portion of the study did not significantly affect the males. In the case of the high-dose F1 females, by the end of their first premating period (test week 52), their body weight was 11% ($p<0.05$) less than the controls; however, by the F1 terminal sacrifice (test weeks 74-77), their body weight still was only10% ($p<0.05$) less.

Parental animals exhibited treatment-related effects in urinary bladder. Unlike the chronic and subchronic studies discussed previously in this RCD, the investigators graded the transitional-cell hyperplasia on a scale of 0 (no hyperplasia) to 5 (severe hyperplasia) instead of expressing the hyperplasia as simple *versus* P/N hyperplasia. The investigators also characterized the hyperplasia using two morphometric measurements: the number of cells across the epithelial layer and the thickness of the epithelial layer. Consistent with the increased ($p<0.05$) incidences of hyperplasia, the male (F0 and F1) and female (F0) groups at 490 mg/kg/day exhibited increases ($p<0.05$) in the number of cells/layer and (or) thickness of the transitional epithelium (Table 39). Because the F0 male and females at 140 mg/kg/day also had one or both of these morphometric measurements increased significantly ($p<0.05$), the investigators identified the mid dose as the LOEL. Also, it can be noted that in the first submission (Eigenberg, 1989a), the investigators reported that four F0 animals exhibited transitional-cell carcinoma in the urinary bladder (one 140 mg/kg/day female, one 490 mg/kg/day female, and one 490 mg/kg/day male) or in an ureter (one 140 mg/kg/day female). However, in the second submission (Eigenberg, 1989b), the investigators reported these lesions as papillomatosis in the case of the females and as multiple, transitional-cell papillomas in the male. Given that urinary-bladder tumors (carcinomas and papillomas) are rarely observed in untreated SD rats (Lang, 1992), especially in rats that are less than a year old, DPR considered these lesions to be possibly treatment-related.

Table 39 Effects of OPP on Urinary Bladder Epithelium in the First Two-Generation Reproductive Study of OPP Using Sprague-Dawley Rats (Eigenberg, 1989b)

Parameters	mg/kg/day			
	0	40	140	490
F0 Males				
Mean Number of Cells/Layer[a]	1.91	1.89	2.32	3.46*
Mean Thickness (μm)[a]	23.6	26.1	34.9*	57.1*
Transitional Cell Hyperplasia[b]	3/35 (9%)	0/35 (0%)	4/35 (11%)	23/35 (66%)*
F0 Females				
Mean Number of Cells/Layer[a]	2.37	2.29	3.06[c,*]	3.14*
Mean Thickness (μm)[a]	33.6	33.6	50.7[c,*]	50.4*
Transitional Cell Hyperplasia[b]	1/35 (3%)	0/35 (0%)	5/35 (14%)	9/35 (26%)*
F1 Males				
Mean Number of Cells/Layer[a]	2.33	2.11	2.37	2.63
Mean Thickness (μm)[a]	23.1	24.3	31.1	37.5*
Transitional Cell Hyperplasia[b]	1/27 (4%)	1/35 (3%)	4/35 (11%)	15/35 (43%)*
F1 Females				
Mean Number of Cells/Layer[a]	3.21	2.74	2.36**	2.88
Mean Thickness (μm)[a]	42.7	39.6	35.2	38.6
Transitional Cell Hyperplasia[b]	6/29 (21%)	4/35 (11%)	1/34 (3%)	6/33 (18%)

[a] Microscopically, the number of cells across the transitional epithelial layer were counted and the thickness of the transitional epithelium was measured with a micrometer under 10x magnification for each animal.

[b] Transitional-cell hyperplasia was graded from 0 (no lesion) to 5 (severe). The incidences of animals with lesions of grade 1 or higher are presented in this row.

[c] The data for a female with a lesion in the urinary bladder described as papillomatosis were not included in the calculations.

[*,**] Significantly different from the negative-controls at $p<0.05$ and $p<0.01$, respectively, as reported by the investigators.

Other organs that OPP may have affected were kidneys and ovaries. In males, the kidney effects occurred exclusively in the F0 males and only at the high dose (i.e., 490 mg/kg/day). At histology, increased incidences ($p<0.05$) of kidney lesions were (incidences in parentheses): renal pelvis hyperplasia (51%), renal calculi (37%), and pyelonephritis (14%, not statistically significant). At necropsy, 20% had renal calculi and 17% had hemorrhage (both at $p<0.05$). Also, in the F0 males, absolute kidney weight did not change significantly at 490 mg/kg/day despite the reduction of body weight; however, there was a significant ($p<0.05$) increase in the relative weight (7%). In females, the incidences of ovaries with cysts were 0%, 9%, 9%, and 14% ($p<0.05$) in the F0 groups exposed to 0, 40, 140, 490 mg/kg/day, respectively. The cysts observed in the 490 mg/kg/day F0 dams measured 2-8 mm, and four of the five dams with cysts had ovary absolute weights elevated. In the F1 dams, the respective incidences of ovaries with cysts were 3%, 0%, 11%, and 0%. The F0 dams were about 1 year old at sacrifice while the F1 dams were ≤40 weeks old. The F0 dams had OPP exposure for a longer duration. These F0 dams also had the oviduct and fatty tissue trimmed away, but not in the case of the F1 dams. The trimming used with F0 ovaries may have made it easier to recognize cysts at necropsy, which, in turn, precipitated the histological examination. Any cysts that did exist in the F1 ovaries would be expected to be smaller in size due to the younger age of the F1 dams and (or) the shorter duration of OPP exposure.

As discussed previously, F0 control animals exhibited problems in procreating. Upon inspecting the individual mating data, it became apparent that the cohabiting of animals during the mating periods was irregular and incomplete. As a result, DPR considered that the assessments on fertility in the study were inconclusive.

Pups also exhibited treatment-related effects. The main effect observed in the study was reduced pup weights during lactation (Table 40). The investigators speculated that the reduced pup weights at 490 mg/kg/day were the result of the pups "overdosing" by eating the feed. In Table 40, the reduced pup weights observed on lactation day 14 represent the combined effects of *in utero* exposure to OPP and (possibly) exposure *via* breast milk. On lactation day 21, there would be the added exposure due to the pups consuming feed containing OPP.

Another effect exhibited by the pups was kidney dilation. F2a pups in all groups receiving OPP had elevated incidences of kidney dilation (4/25, 12/31, 11/28, and 15/31 for the control, low-, mid-, and high-dose groups, respectively). Similarly, F2b pups of the 40 and 140 mg/kg/day groups also exhibited the effect (2/20, 12/24, 7/18, and 5/24 for the control, low-, mid-, and high-dose groups, respectively). However, since the investigators found no such pup kidney effect in the second reproductive study (Eigenberg and Lake, 1995), it is questionable whether the finding in the first study was treatment related.

In conclusion, this study documented that OPP affected both the pups and parents and that the pup effects occurred at doses higher than those causing the parental toxicity. Based on the morphometric data for the transitional epithelium in the urinary bladder, the systemic parental NOEL was 40 mg/kg/day. The pup NOEL was 140 mg/kg/day, based on reduced pup weights. Because of the deficiencies in this study relating to the assessment of reproductive

Table 40 Pup Body Weights (grams)[a] in the First Two-Generation Reproductive Study of OPP Using Sprague-Dawley Rats (Eigenberg, 1989b)

Mating Trial/ Lactation Days	mg/kg/day			
	0	40	140	490
F1a				
0	6.3	6.4 (102%)[b]	6.5 (103%)	6.2 (98%)
7	14.9	15.0 (101%)	15.2 (102%)	13.9 (93%)
F1b				
0	5.7	6.2 (109%)	6.4 (112%)	5.7 (100%)
7	15.6	16.6 (106%)	16.3 (104%)	14.3 (92%)
14	31.0	32.7 (105%)	31.0 (100%)	27.3 (88%)*
21	49.1	50.4 (103%)	48.4 (99%)	40.1 (82%)*
F2a				
0	6.6	6.7 (102%)	6.9 (105%)	6.6 (100%)
7	15.8	16.0 (101%)	16.1 (102%)	15.3 (97%)
14	32.4	32.7 (101%)	32.2 (99%)	29.7 (92%)*
21	49.8	51.1 (103%)	50.7 (102%)	44.3 (89%)*
F2b				
0	6.7	7.0 (104%)	6.8 (101%)	6.7 (100%)
7	15.3	16.3 (106%)	16.1 (105%)	15.4 (101%)
14	31.2	32.3 (104%)	32.2 (103%)	30.0 (96%)
21	50.5	50.1 (99%)	51.3 (102%)	45.2 (90%)*

Note: Shading identifies the only time in the study when adult animals were underdosed. The investigators stated that, in the second and third weeks of the F1a lactation period (days 7-14 and 14-21, respectively), the females received OPP at 50% and 33%, respectively, of the indicated levels to avoid overdosing the neonates. After that, however, the investigators considered the adjustment was unnecessary.

[a] The body weight data of both sexes combined.

[b] The value as a percent of the controls is given in parentheses.

* Significantly different from the controls at $p<0.05$, as reported by the investigators.

performance, DPR derived no reproductive NOEL and considered this study unacceptable.

Eigenberg and Lake (1995)

Four groups of SD rats (30 animals/sex/dose) received diets containing nominal doses of OPP at 0, 20, 100, or 500 mg/kg/day. The exposures to OPP in F0 rats occurred for 10 and ~21 weeks before the 1st and 2nd matings, respectively, and for a total of 25-30 weeks before sacrifice. The exposure to OPP in F1 rats (F1b offspring of F0 parents) occurred for 12 and ~22 weeks before their 1st and 2nd matings, respectively; they were 34-37 weeks old when sacrificed. It should be noted that the selection of F1b weanlings to become F1 adults involved two groups: 30/sex/dose group plus 2/sex/dose group. The investigators did not provide reason for the latter; possibly, these animals served as some sort of replacement group until their sacrifice occurred at about three weeks after the start of the F2a mating phase.

Both sexes of F0 animals dosed at 500 mg/kg/day showed reduced ($p<0.05$) body weight, starting after three weeks of treatment in the females and 10 weeks in the males. At 500 mg/kg/day, F1 animals exhibited reduced body weight as weanlings and premating period. In the F1 males, the body weight stayed reduced by 10-11% ($p<0.05$) throughout the F1 portion of the study, including at the F1 terminal sacrifice. In the F1 females, by the end of their first premating period, the reduction in body weight was 9% ($p<0.05$); however, by the F1 terminal sacrifice, the reduction in body weight was only 4% (statistically not significant). The only treatment-related clinical observation in adults was an increase of urine stain in the 500 mg/kg/day male groups (F0 and F1). Urine staining tended to start at study week 18 and to last until termination.

Parental animals exhibited treatment-related effects in urinary bladder. Increased ($p<0.001$) incidences of simple hyperplasia occurred in the F0 and F1 males at 500 mg/kg/day (73% and 93% incidences, respectively). Of the animals with simple hyperplasia, 15 F0 males (50% incidence) and 18 F1 males (60% incidence) also exhibited P/N hyperplasia. About half of the 500 mg/kg/day males exhibiting simple and (or) P/N hyperplasia also had chronic inflammation in the urinary bladder. At 500 mg/kg/day, OPP also appeared to affect the kidneys and ureters in the F0 and F1 males. The effects identified were dilatation and hyperplasia of the ureters, chronic active inflammation in the kidneys, and debris in the renal pelvis. Although the incidence of each of these effects by itself was not statistically significant, when viewing these lesions altogether in each organ, it would suggest a treatment-related effect on the kidneys and ureters. No other males in this study, including the controls, exhibited lesions in the kidney and ureters.

Regarding the reproductive effect of OPP in F0 and F1 dams at 500 mg/kg/day, an increased ($p<0.05$) fertility index (number of pregnant females/number of sperm positive and (or) pregnant females that were not sperm positive) occurred in F1 dams for breeding F2a and F2b litters (by 53% and 43%, respectively). However, the treatment did not affect other mating and gestation indexes in this and other dose groups. OPP also appeared to have no effects on

other measures of reproductive functions (i.e., estrous cycle length and periodicity, sex ratio, live birth index, viability index, and lactation index) and performances (i.e., gestation length, mating index, and gestation index). In addition, there was no evidence of pathological changes in reproductive organs (ovaries and testes) at any dose level.

OPP also affected the pups. Pups from the F0 and F1 dams at 500 mg/kg/day had reduced ($p<0.0.5$) body weights in each of the four mating trials on lactation day 21, with lesser reductions being present on lactation day 14, which were also statistically significant in the F2a and F2b mating trials (Table 41). In each of these periods, the increased ($p<0.05$) incidence of lactation day-21 weanlings with stunted growth occurred at 500 mg/kg/day and smaller increase occurred at 100 mg/kg/day (Table 42).

In conclusion, this study documented that OPP affected both the pups and parents and that the pup effects occurred at the same doses as those causing parental toxicity. The parental NOEL was 100 mg/kg/day, based on decreased body weights (F0 and F1 dams and F1 males), increased incidences of urinary bladder chronic inflammation and simple hyperplasia, increased incidences of renal chronic active inflammation and debris, and increased incidences of ureter dilation and hyperplasia of F0 and F1 males at 500 mg/kg/day. The pup NOEL also was 100 mg/kg/day, based on decreased body weights and stunting in the F1 and F2 pups. The reproductive NOEL was >500 mg/kg/day. DPR considered this study acceptable for filling the SB950 reproductive and fertility effects requirement based on FIFRA guidelines. The USEPA established the same parental and reproductive NOELs based on the noted effects (USEPA, 2006).

Table 41 Pup Body Weights (grams)[a] in the Second Two-Generation Reproductive Study of OPP Using Sprague-Dawley Rats (Eigenberg and Lake, 1995)

Mating Trial/ Lactation Days	mg/kg/day			
	0	20	100	500
F1a				
0	6.7	6.5 (97%)[b]	6.6 (99%)	6.6 (99%)
7	17.8	17.7 (99%)	17.6 (99%)	16.9 (95%)
14	34.5	34.7 (101%)	34.2 (99%)	32.6 (94%)
21	54.6	55.5 (98%)	54.9 (101%)	47.8 (88%)**
F1b				
0	6.5	6.6 (102%)	6.7 (103%)	6.7 (103%)
7	15.9	16.5 (104%)	15.5 (97%)	16.1 (101%)
14	31.7	32.4 (102%)	31.1 (98%)	30.8 (97%)
21	50.1	51.1 (102%)	49.6 (99%)	45.0 (90%)**
F2a				
0	6.3	6.6 (105%)	6.5 (103%)	6.5 (103%)
7	16.7	17.2 (103%)	17.0 (102%)	16.5 (99%)
14	33.0	33.8 (102%)	32.7 (99%)	31.1 (94%)*
21	50.8	52.4 (103%)	51.6 (102%)	45.4 (89%)**
F2b				
0	6.5	6.7 (103%)	6.5 (100%)	6.4 (98%)
7	16.6	17.7 (107%)	16.5 (99%)	15.9 (96%)
14	33.0	34.3 (104%)	33.2 (101%)	30.7 (93%)*
21	53.1	54.7 (103%)	52.2 (98%)	46.7 (88%)**

[a] The body weight data of both sexes combined.
[b] The value as a percent of the negative controls is given in parentheses.
*,** Significantly different from the negative controls at $p<0.05$ and $p<0.01$, respectively, as reported by the investigators.

Table 42 Distribution of Stunted Pups on Lactation Day 21 in the Second Two-Generation Reproductive Study of OPP Using Sprague-Dawley Rats (Eigenberg and Lake, 1995)

Mating Trial/ Parameters	mg/kg/day			
	0	20	100	500
F1a				
No. of Stunted Pups[a]	4	2	11	16
Litter Incidence[b]	2/25 (8%)	2/20 (10%)	4/22 (18%)	8/26 (31%)[#]
F1b				
No. of Stunted Pups[a]	11	5	13	33
Litter Incidence[b]	4/25 (16%)	3/20 (15%)	5/20 (25%)	9/24 (38%)
F2a				
No. of Stunted Pups[a]	3	0	0	18
Litter Incidence[b]	2/15 (13%)	0/17 (0%)	0/20 (0%)	10/23 (44%)[#]
F2b				
No. of Stunted Pups[a]	2	0	2	13
Litter Incidence[b]	2/18 (11%)	0/19 (0%)	2/22 (9%)	6/28 (21%)
Total Number of Stunted Pups Over the Four Mating Trials	20	7	26	80

[a] DPR defined stunted pups as body weight of ≤40 g at lactation day 21. The cutoff value of 40 g was selected because it was the approximate weight of two weanlings on lactation day 21 that the Registrant identified as being "obviously stunted" in comparison to a normal-size weanling appeared in the photograph submitted to DPR (Eigenberg and Lake, 1998).

[b] Litter incidence is presented as the number of affected litters per the total number of litters at that dose level.

[#] Fisher exact test, as calculated by DPR; significant at $p \leq 0.05$.

III.G. DEVELOPMENTAL TOXICITY

Summary : Results of prenatal developmental-toxicity testing are available in the rat, rabbit, and mouse. In rats, the effects were decreased fetal body weight and an increased incidence of resorptions. In rabbits, there was an association between exposure to OPP and an increased incidence of resorptions in the range-finding and full studies. Fetuses from mice exposed to SOPP exhibited reduced body weight and an increased incidence of cleft palate. In these studies, maternal toxicity was either not observed (rabbits) or appeared to be minimal (mice) at the lowest dose whereat their fetuses exhibited some developmental effects.

III.G.1. *ortho*-Phenylphenol

III.G.1.a. Gavage – Rat

Two developmental-toxicity studies are on file at DPR: Kaneda *et al.* (1978) and John *et al.* (1978). A version of the latter also appeared in print (John *et al.*, 1981); however, this document discusses the unpublished report because it was more comprehensive in its reporting of the data.

Kaneda *et al.* (1978)

Five groups of pregnant Wistar rats (18-20 dams/dose, except only 11 dams in the highest dose group) were treated by gavage at 0 (aqueous gum arabic), 150, 300, 600, or 1200 mg/kg/day OPP (99.7% pure) on gestation days (GD) 6 through 15 and sacrificed on GD 20. At 1200 mg/kg/day, 10 females died 3-9 days after initiation of the dosing. It should be noted that the median lethal dose (LD_{50}) of OPP in rats is ~2500 mg/kg (see III.B. ACUTE TOXICITY). Hence, the high mortality observed may have been due to the toxic effects of OPP.

Maternal effects occurred in OPP-treated dams, starting at 300 mg/kg/day, but the report contained insufficient information regarding most of the observations. The investigators stated that dams dosed at ≥300 mg/kg/day had ataxia that lasted several hours and whose severity increased with dose. Neither deaths nor clinical signs occurred at 150 mg/kg/day whereas all but one of the dams died at 1200 mg/kg/day. However, the report gave no information about whether deaths occurred at 600 or 300 mg/kg/day. The investigators reported body-weight gain data for GD 6, 9, 12, 15, and 20 but not the intervals covered by the data; based on the data presented, dams dosed at ≥300 mg/kg/day exhibited reduced body-weight gain that became evident starting on GD 9.

OPP also affected the fetuses. Dams in the 600 mg/kg/day group exhibited an increased incidence of resorptions and reduced fetal body weights (both sexes) (Table 43). Although the investigators identified both changes as being statistically significant ($p<0.01$), it would appear that fetus (and not the litter) was the experimental unit for the statistical analysis of resorptions.

Table 43 Maternal and Fetal Effects Observed in a Developmental-Toxicity Study of OPP using Wistar Rats (Kaneda *et al.*, 1978)

Parameters	mg/kg/day			
	0	150	300	600
Maternal Effects				
Number Pregnant	20	20	20	18
Maternal Body-Weight Gain (gram)[a]				
GD 6	21	23 (110%)	22 (105%)	19 (90%)
GD 9	30	31 (103%)	25 (83%)*	12 (40%)***
GD 12	45	42 (93%)	37 (82%)**	22 (49%)***
GD 15	61	56 (92%)	44 (72%)***	23 (38%)***
GD 20	121	111 (92%)	97 (80%)***	65 (54%)***
Fetal Effects				
Number Examined				
Fetuses	230	230	237	188
Litters	20	20	20	18
Fetal Body Weight (gram)[a,b]				
Males	4.11	4.12 (100%)	4.04 (98%)	3.87 (94%)**
Females	3.87	3.78 (98%)	3.71 (96%)	3.55 (92%)**
Resorptions	13.9%	13.9%	15.4%	25.7%

[a] The value as a percent of the negative controls is given in parentheses.
[b] Fetal body weight measured on GD 20.
[d] The type of resorptions (early *vs.* late) were not addressed.
*,**,*** Significantly different from the negative controls at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, as reported by the investigators.

In conclusion, this study documented OPP affected both the fetuses and dams and that the fetal effects occurred at doses higher than those causing maternal toxicity. The maternal NOEL was 150 mg/kg/day based on decreased body-weight gain and the appearance of ataxia at 300 mg/kg/day. The developmental NOEL was 300 mg/kg/day based on reduced fetal body weights (and possibly an increased incidence of resorptions) at 600 mg/kg/day. DPR considered this study unacceptable because of inadequate reporting, including the lack of individual data.

John *et al.* (1978)

Four groups of pregnant Sprague-Dawley (SD) rats (24-26 dams/dose; except for the negative controls which had 36 animals) were dosed by gavage at 0 (cottonseed oil), 100, 300, or 700 mg/kg OPP (99.69 % pure) on GD 6 through 15 and sacrificed on GD 21. The investigators chose the dose levels based on the results of a range-finding study. In the range-finding study, sperm-positive dams (5-6 dams/group [John *et al*., 1981]) were dosed by gavage at 0, 250, 400, 800, 1200, or 2000 mg/kg during gestation. Deaths occurred at 2000 mg/kg; however, the investigators neither addressed the number, time, and cause of deaths nor whether the treatment affected the fetuses. Dams exposed to 800 or 1200 mg/kg/day exhibited gastric irritation and changes in maternal body weight, food consumption, and water consumption. On this basis, the investigators assumed that 700 mg/kg/day would constitute the maximum tolerated dose for testing in the full study.

Six pregnant dams left the full study for the following reasons. Erroneous dosing occurred in two control dams; two high-dose dams delivered litters on GD21 before sacrifice; one high-dose dam died from a gavaging error; and one high-dose dam died from not being able to obtain water. The report did not provide individual data for these 6 dams, maternal as well as fetal. Otherwise, there were no indications of any clinical findings associated with exposure to OPP.

Maternal effects occurred almost exclusively at 700 mg/kg/day and were slight in nature. Reduced ($p<0.05$) maternal body weight occurred on GD 10 and 16 (by 6%). However, some of the reduction may not have been due to treatment since a slight reduction (3%) occurred also on GD 6, before OPP exposure was initiated. Other evidence indicating a maternal effect in the high-dose dams included reductions ($p<0.05$) in body-weight gain on GD 6-9 (by 36%) and feed consumption on GD 9-11 (by 9%) and increased ($p<0.05$) water intake on GD 12-14 and 15-17 (by 26% and 16%, respectively). Increased ($p<0.05$) water intake also occurred on GD 12-14 in the 300 mg/kg/day group (by 17%). Although reduced ($p<0.05$) absolute liver weight occurred at 700 mg/kg/day, the relative weight reduction was not significant.

There were no significant effects produced in the fetuses. OPP had no significant effects on fetal body weight or crown-rump length. There were no treatment-related malformations observed in the external examination or in the soft-tissue examination; however, the latter only involved about 35% of the fetuses in each treatment group, as opposed to the ≥50%

recommended in the FIFRA guideline for this study type. The authors of the study identified three skeletal anomalies as being possibly elevated in incidence at the high dose: delayed ossification of sternebrae, pinpoint holes in the occipital or interparietal plates in the skull; and skull bone-island. For each endpoint, the incidence of affected litters had increased from 13-16% in the controls to 30% in the 700 mg/kg/day group. However, when DPR analyzed these data based on the proportion of affected fetuses per litter (Haseman and Peigorsch, 1994), the increases were not statistically significant. The developmental NOEL was 700 mg/kg/day and the maternal NOEL was 300 mg/kg/day based on reduced body weight and feed consumption at 700 mg/kg/day. DPR considered this study acceptable for fulfilling the developmental toxicity data requirement based on FIFRA guideline. The USEPA established a developmental NOEL of 700 mg/kg/day; however, the maternal NOEL was 100 mg/kg/day based on the decreased body weight grains, food consumption, and food efficiency at 300 mg/kg/day (USEPA, 2006).

III.G.1.b. Gavage – Rabbit

Zablotny *et al.* (1991a)

This was a range-finding (i.e., probe) study wherein 7 inseminated females/group were dosed by gavage once daily at 0 (corn oil), 250, 500, or 750 mg/kg/day on GD 7-19 with sacrifice on GD 20 (as opposed to GD 28 in the full study [Zablotny *et al.*, 1991b]). In the 750 mg/kg/day group, three animals died on GD 10: two of these had aspirated dosing solution on GD 8; and one animal died on GD 11 with a hemorrhagic focus in the gastric mucosa. One animal died on GD 14; it exhibited noisy respiration on GD 12-13 and, at necropsy, erosion sites were present in the stomach. The final unscheduled death occurred on GD 18; at that time, there was blood in the pan. This animal also had a hemorrhagic site at the lower sphincter of stomach, hemolyzed blood in the small and large intestines, and blood in the vaginal wall at the site of a hematoma. The only 750 mg/kg/day animal to survive till schedule sacrifice exhibited blood in the pan on GD 17-18. The stomach had several hemorrhagic sites and its kidneys were pale at necropsy; the kidneys also exhibited moderate-grade renal tubular degeneration at histology. The uterus contained only two resorptions with no classification in terms of early *versus* late events.

In the 500 mg/kg/day group, two animals died on GD 10, one due to gavage error. One animal aborted two fetuses on GD 20, before sacrifice; it exhibited hemorrhagic foci in the stomach and pale kidneys at necropsy and moderate-grade renal tubular degeneration at histology. Of the four 500 mg/kg/day animals that survived until scheduled sacrifice, three had kidneys that were unremarkable at necropsy but exhibited slight-grade tubular degeneration at histology. In the 250 mg/kg/day group, one animal, who passed blood-stained feces on GD 19, died on GD 20. Hemorrhagic lesions were present in its stomach; the report did not describe the uterine contents, except to indicate that the animal was pregnant. None of the 250 mg/kg/day animals had abnormal kidney findings at necropsy, but two animals did exhibit slight-grade tubular degeneration at histology. Reduced maternal body weight and body-weight gain occurred at ≥500 mg/kg/day. None of the 28 animals on test had gastric hairballs at necropsy.

Although not discussed by the investigators, increased incidence of litters having resorptions occurred in the OPP-treated groups: the incidences were 43% (5/7), 83% (5/6) and 60% (3/5) for the 0, 250, and 500 mg/kg/day groups, respectively. The report did not provide data on fetal examination. Based on these results, the investigators selected 250 mg/kg/day as the high dose for the full study.

Zablotny *et al.* (1991b)

This study consisted of two phases. In the first phase, four groups of artificially inseminated New Zealand White rabbits (16 animals/dose) were dosed by gavage at 0 (corn oil), 25, 100, or 250 mg/kg OPP (99.8% pure) on GD 7 through 19 and sacrificed on GD 28. After the first phase, there were only 10 litters with live fetuses in the 250 mg/kg/day group, for the reasons shown in Table 44. However, the FIFRA guidelines for this study type recommended ≥12 litters with live fetuses per dose group. To compensate for this deficiency, the investigators conducted a second phase. Two and eight inseminated females received OPP at 0 and 250 mg/kg/day, respectively. The animals used in the two phases came from the same company, but from different facilities (Kalamazoo, MI *vs.* Denver, PA). The insemination of second-phase animals occurred about five days after the last C-section day in the first phase; consequently, the C-sectioning in the second phase was about one month after the last C-sectioning in the first phase. The report combined the data from the second phase with the data from the first phase in its final presentation.

As in the probe study (Zablotny *et al.*, 1991a), OPP had no effect on maternal body weight or body-weight gain in animals dosed up to 250 mg/kg/day. Also, the treatment had no effect on maternal absolute and relative organ weights for the liver and kidneys. The investigators considered the following to constitute evidence of maternal toxicity at 250 mg/kg/day: deaths, ulceration and hemorrhage in the stomach, hemolyzed blood in the intestines, and renal tubular degeneration and inflammation. However, except for the renal lesions, it is not clear whether OPP solely induced these findings, for the reason discussed below.

One moribund sacrifice and three unscheduled deaths occurred in the 250 mg/kg/day group (Table 44). In the former, histological examination in kidneys found moderate-grade tubular degeneration; however, the renal lesions probably were unrelated to the animal's moribund state that the report described as "no movement of hind legs." In the unscheduled deaths, all occurred in the same intervals (GD15-16) and each was preceded by cageside observations of blood. Two of the animals had a gastric hairball. By contrast, although the probe study used doses of 250-750 mg/kg/day, only one unscheduled death was preceded by cageside observations of blood: in the only unscheduled death in the 250 mg/kg/day group, the animal excreted blood-tainted feces on GD19 before being found death on GD20. As noted earlier, in the probe study, none of the 28 animals on test had gastric hairballs at necropsy. This raises the possibility that the presence of hairballs in the full study affected the toxicity of OPP; e.g., a hairball may act to prolong the transit time of the OPP-dosing solution in the stomach and intestines, which in turn results in hemorrhagic lesions in the stomach and intestine. That large

Table 44 Maternal Outcomes in the First and Second Phases of a Developmental-Toxicity Study of OPP using New Zealand White Rabbits (Zablotny *et al*., 1991b)

Event/Outcome	mg/kg/day					
	0		25	100	250	
	1st Phase	2nd Phase	1st Phase	1st Phase	1st Phase	2nd Phase
Inseminated females on GD7	16	2	16	16	16	8
Not pregnant,[a] discovered at:						
• C-section (GD28)	1	0	0	2 (one animal, BIP: 19)	1 (BIP: 23)	0
• moribund sacrifice	0	0	0	0	1 (16)[b]	0
Pregnant when found dead	1 (16)[c]	0	1 (23)[d]	1 (14)[e]	2 (15 [BIF: 14])[f], (16 [RU,BIP: 13-15])[g]	1 (15 [BIP:11; BIF: 12-14])[h]
Aborted before GD28	1 (24)	0	1 (23)[i]	0	1 (21)[j]	0
Litters at C-section	13	2	14	13	11	7
Litters with resorption(s) only	1 (RU: 18-19)[k]	0	1 (BIP: 24)[l]	0	1 (BIP: 20)[m]	0
Litters with live fetuses	12	2	13	13	10	7

Note: The gestation day when an animal was found dead, was sacrificed, or aborted fetuses is noted in parentheses. For animals with these fates as well as those whose litters contained only resorptions, the types and times of cageside observations involving blood also are noted (blood in pan, BIP; blood in association with feces, BIF; and reddish urine, RU); i.e., most but not all blood observations occurring in the study are identified in this table. Footnotes are for abnormal necropsy findings and related comments:

[a] Non-pregnancy confirmed by sodium sulfide staining. Not necrospied, unless noted.

[b] No movement of hind legs (GD16) suggests a broken back. Stomach: hairball, hemolyzed blood. Colon: dark mucosa. Kidneys (histology): tubular degeneration, moderate grade.

[c] Umbilical hernia with volulus.

[d] Stomach: large hairball, mucosal lesions. Left kidney: hypertrophy, dilated pelvis, pale areas in cortex.

[e] Gavage error (lungs).

[f] Colon: hemolyzed blood, mucosal hemorrhage.

[g] Stomach: hairball, hemorrhage. Kidney (histology): tubular degeneration, moderate grade.

[h] Perineal blood staining. Stomach: small hairball. Intestines: blood.

[i] Perineal blood staining. Stomach: lumen occluded by hairball, mucosal lesions.

[j] Kidneys: pale cortices. Kidneys (histology): tubular degeneration, moderate grade. Stomach: mucosal lesions.

[k] 8 early resorptions.

[l] This animal delivered one resorbed fetus on GD28 prior to C-section. Uterus was classified as having one implantation site, an early resorption. However, an expelled fetus would not leave an early resorption. Therefore, there is uncertainty over the number or types of implantations for this animal.

[m] 1 early resorption.

hairballs were present in an unscheduled death and an aborting female in the 25 mg/kg/day group (see footnotes d and i in Table 44) can be noted.

Except for the kidneys, the designs of the probe and full studies exhibited a major difference that made the comparison of their pathology data difficult. In the probe study, all animals on test underwent a complete necropsy whereas in the full study, the only animals to undergo a complete necropsy were the unscheduled deaths, the moribund sacrifices, and those terminated because they had aborted conceptuses before their scheduled C-section (GD 28). The lack of complete necropsy data for the full study makes it questionable to assume that OPP induced intestinal hemorrhaging in two 250 mg/kg/day animals (identified by footnotes f and h in Table 44). Moreover, in the probe study, no animals in the 250 or 500 mg/kg/day groups exhibited this finding and in the one case that did occur in the 750 mg/kg/day group, it is likely that the intestinal blood originated from a hemorrhagic site in the stomach at the lower sphincter.

All animals on test in the full and probe studies had the kidneys examined microscopically. In the full study, essentially no lesions occurred in the 0, 25, or 100 mg/kg/day groups. In the 250 mg/kg/day group, 8 animals exhibited renal tubular degeneration (33% [8/24] incidence); five were slight-grade lesions and three were moderate-grade lesions (identified by footnotes b, g, and j in Table 44). In the probe study, renal tubular degeneration occurred at each dose level. The incidence was 33% (2/6) at 250 mg/kg/day and all were slight-grade lesions. At 500 mg/kg/day, the incidence was 80% (4/5); almost all were slight-grade lesions, except for a single case that was moderate grade. At 750 mg/kg/day, the one animal to survive to scheduled sacrifice (GD 20) exhibited moderate-grade renal tubular degeneration. The 250 mg/kg/day animal with pale kidneys and moderate-grade tubular degeneration in the full study was similar to a 500 mg/kg/day animal with pale kidneys and moderate-grade lesion in the probe study: both animals aborted fetuses at about the same gestation time, GD 20-21. This raises the possibility that OPP's renal toxicity in pregnant rabbits may be associated with a particular type of reproductive failure.

The pattern of cageside observations of blood occurring in Table 44 deserves comment. Although hematuria and perigenital-blood staining are findings with OPP-induced urinary tract toxicity in rats, these findings did not associate with renal toxicity of OPP in pregnant rabbits in this study design. Rather, there appears to be two sources for the blood. One source relates to the finding of intestinal blood, which, as discussed previously, may be associated with gastric hairballs (footnotes f, g, h, and i in Table 44). A second source relates to the finding in three litters that consisted only of early resorptions (identified by footnotes k, l, and m in Table 44). Further review of the cageside-observation data for the full study indicates that other animals also had blood in the collecting pan (two 100 mg/kg/day animals) or blood in association with the feces, along with perineal-blood staining (one 250 mg/kg/day animal). In each of these three cases, the observation of blood occurred on GD 25 and upon sacrifice on GD 28, the animals had one or two late resorptions[50]. These blood-observation data suggest that resorptions can lead to

[50] The 250 mg/kg/day animal also had two early resorptions (i.e., the uterus contained one late resorption, two early resorptions, and 6 live fetuses).

the appearance of blood detected by the cageside observation (in the pan, in the feces, or in the urine). The observation of blood in the pan with two of the three animals that were supposedly not pregnant in Table 44, one 100 mg/kg/day animal and one 250 mg/kg/day animal (1st phase), raises the possibility that these animals were pregnant but suffered resorptions. That the practice of staining the uterus with sodium sulfide to confirm nonpregnancy is considered less reliable in rabbits than it is in rats should be noted.

OPP exerted no significant effect on fetal body weight or litter size. Also, OPP did not induce external, soft tissue, or skeletal anomalies or malformation. Statistical analyses conducted by DPR indicated that the main developmental effect of OPP was to increase the incidence of litters with resorptions. In the first phase (unshaded entries in Table 45), the incidences of litters with resorptions (any type) for the 0, 25, 100, and 250 mg/kg/day groups were 31% (4/13), 57% (8/14), 77% (10/13, $p<0.05$), and 82% (9/11, $p<0.05$), respectively. The incidence for the 250 mg/kg/day group for the first phase, 82%, was comparable to the probe study at this dose, 83%. For the first phase, the incidences of litters with early resorptions were 23% (3/13), 43% (6/14), 62% (8/13, $p<0.05$), and 45% (5/11), respectively, whereas the incidences for late resorptions were 8% (1/13), 14% (2/14), 23% (3/13), and 45% (5/11, $p<0.05$), respectively. Therefore, OPP increased both early and late resorptions, starting at 100 mg/kg/day. In the report, although resorption incidences were the combined early- and late-effect data for both phases with no statistic analysis results presented, the combined data indicate the same conclusions. Analysis of the combined data conducted by DPR indicates statistically significant effects, starting at 100 mg/kg/day (Table 45). Also, historical control data regarding litters with resorptions (any types) that the Registrant submitted (Breslin *et al.*, 1992) support the conclusion that at the conducting laboratory in a contemporary time frame (1982-1991), it was unprecedented to have a 70+% incidence of litters with resorptions in untreated groups.

In conclusion, this study documented that OPP-treated rabbits exhibited increased resorption at the dose level whereat the maternal toxicity was not observed. Based on the increased litter incidence of resorptions at 100 mg/kg/day, the developmental NOEL is 25 mg/kg/day. The maternal NOEL is 100 mg/kg/day based on increased incidences of renal tubular degeneration at 250 mg/kg/day. DPR considered this study acceptable for fulfilling the developmental toxicity data requirements based on the FIFRA guidelines. The USEPA also established a maternal NOEL of 100 mg/kg/day based on the noted renal effects. However, the USEPA conducted no statistical tests on the incidence of resorptions (early or late or both) and determined the developmental NOEL was ≥250 mg/kg/day based on no statistically or biologically significant treatment-related differences in the incidence of fetal malformation or variations in all dose groups tested (USEPA, 2006).

III.G.1.c. Gavage – Mouse

Ogata *et al.* (1978b)

Table 45 Occurrence of Litters with Resorptions in a Developmental-Toxicity Study of OPP using New Zealand White Rabbits (Zablotny *et al.*, 1991b)

Litters[a]	mg/kg/day							
	0		25		100		250	
	ER	LR	ER	LR	ER	LR	ER	LR
1	8/8[b]	0/8	4/11	0/11	4/8	0/8		
2	0/9	2/9	0/10	2/10	3/5	0/5		
3			1/11	0/11	0/8	2/8	1/7	1/7
4	1/7	0/7	0/7	1/7	1/9	1/9	0/8	2/8
5	1/8	0/8	1/11	0/11	2/10	0/10		
6	0[c]	0	1/1	0/1	0/8	1/8	0/11	1/11
7	0	0	1/9	0/9	1/10	0/10	0/6	1/6
8	0	0	1/3	0/3	1/6	0/6	1/1	0/1
9	0	0	0	0	1/8	0/8	1/6	0/6
10	0	0	0	0	1/5	0/5	1/7	0/7
11	0	0	0	0	0	0	0/11	1/11
12	0	0	0	0	0	0	1/8	0/8
13	0	0	0	0	0	0		
14	0	0	0	0			0	0
15							0	0
16								
17								
18								
Incidence of Litters Exhibiting:								
Early Resorptions	4/15 (26%)		6/14 (43%)		8/13 (62%)		9/18 (50%)	
Statistics[d]			NS		NS[e]		NS	
Late Resorptions	1/15 (7%)		2/14 (14%)		3/13 (23%)		7/18 (39%)	
Statistics[d]			NS		NS		$p<0.05$[e]	
Any Type of Resorption	5/15 (33%)		8/14 (57%)		10/13 (77%)		13/18 (72%)	
Statistics[d]			NS		$p<0.05$[e]		$p\leq0.05$[e,f]	

Abbreviations: ER: early resorption; LR: late resorption; NS: not significant. Shading identifies data from the second phase of testing.

[a] In columns 2-9, litters are presented in an ordered fashion. The first column only provides a visual aid for showing the number of litters per group.

[b] Fraction of implantations that were resorptions, as reported by the investigators (Zablotny *et al.*,1991b, Breslin *et al.* 1992); e.g., 8/8 means that 8 of the 8 implantations were early resorptions

[c] Litter with no resorptions of any type.

[d] In the columns for the treated groups are the p-values corresponding to comparisons between the controls and the treated groups (Shirley, 1977). The proportion of affected fetuses per litter was used as an experimental unit for the statistical analyses (Haseman and Peigorsch, 1994).

[e] The litter incidence of resorptions was significant at $p<0.05$ when the statistical analysis was conducted using only the data from the first phase.

[f] Calculated t-value (1.68) was comparable to the table value of 1.72 at $\alpha=0.05$ (William, 1972).

This report consisted of two studies. The second study was with SOPP (see below). In the first study, four groups of Jcl:ICR mice bearing vaginal plugs (21 animals/dose) were dosed by gavage at 0 (olive oil), 1450, 1740, and 2100 mg/kg/day OPP (purity not specified) on GD 7 through 15 and sacrificed on GD 18. Maternal deaths occurred at all dose levels and followed a dose response (Table 46). The only clinical sign reported was bleeding from the vaginal orifice seen in two females at 2100 mg/kg/day that died prior to scheduled sacrifice. Although maternal deaths occurred at each dose level, inhibition of maternal body-weight gains[51] occurred only at 1740 and 2100 mg/kg/day. Therefore, the evidence for maternal toxicity at 1450 mg/kg/day was the four maternal deaths.

OPP also affected the fetuses. Statistical analyses by the investigators indicated that reduced ($p<0.01$) fetal body weight and increased ($p<0.01$) incidence of cervical ribs occurred in each of the OPP-treated groups, with both changes showing dose dependency (Table 46). Increased ($p<0.05$) overall incidence of external deformities also occurred at the low and mid doses. At the high dose, the overall incidence of deformities still showed an increase, albeit only five litters were available for examination at C-section. Despite the increased incidences of external deformities, the investigators concluded that OPP was not teratogenic in the testing. Apparently, the reasons for ignoring the external deformities were the following: OPP induced no unique type of deformity (i.e., both the control and treated groups had the same type of external deformities); increased ($p<0.05$) incidences at the low and mid doses were marginal; and there was no dose response. It should be noted that the investigators did not address whether the dose response for maternal deaths could have affected the dose response for deformities. No NOELs could be determined from this study because both maternal and fetal effects occurred at the lowest dose tested. DPR considered this study unacceptable because of poor dose selection which resulted in many maternal deaths and, consequently, inadequate numbers of fetuses/group being available for visceral and skeletal examinations.

III.G.2. Sodium *ortho*-Phenylphenol

Gavage – Mouse

Ogata *et al.* (1978b)

This report consisted of two studies. The first study with OPP was discussed above. In this second study, four groups of Jcl:ICR mice bearing vaginal plugs (20 animals/dose) were dosed by gavage at 0 (water), 100, 200, or 400 mg/kg/day SOPP (purity not specified) on GD 7 through 15 and sacrificed on GD 18. Maternal deaths occurred during GD 11-18 at 200 and 400 mg/kg/day (4 and 16 deaths, respectively). The investigators indicated that each of the SOPP-treated groups had inhibition of the maternal body-weight gain[51]; the onset times were GD 12-

[51] The investigators presented data on body-weight gain in the control and treated groups only in a graph. Also, the investigators did not report any results regarding statistical analyses of the body-weight gain data.

Table 46 Maternal and Fetal Outcomes in a Developmental-Toxicity Study of OPP Using Jcl:ICR Mice (Ogata *et al.*, 1978b)

Outcomes	mg/kg/day			
	0	1450	1740	2100
Maternal				
Mated Females at Start of Dosing	21	21	21	21
Unscheduled Deaths	0	4	7	16
Impregnated Based on C-Section	20[a]	14[a]	14	5
Litters With Resorptions Only	0	0	0	0
Litters With Live Fetus(es)	20	14	14	5
Mean Corpora Lutea/Dam	13.5±3.1	14.5±3.0	13.1±2.1	13.2±1.8
Fetal				
Mean Implantation Scars/Dam	12.4±2.9	12.6±2.2	11.0±1.2	12.8±1.9
Mean Litter Size (Live Fetuses)[b]	10.9±3.2	11.8±2.5	10.6±1.6	11.2±1.1
Fetal Body Weight (g)[c,d]				
• Male	1.35	1.30 (96%)**	1.28 (95%)***	1.08 (80%)***
• Female	1.28	1.18 (92%)***	1.23 (96%)***	1.02 (80%)***
Skeletal Variations				
• Frequency of Fetuses With Cervical Ribs[e]	0	6.6±10.1**	8.9±12.2**	17.0±28.2***
External Malformations[f]				
• Cleft Palate	1 [1] (5%)	1 [1] (7%)	4 [4] (29%)	1[1] (20%)
• Open Eyelids	1 [1] (5%)	4 [7] (29%)	6 [6] (43%)[#]	1 [1] (20%)
• Exencephalia	0	3 [6] (21%)	0	0
Frequency of Fetuses with Externally Visible Deformities (All Types Combined)[e]	0.67±2.05	6.21±8.03*	6.14±5.96*	3.64±4.98

[a] The investigators explained that the difference between the number of mated females surviving to C-section and the number found pregnant at C-section represented those mated females that did not become pregnant (no implantation sites).
[b] Means ± one standard deviation, as reported by the investigators.
[c] The value as a percent of the value for the negative controls is given in parentheses
[d] Body weight measured on GD 18.
[e] Mean proportion of fetuses affected per litter ± one standard deviation for groups of 5-20 litters, as reported by the investigators. The investigators stated that a fetus with more than one malformation was counted only once.
[f] Number of affected litters, with number of affected fetuses in brackets and the percent of litters affected in parentheses, as reported by the investigators (Ogata *et al.*, 1978b).
[*,**,***] Significantly different from the control at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, as reported by the investigators.
[#] Fisher exact test, $p=0.01$; as calculated by DPR.

13, GD 11, and GD 8 for the 100, 200, and 400 mg/kg/day groups, respectively. Vaginal bleeding was the only clinical sign noted; the report stated that this occurred in almost all animals that died. The investigators related the vaginal bleeding to "abortions" without explaining their reasoning. There was no discussion on the detection times for the blood or the conditions of the uterine contents.

SOPP affected the fetuses. Reduced ($p<0.001$) fetal body weight occurred in each of the treated groups, albeit the magnitude of the reductions did not increase with dose (Table 47). Decreases ($p<0.05$) in the number per litter for implantation sites and live fetuses occurred at 200 mg/kg/day; comparable decreases also occurred at 400 mg/kg/day, albeit only four litters were available for examination at C-section. The numbers of corpora lutea per dam were comparable among the four groups. Therefore, the decreases in the numbers of implantation sites per dam at 200 and 400 mg/kg/day are consistent with preimplantation loss. Alternatively, since treatments commenced on GD 7, which was after the interval that implantations occurred (GD 4.5- 5 [Brinster, 1975]), the apparent preimplantation loss might reflect very early postimplantation loss that went unrecognized in the study. While increased incidences of cervical ribs occurred in the SOPP-treated groups, these were not statistically significant (Table 47). Increase in the overall incidence of external deformities occurred at 100 mg/kg/day. As discussed below, although the increase did not achieve statistical significance, the fact that the increase essentially involved one type of malformation, cleft palate, may indicate a biological significance.

The investigators concluded that SOPP, like OPP, was not teratogenic. However, this conclusion did not consider the frequency of 28 cases of cleft palate in a single treatment group. Also, the report expressed little concern regarding the significant reductions in fetal body weight, which occurred in both studies starting at the low dose. Apparently, the investigators dismissed the cleft palate as a possible treatment effect for three reasons. First, there was no dose response at the higher dose levels including the higher level represented by the dosing in the OPP study. Second, SOPP induced no unique type of deformity (i.e., cleft palate occurred in the fetuses from both the control and treated groups). Third, 15 of the 28 affected fetuses in the 100 mg/kg/day group originated from a single dam (footnote g Table 47). However, these objections are not sufficient for dismissing the possible toxicological significance of the cleft palate based on the following considerations.

First, although Ogata *et al.* (1978b) effectively exposed the animals to OPP in both studies, these studies also need to be considered separately given the inconsistencies in their findings. For example, since 400 mg/kg/day of SOPP, which is equivalent to 258 mg/kg/day of OPP[52], caused 80% of the dams to die (Table 47), one would have expected >80% mortality at the low dose in the OPP testing, 1450 mg/kg/day; instead, the mortality rate was only 19% (4/21) (Table 46). A similar inconsistency occurs in comparing the fetal body-weight data from the two studies. One possibility is that the use of olive oil in the OPP testing may have altered the uptake (and metabolism) of OPP in relation to SOPP, which was delivered as aqueous solutions.

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[52] $\frac{400 \text{ mg/kg/day SOPP}}{264.3 \text{ (Molecular Weight of SOPP)}} \times 170.2 \text{ (molecular weight of OPP)} = 258 \text{mg/kg/day OPP}$

Table 47 Maternal and Fetal Outcomes in a Developmental-Toxicity Study of SOPP Using Jcl:ICR Mice (Ogata *et al.*, 1978b)

Outcomes	mg/kg/day			
	0	100	200	400
Maternal				
Mated Females at Start of Dosing	20	20	20	20
Unscheduled Deaths	0	0	4	16
Impregnated Based on C-Section	17[a]	19[a]	15[a]	4
Litters With Resorption(s) Only	0	0	2	0
Litters With Live Fetus(es)	17	19	13	4
Mean Corpora Lutea/Dam	13.5±6.4	13.1±2.2	14.5±2.0	14.5±2.1
Fetal				
Mean Implantation Scars/Dam	13.2±1.6	12.9±2.1	11.0±3.9*	11.3±5.9
Mean Litter Size (Live Fetuses)[b]	12.6±1.8	11.9±2.9	10.0±4.0*	10.5±6.0
Mean Fetal Body Weight (g)[c,d]				
• Male	1.4	1.1 (79%)***	1.3 (93%)***	1.2 (86%)***
• Female	1.3	1.1 (85%)***	1.2 (92%)**	1.1 (85%)***
Skeletal Variations				
• Frequency of Fetuses With Cervical Ribs[e]	1.2±2.7	2.9±9.0	3.9±6.5	4.2±4.9
External Malformations[f]				
• Cleft Palate	1 [1] (6%)	6 [28][g] (32%)[#]	1 [1] (8%)	1 [3] (25%)
• Open Eyelids	5 [6] (29%)	1 [3] (5%)	1 [1] (8%)	0
• Exencephalia	0	1 [1] (5%)	0	0
Frequency of Fetuses with Externally Visible Deformities (All Types Combined)[e]	3.3±5.9	12.5±23.6	3.2±8.0	5.8±11.5

[a] The investigators explained that the difference between the number of mated females surviving to C-section and the number found pregnant at C-section represented those mated females that did not become pregnant (no implantation sites).
[b] Means ± one standard deviation, as reported by the investigators.
[c] The value as a percent of the value for the negative controls is given in parentheses
[d] Body weight measured on GD 18.
[e] Mean proportion of fetuses affected per litter ± one standard deviation for groups of 4-19 litters, as reported by the investigators. The investigators stated that a fetus with more than one malformation was counted only once.
[f] Number of affected litters, with number of affected fetuses in brackets and the percent of litters affected in parentheses, as reported by the investigators (Ogata *et al.*, 1978b).
[g] In one of the 6 affected litters, 15 of the16 fetuses exhibited cleft palate.
*,*** Statistically significant at $p<0.05$ and $p<0.001$, respectively, as reported by the investigators.
[#] Fisher exact test, p=0.06; as calculated by DPR.

Second, there is no reason to expect that if OPP and (or) SOPP were developmental toxicants, they would induce a type of malformation that does not occur "spontaneously" in fetuses from control animals.

Third, cleft palate had a low spontaneous incidence in this strain of mice at the conducting laboratory[53]. At 100 mg/kg/day, there were 28 fetuses with cleft palate, involving 6 of the 19 litters (32% litter incidence). One of the 6 affected litters consisted of 15 cleft-palate-bearing fetuses and one cleft-palate-free fetus. Therefore, in addition to one greatly affected litter, there were five affected litters containing a total of 13 cleft-palate fetuses. By contrast, there was only a single fetus with cleft palate in the control (water) group (6% litter incidence). A single fetus with cleft palate also occurred in the olive-oil group in the OPP testing (5% litter incidence) (Table 46). Without the individual data, the only statistical comparison that is available is a Fisher exact test using litter incidences; the resulting p-value is 0.06 (1/17 *vs.* 6/19).

Based on reduced fetal body weight (both sexes) and an increased incidence of cleft palate, the developmental LOEL was 100 mg/kg/day. Reduced body-weight gain could be the basis for the maternal LOEL; however, there are insufficient data in the report for the reduced maternal body weight to be distinguished unambiguously from that associated with 15-21% fetal body weight reduction which also occurred at this dose (Table 47). Otherwise, there were no deaths or clinical signs in the dams at 100 mg/kg/day.

In conclusion, this study documented that SOPP affected the fetuses and that the increased toxicity of fetuses occurred at the same or lower doses as those causing parental toxicity. Because of inadequate reporting, including no individual data, DPR judged this study unacceptable for filing SB950 developmental toxicity data requirements.

[53] The following negative-control data were reported by Ogata *et al.* (1984) as part of their testing of another fungicide: 4 cleft-palates were observed among 412 fetuses from 34 litters. Although the litter incidence of cleft palate in the negative controls was not stated, the maximum litter incidence would be 12% (4/34) if one per litter.

IV. RISK ASSESSMENT FOR DIETARY EXPOSURE

IV.A. HAZARD IDENTIFICATION

IV.A.1. Introduction

This section provides discussion of selecting critical toxicity endpoints and quantitative dose-response methods to characterize non-oncogenic and oncogenic risk. In the former, the selection involved the identification of a no-effect level (i.e., threshold) based on the lowest dose in the toxicological study (i.e., LOEL) whereat the critical toxicological effect(s) occurred. In the latter, DPR employs the guidelines for carcinogen risk assessment of the United States Environmental Protection Agency (Meek *et al.*, 2003; USEPA, 2005) for selecting the method needed.

IV.A.2. Selection of Toxicity Endpoints

Results of experimental or epidemiological studies in humans on oral exposures to OPP and SOPP are not available. Hence, this risk assessment extrapolates the results of oral animal toxicity studies to humans, assuming the effects observed in the animals could occur in humans. The major toxicity for OPP and SOPP in experimental animals was developmental, urinary tract, and heart effects. In general, the NOELs for these effects were at or below the NOELs for other endpoints except for carcinogenicity (Tables 48 and 49). Therefore, DPR concluded that these are the critical endpoints for risk assessment. Also, the use of NOELs for these endpoints in risk characterization would protect against effects at higher doses (e.g., liver [focal necrosis], spleen [atrophy], pancreas [focal atrophy of acinar cells], and pup toxicity [body weight reduction]).

IV.A.2.a. Developmental Effects

Experimental animals (rats, rabbits, and mice) treated with OPP and (or) SOPP exhibited developmental effects (Kaneda *et al.*,1978, Ogata *et al.*, 1978b, Zablotny *et al.*, 1991b). In Wistar rats, the effects were decreased fetal body weight and increased incidence of resorptions (Kaneda *et al.*, 1978). In New Zealand White rabbits, exposure to OPP was associated with increased incidence of resorptions. Fetuses from Jcl:ICR mice exposed to SOPP exhibited reduced mean body weight and an increased incidence of cleft palate (Ogata *et al.*, 1978). In the mouse and rabbit studies, maternal toxicity was either not observed (rabbit) or appeared to be minimal (mice) at the lowest dose whereat fetal effects occurred. Hence, the resorption in rabbit is a sensitive developmental toxicity endpoint suitable for risk assessment.

IV.A.2.b. Urinary Tract Effects

OPP and SOPP affected both the urinary bladder and kidneys in experimental animals after the oral exposure. Also, OPP and SOPP induced polydipsia in rats and mice.

IV.A.2.b.1. Urinary Bladder

Two different strains of rat exhibited the bladder toxicity (including nonneoplastic and neoplastic lesions): F344 and SD rats. The bladder effects appeared to be more severe in the males than females (Hiraga, 1983; Hiraga and Fujii, 1984; Iguchi *et al.*, 1979,1984; Eigenberg, 1989b; Eigenberg and Lake, 1995; Wahle and Christenson, 1996). The nonneoplastic effects identified at the LOEL or above were increases in incidences of simple hyperplasia, epithelial cell proliferation, and absolute organ weight (see IV.a.4. ONCOGENICITY for discussion of the neoplastic effects).

IV.A.2.b.2. Kidneys

Available data from animal toxicity studies (rats, mice, and rabbits) indicated that both OPP and SOPP affected the kidneys. Studies investigating the kidney effects of OPP and SOPP showed that similar histologic lesions occurred in the male F344 rats over different doses and exposure durations (i.e., subchronic and chronic). These effects included nephritis and hyperplasia (papilla and [or] pelvis) (Hiraga, 1983; Hiraga and Fujii, 1984; Iguchi *et al.*, 1984; Shibata *et al.*, 1989; Christenson *et al.*, 1996a; Wahle and Christenson, 1996; Niho *et al*., 2002). Other effects identified in the males included increased absolute kidney weight (Nakamura *et al.*, 1981; Iguchi *et al.*, 1979, 1984; Hiraga and Fujii, 1984, Christenson *et al*., 1996b), increased BUN, and decreased urine pH (Nakamura *et al.*, 1981; Iguchi *et al.*, 1984; Wahle and Christenson, 1996). The decreased pH may be related to the acidification of urine due to nephritis (Fujii *et al.*, 1987). In the aforementioned studies wherein female rats also were involved, essentially the same kidney effects were found (histology, necropsy, and clinical pathology); however, the effect appeared to be more severe in the females than males under the chronic exposure protocol (Iguchi *et al.*, 1984; Hiraga, 1983; Whale and Christenson, 1996).

In mice, chronic oral toxicity studies showed that OPP induced different lesions in the kidneys: renal tubular dilation, tubular epithelial degeneration and necrosis, ductular epithelium necrosis and degeneration, and necrosis of transitional cells in papilla and (or) cell debris in pelvis (Mikuriya *et al.*, 1989a). Another effect identified in the mice was increased absolute kidney weight (Ito *et al.*, 1983; Quast and McGuirk, 1995). An additional support for the nephrotoxic effect of OPP in mice is that OPP *via* dermal absorption also caused effects (e.g., renal tubular dilation) in the kidneys (NTP, 1986).

In OPP-treated pregnant rabbits, renal tubular degeneration occurred in as little as 12 days after the dosing was initiated (Zablotny *et al.*, 1991a,b). Viewing altogether, kidney toxicity is a well-supported endpoint for assessing health risk associated with the exposure to OPP.

IV.A.2.b.3. Polydipsia (i.e., Increased Water intake)

In addition to the kidney effects, results of multiple studies indicated that rats and mice that exposed subchronically or chronically to OPP or SOPP exhibited polydipsia (Nakamuara *et al*., 1981; Iguchi *et al.*, 1979,1984; Ito *et al.*, 1983; Fukushima *et al.*, 1983; Hiraga and Fujii *et al.*, 1984; Fujii *et al.*, 1987; Wahle and Christenson, 1996; Mikuriya *et al.*, 1989a). Clinical pathological observations that may be associated with the polydipsia were increase in urine volume, dilution of urinary constituents (e.g., protein and electrolytes [e.g., sodium, potassium, and chloride ions]), and decrease in urinary specific gravity (or osmolality). As discussed in the III TOXICOLOGY PROFILE, the increased water intake may have been due to the effect of OPP on water metabolism. Alternatively, since kidneys are responsible for maintaining water-balancing function and the treated animals that exhibited kidney lesions (e.g., nephritis) also had increased water intake (Hiraga and Fujii, 1984; Iguchi *et al.*, 1984; Mikuriya *et al.*, 1989a), the affected water metabolism might instead reflect the OPP-induced damage in kidneys.

IV.A.2.c. Heart Effects

Experimental animals that were exposed chronically to OPP or SOPP in the diet exhibited effects in the heart. The heart effects appeared to be more severe in the females than males (Ito, 1983, Wahle and Christenson, 1996). In female F344 rats, the effect was cardiac degeneration and (or) fibrosis (i.e., cardiomyopathy) (Wahle and Christenson, 1996). Female B6C3F1 mice exhibited increased heart weight after exposure to SOPP (Ito, 1983).

IV.A.3. Selection of Critical NOELs

DPR established critical NOELs for acute and chronic toxicity of OPP from the oral exposure. An addendum to this document will address the critical NOEL selection for assessing risk associated with exposures from non-dietary sources (e.g., residential and occupational exposure).

IV.A.3.a. Acute Toxicity

For acute exposure, the critical NOEL was 25 mg/kg/day for increased resorption at 100 mg/kg/day (i.e., LOEL) in a rabbit developmental toxicity study with OPP (Zablotny *et al.*, 1991b). The justification of using prenatal developmental toxicity data for acute hazard identification is the assumption that the developmental effects could result from an acute (i.e., single) exposure (USEPA, 1991). The other developmental study that supported this NOEL was the study of SOPP involving mice (Ogata *et al.*, 1978b). In that study, the reported effect was an elevated incidence of cleft palate; the lowest dose tested was the LOEL (i.e., 100 mg/kg/day SOPP). Although the SOPP mouse study had a slightly lower LOEL (and therefore an estimated NOEL) in terms of mg/kg/day OPP, the selection of rabbit over mice studies for critical NOEL

determination was because the former had a lowest observable NOEL. It should be noted, however, that the current state of science considers that developmental effect is an *in utero* effect; i.e., this occurs only during pregnancy. Accordingly, the selected critical NOEL would only be applicable for characterizing health risk in women in their childbearing years (i.e., females at 13-49 yrs). That is, it is not suitable for quantifying the risk in the general population, including infants, children, and adult males.

For assessing the risk associated with acute exposure to OPP in the general population, the critical NOEL was 150 mg/kg/day. This NOEL was based on the maternal toxicity in a rat developmental-toxicity study wherein dams exhibited ataxia and decreased ($p<0.05$) maternal body-weight gain 3 days after the dosing was initiated. DPR selected this as an appropriate endpoint for acute effect because of the early occurrence following treatment initiation (USEPA, 1991).

IV.A.3.b. Chronic Toxicity

For chronic exposure, the effects in rats (i.e., kidney, bladder, and heart) and mice (i.e., heart) showed different sensitive endpoints to OPP in males and females. DPR propose two critical NOELs for quantifying the risk of chronic exposure to OPP in diet: 39 mg/kg/day for the males and 4.9 mg/kg/day for the females. The critical NOEL of 39 mg/kg/day for males was based on a LOEL of 200 mg/kg/day for the urinary tract effects in a combined chronic oral toxicity/oncogenicity study with OPP in rats (Wahle and Christenson, 1996). The critical endpoints were simple hyperplasia in urinary bladder and a set of clinical pathological observations associated with polydipsia and kidney effects; i.e., decreased urinary protein concentration, decreased urine specific gravity, and increased BUN. The supports for urinary tract effect for risk assessment of OPP are: First, the value of 39 mg/kg/day is the lowest experimentally observable NOEL. Further support of this value was from a two-generation reproductive toxicity study wherein the NOEL was 40 mg/kg/day OPP; this was also based on the effect in the urinary bladder of rats (Eigenberg, 1989b). Second, the LOEL (i.e., 200 mg/kg/day) at which OPP produced the toxicologically significant effect in the study by Wahle and Christenson (1996) is within a range of LOELs reported in other studies in rats (i.e., 144-269 mg/kg/day OPP) and mice (i.e., 92-309 mg/kg/day OPP).

The critical NOEL of 4.9 mg/kg/day for females was an estimated No-Observed-Effect-Level (ENEL) value based on a LOEL of 49 mg/kg/day for the cardiac degeneration and (or) fibrosis and a 10x uncertainty factor. Among all studies in Table 49, the LOEL of this heart effect was the lowest and was reported coincidentally in the same study by Wahle and Christenson (1996).

IV.A.4. Mode of Action for Urinary Bladder Tumors

Exposure to OPP or SOPP resulted in urinary bladder tumors (papilloma and carcinoma) and kidney tumors (renal papilla and pelvis carcinoma) in F344 rats (Hiraga and Fujii, 1981,

Table 48 Selected Acute NOELs and LOELs of OPP and SOPP

Species/ Chemical	Route (Duration)	Toxicity Endpoints (Effects observed at LOEL)	NOEL	LOEL	Ref.
		Developmental Toxicity Studies	mg/kg/day		
Wister Rats/ OPP	**Gavage (9 days)**	**Dams: ↓ mean body-weight gain (onset day 3) & ↑ ataxia**	**150**	**300**	**1**
		Fetuses: ↓ mean body weight & possibly ↑ resorptions	300	600	1
Jcl:ICR Mice/SOPP	Gavage (8 days)	Fetuses: ↑ cleft palates	<100 (<64)[a]	100 (64)[a]	2
NZW Rabbits/OPP	**Gavage (12 days)**	**Fetuses: ↑resorptions**	**25**	**100**	**3***

Ref: 1-Kaneda *et al.* (1978), 2-Ogata *et al.* (1978b), and 3-Zablotny *et al.* (1991b).
[a] OPP equivalent (mg/kg/day) is calculated by the following formula:
[(SOPP mg/kg/day)/(SOPP molecular weight)] x OPP molecular weight
* Study that was acceptable to DPR based on the FIFRA guideline.

Table 49 NOELs and LOELs of OPP and SOPP from Selected Chronic-Toxicity and Two Generation Reproductive-Toxicity Studies

Species/ Chemical	Route (Duration)	Toxicity Endpoints (Effects observed at LOEL)	NOEL or ENEL	LOEL	Ref.
		Chronic-Toxicity Studies	Mg/kg/day		
F344 Rats/OPP	**Oral (104 wks)**	**Both sexes: ↑serum BUN, ↓urinary protein concentration, ↓specific gravity; Male: ↑ simple hyperplasia (bladder)**	**39**	**200**	**1**
		Females: ↑ cardiac degeneration and (or) fibrosis	**4.9**	**49**	**1**
F344 rats/SOPP	Oral (104 wks)	Females: ↑interstitial nephritis, ↑ pancreatic acinar cells focal atrophy	<224 (<144)[a]	224 (144)[a]	2*
B6C3F1 mice/OPP	Oral (52 wks)	Males: ↑ water intake, ↑ renal tubular epithelium degeneration, ↑ relative liver weight, ↑ spleen atrophy	<92	92	3
B6C3F1 mice/OPP	Oral (104 wks)	Males: ↑ relative liver weight, ↑ ALP	<250	250[c]	4*
B6C3F1 mice/SOPP	Oral (96 wks)	Females: ↓body weight, ↑absolute weights of heart and kidneys, ↑ ALP	<480 (<311)[a]	480 (309)[a]	5
Beagle Dogs/OPP	Gavage (52 wks)	None	≥300	-	6*
		Two Generations Reproductive-Toxicity Studies			
SD Rats/OPP	Oral (43 wks)	F0 Parents and F1 Males: ↑mean epithelium thickness (bladder)	40	140	7
		F0 Females: ↑mean number of cells/layer (bladder)			
		Pups: ↓pup body weights	140	490	7
SD Rats/OPP	Oral (37 wks)	Both Sexes: ↓ body weight (F0-F1 dams, F1 males)	100	500	8*
		Males: ↑ chronic inflammation and debris (kidneys), hyperplasia and dilation (ureter), and chronic inflammation and transitional epithelium hyperplasia (bladder).			
		Pups: ↓ body weight and ↑ stunting	100	500	8*

Abbreviation: ENEL: estimated No-Observed-Effect-Level.
Ref.: 1-Wahle & Christenson (1996), 2-Hiraga (1983), 3-Mikuriya *et al.* (1989a), 4-Quast & McGuirk (1995), 5-Ito (1983), 6- Cosse *et al.* (1990), 7-Eigenberg (1989b), 8-Eigenberg & Lake (1995).
[a] OPP equivalent (mg/kg/day) is calculated by the following formula:
[(SOPP mg/kg/day)/(SOPP molecular weight)] x OPP molecular weight.
* Study that was acceptable to DPR based on the FIFRA guideline.

1984; Hiraga, 1983; Wahle and Christenson, 1996; Niho *et al.*, 2002) and liver tumors (adenoma, carcinoma, and hepatoblastoma) and circulatory system tumors (hemangiomas) in B6C3F1 mice (Quast and McGuirk, 1995; Ito, 1983). However, the following discussion on carcinogenic mode of action pertains only to urinary bladder in rats, which alone has sufficient data for postulating the mode of action (MOA).

IV.A.4.a. Two possible MOAs

Results from multiple studies indicated that increased incidences of urinary bladder papilloma and carcinoma occurred in F344 rats exposed to OPP or SOPP (Hiraga and Fujii, 1981, 1984; Hiraga, 1983; Wahle and Christenson, 1996; Niho *et al.*, 2002). The characterizing features of the tumor dose response relationship were zero occurrences at relatively low doses and a steep increase in the tumor incidence at higher doses. Also typical of the bladder carcinogenicity of OPP and SOPP is the apparent shortened time-to-tumor with increasing dose, such that detection of tumors occurred as early as 13 weeks of exposure at relatively high dose levels (Hiraga and Fujii, 1981, 1984). Two possible mode-of-actions (MOA) may be involved: non-genotoxic and genotoxic MOA.

(1) Non-Genotoxic MOA This MOA was suggested by the appearance of nonlinear dose response curves (Bomhard *et al*., 2002; Dow and Bayer, 2004). The argument to support this proposal included the assertion of negative genotoxicity (Brusick, 2005) and the decreased urinary bladder hyperplasia after cessation of 13 weeks of exposure to OPP (i.e., implying that the effect is reversible) (Christenson *et al.*, 1996a). Within this MOA, the high-dose cancer effect may be due to increased formation of cytotoxic quinone metabolites caused by the saturation of Phase II detoxification enzyme pathways (Reitz *et al.*, 1983). With continuing exposure to high doses OPP, the cytotoxicity may result in epithelial hyperplasia (Christenson *et al.*, 1996a), which progresses into P/N hyperplasia, papilloma, and transitional cell carcinoma (Wahle and Christenson, 1996). Associated with this proposed non-genotoxic MOA was the further speculation that since the carcinogenicity data were weak (predominately only in male rats, not in mice and dogs), it is considered not relevant to the level of anticipated human exposures (Bomhard *et al*., 2002; Dow and Bayer, 2004). However, it should be noted that although OPP did not cause urinary tumors in mice, its exposure resulted in other tumors in mice. Also, the available study in dogs was not designed for oncogenicity evaluation as the study was carried on for only 1 year.

(2) Genotoxic MOA A two-event carcinogenicity model proposed by Greenfield *et al.* (1984) based on the carcinogenicity data of N-[4-(5-nitro2-furyl)-2-thiazolyl]formamide (FANFT) entails both genotoxic initiation as well as tumor promotion through cell proliferation. A more detailed discussion regarding the support, or the lack thereof, for these two MOAs is presented below.

Genotoxicity of OPP, SOPP and their Metabolites: Many of the Registrant-submitted studies showed weak or negative genotoxicity. However, *in vivo* and *in vitro* studies published in the open literature support the genotoxic potential of OPP, SOPP, and their metabolites (e.g.,

phenylhydroquinone [PHQ] and phenylbenzoquinone [PBQ]), with the metabolites showing greater genotoxic potential. Details of the genotoxicity database are presented in Section III. E. GENOTOXICITY).

Role of Reactive Metabolites: In spite of the evidence of genotoxicity of PHQ (see III. E. GENOTOXICITY), two crucial aspects of the involvement of PHQ and PBQ in tumor formation separate the two proposed MOA that support either threshold (non-genotoxic MOA) or non-threshold (genotoxic MOA) oncogenicity dose-response relationship.

(a) Forms of PHQ and PBQ The threshold (non-genotoxic) MOA argues that bladder tumors only occur at high dose, after the sulfation and glucuronidation conjugation pathways are saturated. This argument was based on the observation in the repeated dosing study by Smith *et al.* (1998) that the sulfate and glucuronide conjugation of OPP in male rats reduced from 87% of total administered OPP at the low dose of 56 mg/kg/day to 64% at the high dose of 924 mg/kg/day. However, this argument did not address the presence of genotoxic PHQ and PBQ in the urine.

Metabolic studies showed that unchanged OPP and its hydroquinone metabolites are largely present in the urine in conjugated forms (see Section III.A. PHARMACOKINETICS). Nevertheless, a small fraction of the metabolites that existed as unconjugated forms is of biological concern. Unconjugated metabolites are reported by Morimoto *et al.* (1989) and Bartels *et al.* (1998). In fact, within the dose range of carcinogenicity bioassay, the results of these studies indicated that the ratio of urinary OPP to the unconjugated PHQ was independent of OPP dose; 800-12500 ppm OPP in Bartels *et al* (1998) and 5000-20000 ppm SOPP in Morimoto *et al.* (1989). Thus, the total amount and the concentration of free PHQ increased within the range of tested dose. It is interesting to note that, although the unconjugated form is only a small fraction of the total urinary metabolites (<2%), the concentration of urinary PHQ (~150-1500 μM [Morimoto *et al.*, 1989]) was similar to that reported to induce DNA breakage, nucleotide oxidation, and (or) chromosomal aberrations *in vitro* (see III.E. GENOTOXICITY).

(b) Oncogenicity Correlations of PHQ and PBQ Based on the lack of apparent correlation between urinary PHQ and PBQ and bladder epithelial alterations, Hasegawa *et al.* (1991) dismissed their role in the induction of urinary bladder tumors in OPP-treated rats. However, a closer look at the data reported by these authors and those reported by Morimoto *et al.* (1989), Kwok and Eastmond (1997) showed a striking linear correlation ($r^2 = 0.8$) between the amount of reactive species generated through pH-dependent PHQ autoxidation and the incidences of preneoplastic and neoplastic lesions. Thus, the pH-dependent autoxidation of unconjugated urinary PHQ remains a viable factor in the tumor formation. Subsequently, Kwok *et al.* (1999) demonstrated good correlations of the autoxidized PHQ to the radiolabels retained by urinary bladder proteins ($r^2=0.94$; $p<0.05$) in male F344 rats exposed to [^{14}C]-OPP. In fact, as shown in Figure 2, a good correlation between the amount of autoxidized PHQ and the bladder protein binding can also be demonstrated with data reported by Reitz *et al.* (1984). In addition, the higher concentration of urinary PHQ in male than female rats (Nakao *et al.*, 1983, Morimoto *et al.*, 1989, Hasegawa *et al*., 1991) corresponded well to the much higher bladder tumor

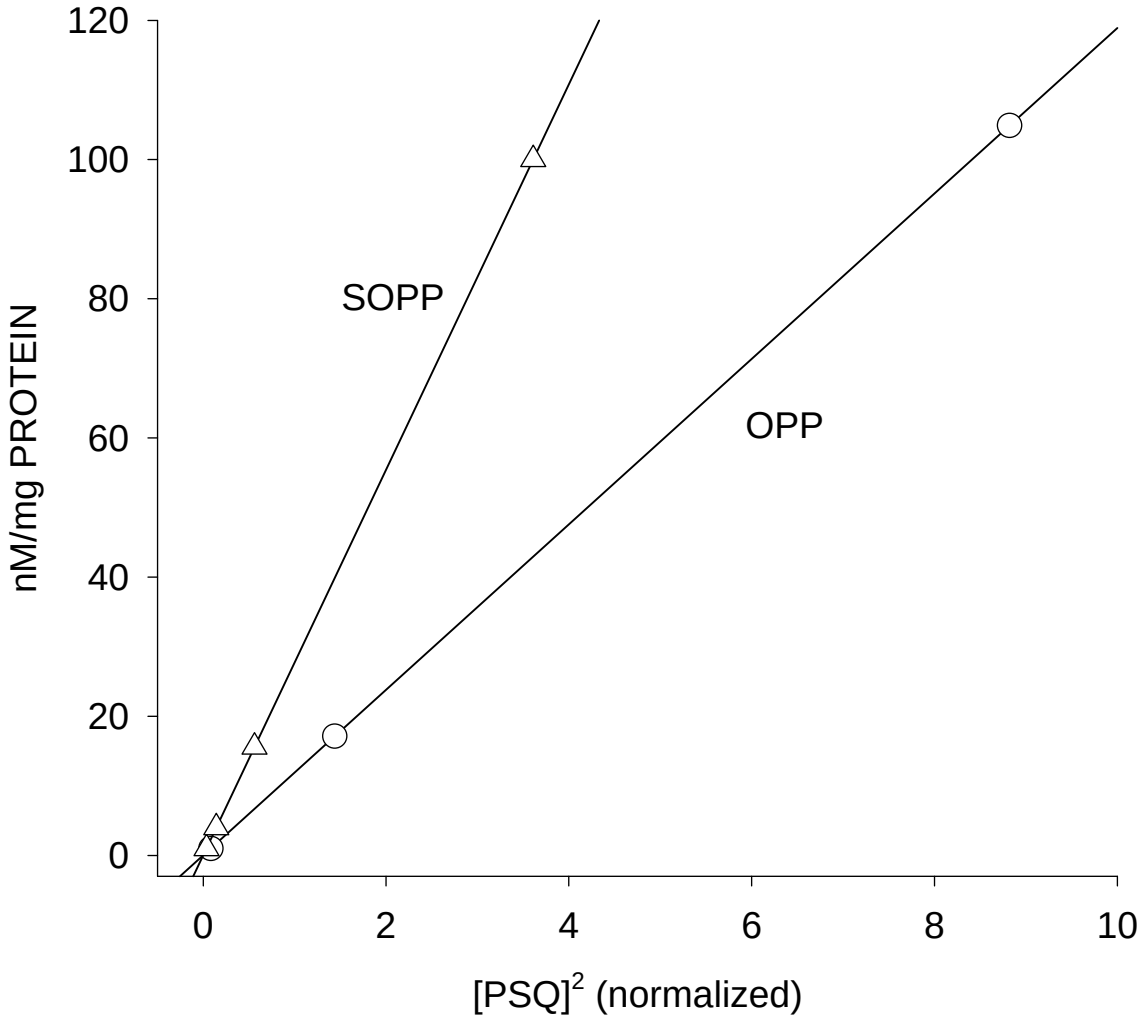

Figure 2 Plot of covalent binding of ^{14}C-OPP and ^{14}C-SOPP to proteins in the urinary bladder of rats from study by Reitz *et al.* (1984) *versus* the predicted amount of PHQ autoxidized (i.e., $[PSQ]^2$).

incidence in the male (Wahle and Christenson, 1996). Finally, lower reactive species formed from PHQ autoxidation under acidic environment (Kwok and Eastmond, 1997) may contribute to the inverse relationship between nephritis and bladder tumors in rats (Hiraga and Fujii, 1981, 1984) as metabolic acidosis could accompany kidney damage caused by high dose of OPP or SOPP.

Cell Proliferation: Evidence to support the cell proliferation mechanism was based on the report by Christenson *et al.* (1996b). In the study by Christenson *et al.* (1996b), the number of cells undergoing DNA replication (as indicated by the BrdU-labeling index) in male rat urinary bladder after 13-week of OPP exposure at 8000 ppm in the diet was increased by 5-fold ($p<0.05$) whereas the increase at 4000 ppm was not significant. Assuming that a threshold exists for the proposed non-genotoxic MOA, this 4000 ppm can be considered as the pre-neoplastic threshold (Dow and Bayer, 2004). On the other hand, data from long-term studies would argue against setting the 4000 ppm as a threshold. Although Wahle and Christenson (1996) reported markedly increased incidences of cell proliferation and tumors and shortened latency of tumor development in male rats from 4000 to 8000 ppm OPP in the diet, the effects at 4000 ppm cannot be dismissed. After 2 years of exposure, the incidence at 4000 ppm was 12% (6/50) for simple hyperplasia and 4% (2/50) for carcinoma whereas at 8000 ppm, 84% had simple hyperplasia and 80% had bladder tumors (papilloma and carcinoma combined) (Wahle and Christenson, 1996).

A key component for advocating threshold for an MOA that involves cell proliferation is the "reversibility" of the cellular events. Christenson *et al* (1996a) reported decreased incidence of hyperplasia four weeks after the cessation of 13 weeks of dietary exposure at 12500 ppm OPP. However, reversible hyperplasia is not supportable by the observations by Niho *et al.* (2002). After a 88-week recovery period, rats that had received 20000 ppm of SOPP in the diet for 24 weeks exhibited simple hyperplasia, papillary and (or) nodular hyperplasia, as well as carcinoma. These results would argue for a genotoxic mechanism that irreversibly altered the urothelial cells during the 24 weeks of exposure and progressed into lesions, including carcinoma.

Conclusions Collectively, data in the above three areas strongly suggested that OPP carcinogenic MOA in the rat urinary bladder may be operated mainly through a two-event model as proposed by Greenfield *et al.* (1984). That is, reactive species, including ROS (reactive oxygen species) derived mainly from the pH-dependent PHQ autoxidation may induce genetic damage in the urinary bladder of rats at lower doses. At higher doses, the proliferation of bladder epithelial cells magnified the genetic damage, an effect that may have been due to the cytotoxic effects of PHQ and other metabolites (e.g., PBQ) that also were found in the urine of rats treated with OPP or SOPP. Bioassay data showed that OPP was capable of inducing urinary bladder tumors in at least two stains of rats: F344 and Sprague-Dawley (SD).

IV.A.5. Oncogenicity Weight of Evidence of OPP and SOPP

The following three areas are used in considering the overall weight of evidence (WOE) considerations (USEPA, 2005):

- Tumor findings in humans and laboratory animals
- *In vivo and in vitro* Genotoxicity data
- Potential MOAs and human relevance

1. Tumor findings in humans and animals: No data in humans are available for assessing the oncogenicity potential of OPP and SOPP. Multiple oncogenicity studies in F344 rats showed that repeated exposure to OPP or SOPP in the diet resulted in urinary bladder tumors (papilloma and carcinoma) in both sexes and, at relatively high dose levels, kidney tumors (renal papilla and pelvis carcinoma) in the males. Both urinary and kidney tumors are rare tumors with very low historical incidence of 2/409 (0.5%) (Whale and Christenson, 1996) and 2/1928 (0.1%) (Haseman *et al.*, 1990), respectively. Urinary bladder tumors occurred in SD rats treated with OPP. Oncogenicity studies in B6C3F1 mice showed that dietary exposure to OPP caused increased liver tumors (adenoma, carcinoma, and hepatoblastoma [rare carcinoma variant]) and (or) tumor multiplicity in both sexes, and circulatory system tumors (hemangiomas) in the male. Overall, these animal studies showed that OPP and SOPP exhibited strong evidence of carcinogenicity (i.e., neoplasms occurred in multiple species, strains, sexes, and sites).

2. Genotoxicity data: The available *in vitro* data indicated OPP itself was positive for intercalation and complex formation with isolated DNA, for nonspecific DNA damage in bacteria, and for sister chromatid exchange (SCE), gene mutation, and chromosomal aberrations in mammalian cells. *In vitro* tests also showed that metabolic transformation of OPP resulted in the formation of highly reactive species (e.g., phenylhydroquinone [PHQ], phenylbenzoquinone [PBQ], and reactive oxygen species [ROS]) that interact with macromolecules including DNA. These reactive species may have contributed to the enhanced OPP genotoxicity in the presence of metabolic activation *in vitro* as well as damages to DNA (i.e., adduction and breakage) and chromosomes (i.e., clastogenic and aneugenic effects) in rat urinary bladder (i.e., target organ) *in vivo*. The genotoxic effect of OPP and its metabolites are consistent with the agent's multi-sites and multi-species carcinogenic activity.

3. Potential MOA and Human Relevance: Of all tumor endpoints identified, data are only sufficient for evaluating the MOA for the urinary bladder tumors. A detailed discussion has been presented in the previous section. In summary, two potential MOA have been proposed that implicated opposing assumptions to the subsequent step of dose-response modeling. One MOA considered that the tumor induction was due to regenerative cell growth caused by cytotoxic OPP metabolites produced only at the higher doses. This mechanism, however, failed to address the experimental results that showed the conversion of OPP to PHQ in dose-dependent manner at all doses tested and that urinary concentration of PHQ in the rat was similar to that reported to induce DNA breakage, nucleotide oxidation, and (or) chromosomal aberrations *in vitro*. The alternative MOA entailed both genotoxic initiation as well as tumor promotion through cell proliferation. Evidence indicated that reactive species derived from pH-dependent autoxidation of PHQ

may have induced genetic damage in the urinary bladder of rats at lower doses and the proliferation of bladder epithelial cells magnified the genetic damage due to the cytotoxic effects of PHQ (and other metabolites [e.g., PBQ]) at higher doses. Currently available information is insufficient to clearly identify the mutagenic events at lower doses *in vivo*. However, a genotoxic MOA is consistent with the concerns that genotoxic PHQ increased with dose and that its enzymatic and non-enzymatic transformations resulted in multiple reactive species (e.g., PBQ and ROS) that can interact with cellular macromolecules including DNA.

Regarding human relevance of the above genotoxic MOA, the existing human data showed: (1) urinary bladder was a target organ after an acute exposure to phenylphenols; (2) excretion of the absorbed OPP occurred mainly *via* urine; (3) the OPP-to-PHQ transformation was operative in humans; and (4) some individuals excreted urine with pH ≥7 and dietary factors could modify the pH (Kadlubar *et al.*, 1977; Derelanko and Hollinger, 1995; Remer and Manz, 1995).

Taken together, data from the above three areas, and according to USEPA's 2005 carcinogen classification, OPP belongs to the category of Likely to be Carcinogenic in Humans.

USEPA's 1994 and 2006 Carcinogenicity Review

Based on evidence of multiple tumor types in multiple studies, USEPA published a review in 1994 and classified the carcinogenic potentials of OPP and SOPP according to the 1986 Guidelines for Carcinogen Risk Assessment (USEPA, 1986) as Group B2 (i.e., probable human carcinogen) (Rinde and Dapson, 1994). In the review, USEPA considered that the linear extrapolation model for projecting human health risk was inappropriate. This was based on the indication that PHQ, a penultimate carcinogen, was formed only at ≥250 mg/kg/day dietary OPP (i.e., 5000 ppm) and that the tumorigenic response in rats also occurred only at or above this level of OPP in the diet. Hence, USEPA considered the tumorigenic effect observed in rats irrelevant to the anticipated human exposure and applied a threshold approach to the dose-response and risk assessment (Rinde and Dapson, 1994).

In the 2006 OPP risk assessment (USEPA, 2006), USEPA applied the agency's 2005 Guidelines for Carcinogen Risk Assessment (USEPA, 2005) and changed OPP and SOPP from the 1994 carcinogenicity designation of Group 2B to a new classification - "likely to be carcinogenic to humans at high doses, unlikely at low doses" (Kidwell, 2005; USEPA, 2006). As in 1994, USEPA retained the threshold approach to characterize the carcinogenic risk in humans but moved the threshold from 250 mg/kg/day to 200 mg/kg/day OPP. The rationale is that PHQ (the penultimate carcinogen) and (or) PBQ was formed in rats at higher (>200 mg/kg/day) OPP doses due to the saturation of Phase II detoxification enzyme pathways and that the urinary tumors were caused by sufficient amount of urinary quinone metabolites, not direct genotoxicity. Regarding the mouse liver tumors, USEPA determined that a threshold risk characterization approach was also applicable although the Agency considered its MOA as unknown. Without detail discussion, the Joint FAO/WHO Meeting on Pesticide Residues

(JMPR) also concluded that urinary bladder tumors in rats and liver tumor in mice operated *via* a threshold MOA (FAO and WHO, 2000).

VI.A.6 Carcinogenic Dose response Assessment

As described previously (IV.A.4. Mode of Action for Urinary Bladder Tumors), the overall data indicated that there is insufficient evidence to conclude that non-genotoxic MOA is the only probable pathway for the oncogenicity of OPP and SOPP. In fact, this Risk Characterization Document (RCD) presented a strong argument that the initial stage of bladder tumors involved a genotoxic mechanism. The latest USEPA Guideline for Carcinogen Risk Assessment stated that "*linear extrapolation is used as a default approach for characterizing the cancer risk when the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data* (USEPA, 2005)." Applying this same guidance, the lack of MOA information on the liver tumors in mice also points toward a low-dose linear default. Therefore, DPR determined that the low-dose linearity model should be used for characterizing the human risk associated with exposures to OPP or SOPP.

Based on the Guidelines for Carcinogen Risk Assessment (USEPA 2005), DPR employed the benchmark dose (BMD) approach for evaluating the carcinogenic risks of OPP. This analysis applied all available models (Multistage, Logistics, Probit, Weibull, Quantal-Linear, and Quantal Quadratic) in the U.S. EPA Benchmark Dose software version 1.3.1 (USEPA, 2000) to the combined incidence of urinary bladder papilloma and carcinoma in rats from the study by Wahle and Christenson (1996). DPR chose the Logistic model as the best fit based on several criteria that included considerations of Chi-square goodness-of-fit ($p>0.05$), Akaike's Information Criterion (lowest value), and Chi-square residual (DPR MT-1, 2004). The modeling was based on "extra risk" above the background rate. Determination of potency slopes at the point-of-departure (POD) of 10% response was at the effective dose (ED_{10}) and its 95th lower bound (LED_{10}), i.e., the best estimate of slope at $0.1/ED_{10}$ and its upper bound at $0.1/LED_{10}$. Based on the ED_{10} of 222.8 mg/kg/day and LED_{10} of 185.2 mg/kg/day, the best estimate and upper bound potency slope was 4.5×10^{-4} (mg/kg/day OPP)$^{-1}$ and 5.4×10^{-4} (mg/kg/day OPP)$^{-1}$, respectively. Using the default assumption that body weight to the ¾ power is the basis for interspecies dose equivalence (USEPA, 1992), the extrapolation of potency slope to humans was calculated by applying a scaling factor of body weight ratio to the ¼ power ($[BWt_H/BWt_A]^{0.25} = [70kg/0.35kg]^{0.25} = 3.76$]. Thus, the DPR estimated human potency was 1.7×10^{-3} (mg/kg/day OPP)$^{-1}$ at the best estimate and its upper bound at 2.0×10^{-3} (mg/kg/day OPP)$^{-1}$. It is noted that SOPP exhibited a higher tumorigenicity than OPP in the urinary bladder of rats. In 1984, USEPA (1984) derived an OPP-equivalent human potency slope of 1.94×10^{-3} (mg/kg/day OPP^{-1})[54] based on linearized multistage model and urinary bladder tumor in SOPP-treated rats from an open literature study (Hiraga and Fujii, 1981). In this risk assessment, DPR derived an OPP-equivalent human potency slope of 1.4×10^{-3} (mg/kg/day OPP^{-1}) using a best

[54] The same value also was obtained by OEHHA of the CalEPA (OEHHA, 1992)

fitted BMD model (i.e., Probit model) and urinary bladder tumor in SOPP-treated rats from another published study (Niho *et al.*, 2002). Compared to the potency slope derived from the study of OPP-treated rats, these values from the SOPP studies were not higher. Therefore, until additional data are available, this risk assessment uses 2.0 x 10^{-3} (mg/kg/day OPP^{-1}) for quantifying the cancer risk associated with OPP and SOPP exposures in humans.

As described in the III.D. CHRONIC TOXICITY/ONCOGENICITY, there appeared to be an early onset of urinary bladder tumors in OPP- and SOPP-exposed rats. Hence, DPR also analyzed the tumor data of Wahle and Christenson (1996) using a time-to-tumor multistage model (i.e., multistage-Weibull model) that has the following form.

$$\frac{[P(d)-P(0)]}{[1-P(0)]} = 1-\exp[-(q_o + q_i d + ... + q_k d^k)(t-t_o)^c]$$

where $c \geq 1$ and $t_o \geq 0$, and $q_i \geq o$ for I=0, 1, …, k, where k = the number of dose groups –1. This analysis assumes that all tumors are either lethal or incidental because the study pathologist made no determination on the cause of death. Using the lethal assumption, the best estimate of potency slope from the time-to-tumor multistage model was 1.6 x 10^{-4} and its 95% upper bound was 6.3 x 10^{-4} (mg/kg/day $OPP)^{-1}$ whereas the best estimate using the incidental assumption was 1.1 x 10^{-4} and its 95% upper bound was 4.6 x 10^{-4} (mg/kg/day $OPP)^{-1}$. As these values are similar to the model output without time-to-tumor considerations, the apparent early tumor onset did not impact potency slope determination.

IV.B. EXPOSURE ASSESSMENT

IV.B.1. Introduction

The Department of Pesticide Regulation (DPR) conducts dietary exposure assessments for evaluating the risk of human exposure to a pesticide in food and water in California (Bronzan and Jones, 1989). Currently, DPR use two approaches to assess the human exposure: the total dietary exposure based on measured residue levels on all label-approved commodities and the exposure to an individual commodity at the tolerance level (DPR MT-3, 2006).

IV.B.2. Consumption Data and Dietary Exposure

Dietary exposure is a product of the concentration of pesticide residue in food and the amount of food consumed; the latter varies with the individual's age, gender, ethnicity, physiological status, and (or) physical locality. Both acute and chronic dietary exposures to the pesticide-containing foods can occur in humans. For estimating the acute exposure in a population, DPR employ two approaches: point estimate analysis and distributional analysis (see also IV.B.4. ACUTE EXPOSURE). In the point estimate analysis, the calculation uses fixed residue level for each commodity and distribution of per-user (i.e., consumers) consumption rates (e.g., 95th, 97.5^{th}, and 99th percentiles). In a distributional analysis, the residue level is a distribution for a particular commodity and the exposure is calculated using Monte Carlo method. For estimating the chronic exposure in a population, the calculation uses the average residue value for each commodity and per-capita (i.e., both consumers and non-consumers) average daily consumption. In this assessment, the dietary exposures (and the subsequent risk estimates) covered the average U.S. population and 15 selected-population subgroups.

DPR uses a computer program to calculate the dietary exposure (and subsequent risk). In this exposure assessment, the computer program was Dietary Exposure Evaluation Model (DEEM™ v. 7.74, Exponent Inc.). The program calculated the exposure using user-input residue data (see below IV.B.3. Residue Data Sources) and food consumption pattern based on data generated by the United States Department of Agriculture (USDA) during the 1994-1998 Continuing Survey of Food Intake by Individuals (CSFII). The CSFII 1994-98 is the most recent and representative consumption database, which provides information on a 2-day food intake by 20,607 individuals of all ages from 62 geographical areas. The database consists of the 1994-1996 food consumption survey, along with the 1998 Supplemental Children's Survey (CSFII 1998), which includes additional 5,559 children from birth to 9 years old.

In addition to the dietary exposure, the DEEM™ has an Acute Module, which allows the determination of those foods having the greatest contribution to the total exposure of individuals. Critical Exposure Commodity (CEC) analysis in the Acute Module is an analytical tool that provides information on the amount of food consumed, body weight, age, residue values, and the exposure estimate by individual food item. Based on this information, the module identifies

individuals at the high end of dietary exposure (in the top 5% or less) as well as the commodities contributing to this level of dietary exposure.

IV.B.3. Residue Data Sources

Currently, USEPA has established tolerances for combined residues of OPP and SOPP on 22 raw agricultural commodities (RAC): apple, cantaloupe, carrots, cherry, citrus, citron, cucumber, grapefruit, kiwifruit, kumquat, lemon, lime, nectarine, oranges, bell pepper, peach, pear, pineapple, fresh plum and prune, sweet potato, tangerine, and tomato (CFR, 2005). In California, three OPP- and eight SOPP-containing products are available for post-harvest treatments of these RAC (DPR, 2006). The monitoring programs for pesticide residue that DPR could use in the dietary risk assessment (DPR MT-3, 2006) are the following (listed in the order of preference).

- USDA Pesticide Data Program (PDP)
- DPR Priority Pesticide and Market Basket Surveillance Programs
- Food and Drug Administrations (FDA) Regulatory Residue Monitoring Program

For pesticide exposure from drinking water, the PDP is also one of main sources. Another data source is the DPR Groundwater and Surface Water Monitoring Program (DPR, 2004).

IV.B.3.a Pesticide Data Program (PDP)

For pesticide residue analyses, the USDA's PDP Program collects samples at product markets and chain-store distribution centers close to the consumer level in ten states, including California. In addition, the analyses employed sample preparation procedures similar to the typical consumer practices to more closely represent actual exposure to residues (e.g., oranges are peeled). Hence, the residue data generated are the most representative for risk assessment. For OPP- and SOPP-treated commodities, PDP reported only residue of OPP (see also IV.B.4. ACUTE EXPOSURE).

Over the 1996-2002 monitoring periods, PDP examined thirteen RAC with legal tolerances for OPP and seven processed products among ten participating states (USDA 1998-2004). These RAC and processed products had detectable residues, except for cherries (Table 50), and the limit of detection (LOD) values varied from 0.003 to 0.066 ppm among the national laboratories contracted by USDA to perform these analyses. Among the RAC with detected residues, the contracted laboratory in California reported the highest residues (unit in ppm) in carrots (0.017), cucumbers (4.7), sweet bell peppers (0.094), pears (11), canned pears (0.017), and pineapples (0.017); lowest in orange juice, canned peaches, and sweet potatoes (0.017 in all); but none in peaches. Also, the California laboratory analyzed no samples of apple, apple-sauce, and canned tomato paste.

Table 50 Anticipated OPP Residues Used for Acute and Chronic Dietary Exposure Assessments

Commodity	Source of Data	Year	Number Samples	Number Detected Samples	Detected Residues (ppm)	Range LOD (ppm)	Adjustment Factor[a]	Acute Point Estimate Residue (ppm)	Chronic Average Residue (ppm)
Apple	PDP	2002	556	16	0.018-0.2	0.011-0.025	8 for dry apple	0.2	0.0125
		2001	736	7					
		2000	184	2					
RTS Apple Juice	PDP	2002	729	8	0.005-0.038	0.003-0.015	3 for juice concentrate	0.038	0.0048
		1998	603	10					
		1997	590	32					
Apple Sauce[55]	PDP	2002	358	13	0.017	0.01	1	0.017	0.0054
Cantaloupes	PDP	2000	120	3	0.005-0.21	0.003-0.015	1	0.21	0.0063
		1999	626	25					
		1998	354	29					
Carrots	PDP	2002	554	17	0.005-0.017	0.003-0.01	1	0.017	0.0026
		2001	739	4					
		2000	184	1					
Cherries	PDP	2001	286	0	No detectable residue	0.003-0.015	1.5 for cherry juice; 4 for dry fruit	0.015	0.0046
		2000	275	0					
Citrus and Citron	PDP data for oranges (Citrus Fruit Group 10)[b]						1		
Cucumbers	PDP	2001	183	2	0.017-4.7	0.01-0.025	1	4.7	0.0283
		2000	737	32					
		1999	551	44					
Grapefruit	PDP data for oranges and orange juices (Citrus Fruit Group 10)[b]						4 for juice concentrate		
Kiwifruit	Tolerance						1	20	10

[55] The DEEM™ v. 7.74 does not have applesauce as one of the food-form entries. Hence, this exposure assessment did not use the data.

Table 50 Anticipated OPP Residues Used for Acute and Chronic Dietary Exposure Assessments (Continued)

Commodity	Source of Data	Year	Number Samples	Number Detected Samples	Detected Residues (ppm)	Range LOD (ppm)	Adjustment Factor[a]	Acute Point Estimate Residue (ppm)	Chronic Average Residue (ppm)
Kumquats	PDP data for oranges (Citrus Fruit Group 10)[b]						1		
Lemons	PDP data for oranges and orange juice (Citrus Fruit Group 10)[b]						2 for lemon juice; 5.7 for juice concentrate		
Limes	PDP data for oranges and orange juices (Citrus Fruit Group 10)[b]						3 for juice concentrate		
Nectarines	PDP	2001	359	4	0.011-0.022	0.01	1	0.022	0.0051
		2000	345	0					
Oranges	PDP	2001	745	14	0.006-3.6	0.005-0.066	3.72 for juice concentrate	3.6	0.0143
		2000	744	33					
		1996	454	66					
RTS Orange Juice	PDP	1998	611	5	0.017-0.033	0.01-0.05	1	0.033	0.0082
		1997	601	16					
Sweet Bell Peppers	PDP	2002	186	5	0.005-0.094	0.003-0.02	1	0.094	0.0053
		2000	308	11					
		1999	730	9					
Peaches	PDP	2002	563	41	0.005-0.046	0.003-0.021	7 for dry peaches	0.046	0.0029
		2001	529	11					
		1996	229	0					
Peaches, Canned	PDP	1997	654	21	0.017-0.033	0.01-0.021		0.033	0.0083
Pears	PDP	1999	270	54	0.005-11	0.003-0.015	6.25 for dried pears	11	0.1896
		1998	604	120					
		1997	614	154					

Table 50 Anticipated OPP Residues Used for Acute and Chronic Dietary Exposure Assessments (Continued)

Commodity	Source of Data	Year	Number Samples	Number Detected Samples	Detected Residues (ppm)	Range LOD (ppm)	Adjustment Factor[a]	Acute Point Estimate Residue (ppm)	Chronic Average Residue (ppm)
Pears, Canned	PDP	2000	366	16	0.013-0.017	0.008-0.01		0.017	0.0048
		1999	281	7					
Pineapples	PDP	2002	360	8	0.017	0.01	5 for dry pineapple; 1.7 for juice; 6.3 for juice concentrate	0.017	0.0051
		2001	730	8					
		2000	364	0					
Plums	PDP data for peaches (Stone Fruit Group 12)[c]						5 for dry plums; 1.4 for juice		
Sweet potatoes	PDP	1998	309	1	0.017-0.1	0.003-0.065	1	0.1	0.0051
		1997	671	10					
		1996	507	4					
Tangerines	PDP data for oranges and orange juices (Citrus Fruit Group 10)[b]						3.2 for juice concentrate		
Tomatoes	PDP	1999	364	4	0.005-0.51	0.003-0.05	14.3 for dried tomato	0.51	0.0085
		1998	626	28					
		1997	627	39					
Tomatoes, Canned	PDP	2000	369	7	0.007-0.025	0.004-0.02	1.5, 2.5, & 3.3 for tomato juice, catsup & puree, respectively.	0.025	0.0081
		1999	368	34					
Tomato Paste, Canned	PDP	2001	369	11	0.021-0.055	0.02	1	0.055	0.0106

Abbreviations: LOD, limit of detection; N/A, not available.

[a] DEEM™ default factors to account for changes in the hydration state of fruits and vegetables.

[b] Citrus Fruit Group 10 as defined in the 40 CFR 180.41: calamondin; citrus citron; citrus hybrids (includes chironja, tangelo, tangor); grapefruit; kumquat; lemon; lime; mandarin (tangerine); orange, sour; orange, sweet; pummelo; Satsuma mandarin.

[c] Stone Fruit Group 12 as defined in 40 CFR 180.41: apricot; cherry, sweet; cherry, tart; nectarine; peach; plum; plum, Chickasaw; plum, Damson; plum, Japanese; plumcot; prune (fresh).

PDP also monitored pesticide residue in drinking water systems starting in the year 2001 (USDA, 2003). At present, however, OPP is not on the pesticide-monitoring list.

IV.B.3.b. DPR Monitoring Programs

Regarding the DPR monitoring programs, Priority Pesticide and Marketplace Surveillance do not routinely monitor OPP. Also, OPP is not on the monitoring list of Pesticide Groundwater and Surface Water Programs (DPR, 2004). Hence, no OPP residue data are available in these DPR databases.

IV.B.3.c. FDA Regulatory Residue Monitoring Program

Another source of OPP residue data is FDA Surveillance Monitoring Program. Unlike PDP, the design for FDA sampling is for tolerance enforcement purposes; hence, the samples that are collected at large-scale distribution center often represent "farm gate" residues. In addition, the analyses employ procedures that involve no washing or peeling, as opposed to typical consumer practices. Because of these differences, DPR only use the FDA data when there are no data available from the PDP. In this exposure assessment, while some residue data are available from the FDA monitoring program, DPR did not use the data because of inadequate sample sizes (i.e., <100).

IV.B.4. Acute Exposure

For estimating the acute dietary exposure to a pesticide, DPR employs a tiered approach for the selection of appropriate residue values (DPR MT-3, 2006). The approach begins with the point estimate (deterministic) assessments (i.e., Tiers 1 to 2), which are generally less time-consuming and less labor intensive than the refining assessment (Tier 3). The Tiers 1 and 2 point estimate assessments employ the tolerance and the highest measured value (or the mean residue value), respectively. If needed, Tier 3 Monte Carlo probabilistic assessment allows refinement of the exposure estimates, taking into account the occurrence and distribution of residue levels, and provides the probability distribution of exposure.

For the acute deterministic exposure assessment, DPR establish two thresholds to determine whether next tier of assessment is needed (DPR MT-3, 2006). These thresholds are the following: (1) the margin-of-exposure (MOE) at the 99th percentile for all foods is within 5-fold of the health protective level; or (2) the MOE at the 97.5th percentile exposure for all food and at the 95th percentile exposure for each of the two high exposure commodities are 10-fold higher than the health protective level. In general, for NOEL based on laboratory animal studies, the acceptable MOE is 100. Therefore, for any population subgroup, the threshold MOE for the next tier of assessment would be ≤500 at the 99th percentile exposure. These thresholds should provide sufficient margin of safety for exposures from other potential routes and the 5-and 10-

fold distance from the acceptable MOE ensures the likelihood that dietary exposure would not be a major contributor to the aggregate risk. In the event that this is not the case, the next tier of exposure may be needed.

In this acute (and also chronic) exposure assessment, DPR assume that

the high pKa value of OPP (9.55 [Dean, 1987]) and the poorly buffered environment employed during the application of SOPP (

Tier 1 Point Estimate Assessment

This model assumes that all foods consumed in a given day contain pesticide residues at the tolerance level. For OPP, using estimated dietary exposure values in each of the population subgroups, this analysis produced exposures ranging from 0.12 to 1.05 mg/kg/day at the 95th percentile, 0.16 to 1.39 mg/kg/day at the 97.5th, and 0.21 to 1.72 mg/kg/day at the 99th percentiles (Table 51). As discussed later (IV.C.2. Dietary Risk), these exposures resulted in MOEs in all of the population subgroups below the threshold for initiating next tier of exposure assessment. Therefore, the next step used was Tier 2 point estimate.

Tier 2 Point Estimate Assessment

For the Tier 2 assessment, DPR employs estimated dietary exposure values in each of the population subgroups and several assumptions. These assumptions are the following: (1) all consumed foods contain the highest reported residue below the tolerance; (2) pesticide residues below the LOD are equal to that limit; (3) all crops are pesticide treated; and (4) residue concentrations do not vary from time of sampling to the time of consumption. Consequently, in the case of OPP, the residue selection (i.e., acute point estimate residues [ppm]) for RAC within the PDP database was either highest measured values or highest LOD (Table 50). For commodities where regulatory monitoring data were either not available or the residue information was not sufficient to be used in the dietary analysis, the DPR guideline for dietary exposure assessment recommends the use of residues on surrogate commodities (DPR MT-3, 2006). Based on the classification of related raw agricultural commodities into crop groups (as established in 40 CFR 180.40) and the agricultural practice specified in the product labels, suitable surrogate for tangerine, lime, lemon, kumquat, and citrus citron was orange (all belong to Citrus Fruit Group 10) and surrogate for plum was peach (both belong to Stone Fruit Group12). Hence, DPR assume that the residues in tangerine, lime, lemon, kumquat, and citrus citron are the same as orange and the residue in plum is the same as peach. For kiwifruit, there are no regulatory monitoring data and suitable surrogate. In the United States, ≥97% of the kiwifruit production occurred in California at 2002-2003 (CDFA, 2002; CASS, 2003); however, the State's growers reported no use of OPP and (or) SOPP (DPR, 2006). It should be noted that the

Table 51 Acute Dietary Exposure Estimates for OPP

Population Subgroup	Acute Dietary Exposure[a] (mg/kg/day)					
	Point Estimate Tier 1[b] (percentile)			**Point Estimate Tier 2[b] (percentile)**		
	95th	97.5th	99th	95th	97.5th	99th
US Population (all seasons)	0.244	0.386	0.634	0.012	0.023	0.037
Western Region	0.255	0.395	0.652	0.018	0.030	0.047
Hispanics	0.297	0.433	0.693	0.018	0.030	0.045
Non-Hispanic Whites	0.228	0.365	0.616	0.012	0.021	0.033
Non-Hispanic Blacks	0.274	0.435	0.695	0.008	0.018	0.031
Non-Hispanic Other	0.294	0.431	0.700	0.027	0.037	0.053
All infants	0.897	1.060	1.354	0.013	0.019	0.047
Infants (nursing, <1yr.)	0.842	0.938	1.200	0.008	0.026	0.067
Infants (non-nursing, <1yr.)	0.915	1.099	1.529	0.013	0.019	0.046
	1.050	1.385	1.724			
	0.705	0.875	1.198			
Children (6-12 yrs)	0.307	0.420	0.601	0.020	0.037	0.055
Youth 13-19 yrs	0.172	0.243	0.316	0.007	0.013	0.031
Adults 20-49 yrs	0.127	0.166	0.220	0.009	0.015	0.025
Adults 50+ yrs	0.123	0.155	0.213	0.013	0.022	0.030
Females (13-49 yrs)	0.138	0.181	0.260	0.009	0.015	0.028

Note: Shading identifies the two highest exposure population subgroups.
[a] DEEM™ program was used for the analysis with the USDA CSFII from 1994-1998.
[b] DPR calculated the acute Point Estimate dietary exposure for all population subgroups from all commodities with OPP registrations using user-days consumption instead of per-capita consumption that was used by the USEPA.

domestic grown kiwifruit only accounted for 28-36% the total sale in the United States in 1998-2000 (FAS, 2000). Hence, because the anticipated residue on kiwifruit due to the imports from other countries (e.g., New Zealand) cannot be ignored, the Tier 2 exposure analysis used tolerance value to represent the residue.

In addition to the pesticide application, changes in the state of hydration could alter the residue concentration in food. The DEEM™ Acute (and Chronic) Module has default adjustment factors to account for concentration of OPP due to change in the food hydration. The computer program calculates the residue values of dehydrated foods using the adjustment factors and RAC residues in Table 50. The commodities with adjustment factor applied were dried fruits (apple, cherry, peach, plum, pineapple, and tomato), juices (cherry, lemon, grapefruit, and lime), and different processed tomato products (paste, catsup, and puree) (Table 50). However, for dried pear and kiwifruit, the application of hydration factor resulted in their OPP residues at or above the tolerance; therefore, the Tier 2 exposure analysis used no hydration factor for these commodities.

Based on the Tier 2 assessment, the 95th percentile user-day exposures to OPP ranged from 0.007-0.04 mg/kg/day. At the 97.5th and 99th percentiles, the exposures ranged from 0.013-0.07 mg/kg/day and 0.025-0.116 mg/kg/day, respectively. In each of these percentiles, "Children 1-2 years" followed by "Children 3-5 years" were the two most highly exposed population subgroups (Table 51). Results of the acute CEC analysis indicated that pear, orange, kiwifruit, and (or) cucumber contributed substantially (84-96%) to the dietary exposure to OPP in each of the population subgroups.

Pears in the food-forms of uncooked, cooked, uncooked juice, and (or) canned juice appeared to be the most significant contributor to the dietary OPP exposure in the following population subgroups (percent contribution in parentheses): U.S. Population, Western Region (49%), Hispanics (44%), Non-Hispanics Whites (52%), Non-Hispanics Blacks (47%), Non-Hispanics Others (59%), All Infants (66%), Nursing Infants (74%), Non-Nursing Infants (63%), Children 1-2 yrs (68%), Children 3-5 yrs (58%), Children 6-12 yrs (48%), Youth 13-19 yrs (22%), Adults 20-49 yrs (41%), Adults 50+ yrs (59%), and Females 13-49 yrs (36%).

Like the pears, peeled orange (uncooked) made a significant contribution to the dietary exposures for these same population subgroups. The respective percent contributions were 29% (U.S. Population, Western Region), 40% (Hispanics), 18% (Non-Hispanics Whites), 36% (Non-Hispanics Blacks), 19% (Hispanics Others), 17% (All Infants), 13% (Nursing Infants), 18% (Non-Nursing Infants), 16% (Children 1-2 yrs), 20% (Children 3-5 yrs), 26% (Children 6-12 yrs), 27% (Youth 13-19 yrs), 21% (Adults 20-49 yrs), 11% (Adults 50+ yrs), and 22% (Females 13-49 yrs).

Cucumber in its food-forms of uncooked and canned (cured) accounted for less than 4% of the dietary exposure to OPP of Hispanics and children under the ages of ≤2 including infants. However, it contributed significantly to the dietary OPP exposure in the following population subgroups (percent contribution in parentheses): U.S. Population, Western Region (6%),

Non-Hispanic White (11%), Non-Hispanic Blacks (6%), Non-Hispanic Others (11%), Children 3-5 yrs (8%), Children 6-12 yrs (9%), Youth 13-19 yrs (25%), Adults 20-49 yrs (14%), Adults 50+ yrs (8%), and Females 13-49 yrs (15%).

Except for the non-Hispanics Blacks and Nursing-Infants subgroups wherein kiwifruit (uncooked) contributed <3% dietary exposure to OPP, the exposure in all other population subgroups were 5-13%. Regarding the kiwifruits, the high exposure, however, may be in part due to the use of tolerance value. As discussed later (IV.C.2. Dietary Risk), the Tier 2 analysis produced exposures resulting in MOEs in all of the population subgroups above the threshold for initiating next tier of exposure assessment. Therefore, the estimation of OPP exposure needs no further refinement.

IV.B.5. Chronic Exposure

In chronic exposure, DPR employ a default assumption that for repeated exposure over time, the residue for all commodities for which a tolerance has been established can be equivalent to some average level at or below tolerance (DPR MT-3, 2006). Also, DPR assume that the population average daily consumption distribution calculated from the 2-day cross-sectional consumption survey in a population reflects the longitudinal consumption patterns. Hence, DPR use the arithmetic mean of the measured pesticide concentrations to estimate the combined exposure from all commodities on which OPP can be used. For RAC with no detectable residue, DPR assign 1/2 of the LOD to the samples with residue below the limit of detection to account for possible quantifiable exposures. For RAC with no residue data available, the default assignment is 1/2 tolerance. Also, assumed percent-crop-treated (PCT) is 100%. Table 50 summarizes the anticipated OPP residues (i.e., chronic average residues [ppm]) that DPR calculates using the residue data reported by the PDP participating laboratories in 10 states, along with the LOD. Based on the paradigm used in this analysis, the estimated chronic exposures to OPP ranged from 0.051 (nursing infants) to 0.417 μg/kg/day (Children 1-2 yrs) (Table 52), which is almost identical to the values obtained by using California-specific data (i.e., 0.055 [nursing infants] to 0.422 μg/kg/day [Children 1-2 yrs]).

IV.B.6. Lifetime Exposure

Dietary exposure assessment for oncogenic endpoint is identical to the assessment of chronic exposure except for the assumption that combined consumption of the entire U.S. population represents daily exposure for individuals over a lifetime (DPR MT-3, 2006). With DEEM, the lifetime exposure (i.e., amortized age-based exposure) is the same as the chronic exposure output for the "U.S. population (all seasons)." As can be seen in Table 52, the estimated lifetime exposure of the U.S. population (all seasons) was 0.121 μg/kg/day.

Table 52 Chronic Dietary Exposure Estimates for OPP

Population Subgroup	Chronic Exposure (μg/kg/day)[a]
US Population (all seasons)	0.121
Western Region	0.153
Hispanics	0.117
Non-Hispanic Whites	0.126
Non-Hispanic Blacks	0.074
Non-Hispanic Other	0.176
All infants	0.103
Infants (nursing, <1yr.)	0.051
Infants (non-nursing,<1yr.)	0.123
Children (3-5 yrs)	0.335
Children (6-12 yrs)	0.225
Youth 13-19 yrs	0.1
Adults 20-49 yrs	0.075
Adults 50+ yrs	0.091
Females (13-49 yrs)	0.093

Note: Shading identifies the highest exposure population subgroup.
[a] The DEEM™ program was used for the analysis with the USDA CSFII from 1994-1998.

IV.C. RISK CHARACTERIZATION

DPR uses two approaches to characterize the dietary risk associated with exposure to OPP and SOPP in humans: margin-of-exposure (MOE) for acute and chronic exposures and excess cancer risk for the lifetime exposure. The MOE is a ratio of the exposure-specific critical NOEL derived from an experimental toxicity study and an estimate of a human exposure. For evaluating the toxicity due to acute exposure, this assessment used two critical NOELs: 25 mg/kg/day for characterizing health risk in women in their childbearing years (i.e., effect that occurred during pregnancy) and 150 mg/kg/day for characterizing health risk in the general population including infants, children, and adult males. The corresponding toxicological endpoints that DPR determined as the most critical (i.e., "sensitive") were resorption in rabbits (i.e., *in utero* effect) and ataxia and decreased body weight gain in rats (acute toxic effects). For the chronic exposure, this risk assessment also used two critical NOELs: 39 mg/kg/day for characterizing health risk in the males and 4.9 mg/kg/day in the females. In the former, the toxicological endpoints were clinical pathology associated with polydipsia and kidney effects and simple hyperplasia in the urinary bladder of rats whereas in the later, the toxicological endpoint was cardiomyopathy. For the cancer risk assessment, DPR calculated a slope factor of 0.002 $(mg/kg/day\ OPP)^{-1}$ [in human equivalent] using low-dose extrapolation method that modeled tumor dose-response in the urinary bladder of rats.

IV.C.1. Acute Exposure

Tier 1 Point Estimate Assessment

As mentioned previously, this model assumes that all foods consumed in a given day contain pesticide residues at the tolerance level. For OPP, using the NOEL of 150 mg/kg/day and estimated dietary exposure values in each of the population subgroups, this analysis produced MOEs ranging from 142 to 1215 at the 95th, 108 to 965 at the 97.5th, and 87 to 704 at the 99th percentiles in population subgroups excluding Females 13-49 yrs (Table 53). The MOEs of Females 13-49 yrs were 181, 133, and 96 at 95th, 97.5th and 99th percentiles, respectively, based on the NOEL of 25 mg/kg/day. These MOEs were below the thresholds at the 97.5th (i.e., 1000) and (or) 99th percentiles (i.e., 500) for all of the population subgroups (Table 53). Therefore, the next step used was Tier 2 point estimate (DPR MT-3, 2006).

Tier 2 Point Estimate Assessment

Tier 2 assessment assumed that all consumed foods contained the highest residue at or below the tolerance. Using the acute NOELs (i.e., 150 mg/kg/day and 25 mg/kg/day) and estimated dietary exposure values in each of the population subgroups, the calculated MOEs ranged from 2778 to 20446 at the 95th, 1718 to 11440 at the 97.5th, and 893 to 15989 at the 99th percentiles (Table 53). Since the lowest MOEs were ~10-fold greater than the acceptable MOE (i.e., 100), the acute dietary exposure to OPP appears to pose no significant health concerns on these evaluated population subgroups.

Table 53 Acute Dietary Risk Estimates for OPP

Population Subgroup	Acute Dietary MOE[a]					
	Point Estimate Tier 1[b] (percentile)			**Point Estimate Tier 2[b] (percentile)**		
	95th	97.5th	99th	95th	97.5th	99th
US Population (all seasons)	613	388	236	12166	6660	4100
Western Region	587	380	230	8530	4965	3220
Hispanics	504	346	216	8362	4969	3348
Non-Hispanic Whites	658	410	243	12747	7163	4485
Non-Hispanic Blacks	547	345	215	17872	8435	4911
Non-Hispanic Other	509	347	214	5457	4077	2820
All infants	167	141	110	11863	7700	3188
Infants (nursing, <1yr.)	178	159	125	18793	5689	2246
Infants (non-nursing, <1yr.)	163	136	98	11572	7992	3253
	142	108	87			
Children (3-5 yrs)	212	171	125	4519	2687	15989
Children (6-12 yrs)	488	357	249	7548	4097	2749
Youth 13-19 yrs	869	616	475	20446	11440	4903
Adults 20-49 yrs	1177	905	608	17449	10066	6070
Adults 50+ yrs	1215	965	704	11123	6852	5023
	181	133	96			

Note: Shading identifies the lowest MOEs.

[a] DEEM™ program was used for the analysis with the following input parameters: (*i*) USDA CSFII from 1994-1998, (*ii*) acute NOEL of 25 mg/kg (females 13-49 yrs), and (iii) 150 mg/kg/day (others population subgroups). MOE is defined as NOEL/Acute Dietary Intake.

[b] DPR calculated the acute dietary exposure for all population subgroups using user-days consumption instead of per-capita consumption that was used by the USEPA.

IV.C.2. Chronic and Lifetime Exposure

Using the chronic NOEL of 39 mg/kg/day and estimated dietary exposure values in each of the population subgroups (except Females [13-49 yrs]), the estimated MOEs ranged from 962,967 for Nursing Infants to 93,595 for Children 1-2 yrs (Table 54). For Females (13-49 yrs) subgroup, the estimated MOE was 52,884 based on the estimated NOEL of 4.9 mg/kg/day. For lifetime exposure, the estimated cancer risk of the average U.S. population was 2.4×10^{-7}. The negligible upper-bound risk of cancer, by convention, is $<1 \times 10^{-6}$ (i.e., one case in a population of a million), thus indicating that the lifetime dietary exposure to foods with legally allowed residues of OPP would not pose a significant carcinogenic concern.

IV.D. COMPARISON WITH U.S. ENVIRONMENTAL PROTECTION AGENCY RISK ASSESSMENT

This section is to provide a comparison between DPR and USEPA risk assessments for OPP and its salts (USEPA, 2006). Although the USEPA risk assessment discussed exposures from both dietary (i.e., direct-food and indirect-food uses [e.g., disinfecting food-processing utensils]) and non-dietary means (i.e., inhalation and dermal), the comparison will only address the risk associated with direct dietary exposure because this was the focus of DPR's risk assessment. For the salts of OPP, USEPA risk assessment considered both SOPP and potassium *ortho*-phenylphenate (KOPP) whereas DPR risk assessment considered only SOPP because the established tolerances for postharvest applications of foods (i.e., dietary exposure) are combined residues of OPP and SOPP. Also, DPR find no labels of products registered in California have post-harvest treatment application of KOPP. An addendum to this document will address the potential exposure to OPP and its salt from other sources.

I.V.D.1. Hazard Identification

Table 55 shows the critical endpoints and NOELs selected by DPR in comparison to those used by USEPA.

1. DPR determined two acute NOELs from a developmental toxicity study for woman in their childbearing years and for general populations (i.e., infants, children, and adults), respectively. In contrast, USEPA considered that there was no need to address dietary risk associated with the acute exposure because there was no appropriate endpoint identified that represents a single dose effect.

Table 54 Chronic Dietary Risk Estimates for OPP

Population Subgroup	Chronic MOE[a]
US Population (all seasons)	323,236
Western Region	254,537
Hispanics	332,048
Non-Hispanic Whites	309,841
Non-Hispanic Blacks	527,686
Non-Hispanic Other	221,800
All infants	377,096
Infants (nursing, <1yr.)	762,967
Infants (non-nursing, <1yr.)	316,380
Children (3-5 yrs)	116,450
Children (6-12 yrs)	173,425
Youth 13-19 yrs	388,390
Adults 20-49 yrs	518,812
Adults 50+ yrs	426,726
Females (13-49 yrs)	52,884

Note: Shading identifies the lowest MOE.

[a] The DEEM™ program was used for the analysis with the following input parameters: (i) USDA CSFII 1994-1998 and (ii) chronic NOELs of 39 mg/kg/day for the general population and 4.9 mg/kg/day for Females (13-49 yrs) (Wahle and Christenson, 1996). MOE is defined as NOEL/Chronic Dietary Intake.

Table 55 Comparisons of Critical Endpoints and NOELs for OPP Risk Characterization Between Department of Pesticide Regulation (DPR) and U.S. Environmental Protection Agency (USEPA).

DPR		USEPA	
Duration	Endpoint and NOEL	Duration	Endpoint and NOEL
Acute (1-2 days)	Developmental toxicity in rabbits: increased resorption (Zablotny *et al.*, 1991b) NOEL (fetal): 25 mg/kg/day (for women of childbearing years); Developmental toxicity in rats: ataxia & reduced body weight gain (Kaneda *et al.*, 1978) NOEL (maternal): 150 mg/kg/day (for infants, children, and adults)	No toxicity endpoint for single exposure.	
Chronic	Combined oral toxicity/carcinogenicity study in rats: simple hyperplasia (urinary bladder), decreased urinary protein concentration, decreased urine specific gravity, and increased BUN (Wahle and Christenson, 1996). NOEL: 39 mg/kg/day (for males) Combined oral toxicity/carcinogenicity study in rats: cardiac degeneration and (or) fibrosis in the females. Estimated NOEL: 4.9 mg/kg/day (for females)	Long-term (>6 months)	Combined oral toxicity/ carcinogenicity study in rats: decreased body weight, body weight gain, food consumption and food efficiency, and increased clinical and gross pathological signs of toxicity (Wahle and Christenson, 1996). NOEL: 39 mg/kg/day
Lifetime	Combined oral toxicity/carcinogenicity study in rats: urinary bladder tumors (Wahle and Christenson, 1996) Cancer Potency Factor: 2.0×10^{-3} $(mg/kg/day)^{-1}$	No need for quantification of cancer risk.	

2. For chronic dietary exposure, in addition to a chronic NOEL (i.e., 39 mg/kg/day), DPR quantified the human cancer risk of OPP by deriving a potency factor of 2.2 x 10^{-3} $(mg/kg/day)^{-1}$ from a combined oral toxicity/oncogenicity study in rats with urinary bladder tumors as the endpoint. DPR considered that the weight of evidence for carcinogenic and genotoxic activities is sufficient to warrant the quantitative risk assessment. In contrast, while USEPA classified OPP and SOPP 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, the USEPA considered that there is no need for quantification of cancer risk. The rationale of this determination was that the chronic reference dose (i.e., 39 mg/kg/day) is protective of the precursor events leading to the development of bladder tumors that occur at doses above 200 mg/kg/day and liver tumors that occur above 500 mg/kg/day.

I.V.D.2. Exposure Assessment and Risk Characterization

The chronic dietary exposure estimates derived by DPR and USEPA showed differences. These differences were primarily due to the different extent of exposure refinements used. While both agencies conservatively estimated the exposure by a deterministic approach using the highest residue value, for each of the raw agricultural commodities, DPR based its estimate on highest measured residue value (i.e., Tier 2 assessment) whereas USEPA based its estimate on residue-level tolerance (i.e., Tier 1 assessment). Accordingly, the DPR's exposure estimates were lower than those of USEPA were and hence, the margins of exposures were higher.

V. RISK APPRAISAL

V.A. INTRODUCTION

This health risk assessment for OPP evaluated the risk to the average U.S. population and to 15 population subgroups from potential residue in foods under acute and chronic exposure scenarios. As in all risk assessments, there are limitations in the knowledge to estimate the potential risk of OPP to human health. Hence, the common practice is to apply assumptions and extrapolations when the available data are insufficient to identify the hazard, to characterize the dose-response, or to assess the exposure. These, in turn, result in uncertainty in the risk characterization, which integrates all the information from the previous three processes. The following discusses specific areas of uncertainty associated with this risk assessment for OPP.

V.B. HAZARD IDENTIFICATION

V.B.1. Acute Toxicity

The uncertainties in hazard identification were associated with the paucity of acute toxicity studies to define the NOEL of a single day exposure. For estimating the risk of acute dietary exposure to OPP in the general population (including infants, adult males, and children), the critical NOEL was based partly on an effect observed after several days of dosing in a rat-developmental toxicity study (i.e., decreased mean body weight gain 3 days after the exposure initiated) (Kaneda *et al*., 1978). The other endpoint was based on a clinical observation (i.e., ataxia) for which no incidence information was provided. Because of these deficiencies, DPR assumed that the body weight effect and clinical sign that occurred in this short-term study, may have been resulted from a single (acute) exposure. The acute single-day oral NOEL could be higher than the 150 mg/kg/day value if the effect reported were a result of several days of repeated exposure.

For estimating the risk of acute dietary exposure to OPP in women in their childbearing years, the critical NOEL selected was based on an increased incidence of fetal resorptions (Zablotny *et al.*, 1991b). Consistent with the U.S. EPA Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), DPR assumed that the developmental effect observed at the end of the gestation was due to a single-day exposure. However, if the effect was due to repeated exposure, the actual NOEL for a single-day exposure may be higher.

V.B.2. Chronic Toxicity/ Oncogenicity

There were uncertainties associated with the magnitude of the critical NOEL for males and ENEL for females used to calculate the chronic MOEs. The selection of the NOEL of 39 mg/kg/day for males was based on the histopathological observation that there was a dose-dependent increase in the incidence of simple hyperplasia in the urinary bladder of OPP-treated

male rats (Wahle and Christenson, 1996). As detailed in the III.C. SUBCHRONIC TOXICITY, there were more animals with lesions in the bladder as indicated by scanning electron microscopy (SEM) than by light microscopy (Christenson *et al*., 1996a,b). Hence, the chronic NOEL based on histopathology could be lower than the 39 mg/kg/day value if the effect reported were based on SEM analysis.

In the derivation of ENEL, DPR applied a default 10-fold uncertainty factor (UF) to the chronic LOEL for females whereat increased ($p<0.05$) incidence of cardiomyopathy occurred at 49 mg/kg/day (i.e., the lowest tested dose). Because of the response magnitude at the LOEL was large (i.e., extra risk = [P(d) – P(0)]/[1-P(0)] = [0.84-0.54]/[1-0.54] = 0.65, where P(d) and P(0) are responses at dose d and 0, respectively), the LOEL-to-NOEL extrapolation may require a larger UF (Douron *et al.*, 1996). Benchmark dose approach could be used for estimating the NOEL; however, the available cardiomyopathy incidence data are not amendable to BMD analysis due to the modifying effect of feed restriction/body weight reduction on the cardiomyopathy incidences at the mid and high doses (see III.D. CHRONIC TOXICITY/ONCOGENICITY).

Another related issue in the use of this NOEL was the interspecies variability in OPP pharmacokinetics between rats and humans. For the most part, pharmacokinetics of OPP in humans was comparable to the rats. The most important difference was the *in vitro* results that human liver samples conjugated OPP with sulfate but not glucuronide (Temellini *et al.*, 1991) whereas the rat liver samples formed both conjugates (Nakagawa and Tayama, 1989). Because of this, USEPA suggested that humans with lower levels of sulphotransferase (i.e., reduced ability for the Phase II detoxification) may exhibit a higher susceptibility to the effects of OPP (Rinde and Dapson, 1994).

V.C. DIETARY EXPOSURE ASSESSMENT

The uncertainty in the exposure assessment is classified in three major categories: (i) parameter uncertainty, (ii) model uncertainty, and (iii) scenarios uncertainty (Peterson *et al*., 2001).

Parameter Uncertainty

Sources of parameter uncertainty in the dietary exposure assessment include completeness of the food residue database, use of surrogate data (including tolerance), and measurement errors (sampling and or reporting). Other areas of uncertainty are the assumptions of no exposure from commodities without legally established tolerance (e.g., banana, celery, grape, green bean, and mushroom [USDA, 1998-2004]) or co-exposure to other pesticides at concentrations that may modify the toxicity of OPP or SOPP (e.g., thiabendazole).

Residue data were available for slightly more than half of the commodities with legally allowed tolerance (i.e., 13 out of 22); these were actual monitoring results of USDA's PDP

Program 1996-2002. Of the commodities with monitoring data, some (i.e., cucumber, orange, and pear) contributed significantly to the total dietary exposure to OPP.

Regarding the use of surrogate data in this assessment, orange was the surrogate for citrus, citron, grapefruit, kumquat, lemon, and lime whereas peach was the surrogate for plum because of the lack of residue data in these commodities. Except for grapefruit, the commodities for which orange and peach were surrogates did not emerge as major contributors to the total dietary exposure to OPP. For grapefruit, the surrogate residue used (orange: 3.6 ppm) was ~4 times higher than the maximum detected value (0.91 ppm) by FDA. Therefore, DPR considered the orange residue data to be a conservative measure of actual residue on grapefruits. Because of the lack of suitable surrogate, kiwifruits residue value used in this assessment was the tolerance. It should be noted that establishment of tolerance involved the use of field trial-measured residue, following maximum application rate and frequency. Typically, these conditions do not reflect the actual use pattern. Although the results indicated that kiwifruit was one of the high contributors to the total acute exposure to OPP, it was likely reflective of the use of the tolerance value.

The important measurement errors included food consumption data. Although the food consumption data employed (1994-1998 USDA CSFII survey) in the exposure assessment are the latest of such survey available, uncertainties in exposure estimates may result from under representation of actual dietary consumption, reporting errors, response and variation in the culinary habits over the consumption period.

Model and Scenario Uncertainty

DPR evaluated several dietary exposure scenarios for OPP under acute and chronic conditions. For estimating the acute dietary exposure, a tier approach was the method used in the selection of appropriate residue values.

Acute Dietary Exposure

According to the DPR guideline for dietary exposure assessment, the tier approach began with legally allowed maximum residue value (i.e., tolerance; Tier 1) and progressed to residue distribution in the probabilistic exposure assessment (Tier 4), if necessary. Tier 1 Point Estimate analysis assumed that the concentration of OPP on each of the commodities was equal to tolerance. The results indicated that all population subgroups had MOEs below 1000 at the 97.5^{th} percentile. It should be emphasized that the likelihood that each of the commodities consumed in a given day that contain OPP at the highest legally allowed residue level is very low. Nevertheless, because of the low MOEs, the DPR guideline for dietary exposure assessment recommends additional refinements of the acute scenario.

Tier 2 acute analysis assumed the following: (1) residue of OPP occurred in all commodities at the highest LOD, measured levels, or tolerance (kiwifruits only); (2) all crops

received OPP treatment, and (3) a single value represented the residues for each crop. The Tier 2 analysis represents a "worst case" exposure estimate because the low probability of consuming all 22 food items, in a single day, containing upper-bound OPP residue would like be small. Nevertheless, this analysis produced MOEs ranged from 10^2-10^4 at the 95th, 97.5th, and 99th percentiles.

Chronic dietary Exposure

Like the acute assessment, chronic dietary assessment used assumptions. These assumptions are the following: (1) 100% crops received OPP treatment, because of the lack of percentage crop-treated (PCT) information; and (2) chronic-residue levels below the LOD were 1/2 of that limit. Although these assumptions may result in overestimating the exposure, this analysis produced MOEs estimates, which ranged from 10^4 to 10^5.

V.D. RISK CHARACTERIZATION

This section discusses the uncertainties of estimated acute and chronic MOEs. MOE is a ratio of the critical NOEL to the estimated exposure level. However, the MOE does not provide an estimate of population risk; it simply describes the relative distance between the exposure level and the NOEL. Another area of uncertainty pertaining to the MOE approach with no available human data is the selection of a NOEL from animal experiments. Two uncertainty factors are required: one for consideration of interspecies variation due to animal-to-human extrapolation and another for intraspecies variation in humans. Each of these uncertainty factors is given a value of 10, by default.

V.E. ISSUES RELATED TO FOOD QUALITY PROTECTION ACT

The Food Quality Protection Act of 1996 mandated U.S. EPA to "upgrade its risk assessment process as part of the tolerance setting procedures" (U.S. EPA, 1997a and b). The improvements to risk assessment were based on the recommendations from the 1993 National Academy of Sciences report, "*Pesticides in the Diets of Infants and Children*" (NRC, 1993). The Act required an explicit finding that tolerances are safe for children. The U.S. EPA was required to use an extra 10-fold safety factor to take into account the potential increased sensitivity of infants and children and the completeness of the data unless U.S. EPA determined, based on reliable data, a different margin would be safe. In addition, U.S. EPA must consider: (1) aggregate exposure from all non-occupational sources; (2) the effects of cumulative exposure to other pesticides with common mechanism of toxicity; (3) the effects of *in utero* exposure; and (4) the potential for endocrine disrupting effects.

V.E.1. Pre- and Post-Natal Sensitivity

USEPA determined that available developmental and reproductive toxicity studies including the study by Zablotny *et al.* (1991) showed no evidence of increased toxicity to offspring at the same or lower doses as those causing parental/systemic toxicity (USEPA, 2006) and, therefore, concluded that the special hazard-based FQPA factor be reduced to 1x for OPP. In this RCD, however, the potential of higher risk for children, compared to adults, was indicated by a developmental toxicity study in rabbits in which the developmental NOEL was lower than the maternal NOEL; the developmental effect identified was increased resorptions (Zablotny *et al.*, 1991b). Adjusting the NOEL of 25 mg/kg/day by a FQPA safety factor of 10, the Tier 2 acute dietary risk calculations produced MOEs of 277 at the 95th, 171 at the 97.5th, and 91 at the 99th percentiles in population subgroup of Females 13-49 yr. Since the lowest MOE was almost the same as the acceptable MOE (i.e., 100), the acute dietary exposure to OPP appears to pose no significant health concerns on this evaluated population subgroup.

V.E.2. Aggregate Exposure

Because OPP and SOPP have residential and occupational uses, aggregate exposure is possible. For total non-dietary exposures, the DPR considers contributions to risk from various exposure sources: food (indirect), drinking water, air, home, and work places. At present, the only exposure route with monitoring data was the food. An addendum to this document will address the exposures from other sources once the data become available at DPR.

V.E.3. Endocrine Effects

In a recently published risk assessment, USEPA concluded that OPP demonstrates some potential to act as an endocrine disruptor (USEPA, 2006). Hence, the USEPA recommended subjecting OPP to additional screening and (or) testing for better characterization of effects related to endocrine disruption. This recommendation is consistent with the DPR findings that OPP was positive in several studies for endocrine disrupting potential *in vitro* (Soto *et al,* 1997, Routledge and Sumpter, 1997, Rehmann *et al.*, 1999, Blair *et al.*, 2000, Miller *et al.*, 2001). The assay systems used were estrogen-receptor binding (non-competitive), estrogen-induced cell proliferation (e.g., MCF-7 human breast cancer cells), and estrogen-receptor transcription activity in cells (e.g., MVLN cell line).

V.E.4. Cumulative Toxicity

Because USEPA has not initiated a review to determine if there are chemicals that have a mechanism of toxicity common with that of OPP, the Agency assumed that OPP does not have a common mechanism of toxicity with other substances.

VI. TOLERANCE ASSESSMENT

VI.A. BACKGROUND

A tolerance is the legal maximum residue concentration of a pesticide, which may exist in or on a raw agricultural commodity or processed food. U.S. EPA is responsible under the Federal Food, Drug, and Cosmetic Act (FFDCA) for setting tolerances for pesticide residues in raw commodities (Section 408 of FFDCA) and processed commodities (Section 409 of FFDCA). The established levels of tolerances aim at the necessary maximum application rate and frequency and produce no deleterious health effects in humans from chronic dietary exposure (USEPA, 1991b).

The data requirements for the registration of a pesticide and for establishment of tolerances include: (1) residue chemistry, which includes measured residue from field studies, (2) environmental fate, (3) toxicology, (4) product performance such as efficacy, and (5) product chemistry (Code of Federal Regulations, 1996). The field studies must reflect the proposed use with respect to the rate and mode of application, number and timing of applications and the proposed formulations (USEPA, 1982).

In 1996, the Food Quality Protection Act (FQPA) amended the overall regulation of pesticide under FIFRA and FFDCA (USEPA, 1997a and b). One major change was the removal of the Delaney Clause that prohibited residues of cancer-causing pesticides in processed foods. FQPA requires scientific evidence to show that tolerances are safe for children. The USEPA can apply an additional uncertainty factor of up to 10-fold to take into account potential pre- and postnatal developmental toxicity and the completeness of the data.

The FQPA requires the USEPA to reassess all existing tolerances and exemptions from tolerances for both active and inert ingredients by 2006 (USEPA, 1997c). In the evaluation of tolerances, the USEPA uses a tiered approach and the assessment includes all-label-use commodities.

In California, Assembly Bill 2161 (referred to as Food Safety Act) requires DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides" (Bronzan and Jones, 1989). This assessment requires individual evaluation of tolerance for each specific commodity. In the situation where "any pesticide represents a dietary risk that is deleterious to health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance."

VI.B. ACUTE EXPOSURE

The acute tolerance assessment for a commodity evaluates the health protectiveness of the tolerance for that commodity as specified in CFR 180.129 under an acute exposure scenario.

The assessment, however, does not include multiple commodities at their respective tolerance levels, because the probability is low for diets to contain multiple foods with concentrations of a particular pesticide at the tolerance level.

The DPR estimates the acute tolerance exposure as the sum of the estimated 95th percentile exposure in each of the population subgroups for the commodity of concern at the tolerance and a background exposure for selected population subgroups. The added background exposure is to account for residues in other commodities, which may also be treated with the same pesticide (DPR MT-3, 2006). The first step is to set the chronic dietary exposure from total dietary exposures as a surrogate for background exposure. Because the exposure contribution of the commodity of interest is "double counted" (e.g., having pesticide residues at the tolerance and having average "chronic" residues) in this step, exceedance of the benchmark for acceptable exposure (i.e., MOE of 100) by the margin of exposure for any population subgroup would conclude the acute tolerance assessment. Otherwise, the next step is to reassess the background dietary exposure without the commodity that is evaluated for tolerance.

Regarding the commodities of concern, the tolerance assessment evaluates commodities that had a significant impact on the dietary exposure. The commodities that critical exposure commodity analysis (CEC) identified as having substantial acute dietary exposure contribution (i.e., >5%) were pears, oranges, kiwifruits, cucumber, and grapefruits. The tolerance assessment also included foods that young children consumed heavily (apples, carrots, peaches, sweet potatoes, cantaloupes, cherries, and tomatoes [Landrigan *et al.*, 1999]) or foods that, in California, have major uses (lemons and bell peppers [DPR, 2006]).

DPR currently excludes from the tolerance assessment population subgroups with less than 25 user-days in the USDA 1994-1998 CSFII database, because of the high uncertainty associated with consumption data (Chaisson *et al.*, 1999). Accordingly, the population subgroups excluded in this tolerance assessment were (commodity involved in parentheses): Hispanics (kiwifruits), Non-Hispanics Whites, Blacks, and Others (kiwifruits), All Infants (cantaloupes, grapefruits, and kiwifruits), Nursing Infants (bell peppers, cantaloupes, cucumbers, grapefruits, kiwifruits, and lemons), Non-nursing Infants (cantaloupes, grapefruits, and kiwifruits), Children 6-12 yrs (kiwifruits), and Youth 13-19 yrs (kiwifruits and sweet potatoes).

Among the average U.S. population and 15 population subgroups considered, 14 had more than 25, but less than 100 user-days for some of the evaluated commodities. These subgroups were the following (commodity involved in parentheses): Hispanics (sweet potato), Non-Hispanics Blacks (cantaloupe); Non-Hispanics Others (cantaloupe, grapefruit, and sweet potato), All Infants (bell pepper, cucumber, and lemon), Nursing Infants (cherry, orange, pear, peach, sweet potato, and tomato), Non-Nursing Infants (bell pepper, cucumber, and lemon), Children 1-2 and 3-5 yrs (kiwifruit and sweet potato), Children 6-12 yrs (sweet potato), Youth 13-19 yrs (cantaloupe and grapefruit), Adults 20-49 yrs (kiwifruit), Adults 50+ yrs (kiwifruit), and Females 13-49 yrs (kiwifruit and sweet potato). Although the tolerance assessment included all these subgroups, interpretation of the results (i.e., exposure and MOE values) needs caution, because of the relatively small sample size.

Table 56 summarizes the ranges of the exposure and MOE values at the 95th percentile for each of the evaluated commodity at its tolerance level in the background of the chronic dietary exposure. The acute NOELs used for calculating MOE were 25 mg/kg/day for women in their childbearing years (i.e., female 13-49 yrs) and 150 mg/kg/day for the general population (i.e., population subgroups excluding females 13-49 yrs). The MOEs at 95th percentile were above the benchmark of 100 in different population subgroups for the analyzed foods.

VI.C. CHRONIC EXPOSURE

DPR did not conduct chronic exposure assessment using residues equal to the established tolerances for individual or combination of commodities, because it is highly improbable that an individual would habitually consumer single or multiple commodities with pesticide residues at tolerance levels. The federal and DPR monitoring program data that support this conclusion indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (DPR MT-3, 2006).

Table 56 Acute Dietary Risk Estimates for OPP Residues at the Tolerance Level

Commodity	General Population		Females 13-49 yrs		Tolerance (ppm)
	Range of Exposure[a,c] (mg/kg/day)	Range of MOE[b,c]	Exposure[a] (mg/kg/day)	MOE[b]	
Apple	1.094-0.109	137-1375	0.152	164	25
Bell Pepper	0.016-0.003	9296-43541	0.008	3152	10
Carrot	0.314-0.032	477-4722	0.038	664	20
Cantaloupe (edible portion)	0.209-0.046	717-3251	0.072	348	10
Cherry	0.009-0.002	15890-69061	0.004	6345	5
Cucumber	0.069-0.009	2189-17424	0.013	1987	10
Grapefruit	0.152-0.047	985-3162	0.058	430	10
Kiwifruit	0.168-0.032	893[d]-4695[d]	0.052	477[d]	20
Lemon	0.016-0.004	9275-41175	0.006	3912	10
Orange	0.261-0.055	575-2715	0.085	295	10
Pear	0.624-0.067	240[d]-2226[d]	0.092	273	25
Peach	0.267-0.048	562[d]-3129	0.060	417	20
Sweet Potato	0.228-0.040	659[d]-3797	0.053	475[d]	15
Tomato	0.099-0.036	1517-4220	0.036	688	10

[a] Acute dietary exposure assessment was conducted for OPP residues on 14 commodities at a level equal to the USEPA tolerance. For each of the commodity, the estimated exposure was the sum of the estimated 95th percentile exposure in each of the population subgroups for the commodity of concern at the tolerance.

[b] Margin of Exposure (MOE) is defined as NOEL/Acute Dietary Intake; the number of user-days ranged from 0 to 24759 for the general population and 3-3818 for females 13-49 yrs.

[c] 15 general population subgroups considered were U.S. population (all seasons), U.S. population (western region), Hispanics, non-Hispanic whites, non-Hispanic blacks, non-Hispanic others, all infants, nursing infants, non-nursing infants, children 1-2 yrs, children 3-5 yrs, children 6-12 yrs, youth 13-19 yrs, adults 20-49 yrs, and adults 50+ yrs.

[d] Exposure estimates were based on >25 but <100 user-days, therefore the risk estimates may not be representative of the population.

VII CONCLUSION

This health risk assessment evaluated the dietary risk to the average U.S. population and to 15 selected population subgroups from potential residue exposure of OPP and SOPP in food using different acute and chronic scenarios. For the acute exposure, MOEs ranged from ~10^3-10^4 at the 95th, 97.5th, and 99th percentiles. The most highly exposed population subgroup was children 1-2 yrs; the respective MOE at 95th, 97.5th and 99th percentiles were ~4000, ~2000, and ~1300. For the chronic exposure, MOE in each of the population subgroups was >10^4. The most highly exposed population subgroup also was children 1-2 yrs; the MOE was ~95000. These MOEs were well above the benchmark for determining the level of health protectiveness (i.e., 100). OPP also showed carcinogenic activity in experimental animals; hence, the risk calculation in humans included excess cancer risk due to the lifetime exposure. The estimate lifetime risk was 2.6×10^{-7}, which was lower than the convention that considered negligible effect for cancer risk estimate (i.e., an upper-bound value of $<1 \times 10^{-6}$).

For tolerance assessment, the 95th percentile MOEs for exposure to a tolerance level OPP were above the benchmark of 100 in the population subgroups for the foods analyzed. Taken altogether, this health risk assessment indicates that no appreciable health concern exists (including cancer) due to the exposures to food with legally allowed residues of OPP under acute and chronic conditions.

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IX. APPENDICES

A. Toxicology Summary of OPP & SOPP
B. Benchmark Dose Oncogenicity Computer Model Printout
C. Dietary Exposure Analysis Printout
D. Comments and Responses to Comments from Laxness Corporation and the Dow Chemical Company
E. Comments and Responses to Comments from Pesticide and Environmental Toxicology Branch of the Office of Environmental Health Hazard Assessment
F. Comments and Responses to Comments from Reproductive and Cancer hazard Assessment Branch of the Office of Environmental Health Hazard Assessment

APPENDIX A. TOXICOLOGY SUMMARY OF OPP & SOPP

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

ACTIVE INGREDIENT

O-PHENYLPHENOL* & SODIUM O-PHENYLPHENOL**

*OPP: Chemical code # 448, Tolerance # 129, SB 950 # 090
**SOPP or OPP-Na: Chemical code # 248, Tolerance # 50438, SB 950 # 474

July 29, 1986
Revised: March 30, 1987; August 30, 1989; September 3, 1991; February 19, 1993; April 24, 1996; May 20, 1997; and March 16, 2001

I. DATA GAP STATUS

Chronic rat:	No data gap, possible adverse effects [1]
Chronic dog:	No data gap, no adverse effect
Onco rat:	No data gap, possible adverse effect
Onco mouse:	No data gap, possible adverse effect
Repro rat:	No data gap, possible adverse effect
Terato rabbit:	No data gap, possible adverse effect
Terato rat:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotox:	Not required at this time

[1] See statement on page 2 by J. Gee

Note: toxicology one-liners are attached; these pages contain summaries only; each individual worksheet may contain additional effects.

** indicates acceptable study
Bold face indicates possible adverse effect

Revised file name: T010316
Revised by Stephen J. Rinkus, 3/16/01

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

Note: These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED CHRONIC-ONCOGENICITY, RODENTS

Note: No individual long-term study in the rat has been evaluated as acceptable due to deficiencies in conduct and/or reporting. There are, however, several long-term studies which have examined a number of toxicological parameters in the rat. There is little doubt that ortho-phenylphenol has been associated with oncogenicity as demonstrated in a number of these studies. The data for chronic effects is somewhat less clear for determining a NOEL for non-oncogenic effects. The study that most closely addresses chronic toxicity is record # 145317 in which there was a one-year sacrifice of 20/sex for controls and the high-dose (8000 ppm for males, 10,000 ppm for the females) and 10/sex/dose for the low (800 ppm) and mid (4000 ppm) dose groups. Although there were deficiencies noted in the review of this study, considering all of the collective data, there is no need for another long-term study in the rat at this time and the data gap is considered filled. (Gee, 4/9/01).

Analytical data in the rat chronic toxicity-oncogenicity study (record 145317) and the rat reproduction study (record 141559) indicate that OPP in acetone-corn-oil-feed mixtures at concentrations of 800 ppm or 200 ppm degrade over the span of 1 to 4 weeks whether stored frozen (-23°C) or at room temperature. A similar degradation was not seen with a concentration of 10,000 ppm. (Rinkus, 5/20/97).

129-245 145317 "Technical Grade ortho-Phenylphenol: A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat" (Wahle, B.S. and Christenson, W.R.; Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS.; laboratory project study ID number 92-272-SC; 2/23/96). o-Phenylphenol (OPP), ≥ 99.5% purity, was administered in the diet at 0 ppm (acetone-corn oil treated feed), 800 ppm, 4,000 ppm and 8,000 ppm (males) or 10,000 ppm (females), initially to 70-80 Fischer 344 rats/sex/treatment level. Interim sacrifices were performed after 1 year using 10 (low- and mid-dose groups) or 20 (control and high-dose groups) rats/sex/treatment level. Twelve rats were replaced after the first month of testing. Six of these involved the high-dose female groups (three for the 1-year sacrifice group and three for the 2-year-sacrifice group); these six appeared to have been replaced because they had exhibited perigenital urine staining or a red ocular discharge. Survival was only affected in the high-dose male group: by week 85, 12 animals bearing urinary bladder cancer were dead or had to be sacrificed due to their moribund condition versus two deaths in the male control group. Decreased bodyweight appeared in the first few weeks of the study for both sexes exposed to ≥ 4000 ppm OPP; continued exposure only slightly affected the relative degree of bodyweight reduction. For the 8000 ppm males and 10,000 ppm females, decreased bodyweight paralleled significant decreases in the feed consumed/rat/day. The clinical observation data indicated the following: hematuria (red blood cells in urine; red stained, perigenital fur) presumably associated with the urinary bladder cancer in the 8000 ppm male group; and possibly polyuria (urine-stained, perigenital fur) in each of the OPP-exposed female groups. Ophthalmological testing at the end of the study found significant increases in the incidences of uveitis, corneal vascularization and cataract in the 4000 ppm female survivors and of cataracts in the 8000 ppm male survivors. At each testing period, two or more mean corpuscular indices (i.e., MCV, MCH & MCHC) were affected statistically, sometimes involving the mid- and high-dose groups and both sexes. However, the data for hematocrit, rbc concentration and hemo-

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globin concentration did not indicate that OPP had significantly affected these endpoints. Serum-chemistry changes were seen at multiple testing times and often in both sexes in rats exposed to ≥ 800 ppm OPP; these changes included: decreased creatine kinase; decreased lactate dehydrogenase; decreased calcium; increased albumin/decreased globulin; decreased triglycerides; decreased cholesterol; increased urea nitrogen; and decreased total bilirubin. In some instances, the same serum-chemistry change had been seen in the 4-week, range-finding study. Treatment-related urinalysis findings included: increased pH at the high dose in both sexes; decreased protein at the mid and high doses in both sexes; decreased ketones at the low, mid and high doses in the males and at the mid and high doses in the females; decreased specific gravity at the mid and high doses in both sexes; and decreased leukocytes at the low, mid and high doses in the males and at the mid and high doses in the females. The urinalysis findings, taken together with the urine staining of the perigenital fur, suggest that the OPP-treated rats had polyuria. Absolute-organ-weight changes appeared to be related to bodyweight decreases or the occurrence of tumors (leukemia in the spleen [females]; interstitial cell tumor in the testes). Necropsy data indicated the following: urinary bladder masses, consistent with urinary bladder cancer, in the mid- and high-dose male groups; kidney changes, consistent with kidney damage, in the high-dose female group; and red stained ventrums, consistent with hematuria resulting from urinary bladder cancer, in the high-dose male group. Histological findings in the kidneys included increased incidences of cystic tubular dilatation/degeneration at the high dose in both sexes, cortical infarct at the high dose in the females (and possibly the males), mineralization within the tubules of the papillae at the high dose in the females, tubular hyperplasia at the high dose in the females, and acute inflammation at the high dose in the females. Histological findings in the urinary bladder included increased incidences of simple transitional cell hyperplasia at the high dose in both sexes, nodular/papillary transitional cell hyperplasia at the high dose in the males, transitional cell carcinomas at the mid and high doses in the males, and transitional cell papillomas at the high dose in the males. High-dose males also exhibited the following urinary bladder findings in association with urinary-bladder tumors: congestion, hemorrhage, mineralization within the tissues, necrosis and calculi. Histological findings also included increased incidences of an eye syndrome consisting of cataract, retinal degeneration, optic nerve atrophy and optic chiasma atrophy in the mid-dose females, vascular mineralization in the heart in the mid-dose males, and cardiomyopathy in the low- and mid-dose females. The incidence and (or) spread of mononuclear cell leukemia (MCL) appeared to be increased in the 800 ppm males but supplemental information was needed to complete the evaluation. When first reviewed (1/21/97), this study was considered unacceptable pending the submission of supplemental information (detailed in worksheet W145317.835). In response, the Registrant submitted records 168946 and 169048. Based on the former, it is possible that orbital-sinus bleeding of the right eye was involved in the increased incidences of eye lesions in the 4000 ppm female survivors seen at ophthalmology and in the increased incidence of the aforementioned eye syndrome in the 4000 ppm female group seen at histology. However, other deficits in various data regarding the eyes and optic nerve need to be resolved before deciding these issues. The supplemental information also indicated that the incidence of MCL-bearing animals was statistically increased ($p<0.01$) in the 800 ppm males that survived to terminal sacrifice. However, given the lack of a similar response in the 4000 ppm males and the known variability in the incidence of MCL in F344 males, there is insufficient evidence to conclude that the increased MCL incidence was treatment-related. Whether the onset or spread of MCL was affected by treatments remains unresolved. **Possible adverse effect: urinary bladder cancer. Noncancer NOEL < 800 ppm (polyuria [urine-fur staining, urinalysis changes]; serum-chemistry changes; cardiomyopathy).** This study is still considered **UNACCEPTABLE**. (Rinkus, 10/5/00).

129-281 168946 This record consists of a 36-page narrative section that provides a response to issues raised in worksheet W145317.835, with the following 7 appendices: Appendix I, worksheet W145317.835; Appendix II, individual urinary-volume data taken on study day 22 of the four-week

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range-finding toxicity study; Appendix III, historical negative-control data from the conducting laboratory in the areas of clinical chemistry, hematology and urinalysis; Appendix IV, a compilation of Fisher exact tests conducted on the revised clinical-observation data; Appendix V, clinical-observation summary data excluding the data for the replacement rats; Appendix VI, individual clinical-observation data for the one-year-sacrifice and two-year-sacrifice groups, excluding the data for the replacement rats; and Appendix VII which consists of four parts: Part 1 contains responses to issues raised in section VI in worksheet W145317.835 as well as to related issues that were raised elsewhere in that worksheet; Part 2 contains responses to 10 miscellaneous issues that appeared in section IV of worksheet W145317.835; Part 3 contains analyses of the MCL data and of data related to whether OPP had affected the eyes, optic nerves and optic chiasma; and Part 4 contains 38 tables to which references were made in the previous three parts. **Supplemental information, discussed in worksheet w145317.s01**. (Rinkus, 3/16/01).

129-282 169048 This record consists of two parts. First, there is an 8-page section whose purpose was to correct the year appearing on page 10 of record 168946. The second part began with a letter dated May 13, 1999, from Stan Olosky (Bayer) to Karen Fletcher (DPR), indicating that "raw data" were attached, pursuant to discussions that had occurred between representatives of the Registrant and Medical Toxicology staff at a meeting held on February 10, 1998. Pages are not numbered sequentially in this part. There are the following 8 sections (in order of appearance): 1) use of death-rate and prevalence methods to analyze survival-adjusted mononuclear-cell leukemia (MCL) incidence data; also summary statistics regarding the number of tumorous tissues per animal having MCL; 2) summary statistics of absolute organ weights (spleen, liver, lung) from animals with MCL; 3) survival-analysis tables, Kaplan-Meier plots and summary statistical analyses; also individual animal fate data; 4) a summary table of incidence data for 70 endpoints; the endpoints included histology, necropsy, clinical observations and ophthalmology from the one-year-sacrifice and two-year-sacrifice groups; endpoints were selected on the basis of visual examination of the data for a significant difference when compared to controls; following the summary table, there were individual 2x4 (all groups compared) and 2x2 (controls versus a treatment group) contingency tables with summary statistical analyses; 5) individual contingency tables with summary statistical analyses concerning necropsy observations of the ventrum; 6) individual contingency tables with summary statistical analyses concerning several endpoints; 7) individual contingency tables with summary statistical analyses concerning eye-histology endpoints; and 8) tables listing individual data for organ weights (liver, lungs, spleen) for animals that were either found dead or were sacrificed due to their moribund state. **Supplemental information, discussed in worksheet w145317.s01**. (Rinkus, 3/16/01).

****129-058 065929** "Carcinoginicity Testing of Sodium Orthophenylphenate in F-344/DuCrj Rats," (Kogo Hiraga [author], Tokyo Metropolitan Research Laboratory of Public Health, 1983). Sodium o-phenylphenol (OPP-Na), a stated purity of 95.5%, was given in the feed at concentrations of 0, 0.7 and 2% to 50 male rats and at 0, 0.5 and 1% to 50 female rats for 104 weeks in the 106-week study. OPP-Na also was given in the feed at 0, 0.25, 0.7 and 2% to 25 males and at 0, 0.25, 0.5 and 1% to 25 females for 104 weeks in the lifespan study. After dietary exposure to OPP-Na, rats then were fed basal diet for either two more weeks (106-week study) or until death (lifespan study). Reduced mean bodyweights were observed in the high-dose males and females in the 106-week study and in the high-dose males in the life-span study. Hematuria and early deaths were noted only in the high-dose males and may have resulted from the presence of urinary bladder tumors. These tumors arose in the transitional epithelium of the urinary bladder; some tumors also were induced in the transitional epithelium of the renal pelvis in high-dose males in the the 106-week study. At the high-dose level in both study designs, the incidence of urinary bladder tumors in the males was > 90%. Also, nonneoplastic lesions were seen in the pancreas (females only), the testis and the urinary tract. **NOEL = 224 mg/kg/day as nominal time-**

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weighted average (2500 ppm nominal) (tumors & hyperplasia in the urinary tract of females). This record was considered originally to be unacceptable as a rat oncogenicity study; upgrading required the submission of data testifying to the stability of OPP-Na in the feed under the storage conditions used in this study (Rinkus & Kishiyama, 6/6/89). These analytical data now have been provided in record 091951; as a result, this study has been reclassified as **ACCEPTABLE** only as a rat oncogenicity study. In terms of chronic toxicity testing, this study remains unacceptable because there are insufficient data in the areas of hematology and ophthalmology. (Rinkus, 4/5/91).

129-058 065931 "Carcinogenicity Testing of Sodium Orthophenylphenate in F 344 Rats," (Fujii, T. and Hiraga, K., J. Saitama Med. School, 12: 277-287, 1985). Partial duplicate of 129-058 065929. Materials and Methods section indicates that "test diets were prepared once per three months," an important point which was not mentioned in 129-058 065929. No worksheet. (Rinkus, 7/13/89).

129-010 004164 "Toxicological Studies of Orthophenylphenol (Dowicide 1)," (Dow, 2/52, published article in J. Pharmacol. Experimental Therapeutics 104: 202-210 (1952), Hodge, H. C. et al.). OPP (>98%) fed in the diet for 2 years at 0, 200, 2000 or 20,000 ppm (0.02. 0.2 or 2.0%); 25/sex/group; **UNACCEPTABLE** (limited data, no analysis of diets, no report on clinical findings or hematology/urinalysis/clinical chemistry, inadequate tissues for histopathology). **Positive for adverse effects to the kidneys ("marked tubular dilation" at the high dose).** NOEL=2000 ppm according to text of article. (Remsen (Gee), 4/2/85).

50438-005 038120 "Molecular Mechanisms Involved in the Toxicity of Ortho- phenylphenol and its Sodium Salt," (Dow, 1981, published in: Chem.-Biol. Interactions 43: 99-119 (1983), Reitz, R.H. et al.). Subchronic study (3 to 90 days) with 2% OPP or 2% SOPP in the diet fed to 30 male rats per group for the purpose of studying the effects on kidney and bladder, specifically; OPP, lot MM09250, 99.6% and SOPP, lot MM09220B, 72% SOPP, 25.6% water and 1.05% NaOH; fed in the diet with sacrifices at days 3, 7, 14, 30, 65 and 90, 5-7 per sacrifice group; OPP decreased the food intake in the first week so severely that seven died apparently from starvation. Beginning day 30 on OPP, focal areas of discoloration in kidney were seen upon necropsy and microscopy showed multiple areas of focal tubular collapse and atrophy of the cortex and days 65 and 90 sacrifices showed "cystic degeneration suggestive of obstructive phenomenon." No bladder lesions related to treatment. With SOPP, beginning day 3, increased mitosis was seen in the bladder epithelium. At subsequent times, the proportion reportedly appeared to decrease but remained above normal. Beginning day 14, thickening of the bladder epithelium was seen and increased with time. Reviewed as **supplemental** to chronic/onco studies. (Gee, 3/30/87).

129-032 035998 "Molecular Mechanisms Involved in the Toxicity of Ortho-phenylphenol and Its Sodium Salt," (Reitz et al.; Dow Chemical Company; HET K-1025-(8); 12/10/81). This appears to be the laboratory report which is the basis for record 038120. It contains some data that did not appear in that publication. **Supplementary information. No worksheet.** (Rinkus, 3/29/91).

129-032 036006 "Biochemical Factors Involved in the Effects of Orthophenylphenol (OPP) and Sodium Orthophenylphenate (SOPP) on the Urinary Tract of Male F344 Rats," (Reitz et al., Toxicol. Appl. Pharmacol., 73: 345-349, 1984). **Supplemental information. No worksheet.** (Rinkus, 3/29/91).

129-032 036005 "Follow Up Studies of the Effects of Orthophenylphenol (Dowicide®1) and Sodium Orthophenylphenol (Dowicide A) on the Urinary Tract of F344 Rats," (Reitz et al.; Dow Chemical Company; HET-K-1025-(11); 3/2/83). Male F344 rats (6/group) were fed one of the

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following diets for 30 days: 1) a control diet; 2) a diet containing 1.3% OPP; 3) a diet containing 2% SOPP (this is equimolar in OPP to the 1.3% OPP diet); 4) a diet containing 10% NaCl; 5) a diet containing 10% NaCl plus 0.1% NaOH; and 6) a diet containing 1.3% OPP, 10% NaCl and 0.1% NaOH. Histological examinations of the urinary tract indicated that the addition of sodium to the diet did not affect the toxicity of OPP (i.e., it did not make it SOPP-like). The in vitro microsome-binding data and the in vivo macromolecular binding data in this record are the basis for record 036006. **Supplementary information. No worksheet.** (Rinkus, 3/29/91).

129-032 036009 "Induction of Tumors of the Urinary Bladder in F344 Rats by Dietary Administration of o-phenylphenol", (Journal article (1984) published in: Fd. Chem. Toxic. 22: 865-870, Hiraga K. and Fujii, T.). OPP (>98%, lot no. MM01040) was administered in the diet at 1560, 3130, 6250, 12,500, or 25,000 ppm to male F344/DuCrj rats for 91 weeks; these doses are equivalent to 0.25, 0.5, 1, 2, or 4% OPP-Na, doses used in other studies. OPP stated to be stable in the diet as measured by gas chromatography; 20-24 males per group. The mean intakes in the 91-week study for the 6250, 12,500 and 25,000 ppm groups were 269, 531 and 1140 mg/kg/day, respectively. The report also contains information on a 13-week study in male and female rats at the same dose levels of OPP. In the 91-week study, urinary bladder tumors noted in 96% of animals at 12,500 ppm and 17% of animals at 25,000 ppm; transitional cell carcinomas were identified in 87% of animals in the 12,500 ppm group and 50% in the 25,000 ppm group. Proliferative lesions of the urinary bladder were also seen in the 13-week group with 12/12 (100%) of males in 12,500 ppm group but in no other group - half were transitional-cell papillomas and half were transitional-cell hyperplasias. In the 91-week study, calculi were found in 17/24 (71%) of the 12,500 ppm males and in 14/23 (61%) of the high dose group. In addition, nephritic lesions were also increased significantly in the high dose groups in both studies. Survival was adversely effected in the mid- and high-dose groups. The discussion compares these results with those for OPP-Na in rats with good correlation but with the suggestion that OPP-Na may be a more effective carcinogen - the sodium salt is quite alkaline (pH 11.8) when dissolved in water and this is suggested as a possible factor. **UNACCEPTABLE** (missing information and use of males only) with a **positive adverse effect.** (Gee, 5/30/86).

50438-005 038118. Partial duplicate of 036009.

50438-005 038117, 038119, "Induction of Tumors of the Urinary System in F344 Rats by Dietary Administration of Sodium o-phenylphenate", (Report and Journal article (1981) Published in Fd. Cosmet. Toxicol. 19: 303-310, Hiraga and Fujii). Male Fisher rats (F344/DuCrj) were exposed to sodium salt of OPP (lot MM01044, ≥ 95% (Dow))at levels of 0, 1250, 2500, 5000, 20,000 or 40,000 ppm in the diet for 91 weeks, one analysis of pellets presented; 21/group; increased incidence of transitional cell carcinomas in urinary bladder, renal pelvis and renal papilla at 10,000 (1/21), 20,000 (20/21) and 40,000 (17/20) ppm. All were transitional cell carcinomas with one exception of a carcinosarcoma in the 20,000 ppm group. Severe pyelonephritis occurred in 19/20 of the high dose group. **UNACCEPTABLE** (use of males only, inadequate number of animals at risk, missing data). Individual data in 038117. (Gee, 5/30/86).

129-047 050577. Duplicate of 038119.

50438-005 038116. Addendum (letter - no data) to 038117.

129-032 036007 "Promoting Effect of Sodium o-Phenylphenate and o-Phenylphenol on Two-Stage Urinary Bladder Carcinogenesis in Rats", (Nagoya City University Medical School, in: Gann 74: 625-632 (1983) by Fukushima, S., et al). OPP-Na, 97% (Lot no. 04279A) and OPP, 98% (Lot no. ARO1); 2% of diet (actual level was 1.59% OPP-Na in expt. 1 and 1.50% in expt. 2;

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actual level of OPP was 1.72%); stable for 3 months--conditions not specified; some groups of rats were given 0.01 or 0.05% N-butyl-N-(4-hyroxybutyl)nitrosamine (BBN) in the drinking water for 4 weeks prior to feeding with OPP-Na or OPP for 32 weeks; 30 males per group; OPP-Na had a positive effect both with BBN and alone; OPP was not a promoter. **Supplemental information.** (Pfeifer, 9/4/86 and Gee, 3/9/87).

50438-005 038129. Duplicate of 036007.

129-032 036008 "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans," (IARC Working Group, 3/83, WHO, volume 30). The Working Group concluded that the available data (e.g., record 004164) were inadequate to evaluate the carcinogenicity of OPP in experimental animals but there was "limited evidence" (record 038117/038119) that OPP-Na is carcinogenic to the urinary tract of rats, producing both benign and malignant tumors. The Working Group noted that no data on humans were available. (**Note:** in 1987, IARC [Supplement 7] identified OPP-Na has having "sufficient evidence" that it is carcinogenic in experimental animals; this publication presently is not on file at CDPR). (Rinkus, 9/3/91).

242-051 074911 "Enhancing Effect of Thiabendazole on Urinary Bladder Carcinogenesis Induced by Sodium o-Phenylphenate in F344 Rats" (Fujii et al., Fd. Chem. Toxic. 24: 207-211, 1986). The tumor incidences in males eating a diet containing 1% OPP-Na & 0.2% Thiabendazole for 13 weeks or 65 weeks was 80% in both cases (8/10 and 12/15, respectively). By contrast, in males eating just 1% OPP-Na for either exposure period, no tumors were seen (0/10 and 0/15); and in males just eating 0.2% Thiabendazole for 13 weeks or 65 weeks, the respective tumor incidences were 0% (0/10) and 6.7% (1/15). The tumor incidence in females eating a diet containing 2% OPP-Na & 0.2% Thiabendazole for 65 weeks was 80% (12/15), whereas the respective tumor incidences in females just eating 2% OPP-Na or 0.2% Thiabendazole for 65 weeks were 13% (2/15) and 0% (0/15). These enhanced tumor incidences due to cotreatment with Thiabendazole were statistically significant. This study is also useful for indicating the magnitude of the sex difference in the tumor incidences in rats treated chronically to the same diet concentration of OPP-Na: for 2% diets given for 65 weeks, males and females exhibited 100% (15/15) and 13% (2/15) tumor incidences, respectively. **Supplemental Information.** No worksheet. (Rinkus, 8/30/89).

129-059 065932 This record contains tables (but no text) that were part of the presentation made by the Sponsors at a meeting at CDFA Medical Toxicology Branch on 1/28/88. The tables compare FIFRA requirements for oncogenicity testing in rodents, chronic toxicity testing in two species, teratology testing in two species, and reproduction toxicity testing in rats to the various studies that had been submitted by the Sponsors to fill SB950 data requirements (**note**: most of these studies had been conducted by researchers other than those of the Sponsors, e.g., records 065929, 045322, 065930 & 03813.). **Supplementary information. No worksheet.** (Rinkus, 3/29/91).

129-166 113826 This record contains two letters. The one dated 3/31/92 discusses the status of the following SB 950 data requirements: chronic toxicity in rats, oncogenicity in mice, reproduction in rats, and teratogenicity in rabbits. The other letter is discussed under this record number in the section "Teratology, Rabbit." **Supplemental information. No worksheet.** (Rinkus, 8/4/92).

129-166 113774 This record is a protocol for a dietary combined chronic toxicity-oncogenicity study using B6C3F1 mice. The test material appears to be o-phenylphenol (as opposed to its sodium salt). **Supplemental information. No worksheet.** (Rinkus, 8/4/92).

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ANALYTICAL STUDIES, IN SUPPORT OF CHRONIC STUDIES

Note: With the submission of record 091951, the matter that was discussed in the rebuttal dated 8/30/89 (R890830), regarding inadequate analytical data to support the chronic study in rats (record 065929), can be considered resolved. (Rinkus, 4/5/91).

129-140 091951 "Stability of Ortho-Phenylphenol (OPP) and Sodium Salt of Ortho-Phenylphenol (OPP-Na) in Rodent Chow Used in Japanese Toxicity Studies," (Sowle et al.; The Dow Chemical Company; Laboratory Project Study ID T2.02-195-000-002; 12/18/90). OPP (lot # MM890315) and OPP-Na (lot # 890831), both technical grades and both having ≥ 99% purity, were tested separately for their stability in rodent chow. The chow was obtained from Japan (Clea Diet CE-2, Clea Japan, Inc., Tokyo) and the preparation methodology and storage conditions that were used simulated those used in the chronic studies in rats conducted by Japanese researchers at the Tokyo Metropolitan Research Laboratory of Public Health (e.g., record 065929). This stability study was performed in response to concerns raised by CDFA (R890830) that the Sponsor was using these chronic studies in rats to fill certain SB950 data requirements but the analytical studies to support these studies (records 068363, 068361 & 068362) were inadequate and there was even possible evidence that the test material was unstable in the diet (record 072405). The stability study of both forms of OPP was conducted as follows. Test material was ground to create a fine powder and then combined with pulverized chow to produce eventually diets containing 0% (control), 0.25%, 0.50%, 0.70%, 1.00% and 2.00% (w/w) (**note:** a total of 12 test diets). The powder diets were shipped to Purina Mills in Richmond, Indiana to be made into pellets; the pelletization process involved the addition of water (temperature not stated) to facilitate making the pellets and a subsequent drying step (~100°C for up to 2 h) to reduce the water content. The pelletized diets were shipped back to Dow (Midland, MI) and stored inside closed (the containers were only opened to remove samples for analysis), polyethylene-lined, light-excluding cardboard drums at ambient room temperature for up to 104 days; also, there was no use of a "nitrogen gas" pad to create a deoxygenated enviroment. The technique for analyzing test material involved overnight extraction with 5% acetic acid in methanol, chromatographic separation by HPLC (column type, etc., not stated), and detection by UV-absorption (wavelength not stated). Recoveries of test material from "spiked" samples was ≥ 95% by this method; these extraction efficiencies were applied to sample calculations (p. 21). Homogeneity analysis (10 samples each) of the powder diets containing 0.25% of test material indicated that both OPP and OPP-Na diets were homogeneous; the OPP diet was 100% ± 2% of its target level while the OPP-Na diet was only 90% ± 3% of its target level (pp. 36-37). After being made into pellets, these same diets were 92-93% of their target levels, indicating possibly some slight loss in content due to the pelletization process. Likewise, slight losses in content were observed when the content of the other diets were compared before and after pelletization (pp. 38-41). However, sampling at approximately 1 month, 2 months and 3 months postpelletization indicated that there was no appreciable loss in content for any of the diets containing either test material. Therefore, these analytical studies constitute sufficient analytical data to support the chronic rat studies conducted at the Tokyo Metropolitan Research Laboratory of Public Health (e.g., record 065929). **Supplementary information.** (Rinkus, 3/21/91).

129-129 087132 "Analytical Stability of Ortho Phenyl Phenol (OPP) and Sodium Salt of Ortho Phenyl Phenol (OPP-Na) in Rodent Chow Used in Japanese Toxicity Studies," (The Dow Chemical Company; file number HET T2.02-195-000-002; 3/22/90). This record is the protocol for record 091951. **Supplementary information. No worksheet.** (Rinkus, 3/21/91).

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129-012 068363 "Quantitative Analysis of Sodium o-Phenylphenol Added to the Standard Animal Foods and Effect of Preservation," (Nawai et al., Ann. Rep. Tokyo Metr. Lab. P.H., **29**-2: 97-98, 1978). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables written in English; and an English translation of the former in its entirety. This testing was conducted apparently to provide analytical data in support of ongoing testing in mice for the induction of dominant lethals (however, no feed studies for dominant lethal testing are on file with CDFA). Sodium OPP (Tokyo Kasei Kogyo K.K., Lot AL01, 97.1% purity) was mixed homogeneously into mouse food powder (Nippon Kurea's CE-2) at final target levels of 0.125%, 0.25%, 0.5%, 1%, 2%, and 4%. Mixtures were made into pellets by a process involving: applying steam heat (100°C) on the mixture; compressing the mixture into pellets; and drying the pellets at 100°C for 40-60 min. Prepared pellets were stored in a "food box," but the storage temperature was not specified. In general, it is not clear what were the preservation conditions highlighted in the title of this report. At 0, 10, 20, 30, and 55 days presumably after preparation (a footnote in a table discusses time in terms of days after the start of the dominant lethal testing), pellets were sampled randomly and stored under a nitrogen atmosphere until they could be analyzed. Analysis involved an extraction process using steam distillation and ident-ification by gas chromatography (flame ionization detection). Analytical testing indicated the following: 1) recovery of OPP standard (dissolved in NaOH solution; therefore, this was an OPP-Na standard) from spiked food was ≥ 96%; 2) there was no degradation of OPP-Na over the 55 days for any of the six test diets; and 3) the percent of target levels achieved decreased with decreasing concentration. Regarding item 3, for diets targeted to contain ≤ 0.5% concentrations of OPP-Na, only 72-76% of the targeted levels were achieved. The authors suggested that loss was occurring as a result of heat treatments during pellet preparation. **Supplemental information.** (Kishiyama, 1/10/89; Rinkus, 7/14/89).

129-012 068361 "Quality and Determination of o-Phenylphenol-Na in Animal Feeds," (Mizoiri et al., Ann. Rep. Tokyo Metr. Lab. P.H., **32**-2: 28-32, 1981). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables written in English; and an English translation of the former in its entirety. Analytical testing consisted of two purposes: 1) to characterize the test material; and 2) to determine the recovery of OPP and OPP-Na from separate test diets supplied by the "Toxicity Department" of their organization. Regarding the former, only OPP-Na (Dow Chemical Co.; the lot was not specified, but presumably this was the lot used in the ongoing rodent bioassays that were mentioned in the report) was analyzed, in accordance with the "4th Food Additive Official Form," the meaning of which was not explained. The various data from this analysis were described inadequately, but the text stated that the observed values agreed with what was expected. The one noted exception was the melting point: 49-51°C was observed, when 55-58°C was expected. Although not explained, these melting point values appear to refer to the precipitate that results when an aqueous solution of OPP-Na is acidified: OPP-Na + HCl → OPP (insol.) + NaCl; in which case, the expected value, 55-58°C, is the melting point of pure OPP. Some investigation of the impurities was done, but the methods were not described adequately. Apparently, an aqueous solution of the test material was acidified (therefore, OPP precipitated) and then was extracted wth n-hexane (OPP, but not OPP-Na, is soluble in this solvent; therefore, if the precipitate was not removed, the solvent would solubilize some OPP). The hexane fraction was washed with alkaline water, then plain water, and was concentrated for analysis by gas chromatography-mass spectrometry. Identified "neutral" impurities were: acetophenone, a and b-methyl naphthalenes, biphenyl, OPP, dibenzyl, dibenzofuran, diphenyl ether, phthalic acid n-butyl ester, phenyl [biphenyl-2] ether, and xanthone; why nonphenolic compounds would be contaminants was not discussed but it would seem inconsistent with the probable pathways for synthesizing OPP-Na. The authors concluded that the purity of the test material was 95.5%, with pure test material being the tetrahydrate of OPP-Na. However, the analysis did not account for the alleged 4.5% impurity. Excess NaOH was said to be only 0.72% and the aforementioned neutral impurities amounted to only 0.03% (actually, the latter is an overestimate since

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it counted OPP as a major contaminant, which should not be possible if NaOH was also a contaminant). Consequently, the only useful information provided about the test material was that it was not analytical grade OPP-Na, and therefore it can be considered as some type of technical grade material. Regarding the ability to recover OPP and OPP-Na from feed, content analysis using three separate methods (steam distillation method used in record 68363; a dialysis method with 0.01 M NaOH; and extraction with methanol and detection with HPLC) indicated that >90% of the nominal concentrations were detected. However, since it was not stated how the feeds were prepared and stored and how much time had elapsed since their formulation, these results do not provide any information on the stability of the test materials in feed. One notable observation from the dialysis measurements was that with either OPP or OPP-Na, about 35% of the test material exists in the feed as OPP, which is insoluble in water, but is soluble in methanol or hexane. **Supplemental information.** (Kishiyama, 1/10/89; Rinkus, 7/17/89).

129-012 068362 "Uniformity of Test Article Concentrations in Pellet Diet used [in] Feeding Study," (Kamiya, N. and Hiraga, K., Ann. Rep. Tokyo Metr. Lab. P.H., **33**: 561-563, 1982). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables written in English; and an English translation of the former in its entirety. OPP (ultrapure grade; Tokyo Kasei Kogyo), OPP-Na (purity and source not specified), and thiabendazole (purity not specified; Merck, Sharp & Dhome International) were added to rodent-feed powder (Nippon Kurea) at nominal concentrations of 1.25%, 1%, and 0.2%, respectively; and pellets were made according to the method described in record 68363. For each test material, 18 samples were taken from a rectangular container, representing 6 samples at three different depths. OPP and OPP-Na were extracted from crushed pellets into methanol and then quantitated by high-pressure liquid chromatography using the method described in record 68361. The results indicated that both OPP and OPP-Na were distributed homogeneously through the test diet. However, without data, the authors cautioned that it was difficult to make homogeneous test diets when the target levels were <1%. **Supplemental information.** (Kishiyama, 1/10/89; Rinkus, 7/17/89).

SUBCHRONIC STUDIES, IN SUPPORT OF CHRONIC STUDIES

129-012 068364 "Subacute Toxicity of Sodium o-Phenylphenate by Food Administration to Rats," (Iguchi et al., Ann. Rep. Tokyo Metr. Lab. P.H., **30**-2: 67-79, 1979). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables in English; and an English translation of the former in its entirety. OPP-Na was presented in the diet for 13 weeks at the nominal concentrations of 0%, 0.125%, 0.25%, 0.5%, 1%, 2%, and 4%, to 10 F-344/Ducrj rats/sex/treatment level. Bodyweights as a percent of the control values showed a slight sex-related difference with the 2% diet: males' relative weight was 100%, while females' relative weight was 83%; with the 4% diet, both sexes had relative weights of ~83%. Absolute organ weights for the liver and the kidneys were increased statistically only in males on the 2% and 4% diets; urinary bladder weights were not measured. While serum GPT was decreased clearly in males on the diets containing ≥ 0.5% and in females on the diets containing ≥ 2% and serum GOT also was decreased in males on the diets containing ≥ 1%, the toxicological significance of decreases in these serum enzymes is not obvious. The pH of urine collected before sacrifice tended to be alkaline (pH 8) with increasing dietary concentration, but no occult blood in the urine was noted. The only hematological finding was a tendency towards anemia in females on the diets containing ≥ 0.5%. Based on this subchronic study, the maximum doses for the chronic feeding studies with OPP-Na that were done later (e.g., record 065929) were set to 2% and 1% for males and females, respectively. **NOEL = 1% diet (increased absolute kidney weight). Supplemental information.** (Kishiyama, 1/12/89; Rinkus, 7/19/89).

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129-012 068374 "Subacute Toxicity of o-Phenylphenol by Food Administration to Male Rats," (Nakamura et al., Ann. Rep. Tokyo Metr. Lab. P.H., **32**-2: 33-39, 1981). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables in English; and an English translation of the former in its entirety. OPP was presented in the diet for 13 weeks at the nominal concentrations of 0%, 0.625%, 1.25%, and 2.5%, to 10 male F-344/Ducrj rats/group. Mean bodyweights at the end of the study were 88% and 78% of the control value for the 1.25% and 2.5% groups, respectively. Absolute brain weight was decreased in the 2.5% group and absolute urinary-bladder postfixation weight was increased in the 1.25% and 2.5% groups; the weights of the liver and the kidneys relative to the bodyweight were increased in all groups receiving OPP. RBC count and hemoglobin concentration were decreased only in the 2.5% group. Increasing dietary intake of OPP tended to decrease both the pH of the urine (to pH 6) and the urinary concentration of protein; occult blood was detected in one rat in the 1.25% group and in another rat in the 2.5% group. No histological data was provided. **NOEL = <0.625% (increased relative kidney weight). Supplemental information.** (Kishiyama, 1/11/89; Rinkus, 7/20/89).

129-012 068373 "Subchronic Toxicity of o-Phenylphenol (OPP) by Food Administration to Rats," (Iguchi et al., Ann. Rep. Tokyo Metr. Lab. P.H., **35**: 407-415, 1984). This record consists of two parts: a study in Japanese, with only the abstract, figures and tables written in English; and an English translation of the former in its entirety. OPP was presented in the diet for 13 weeks at the nominal concentrations of 0%, 0.156%, 0.313%, 0.625%, 1.25%, and 2.5%, to 10 F-344/Ducrj rats/sex/treatment level. At the end of the study, the bodyweights of the high-dose males and females as a percent of the control values were 78% and 89%, respectively; no other groups showed any bodyweight effects. In males, the absolute and (or) relative organ weights of the kidneys, urinary bladder (postfixation), and liver increased with increasing intake of OPP, starting with the 0.313% diet for the liver effects; in females on the 2.5% diet, an increase was seen for the liver. In males on the 2.5% diet, brain weights were decreased, while in females on the 2.5% diet, heart, spleen, and possibly uterus weights were decreased; decreased spleen weight also was seen in females on the 1.25% diet as well. The pH of urine tended to be acidic (pH=6) only in the highest dose groups (both sexes); occult blood was detected in the urine of a few males on the 1.25% and 2.5% diets. The only hematological finding was a tendency towards anemia in both sexes on the 2.5% diet, and possibly the 1.25% diet. **NOEL = 0.313% (increased relative kidney weight). Supplemental information.** (Kishiyama, 1/11/89; Rinkus, 7/24/89).

129-012 068365 "Urinalysis of Male F344/DuCrj Rats Fed with Sodium o-Phenylphenate (OPP-Na)," (Tayama et al., Ann. Rep. Tokyo Metr. Lab. P.H., **35**: 425-430, 1984). This record consists of two parts: a study in Japanese, with only the abstract, photograph legends, and tables in English; and an English translation of the former in its entirety. The methods and the results are not described adequately, and the conclusions of the authors sometimes are not supported by the data. OPP-Na (Dow Chemical Co.; Dowicide A; lot no. MM01044) was presented in the diet for 52 weeks at the nominal concentrations of 0% and 2% to 6 and 30 male F-344/DuCrj rats, respectively. Urine was collected forcibly at the following times: weekly for the first 11 weeks, and then in weeks 13, 15, 17, 24, 30, 35, and 52. Urinalysis consisted of the following determinations: color, precipitate, pH, protein, glucose, ketones, and occult blood; however, the number of rats on the 2% diet whose urine actually was analyzed typically was <20. Also, naturally excreted urine that collected below the individually caged rats was checked for pH weekly for the first 26 weeks using a pH meter, and then weekly afterwards using only pH test paper. The authors state that the forcibly excreted urine from the OPP-Na-treated rats always exhibited a higher pH (using a pH meter), in comparison to the urines of the untreated rats. However, based on the data (mean values) presented, this was only obvious in week 1 and may have been suggested by the data for weeks 2-4; in weeks 5-7, the difference in urinary pH between the two groups appears insignificant, possibly because by this time the pH of the control group's urine itself had become more

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alkaline. Positive findings of ≥ 1 forcibly excreted urine with occult blood were made with the OPP-Na-treated rats in weeks 6, 7, 10, 11, 13, 15, 17, 24, 30, 35, and 52; however, individual rats testing positive for occult blood changed with each round of testing. These results contrast with the testing for occult blood in the naturally excreted urine, wherein positive findings were observed with the OPP-Na-treated rats starting week 6 and continuing with each testing till the end of the study. Starting week 44, obviously bloody urines were observed; and the incidence of this hematuria increased in the following weeks. The authors implied that categorically rats exhibiting hematuria had bladder tumors when autopsied; however, the number of rats involved was not stated. Stones were detected macroscopically in weeks 11, 24, 30, 35, and 52. Fluorescence X-ray analysis indicated that the stones contained: Ca, P, Na, Mg, S, Fe, and Al. However, the color of the stones was not stated; therefore, it is not clear if these were the same "dark or greenish brown" stones that have been described in the carcinogenicity bioassays (e.g., record 065929). In general, CDFA can only conclude from this study that rats eating the 2% OPP-Na diet exhibited hematuria (both occult and frank) starting week 6 and many weeks later passed urinary stones that could be seen macroscopically. However, since no correlations among individual treated rats exhibiting acidic urine, occult or frank hematuria, and urinary stones were made, this study has provided little towards understanding the relationship of these findings to OPP-Na-induced carcinogenesis in the urinary tract of rats. **Supplemental information.** (Kishiyama, 1/12/89; Rinkus, 7/25/89).

CHRONIC DOG

129-010 004165 "Toxicological Studies of Orthophenylphenol (Dowicide 1)," (Dow, 2/52, published in J. Pharmacol. Experimental Therapeutics 104: 202 (1952), Hodge, H. C. et al.) OPP (>98%) fed in the diet at 0.02, 0.2 and 0.5 g/kg/day for one year; 2 mongrel dogs at each dose; no adverse effects reported including kidneys; UNACCEPTABLE, not upgradeable. Insufficient information for evaluation. (Remsen (Gee), 4/2/85).

129-012 068360 Protocol. "Ortho-Phenylphenol: Palatability and Two-Week Dietary Toxicity Study in Beagle Dogs." No worksheet.

129-012 068703 Addendum to protocol (129-012 068360). No worksheet.

129-063 069841. Protocol. Ortho-Phenylphenol: One-Year Oral Toxicity Study in Beagle Dogs." No worksheet.

129-141 095220 "Ortho-Phenylphenol: Palatability/Probe, Four-Week and One-Year Oral Toxicity Studies in Beagle Dogs," (Cosse et al.; The Toxicology Research Laboratory/Dow; Report ID K-001024-039; 9/24/90). OPP, 100% purity, was administered by gavage at doses of 0 (peanut oil), 30, 100 and 300 mg/kg/d to 4 beagle dogs/sex/group for 12 months (5 d/w). Dose levels were based on preliminary studies which were included in the report. Two high-dose males died from gavaging errors on test days 137-138. The only effects observed in the full study were the following: vomiting (both sexes), with the frequency and volume being greatest in the high-dose groups; and a decrease in serum phosphate levels for the mid- and high-dose female groups tested at 12 months. The toxicological significance of the decreased serum phosphate levels is not obvious since no other toxicological effects were noted, including none that would be indicative of an effect on the urinary tract. **NOAEL ≥ 300 mg/kg/d. This study is considered **ACCEPTABLE**. (Rinkus, 1/24/91).

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ONCOGENICITY, MOUSE

Note: In record 065930, which was not an acceptable study, there was evidence of a possible carcinogenic effect by OPP-Na at two sites: liver (males: hemangioma/hemangiosarcoma, plus possibly hepatocellular carcinoma) and stomach (females: squamous-cell papilloma) (discussed in worksheet W065930.832). In a second study, record 137329, which is an acceptable study, OPP was hepatocarcinogenic and there is a question about the possible induction of vascular tumors (all sites); but a carcinogenic effect in the stomach was not noted (discussed in worksheet W137329.832). (Rinkus, 4/24/96).

****129-221 137329** "Ortho-Phenylphenol: Two-Year Dietary Chronic Toxicity/Oncogenicity Study in B6C3F1 Mice" (Quast, J.F. and McGuirk, R.J.; The Toxicology Research Laboratory, The Dow Chemical Co.; laboratory project study ID number K-001024-047; 2/1/95). o-Phenylphenol (OPP), ≥ 99.7% purity, was administered in the diet for two years to achieve dose levels of 0, 250, 500 and 1000 mg/kg for ~50 B6C3F1 mice/sex/dose level. Dose levels were chosen based on the results observed in the mouse oncogenicity study of sodium OPP (record 065930). Survival ranged from 74% to 84% in the males and 56% to 72% in the females, was not statistically different among treatment groups and did not follow a dose-related pattern. Bodyweights were comparable among treatment groups at 13 weeks; subsequently, significant bodyweight reductions were noted in the high- and mid-dose groups (both sexes). At the end of the study, the mean bodyweights of the high-dose males, high-dose females, mid-dose males and mid-dose females were 87%, 80%, 93% and 87% of the corresponding control value, respectively. Serum alkaline phosphatase was statistically increased in each of the male groups and in the high-dose female group when assayed at one year, but not at two years. The specific gravity of urine was decreased significantly for the high- and mid-dose females when tested at two years. Significant, dose-related decreases in absolute kidney weight were seen with the high- and mid-dose male groups when measured at one and two years; when expressed as kidney weight relative to bodyweight, no statistical differences were noted. Absolute and/or relative kidney weight were increased in each of the female groups, with the increases in relative kidney weight achieving statistical significance at one year (low-, mid- and high-dose groups) and at two years (mid- and high-dose groups). Significantly increased absolute and/or relative liver weight was seen at each dose level at one year (both sexes) and at two years (females). Statistically significant nonneoplastic findings at two years included: decreased microvacuolation in kidney tubular cells for each of the OPP-exposed male groups; decreased severity of degeneration/regeneration of kidney tubules for each of the OPP-exposed groups (both sexes); accentuated lobular pattern consistent with liver hypertrophy/enzyme induction in each of the OPP-exposed groups (both sexes); decreased fatty change in the liver for the high- and mid-dose male groups; decreased incidence in the liver of foci of necrosis in the high- and mid-dose female groups; decreased incidence in the liver of vacuolated or clear foci of cellular alteration in the high-dose male group; increased incidence of eosinophilic foci of cellular alteration in the high- and mid-dose male groups; and decreased pancreatic islet-cell hyperplasia in the high-dose male group. Neoplastic findings at two years included: dose-related increases in the incidence of liver adenomas in both sexes, with the incidences seen in the high- and mid-dose male groups achieving statistical significance; a 16% (8/49) incidence of liver carcinoma in the low-dose female group vs. 4% (2/48) in the female controls; a 4-12% incidence of hepatoblastoma in the OPP-exposed male groups; a 22% incidence of hemangioma/hemangiosarcoma (any site) in the low-dose male group, based on a partial examination of the spleens for the low- and mid-dose groups (both sexes); and a 18% and at least 14% incidence (latter based on the examination of organs from only 16 mice) of Harderian gland tumors in the control and low-dose male groups, respectively. **NOAEL < 250 mg/kg-d (hepatoblastoma).** How feed restriction/bodyweight reduction may have affected the interpretation of the results is discussed in

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worksheet W137329.832. This study is considered **ACCEPTABLE**. However, if the NOAEL is changed, the matter of the true incidences of hemangioma/hemangiosarcoma in the low- and mid-dose groups (both sexes) will need to be resolved. (Rinkus, 10/3/95).

129-200 130207 This record consists only of a table, entitled "Ortho-Phenylphenol (OPP): Two-Year Dietary Chronic Toxicity/Oncogenicity Study in B6C3F1 Mice--Preliminary, Unaudited Results from Male Mice." This record was accompanied by a letter from Paul A. Wright (Dow) to California Department of Pesticide Regulation, dated May 3, 1994. The letter acknowledges that the preliminary results indicate a tumorigenic response in the liver at all doses. **Supplemental information. No worksheet.** (Rinkus, 4/24/96).

129-032 035994 "Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note," (Bionetics and NCI, published in: J. Nat. Cancer Inst. 42: 1101-1114, 4/30/69, Innes, J. R. M. et al). Two hybrid strains of mice were given 100 mg/Kg of OPP days 7 - 28 of age by gavage followed by 280 ppm in the diet after day 28 for 18 months; OPP was one of 120 other chemicals tested; at the level tested OPP gave no reported significant indication of oncogenicity. **UNACCEPTABLE**, no data. (Gee, 5/30/86).

129-032 035995 "The Tumor-Promoting Action of Phenol and Related Compounds for Mouse Skin," (R.K. Boutwell & D.K. Bosch, Cancer Res. 19:413-424, 1959). **Supplementary information. No worksheet.** (Rinkus, 4/1/91).

50438-007 003880/003883 Summaries of 004164 (chronic rat) and 035994.

129-037 045322 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of ortho-phenylphenol alone and with 7,12-dimethylben(a)anthracene in Swiss CD-1 Mice," (Natl. Tox. Program, 3/86, NTP 84-099, NIH Publication No.85-2557). OPP (>99%, lot MM 09157); 50/sex/group; Swiss CD-1 mice were given dermal applications of 0.1 ml acetone, 55.5 mg/0.1 ml OPP in acetone, DMBA followed by acetone, OPP or TPA, 3 times per week for 102 weeks at the same site as DMBA for promotion; onco NOEL > 55.5 mg/day; no evidence of oncogenicity or promotion due to dermal application of OPP; OPP did cause an increase in non-neoplastic skin lesions over acetone control; **UNACCEPTABLE** (route of exposure with known poor absorption through skin, no indication of time of exposure and whether washed off is not clear). The single concentration used was the maximum soluble in acetone. This is not an oncogenicity study of the usual type and was designed for a different purpose, notably to test whether OPP acts as a promoter following initiation with a known carcinogen, DMBA. The DMBA/TPA combination gave the anticipated results of increased neoplasms and decreased survival. The OPP was irritating to the skin and increased the incidence of skin lesions over the acetone control whether alone or following DMBA. Introductory discussion states that OPP is poorly absorbed though the skin. NCI recommended the study of whether it is a promoter for skin exposure. Study is complete but supplementary only for oncogenicity due to route of administration.
NOTE: From data in Record 036007, OPP-Na might have given a different result. (Gee, 6/2/86).

129-032 036010. Board Draft, 2/85, of 037 045332.

129-058 065930 "Long-Term Toxicity and Carcinogenicity Study of Sodium o-Phenylphenate in B6C3F1 Mice," (Nobuyuki Ito [author], First Department of Pathology, Nagoya City University Medical School, Nagoya, Japan, 1983). Sodium o-phenylphenol (OPP-Na), a stated purity of 97%, was given in the feed at the nominal concentrations of 0, 0.5, 1, and 2% to 50 B6C3F1 mice/sex/treatment group, for 96 weeks, followed by 8 weeks of basal diet until terminal sacrifice. Analytical testing was inadequately described but would indicate that the corresponding OPP-Na

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intake was 0, ~0.5, ~1.2, and ~2.4 grams/kg/day for both sexes. Percent survival at test week 96 was ≥ 80% for all treatment groups of both sexes, except the high-dose males, whose percent survival was 74%. Each of the three female groups eating OPP-Na had mean bodyweights at test week 90 that were depressed by 9-23% relative to the value for the controls; for males at test week 90, only the high-dose group showed any bodyweight depression (9%). The incidence of brain calcification was increased in the high-dose males and the mid- and high-dose females; only the 45% incidence in the high-dose females was statistically significant ($p<0.05$). For each of the three female groups eating OPP-Na, the heart, liver, and kidneys showed increased absolute weights and/or organ weights relative to bodyweight and serum alkaline phosphatase levels were increased; comparable effects in the males were not seen. A decrease in the specific gravity of urine was seen in each of the female groups eating OPP-Na and in the mid- and high-dose males. The incidences of hemangiomas/hemangiosarcomas in the livers of males and of squamous-cell papillomas in the stomachs of females were increased in each of the respective groups eating OPP-Na. **NOEL < 0.5% nominal (equivalent to N0.5 g/kg/day intake).** This record was considered originally (Rinkus, 8/9/89) to be unacceptable but upgradable upon submission of: 1) the OPP-Na stability data requested in record 065929; 2) method, chronology & storage conditions for formulating test diets; 3) explanation of histological data inconsistencies; and 4) complete historical control data from the conducting laboratory for observed tumor types. The requested data in item 1 now has been provided in record 091951, but the study is still considered **UNACCEPTABLE** pending submission of the other requested information. (Rinkus, 4/5/91).

50438-005 038132 "Long-term Toxicity and Carcinogenicity Study of Sodium o-Phenylphenol in B6C3F1 Mice," (Hagiwara et al., Fd. Chem. Toxic. 22: 809-814, 1984). Published version of 129-058 065930; it lacks data given as appendices in 065930. (Rinkus, 8/15/89).

129-032 036008 "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans," (IARC Working Group, 3/83, WHO, volume 30). Document states that inadequate data to evaluate carcinogenicity study reported by Innes et al. (record 035994).

REPRODUCTION, RAT

****129-232 141559** "A Two-Generation Dietary Reproduction Study in Sprague Dawley Rats Using Technical Grade Ortho-Phenylphenol" (Eigenberg, D. A. And Lake, S.G.; Bayer Corporation, Agriculture Division, Toxicology, Study no. 93-672-VX, Sept. 28, 1995). This is the replacement study for record 072405/095639. o-Phenylphenol (OPP), purity ≥ 99.5%, was mixed into the feed such that ~30 CD Sprague-Dawley rats/sex/generation received nominal doses of 0, 20, 100 and 500 mg/kg/day. F0 rats were exposed for 10 weeks and ~21 weeks before the 1st and 2nd mating periods, respectively and were exposed for a total of 25-30 weeks (depending on the sex) before sacrifice. The postweaning exposure of the F1 rats before the 1st and 2nd mating periods lasted 12 weeks and ~22 weeks, respectively; and the F1 rats were 34-37 weeks old when sacrificed (depending on the sex). The only treatment-related, clinical-observation finding in the adults was an increased incidence of urine staining in the 500 mg/kg male groups (F0 and F1). Urine staining tended to start at study week 18 and to last till termination; in some cases, urine staining was also a finding at necropsy. Reduced bodyweight was noted in the F0 and F1 adults (both sexes) at the 500 mg/kg dose level. In the F0 females, the reduction was evident after three weeks of treatment; at 10 weeks and at termination, the mean bodyweight was 92-93% of the respective control values. The F0 males appeared to respond slower than the females; at 10 weeks and at termination, the mean bodyweight was 95% of the respective control values. F1 male and female 500 mg/kg groups exhibited reduced bodyweight as weanlings and began and ended the F1 premat-

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ing period with bodyweights that were 89-91% of the respective control values. Gestation bodyweight gain was not affected by treatment but the lactation bodyweight gain of the F2b 500 mg/kg group may have been increased. Food consumption (amount consumed relative to bodyweight) tended to be increased in the F0 and F1 500 mg/kg groups, with the effect in males being greater than in females. Since an increase in food consumption occurred in the F0 males before the onset of bodyweight reduction, some of the increase in relative food consumption may not be attributable simply to the bodyweight reduction. Mating, fertility and gestation indices were not decreased by treatments. Fecundity (# live deliveries / # cohoused) was lower than expected in the F1 controls and increased with dose in the F2a and F2b periods. Estrous cycling, which was monitored for the last three weeks of the F0 and F1 premating periods, was not affected. The absolute and relative weights of the kidneys, testes and ovaries were not affected by treatment in either generation. The incidences of two necropsy findings were increased in the F1 500 mg/kg male group: urine-stained ventrums and urinary-bladder calculus (color not stated). The following nonneoplastic lesions were observed in the urinary bladder of the F0 and F1 500 mg/kg males: simple hyperplasia, nodular/papillary hyperplasia and chronic inflammation. Incipient effects also may have been produced in the kidneys and ureters of the 500 mg/kg males. No urinary tract cancers were noted. Lymphoma (a rare cancer for young Sprague-Dawley rats) occurred in two F1 500 mg/kg males. The main progeny effect was reduced pup bodyweight in each of the four periods at the 500 mg/kg dose level on lactation day 21, with marginal effects being present on lactation day 14. There was no effect on the following: live litter size, the incidence of stillbirths, perinatal death or the incidence of renal pelvic dilatation in the pups. When first reviewed (May 6, 1997), this study was considered unacceptable and upgrading required the submission of the supplemental information discussed in worksheet W141559.834. In response, the Registrant submitted record 165412. Based on the supplemental information, the following conclusions have been reached (discussed in worksheet W141559.S01). The selection of F1b weanlings to become F1 adults involved two groups: 30/sex/dose group, plus 2/sex/dose group; the latter served as some sort of replacement group until sacrificed (apparently) at about three weeks after the start of the F1 premating phase. In the first review, it was presumed that nude-rat syndrome (a rare trait) occurred in the 20 mg/kg group, involving 7 pups from two F0 dams. Nude-rat syndrome also had been seen in the first study (record 072405/095639) in the 490 mg/kg group, involving 10 pups from three F1 dams. However, upon reinspection of the data, it is questionable that the hypotrichosis occurring in record 141559 qualifies as nude-rat syndrome. The incidence of lactation-day-21 weanlings with stunted growth (≤ 40.0 grams) was increased in the 500 mg/kg groups. Although two reproductive-toxicity studies, with two cases each of lymphoma (a rare cancer) associated with males and the same high dose, suggests of an incipient effect, the evidence is insufficient for concluding that OPP induced lymphoma in these reproductive-toxicity studies. **Parental NOEL = 100 mg/kg (nonneoplastic urinary-bladder lesions). Reproductive NOEL = ≥ 500 mg/kg (no effects at high dose). Progeny NOEL = 100 mg/kg (reduced pup bodyweight, including stunting)**. This study is marginally **ACCEPTABLE**. (Rinkus, 3/15/01).

129-278 165412 This record consists of an 8-page narrative section that provides a response to issues raised in worksheet W141559.834, with the following 10 appendices: Appendix I, a protocol amendment regarding F1b pups retained after weaning but not selected to be F1 adults; Appendix II, discussion and photographs of the hypotrichosis observed in the F0 low-dose group; Appendix III, individual pup bodyweights for lactation days 0, 4, 7, 14 and 21; Appendix IV, nude-rat syndrome bodyweights and necropsy findings; Appendix V, bodyweights and necrospy findings for pups as small as nude-rat-syndrome animals on lactation days 4 or 21; Appendix VI, the randomization scheme for assigning F1b weanlings to be F1 adults; Appendix VII, an amended page 92 from record 141559, indicating now that dam ID number 1120 was sacrificed in a moribund state; Appendix VIII, standard operating procedures for cohousing during mating triasl; Appendix IX, raw data for vaginal-smear inspections during cohousing for the four mating trials (TOX FORM

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57's); and Appendix X, historical negative-control data regarding mononuclear cell leukemia for the conducting laboratory. **Supplemental information, discussed in worksheet w141559.s01.** (Rinkus, 3/16/01).

129-233 141560 "A Two-Generation Dietary Reproduction Study In Sprague-Dawley Rats Using Technical Grade ortho-Phenylphenol: Supplemental Information Requested by California EPA" (Eigenberg, D.A. & Lake, S.G.; Bayer Corp., Agricultural Division Toxicology, Stillwell, Kansas; study number: 93-672-VX; 9/28/95). This record contains the following: protocol amendments, protocol deviations and SOP deviations that apply to record 141559; and the estrous-cycle raw data for record 141559 and the procedure for analyzing these data. **Supplemental information. No worksheet.** (Rinkus, 5/20/97).

129-211 133253 This record contains unaudited data from the F1a, F1b and F2a periods of record 141559. These data were submitted at a meeting held on Nov- ember 18, 1994 between DPR MT staff (Drs. Gee, Iyer and Rinkus) and the Registrant's representatives (Dr. Sangha, Dr. Burin and Ms. Stevens). The meeting was held at the Registrant's request to discuss the low fertility that had occurred with the control and some OPP-treatment groups in the F2a mating trial in record 141559. These data were inspected originally in 1994 but they have not been given a formal review because these data are considered superseded by the data in the full study, record 141559. **Supplemental information. No worksheet.** (Rinkus, 5/20/97).

129-206 132174 "A Two-Generation Dietary Reproduction Study In Rats Using Technical Ortho-phenylphenol: Unaudited Interim Summary Tables" (Eigenberg, D.A.; Miles Inc., Agricultural Div-ision Toxicology, Stillwell, Kansas; study number: 93-672-VX; report is undated [cover letter dated 9/13/94, from Christina L. Cocciardo to Fely Frank]). These data have not been reviewed because this submission was superseded by the submission of the full study, record 141559. (Rinkus, 5/20/97).

129-190 126178 "Protocol: A Two-Generation Reproduction Study in Rats Using Technical Ortho-phenylphenol" (Eigenberg, D.A.; Miles Inc., Agricultural Dvvision, Toxicology, Stillwell, Kansas; study number: 93-672-VX; report is unsigned and undated [it was stamped as received at DPR on 9/20/93]). This is the protocol for the replacement rat reproduction study (record 141559). DPR MT's review is contained in worksheet W126178.834. This worksheet served as the basis for discussions conducted by telephone on October 20, 1993 between DPR MT staff and the Registrant's scientists. **Supplemental information**. (Rinkus, 5/20/97).

129-198 127753 "Protocol: A Two-Generation Reproduction Study in Rats Using Technical Ortho-phenylphenol" (Eigenberg, D.A.; Miles Inc., Agricultural Division, Toxicology, Stillwell, Kansas; study number: 93-672-VX; no report date per se; signature page has 12/3/93 as latest date; ac-companying letter by Christina L. Cocciardo (Miles) is dated 12/16/93. Letter indicates that this protocol is the same as record 126178 except that Dow Chemical is listed as a cosponsor. This was not reviewed. **Supplemental information. No worksheet.** (Rinkus, 5/20/97).

129-047 050575 Protocol for reproduction study at Mobay.

129-048 075867 This record concerns the study in records 072405/095639; its contents include the following: a protocol amendment regarding the shortening of the F1 premating before the F2a mating trial and the rest period before the F2b mating trial; and a progress report discussing the low fertility observed in the F1b mating trial. **Supplementary information. No worksheet.** (Rinkus, 4/1/91).

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129-082 072405 "Two-Generation Dietary Reproduction Study in Rats Using Ortho-phenylphenol," (Mobay Corporation, Corporate Toxicology Department, Study no. 85-671-02, January 13, 1989). When this study was first submitted, it was considered unacceptable and to upgrade it would require the submission of data that addressed the many concerns that CDFA had with this study (Kishiyama, 2/23/89; Rinkus, 7/6/89). Subsequently, this study was resubmitted in its entirety, incorporating a variety of changes; this second submission is record 095639. See the summary to record 095639 for details. (Rinkus, 3/15/91).

129-139 095639 "Two-Generation Dietary Reproduction Study in Rats Using Ortho-phenylphenol--Revised Report" (Mobay Corporation, Corporate Toxicology Department, Study no. 85-671-02, January 13, 1989). This is the second submission of this reproduction study; the first was 129-082 072405. o-Phenylphenol, purity ≥ 99.4%, was mixed into the feed such that Sprague-Dawley rats of both sexes received (analytical) doses of 0, 35, 125 or 457 mg/kg/day. F0 rats were exposed for 15 and ~31 weeks before their 1st and 2nd matings, respectively, and were exposed for a total of 43 weeks before they were sacrificed (age: ~1 y); and F1 rats were exposed for 10 and ~22 weeks before their 1st and 2nd matings, respectively, and were exposed for a total of 31-37 weeks before they were sacrificed (age: 34-40 weeks). Premating bodyweights were decreased by treatments only in the high-dose groups: both sexes for F0 rats; and unequivocally the dams for F1 rats. Lactation BW change was increased for the F1b and F2b litters in the mid- and high dose groups. Mating and fertility indices were not affected by treatments for three of the mating trials; but the reduced fertility in the high-dose group for the F1b mating trial is difficult to interpret due to low fertility in the controls and the reduced estrus cycling observed for each of the treatment groups before the F1b mating trial. Organs whose absolute weights tended to increase with treatments were the testes, ovaries, kidneys (F0 and F1 males), and liver (F1 males). The incidence of ovarian cysts was increased in the F0 high-dose dams. Nonneoplastic lesions were observed in the urinary bladder and kidneys, including 4 cases of papil-lomatosis or papilloma formation in the urinary tract of F0 rats from the mid- and high-dose groups. **Parental NOAEL = 35 mg/kg (one dam exhibited papillomatosis in its urinary bladder after 14 weeks of eating a diet of ≤ 2000 ppm).** Based on the raw data, the incidence of "stillbirths" for the F2a and F2b litters appeared to be increased in the mid- and high-dose groups; therefore, the reproductive NOAEL appeared to be 35 mg/kg. Day-14 and day-21 pup weights were reduced in the high-dose groups for each of the four periods. The incidence of kidney dilatation observed grossly in day-21 weanlings was increased in each of the OPP-treatment groups for both the F2a and F2b litters. **Progeny NOAEL = < 35 mg/kg.** When previously reviewed (3/15/91), this study was considered unacceptable and to upgrade the following had been requested for submission: an appropriate audit of the data; all protocol changes and deviations; and supplementary information, including raw data, in various areas, as detailed in worksheet W095639.834. These data have now been submitted (records 112073, 113297 & 113884) and are discussed in worksheet W095639.S01. Statistical analyses confirm the identification of weanling kidney dilatation as an endpoint for the progeny NOAEL. However, the following clarification regarding the endpoint for the reproductive NOAEL is indicated: what is increased in the F2a and F2b mid- and high-dose groups is perinatal deaths occurring on lactation days 0-4, not simply stillbirths. **Reproductive NOAEL = 35 mg/kg (perinatal deaths).** This study is considered **UNACCEPTABLE AND NOT UPGRADABLE.** (Rinkus, 7/7/92).

129-138 095645 This record contains supplementary information to record 072405. It consists of 6 parts: 1) responses by the Sponsor to the matters raised in the worksheet to record 072405; 2) data and statistical analyses for gestational and lactational bodyweights and litter parameters; 3) necropsy data regarding kidney dilatation in day 21-weanlings (as well as some day-4 culled pups & pups found dead); 4) pretesting serological data attesting to the well being of the rats before being shipped from the supplier; 5) a letter from the American Association for Accreditation of Labor-

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atory Animal Care (AAALAC) indicating that the conducting laboratory is AAALAC accredited; and 6) a letter from the animal supplier (Charles River, Wilmington, MA) noting that hypotrichosis has a low incidence among their rats. **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095663 This record concerns the histological examination of 4 slides of the urinary tract, representing 4 rats from records 072405/095639; the examinations were done by Dr. Cohen (University of Nebraska Medical Center) and Drs. Kociba and Quast (The Dow Chemical Company). **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095664 "Cell Proliferation Induced by Uracil-Calculi and Subsequent Development of Reversible Papillomatosis in the Rat Urinary Bladder," (Shirai et al., Cancer Research, 49:378-383, 1989). This record along with record 095665 are supposed to constitute the "classification scheme" for the histopathological examinations given in record 095663. **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095665 "Uracil-Induced Urolithiasis and the Development of Reversible Papillomatosis in the Urinary Bladder of F344 Rats," (Shirai et al., Cancer Research, 46:2062-2067, 1986). This record along with record 095664 are supposed to constitute the "classification scheme" for the histopathological examinations given in record 095663. **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095666 "Toxic and Non-Toxic Changes Induced in the Urothelium by Xenobiotics," (Samuel M. Cohen, University of Nebraska Medical Center, Paper presented at the 1989 Society of Toxicology meeting). **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095667 This record concerns the occurrence of stones in the urinary tract of untreated SD rats; it consists of two sections: 1) The Pathology of Laboratory Animals, Volume I, p. 159, Benirschke et al. (Eds.), Springer-Verlag, New York, year not stated; and 2) The Laboratory Rat, Volume I, pp. 389-390, Baker et al. (Eds.), Academic Press, New York, 1979. **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-138 095668 This record concerns the occurrence of pinworms in laboratory rats; it consists of two sections: 1) Laboratory Animal Medicine, pp. 111-113, Fox et al. (Eds.), Academic Press, New York, year not stated; and 2) The Laboratory Rat, Volume I, pp. 321-322, Baker et al. (Eds.), Academic Press, Ney York, 1979. **Supplementary information. No worksheet.** (Rinkus, 3/15/91).

129-160 112073 This record contains the following: 1) individual responses to the matters raised in worksheet W095639.834; 2) the protocol to the study presented in records 095639 and 072405; 3) 17 protocol amendments and 7 protocol deviations; 4) raw data regarding lactation for the F1b period; 5) raw data regarding designation of mating status for the entire study; 6) raw data regarding external and gross pathology observations for dams 0069, 2252 and 3259; 7) raw data regarding litter necropsy observations for the litters of dams 0069, 2252, and 3259; 8) corrected tables for stillborn pups and statistical analyses of these corrected data; 9) raw data regarding kidney dilatation in 21-day old pups for the entire study; 10) an overview of kidney dilatation observed in the pups in the study as well as in the historical control data; 11) an evaluation by a consultant to the Registrants of the kidney dilatation observed in weanlings in the study; 12) a table listing corrections to the delivery data presented in record 095639; 13) a summary of stillborn pups in the study and a statistical analysis thereof; 14) 4 articles from the literature regarding the use of the litter as the experimental unit for statistical analyses of pup data; and 15) raw data regarding litter

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necropsy observations for all pups in the study which were older than 21 days when sacrificed. **Supplemental information. No worksheet.** (Rinkus, 7/7/92).

129-165 113297 This record contains a variety of corrections to the data presented in records 095639 and 112073. These corrections were identified by the Registrant in the course of auditing the pathology tables and appendices in record 095639. **Supplemental information. No worksheet.** (Rinkus, 7/7/92).

129-167 113884 This record contains the following: 1) written responses to questions posed by Dr. Rinkus (DPR MT) in a telephone conversation with the Registrant's toxicologists (3/11/92) regarding record 112073; 2) raw data regarding lactation for the F1b period for dams 0063 and 3062-3085; and 3) the standard operating procedure used for the mating trials in record 095639. **Supplemental information. No worksheet.** (Rinkus, 7/7/92).

ANALYTICAL STUDIES, IN SUPPORT OF THE REPRODUCTION STUDY

129-138 095669 "Analytical Chemistry Report: The Evaporation of Methyl Isobutyl Ketone (MIBK) from Rodent Ration Stored in Rat Feeders," (K.D. Moore; Mobay Chemical Corporation; Toxicology Report No. 710; 2/10/86). This record was submitted as evidence that it is highly unlikely that any acetone was present in the diets that the rats ate in the reproduction study (records 072405/095639) even though acetone was used to prepare the diets. **Supplemental information. No worksheet.** (Rinkus, 3/15/91).

129-138 076032 "The Stability of Ortho-phenylphenol in Rodent Ration--A Comparison of Three Methods of Analysis," (K.D. Moore; Mobay Corporation; Laboratory Project ID Report No. 100271; 8/29/90). This stability study was performed in response to concerns raised by CDFA in its review of the first submission of the rat reproduction study, record 072405, wherein the analytical data had indicated that o-phenylphenol (OPP) was not stable in rodent chow. While these analytical findings were not one of the reasons for not accepting record 072405, they were still important because they indicated that OPP-containing diets were not stable enough to be stored for 3-month periods, as had been done in some rat chronic studies conducted by Japanese researchers that the Sponsors had submitted to fill SB950 data requirements (e.g., record 065929). Since a loss of OPP content over time could be the result of the actual OPP degradation as well as an increasing inefficiency in recovering OPP from the diet, the latter possibility was tested by using three different methods to recover OPP from aging diets. A diet made up to contain 5031 ppm (40.241 g OPP mixed into 7880 g chow plus 79 g corn oil [presumably]) was analyzed for content over a 28-day period. The three extraction methods were: 1) methanol extraction (OPP is highly soluble in methanol); 2) 0.1 M NaOH extraction (OPP is ionized in alkaline water, which maximizes its water solubility); and 3) acetonitrile extraction (this was the method used in the analytical studies reported in record 072405). What exactly was done and whether recovery efficiencies were determined for each day that analyses were done were not clearly stated. For some "5000 ppm" diet, the recovery efficiencies were $\geq$ 94% for each of the methods (pp. 488, 493 & 498), but from the day-0 results of the aging study (p. 483), method 2 appeared to recover only 86% of the target value (4303/5031). At the end of 28 days, method 3 only recovered 70% of the original target value (3505/5031); the loss corresponds to what had been observed in record 072405. The proof that this was not OPP degradation was that the recovery of OPP was still 95% with method 1 (4792/5031). Method 2 showed a seeming increase in OPP content over time (4813 ppm on day 28), but this may be artefactual because the recovery efficiency on day 0 may have been abnormally low. Assuming that the 5031 ppm diet and a control diet (0 ppm diet) were prepared the

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way diets were prepared in record 072405 (i.e., with acetone and corn oil) and that there were no interfering peaks coeluting with OPP from control diets over time with method 1, these analytical findings would indicate that OPP in diets prepared in the manner described in record 072405 is stable for at least 28 days. **Supplementary information.** (Rinkus, 3/15/91).

TERATOGENICITY, RABBIT

****129-148 097303** "Ortho-Phenylphenol (OPP): Gavage Teratology Study in New Zealand White Rabbits," (Zablotny et al.; The Toxicology Research Laboratory, Dow Chemical Company; Laboratory Project Study ID number K-001024-045; 4/23/91). In the first phase of testing, OPP was given by gavage on gestation days 7-19 to 16 inseminated NZW rabbits/group at 0 (corn oil), 25, 100 and 250 mg/kg and does were sacrificed on gestation day 28. In supplementary testing, OPP was administered only to 2 and 8 inseminated does/group at 0 and 250 mg/kg, respectively; data from this second phase of testing were combined with the data from the first phase. Treatment levels were chosen on the basis of a probe teratology study (record 097302). Does, especially those in the high-dose group, exhibited several effects (blood in the excrement pans, hairballs in the stomach, increased mortality, ulceration and hemorrhaging in the stomach, hemolyzed blood in the intestines); but it is unclear whether most of these were actually OPP-induced. One effect that did appear to be OPP-induced was renal tubular degeneration and inflammation. **Maternal NOAEL = 100 mg/kg (renal tubular degeneration/inflammation).** The only fetal effect noted in the study was an increase in the frequency of litters with resorptions in the 100 and 250 mg/kg groups. **Fetal NOAEL = 25 mg/kg (increase in resorptions).** When first reviewed (7/16/91), this study was considered UNACCEPTABLE but UPGRADABLE upon submission of: 1) raw data regarding when some does died; and 2) statistical analyses and historical control data regarding resorptions. These data now have been submitted (records 112322 & 113826) and, as discussed in worksheet W097303.S01, the matters that they addressed now are considered resolved. This study now is considered **ACCEPTABLE**. (Rinkus, 8/4/92).

129-162 112322 This record contains the following: 1) individual responses to the matters raised in W097303.833; 2) Registrant's comments about whether rabbits had been randomly assigned to treatment groups and about whether doses of ≥ 250 mg/kg cause multiple toxicological responses in the does; 3) raw data for two high-dose does that died on gestation days 15-16; 4) a 1975 memorandum from Dr. Joseph K. Haseman regarding the uses of the Fisher's exact test in teratology studies; 5) historical control data for the conducting laboratory regarding the incidence of litters with resorptions; 6) statistical analyses of the data regarding resorptions in record 097303; and 7) four articles from the open literature regarding hairballs in rabbits. **Supplemental information. No worksheet.** (Rinkus, 8/4/92).

129-166 113826. This record contains two letters. The one dated 3/19/92 is from the study director for record 097303; the letter explains why the wrong identification number appears in the raw data for doe 90A6484 contained in record 112322 (p. 16). The other letter is discussed under this record number in the section "Combined Chronic-Oncogenicity, Rodents." **Supplemental information. No worksheet.** (Rinkus, 8/4/92).

129-132 095057 "Ortho-Phenylphenol (OPP): Gavage Teratology Study in New Zealand White Rabbits," (The Dow Chemical Company, file number HET K-001024-045; 8/13/90). This record is the protocol to record 097303. **Supplementary information. No worksheet.** (Rinkus, 3/25/91).

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129-148 097302 "Ortho-Phenylphenol (OPP): Gavage Teratology Probe Study in New Zealand White Rabbits," (The Dow Chemical Company; laboratory project study ID: HET K-001024-044; 4/2/91). This study was used to set the dose levels in the full study in record 097303. Seven inseminated does/group were gavaged once daily at 0, 250, 500 and 750 mg/kg on gestation days 7-19 and were sacrificed on day 20. **Supplementary information. No worksheet.** (Rinkus, 7/16/91).

129-130 087182 "Ortho-Phenylphenol (OPP): Gavage Teratology Probe Study in New Zealand White Rabbits," (The Dow Chemical Company, file number HET K-001024-044; 6/29/90). This record is the protocol to record 097302. **Supplementary information. No worksheet.** (Rinkus, 3/25/91).

129-140 091950 This record contains two protocol addenda to the full teratology study in record 097303. The first addendum concerns the addition of 2 does to the 0 mg/kg/day group and 8 does to the 250 mg/kg/day group. Does were added to these groups because the number of litters with viable fetuses in these groups were only 12 and 10, respectively; FIFRA guidelines recommend ≥ 12 pregnant does per dose level. The second addendum concerns histopathological examinations of the kidneys of all rabbits in the study; this was done to establish a NOAEL for the kidney lesions observed in the teratology probe study. **Supplementary information. No worksheet.** (Rinkus, 3/25/91).

129-148 097301 "Ortho-Phenylphenol (OPP): 13-Day Range Finding Oral Gavage Study in New Zealand White Rabbits," (The Dow Chemical Company; laboratory project study ID: HET K-001024-043; 3/19/91). This study was used to set dose levels for the probe teratology study found in record 097302. Two nonpregnant female rabbits/group were gavaged at 0, 100, 500, and 1000 mg/kg for 13 consecutive days and were sacrificed on day 14. **Supplementary information. No worksheet.** (Rinkus, 7/16/91).

129-130 087181 "Ortho-Phenylphenol (OPP): 13-Day Range Finding Oral Gavage Study in New Zealand White Rabbits," (The Dow Chemical Company, file number HET K-001024-043; 5/21/90). This record is the protocol to record 097301. **Supplementary information. No worksheet.** (Rinkus, 3/25/91).

TERATOGENICITY, RODENT

RAT

129-032 036013 "Teratogenicity and Dominant-Lethal Studies with o-Phenylphenol," (Journal article (1978) published in J. Pesticide Sci. 3: 365-370, Kaneda, M. et al). OPP (99.7%); 20 female Wistar rats per group were dosed by gavage with 0, 150, 300, 600 or 1200 mg OPP/Kg on days 6 through 15 of gestation; all but 1 female at high dose died; body weights of dams in the 600 and 300 mg/Kg groups were significantly lower; fetal resorption was elevated and pup weights were reduced in the 600 mg/kg group; abnormalities did not differ among surviving pups; sys NOEL = 150 Mg/Kg (maternal toxicity). From limited information, dev. tox. NOEL appears to be 300 mg/kg. **UNACCEPTABLE** with no adverse teratogenic effect (no analysis of dosing solutions, no individual data). (Gee, 6/3/86).

129-031 028378. Duplicate of 036012 & 036013.

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129-032 036020 "The Effects of Orally Administered Orthophenylphenol on Rat Embryonal and Fetal Development", (Tox Research Lab-Dow, 8/30/78, John, J. A. et al). OPP (>99%); 34 control and 25-27 pregnant Sprague-Dawley rats were given 0, 100, 300, or 700 mg/Kg by oral gavage days 6-15; no adverse effects reported for developmental tox; sys NOEL = 300 mg/Kg (maternal body weight), Dev. NOEL = 700 mg/kg; originally reviewed as unacceptable by Gee, 6/3/86, because of missing data on food consumption and dosing solution preparation. Now upgraded to **ACCEPTABLE (missing data on analysis of dosing material and individual values on food consumption contained in Record # 054898, 129-048). Although the maternal effects reported are marginal in terms of toxicity, 1200 mg/kg to rats was lethal to 10/11 pregnant animals -see 036013 above. (Gee, 6/3/86 and 3/30/87).

129-048 054898 Supplement to 036020. Individual food consumption and records for preparation of dosing solutions.

129-032 036021 Partial duplicate of 036020. Publication in Fundamental and Appl. Toxicol. 1: 282-285 (1981). (Gee, 6/3/86).

129-031 028379 Exact duplicate of 036021.

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50438-005 038133, "Teratological Tests of Ortho-Phenylphenol and Its Sodium Salt in Mice", (Journal article (1978) published in Ann. Rept. Tokyo Metr. Res. Lab. P. H. 29: 89-96, Ogata, A. et al). OPP (Lot FB103) and its sodium salt (lot MM1044); 20/group given 0, 1450, 1740 or 2100 mg/Kg/day OPP in olive oil or 0, 100, 200 or 400 mg/Kg/day OPP-Na in water by oral gavage days 7-15; high mortality at high dose with both test articles - 16/21 with OPP and 16/21 with OPP-Na; some at mid-dose; fetal effects at all doses with cleft palate, open eye and exencephalia being identified - skeletal findings are attributed to maternal toxicity; **positive fetal findings occurred at the low doses where marginal maternal toxicity, especially with OPP-Na**. Systemic NOEL not clearly established due to lack of data. **Dev. NOEL <100 mg/kg. UNACCEPTABLE** (dev. NOEL not established, no individual data, tables hard to read with Japanese and English headings in small print, no historical controls, inadequate number of fetuses for visceral exam, no purity of test articles, dose selection poor), **not upgradeable.** (Gee, 5/30/86 and Parker, 6/13/86).

129-032 036022. Duplicate of 038133.

GENOTOXICITY, GENE MUTATION

Note: Results with gene mutation studies present mixed results in both bacterial and eukaryotic systems. Although no one study is adequate, collectively they contain sufficient data to determine that OPP is not mutagenic in bacteria but is genotoxic in mammalian cells in vitro. (Gee).

Microbial Systems

50438-005 038122 "Molecular Mechanisms involved in the Toxicity and Carcinogenicity of Orthophenylphenol and its Sodium Salt", (Journal article (1983) published in Chem.-Biol. Interactions

3/16/01 4/9/01

43:99-119, Reitz, R. H. et al). OPP-Na (lot MM09220B, 72%, 25.6% water and 1.05% NaOH); Strains TA98, 100, 1535, 1537 and 1538 of Salmonella typhimurium were tested at 0.25, 2.5, 25, 125 and 250 µg OPP-Na/plate with and without S9 with 30 minutes preincubation before plating; no increases in reversion rates were noted at these exposure levels; cytotoxicity at 125 and 250; triplicate plates. UNACCEPTABLE, no repeat trial. (Gee, 5/30/86).

129-032 035998. Duplicate of 038122 in 50438 - same data in report form.

129-032 036002. Exact duplicate of 038122.

129-047 050581. Duplicate of 038122.

129-032 036000. Exact duplicate of 038122.

129-032 036011, "Mutagenicity Evaluation of Ortho-Phenylphenol: Final Report", (Litton Bionetics, 3/31/76, LBI Project No. 2547.). OPP (no purity or lot number) tested in Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 at 0, 0.025, 0.25, 2.5 or 25 µg/plate +/- rat S9; one trial, one plate; no increase in reversion rate reported; UNACCEPTABLE (missing information, no repeat test, no evidence that cytotoxic level achieved), not upgradeable. (Gee, 6/2/86).

129-032 045825 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Orthophenylphenol Alone and with 7,12-Dimethylbenz(a)anthracene in Swiss CD-1 Mice" (Appendix K, Genetic Toxicology of o-phenylphenol), (Natl.Toxicology Prog., 2/85, Board Draft, NTP 84-099 - see 129-037 for final version of report, dated March, 1986). Summary only: Strains TA 98, 100, 1535, and 1538 of Salmonella typhimurium were tested at 0, 3.3, 10, 33, 40, 60, 80, 100 120, 140 or 200 µg OPP/plate +/- S9 (rat and hamster) with 20 minute preincubation before plating; test article was weakly mutagenic at 80 µg/plate and higher in TA 1535 (minus S9). The assay was performed twice with triplicate plates but only the mean and SD of one experiment is presented in a table as an Appendix. UNACCEPTABLE because incomplete report. (JG, 6/2/86).

129-032 036010. Main report of 045825

129-032 036014, "Mutagenicity testing on o-phenylphenol", (summary only--abstract in Mutation Res. 54: 277 (1978), Shirasu, Y., et al). Salmonella host-mediated in mice. No effect reported. No data.

129-032 036018, "Mutagenicity testing on o-phenylphenol", (abstract from Mutation Res. 54: 277 (1978), Shirasu, Y. et al). E. coli WP2 hcr⁻ with and without S9. No data. No effect.

129-047 050580 "Detection of Chemical Mutagens--Use of Concentration Gradient Plates in a High Capacity Screen," (J.C. Cline & R.E. McMahon, Res. Comm. Chem. Path. Pharmacol. 16:523-533, 1977. **Supplementary information. No worksheet.** (Rinkus, 4/1/91).

Mammalian Systems

129-032 036019, "Orthophenylphenol Mutagenicity in a Human Cell Strain", (Journal article (1984): published in Mutation Res. 156:123-127, Suzuki, H. et al). The potential for OPP to induce ouabain-resistant mutants in ultraviolet-sensitive human RSa cells was examined; cells were exposed to 0, 15, 20, 25, or 30 µg OPP/ml with ethanol as solvent for 24 hrs; mutation frequencies increased in a dose-related fashion. No data--only graphs. MF appeared to be about 100x control at 30 µg with a linear increase with concentration. UNACCEPTABLE. (Gee, 6/3/86).

LGR 3/16/01 [illegible] 4/9/01

129-032 045826 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Orthophenylphenol Alone and with 7,12-Dimethylbenz(a)anthracene in Swiss CD-1 Mice" (Appendix K, Genetic Toxicology of o-phenylphenol), (Natl. Tox. Program, 2/85, Board Draft, NTP 84-099--see 129-037 for final version of report dated March, 1986, summary only). L5178Y/TK +/- mouse lymphoma cells were exposed to 0, 0.32, 0.63, 1.25, 2.50 and 5.00 µg/ml OPP with S9 and 0, 20, 30, 40, 50 or 60 µg/ml without S9; weakly mutagenic at levels of 40 µg/ml and above without activation and at 5 µg/ml with metabolic activation. A second trial -S9 was performed but no data presented. UNACCEPTABLE, incomplete. (Gee, 6/2/86).

129-032 036010. Main report of 045826.

129-032 045827 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Orthophenylphenol Alone and with 7,12-Dimethylbenz(a)anthracene in Swiss CD-1 Mice", (Natl. Tox. Program, 2/85, summary only). The sex-linked recessive lethal assay with Drosophila was utilized to test mutagenic potential of OPP; insects were either fed the test article at 250 ppm or received injections of 500 ppm; three broods of 3, 2, 2 days; at these levels the test article failed to increase the incidence of mutations. UNACCEPTABLE (missing data), No adverse effect. (Gee, 6/2/86).

129-032 036010. Main report of 045827.

GENOTOXICITY, CHROMOSOME

Note: This data requirement was considered satisfied previously, with a possible adverse effect indicated based on studies in mammalian cells (record 045828). Since then, at least two more studies have appeared in the open literature which also indicate that OPP itself or its metabolites are capable of causing chromosomal damage in mammalian cells treated in vitro: Tayama-Nawai et al., Mutation Res. 141:95-99, 1984; and Tayama et al., Mutation Res. 223:23-33, 1989. (Rinkus, 8/15/89).

129-032 045828 "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Orthophenylphenol Alone and with 7,12-Dimethylbenz(a)anthracene in Swiss CD-1 Mice" , (Natl. Tox. Program, 2/85, summary only). The potential for OPP to induce sister-chromatid exchange (14.9, 20.0 and 29.9 µg/ml without S9 and 24.9, 49.8 and 75.4 µg/ml + rat liver S9) or chromosomal aberrations (60.0, 70.2 and 80.0 µg/ml without S9 and 70.2, 80.0 and 90.0 µg/ml +S9) in cultured Chinese hamster ovary (CHO) cells was examined; without S9, OPP tended to increase sister-chromatid exchange but not aberrations. SCE/cell for DMSO control was 8.9/cell and 11.4 at 29.9 µg/ml. UNACCEPTABLE (inadequate data and methods description), with weak effect on SCE formation. (Gee, 6/2/86).

129-032 036012 "Teratogenicity and Dominant-Lethal Studies with o-phenylphenol", (Journal article published in J. Pesticide Sci. 3:365-370 (1978), Kaneda, M. et al). Dominant lethal in groups of 15 male CH3 mice were administered 0, 100 or 500 mg OPP (>99%)/Kg by gavage for 5 successive days; immediately following the exposure period each male was caged with 2 females for 1 week; mating trials continued for 6 weeks; pregnant females were killed on day 12 or 13 of gestation to examine for dominant-lethal mutations; increased frequencies were not recorded in test groups. Body weight of males was depressed to 92% of controls after five days at the high dose. UNACCEPTABLE (no analysis of dosing solutions, no individual data), negative

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for adverse effect. (Gee, 6/3/86).

129-032 036016. Partial duplicate of 036012.

129-031 028378. Duplicate of 036012.

129-032 036015 "Mutagenicity Testing of o-phenylphenol", (Abstract in Mutation Res. 54: 277 (1978), Shirasu, Y. et al). Rat in vivo cytogenetics. A single dose at 0, 250, 500, 1000, 2000 or 4000 mg/kg or 5 doses at 0, 50, 100, 200, 400 or 800 mg/kg and sacrificed at 24 hours. No effect was found. UNACCEPTABLE, incomplete.

GENOTOXICITY, DNA/OTHER

Note: Based on the study by Reitz et al. (Chem.-Biol. Interactions 43: 99-119, 1983) the negative findings for Unscheduled DNA Synthesis and for DNA binding were used previously to close this data gap. These findings along with others were presented by the Sponsors in support of the hypothesis of a non-genotoxic mechanism for the formation of bladder tum ors. However, CDFA has noted in its rebuttal of 8/30/89 that a genotoxicity mechanism that does not involve DNA binding also appears plausible. Morimoto et al. (Jpn. J. Cancer Res. 78: 1027-1030, 1987) have shown using the alkaline elution assay that intrabladder injection of the quinone of 2,5-dihydroxybiphenyl, a suspected metabolite of OPP, induced DNA single-strand breaks in the cells isolated from the epithelium of the bladder; also, epi thelial hyperplasia was observed 5 days later in the bladder after a single treatment with this metabolite. These results may indicate that the treatment with the quinone led to the formation of radicals which attacked the DNA to cause the genotoxicity. Morimoto et al. have proposed that the "active oxygen species" were probably responsible for the bladder carcinogensis. Thus, CDFA does not agree necessarily with the position of the Sponsors that the mechanism of action for OPP does not involve genotoxicity. (Rinkus, 8/30/89).

50438-005 038123, "Molecular Mechanisms involved in the Toxicity of Orthophenylphenol and its Sodium Salt", (Journal article (1983) published in Chem.-Biol. Interactions 43: 99-119 (1983), Reitz, R. H. et al). Using cultures of rat hepatocytes, OPP-Na (lot MM0922OB, 72% SOPP, 25.6% water and 1.05% NaOH) was evaluated for its potential to induce UDS; 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M, higher concentrations were cytotoxic; test article did not increase UDS. UNACCEPTABLE (missing data), No adverse effect. (Gee, 5/30/86).

129-032 035999 Duplicate of 038123.

129-032 036003 Duplicate of 038123.

129-032 036017 "Mutagenicity Testing on o-phenylphenol", (Abstract in Mutation Res. 54: 277 (1978), Shirasu, Y. et al). Rec assay in B. subtilis with and without activation; results stated to be negative --no data. (Gee, 6/3/86).

129-032 036001 [identical to 035999], "Molecular Mechanisms Involved in the Toxicity of Ortho-phenylphenol and its Sodium Salt--DNA alkylation," (Dow, 1981, published in: Chem.-Biol. Inter-actions 43:99-119 (1983) by Reitz, R. H. et al). [14C]-OPP (99.8%, lot MM09250) or [14C]-SOPP (lot MM09220OB, 72% SOPP, 25.6% water and 1.05% NaOH), 500 mg/kg, given by oral gavage

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to groups of 8 male rats per test compound; sacrificed after 16 hours and DNA extracted from the bladders--DNA pooled; no details of methods for DNA extraction or for counting the DNA; **two experiments with negative results** reported in both in terms of dpm; samples counted (method not described) for 100 minutes per sample--report states the accumulated counts were sufficient to detect 1-2 dpm at the 95% confidence limit; **unacceptable** as reported due to lack of methods. (Gee, 3/30/87).
EPA memo [copy in document 129-048] of March 22, 1982, indicates the test was evaluated as "acceptable" and "adequately" conducted "within the context of the reservations when using this testing approach...." Whether they reviewed the identical document is not known.

50438-005 038125 "Molecular Mechanisms Involved in the Toxicity of Orthophenylphenol and its Sodium Salt - Cellular Regeneration", (Dow, 1981, published in: Chem.-Biol. Interactions 43: 99-119 (1983), Reitz, R. H. et al). Orthophenylphenol (OPP) and sodium orthophenylphenol (SOPP) were given by oral gavage at 500 mg/kg to 2 (experiment 1) or 4 (experiment 2) male rats; after 4 hours, [3H]-thymidine (sp. act. 20 mCi/mM, approximately 500 µCi/kg) was injected --animals sacrificed after an additional 4 hours (8 hours post-treatment) and the DNA extracted from the bladders; sp. act. of DNA of individual rats determined; no details of methods and results reported as ratio of sp. act. of isolated DNA to mean sp. act. of controls (not defined whether received vehicle or nothing); negative for increased sp. act. after OPP but sp. act. increased 2-3 fold after SOPP; incomplete and, therefore, UNACCEPTABLE. (Gee, 3/30/87).
EPA memo [copy in document 129-048] dated March 22, 1982, reviewed the cellular regeneration test as: "The procedure adopted for demonstrating "cellular regeneration" appears to be adequate and the results acceptable. CDFA has no means of knowing whether EPA reviewed the identical documents on file at CDFA.

NEUROTOXICITY

Not required at this time.

The records shown below also are listed in the CDPR library computer printout of 7/5/91 for OPP (DPN 129) and OPP-Na (DPN 50438). Due to their trivial nature or due to the fact that they are partial or exact duplicates of other records in the Summary of Toxicology Data, these records have not been accorded a regular entry into the Summary of Toxicology Data. They are listed below in order to help the CDPR staff toxicologist verify that the Summary of Toxicology Data accounts for all records on file in the CDPR library.

Records: 004623, 035990, 035991, 050576, 060331, 060332, 096034

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3/19/01

APPENDIX B. BENCHMARK DOSE ONCOGENICITY COMPUTER MODEL PRINTOUT

```
=====================================================================
Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: H:\HAS-RCD\ORTHO-PHENYLPHENOL\BMDS\OPP_WS_LOGISTICS.(d)
Gnuplot Plotting File:  H:\HAS-RCD\ORTHO-PHENYLPHENOL\BMDS\OPP_WS_LOGISTICS.plt
                                        Thu Feb 22 14:34:06 2007
=====================================================================

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

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = COLUMN2
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                   Default Initial Parameter Values
                      background =            0   Specified
                       intercept =     -4.62151
                           slope =    0.0136647

           Asymptotic Correlation Matrix of Parameter Estimates

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

              intercept        slope

 intercept            1        -0.96

     slope        -0.96            1

                           Parameter Estimates

       Variable           Estimate             Std. Err.
      intercept            -6.52761             1.04057
          slope           0.0195036          0.00293299

                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -38.3193
   Fitted model        -39.7825       2.92642      2          0.2315
  Reduced model        -104.101       131.564      3         <.0001

           AIC:          83.565
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0015	0.073	0	50	-0.2704
39.0000	0.0031	0.156	1	50	2.14
200.0000	0.0674	3.371	2	50	-0.7734
402.0000	0.7880	39.400	40	50	0.2078

Chi-square = 5.30 DF = 2 P-value = 0.0708

Benchmark Dose Computation

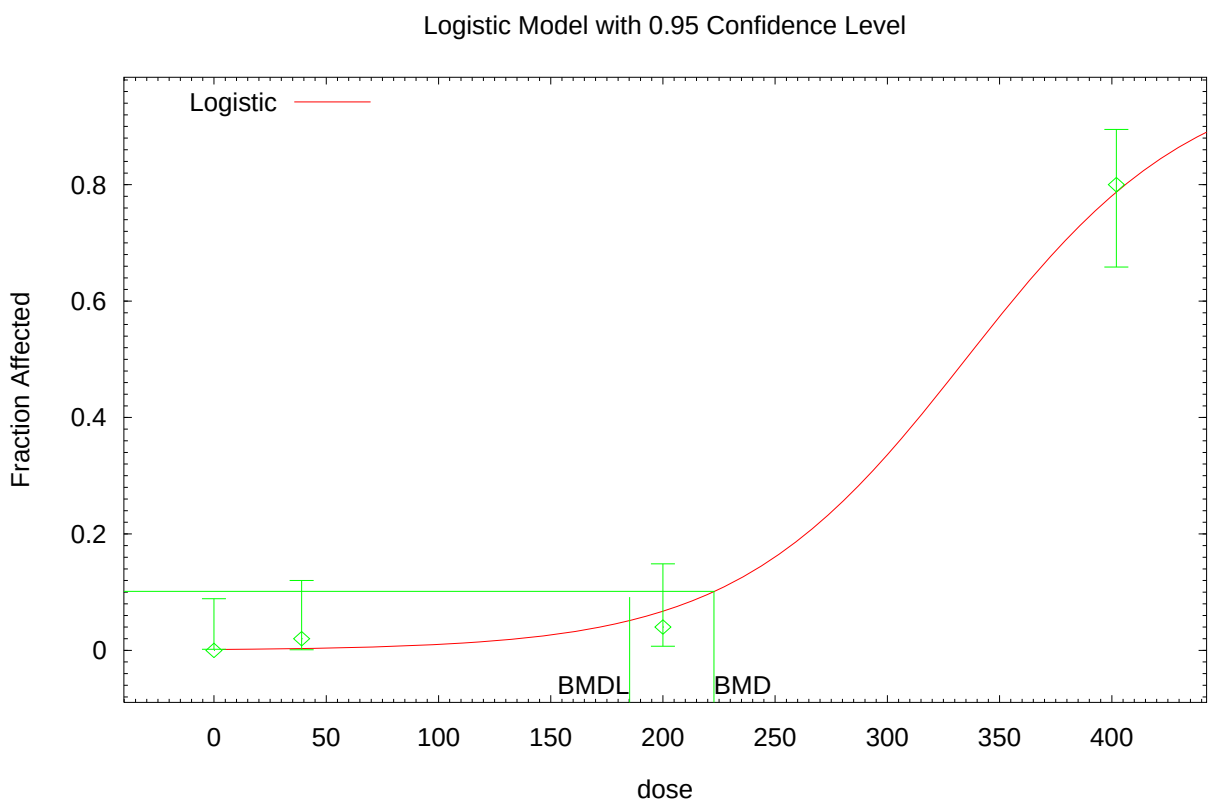
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 222.774

BMDL = 185.208



14:34 02/22 2007

APPENDIX C. DIETARY EXPOSURE ANALYSIS PRINTOUT

C.1. ACUTE DIETARY EXPOSURE: RESIDUES
Filename: H:\HAS-RCD\Ortho-Phenylphenol\DEEM\REVISED VERSION\Acute Tier 2.RS7
Chemical: Ortho-Phenylphenol
RfD(Chronic): 0 mg/kg bw/day NOEL(Chronic): 39 mg/kg bw/day
RfD(Acute): 0 mg/kg bw/day NOEL(Acute): 25 mg/kg bw/day Q*= .022
Date created/last modified: 01-27-2005/09:49:20/14 Program ver. 7.87
Comment: Monitoring Data (High End Values) Tier 2 Analysis

Food Code	Crop Grp	Food Name	Def Res (ppm)	Adj.Factors #1	#2	Comment
52	11	Apples	0.200000	1.000	1.000	PDP (H
		Full comment: PDP (Highest Measured)				
53	11	Apples-dried	0.200000	8.000	1.000	
54	11	Apples-juice/cider	0.038000	1.000	1.000	AF=1 i
		Full comment: AF=1 instead of 1.3 (PDP Measured)				
377	11	Apples-juice-concentrate	0.038000	3.000	1.000	AF=3 a
		Full comment: AF=3 adjust fr. 3.9/1.3				
198	1AB	Carrots	0.017000	1.000	1.000	PDP (H
		Full comment: PDP (Highest measured)				
61	12	Cherries	0.015000	1.000	1.000	PDP Ma
		Full comment: PDP Max. LOD				
62	12	Cherries-dried	0.015000	4.000	1.000	
63	12	Cherries-juice	0.015000	1.500	1.000	
20	10	Citrus citron	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
148	9B	Cucumbers	4.700000	1.000	1.000	PDP (H
		Full comment: PDP (Highest Measured)				
23	10	Grapefruit-juice	0.033000	1.000	1.000	AF=1 i
		Full comment: AF=1 instead of 2.1 (OJ Surrogate)				
441	10	Grapefruit-juice-concentrate	0.033000	4.000	1.000	AF=4 a
		Full comment: AF=4 adjust fr. 8.26/2.1				
448	10	Grapefruit peel	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
22	10	Grapefruit-peeled fruit	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
97	O	Kiwi fruit	20.000000	1.000	1.000	Tolera
		Full comment: Tolerance				
24	10	Kumquats	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
28	10	Lemons-juice	0.033000	1.000	1.000	AF=1 i
		Full comment: AF=1 instead of 2 (OJ Surrogate)				
442	10	Lemons-juice-concentrate	0.033000	5.700	1.000	AF=5.7
		Full comment: AF=5.7 adjust fr 11.4/2				
27	10	Lemons-peel	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
26	10	Lemons-peeled fruit	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
32	10	Limes-juice	0.033000	1.000	1.000	AF=1 i
		Full comment: AF=1 instead of 2 (OJ Surrogate)				
443	10	Limes-juice-concentrate	0.033000	3.000	1.000	AF=3 a
		Full comment: AF=3 adjust fr. 6/2				
31	10	Limes-peel	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
30	10	Limes-peeled fruit	3.600000	1.000	1.000	Orange
		Full comment: Orange Surrogate (PDP)				
141	9A	Melons-cantaloupes-juice	0.010000	1.000	1.000	PDP (A

```
         Full comment: PDP (Average RAC; ND by LOD)
142  9A  Melons-cantaloupes-pulp            0.210000   1.000  1.000  PDP (H
         Full comment: PDP (Highest Measured)
 64  12  Nectarines                         0.022000   1.000  1.000  PDP (H
         Full comment: PDP (Highest Measured)
 36  10  Oranges-juice                      0.033000   1.000  1.000  AF=1 i
         Full comment: AF=1 instead of 1.8 (PDP Highest M)
 33  10  Oranges-juice-concentrate          0.033000   3.720  1.000  AF 3.7
         Full comment: AF 3.72 adjusted fr.6.7/1.8
 35  10  Oranges-peel                      3.600000   1.000  1.000
 34  10  Oranges-peeled fruit               3.600000   1.000  1.000  PDP (H
         Full comment: PDP (Highest Measured)
 65 12   Peaches
            11-Uncooked                     0.046000   1.000  1.000
            12-Cooked: NFS                  0.046000   1.000  1.000
            13-Baked                        0.046000   1.000  1.000
            14-Boiled                       0.046000   1.000  1.000
            31-Canned: NFS                  0.033000   1.000  1.000  PDP (H
           Full comment: PDP (Highest Measured)
            41-Frozen: NFS                  0.046000   1.000  1.000
 66  12  Peaches-dried                      0.046000   7.000  1.000
402  12  Peaches-juice                      0.005400   1.000  1.000  PDP (A
         Full comment: PDP (Average RAC; ND by LOD)
 56 11   Pears
            11-Uncooked                    11.000000   1.000  1.000
            12-Cooked: NFS                 11.000000   1.000  1.000
            13-Baked                       11.000000   1.000  1.000
            14-Boiled                      11.000000   1.000  1.000
            31-Canned: NFS                  0.017000   1.000  1.000  PDP (H
           Full comment: PDP (Highest Measured)
 57  11  Pears-dried                       25.000000   1.000  1.000  AF=1 i
         Full comment: AF=1 instead of 6.25 (Tolerance Exceed)
404  11  Pears-juice                       0.877000   1.000  1.000  PDP (A
         Full comment: PDP (Average RAC; ND by LOD)
155  8   Peppers-sweet(garden)              0.094000   1.000  1.000  PDP (H
         Full comment: PDP (Highest Measured)
 90  O   Pineapples-dried                   0.017000   5.000  1.000
 91  O   Pineapples-juice                   0.010000   1.700  1.000  PDP (A
         Full comment: PDP (Average RAC; ND by LOD)
406  O   Pineapples-juice-concentrate       0.010000   6.300  1.000
 89  O   Pineapples-peeled fruit            0.017000   1.000  1.000  PDP (H
         Full comment: PDP (Highest Measured)
 67  12  Plums (damsons)                    0.046000   1.000  1.000  Peache
         Full comment: Peaches Surrogate (PDP)
 68  12  Plums-prunes (dried)               0.046000   5.000  1.000
 69  12  Plums/prune-juice                  0.005400   1.400  1.000  Peach
         Full comment: Peach Juice Surogate (Average RAC)
218  1CD Sweet potatoes (incl yams)         0.100000   1.000  1.000  CA PDP
         Full comment: CA PDP (Highest Measured)
 38  10  Tangerines                        3.600000   1.000  1.000  Orange
         Full comment: Orange Surrogate (PDP)
 39  10  Tangerines-juice                  0.033000   1.000  1.000  AF=1 i
         Full comment: AF=1 instead of 2.3 (PDP OJ Surr.)
420  10  Tangerines-juice-concentrate       0.033000   3.200  1.000  AF=3.2
         Full comment: AF=3.2 adjust fr. 7.35/2.3
163  8   Tomatoes-catsup                    0.025000   2.500  1.000  Canned
         Full comment: Canned Tomatoes Surrogate (PDP)
```

```
423  8   Tomatoes-dried                      0.510000  14.300  1.000
160  8   Tomatoes-juice                      0.014700   1.500  1.000  PDP (A
         Full comment: PDP (Average RAC; ND by LOD)
162  8   Tomatoes-paste                      0.055000   1.000  1.000  AF=1 i
         Full comment: AF=1 instead of 5.4 PDP (Highest)
161  8   Tomatoes-puree                      0.025000   3.300  1.000  Canned
         Full comment: Canned Tomatoes Surrogate (PDP)
159 8    Tomatoes-whole
            11-Uncooked                      0.510000   1.000  1.000
            12-Cooked: NFS                   0.510000   1.000  1.000
            13-Baked                         0.510000   1.000  1.000
            14-Boiled                        0.510000   1.000  1.000
            15-Fried                         0.510000   1.000  1.000
            31-Canned: NFS                   0.025000   1.000  1.000  PDP (H
           Full comment: PDP (Highest Measured)
            32-Canned: Cooked                0.025000   1.000  1.000
            33-Canned: Baked                 0.025000   1.000  1.000
            34-Canned: Boiled                0.025000   1.000  1.000
            42-Frozen: Cooked                0.510000   1.000  1.000
```

C.2. ACUTE DIETARY EXPOSURE: RESULTS
California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"
==========

Summary calculations (per capita):

	95th Percentile		99th Percentile		99.9th Percentile	
	Exposure	MOE	Exposure	MOE	Exposure	MOE
U.S. Population:	0.011491	13053	0.035138	4268	0.098498	1522
Western region:	0.016610	9030	0.045037	3330	0.115550	1298
Hispanics:	0.017109	8767	0.043083	3481	0.116430	1288
Non-hispanic whites:	0.011017	13614	0.032251	4651	0.091698	1635
Non-hispanic blacks:	0.007425	20202	0.028381	5285	0.096527	1553
Non-hisp/non-white/non-black:	0.024588	6100	0.051592	2907	0.174554	859
All infants:	0.006218	24121	0.024537	6113	0.115070	1303
Nursing infants (<1 yr old):	0.003896	38499	0.026370	5688	0.105257	1425
Non-nursing infants (<1 yr old):	0.007315	20505	0.023943	6264	0.115561	1298
Children 1-2 yrs:	0.038354	3910	0.116604	1286	0.220557	680
Children 3-5 yrs:	0.032524	4611	0.090898	1650	0.200330	748
Children 6-12 yrs:	0.018764	7993	0.053298	2814	0.113075	1326
Youth 13-19 yrs:	0.006655	22540	0.028246	5310	0.064687	2318
Adults 20-49 yrs:	0.007951	18866	0.024070	6231	0.047906	3131
Adults 50+ yrs:	0.012375	12120	0.029490	5086	0.056449	2657
Females 13-49 yrs:	0.008144	18419	0.027439	5466	0.052182	2874

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

U.S. Population	Daily Exposure Analysis /a (mg/kg body-weight/day) per Capita	per User
Mean	0.002412	0.002628
Standard Deviation	0.007969	0.008284
Standard Error of mean	0.000039	0.000043
Margin of Exposure 2/	62,187	57,069

Percent of Person-Days that are User-Days = 91.77%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000023	>1,000,000	90.00	0.006329	23,698
20.00	0.000078	>1,000,000	95.00	0.012328	12,166
30.00	0.000161	930,180	97.50	0.022520	6,660
40.00	0.000286	525,321	99.00	0.036579	4,100
50.00	0.000454	330,185	99.50	0.050757	2,955
60.00	0.000690	217,357	99.75	0.069071	2,171
70.00	0.001074	139,693	99.90	0.103076	1,455
80.00	0.001995	75,196			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000004	>1,000,000	90.00	0.005633	26,630
20.00	0.000035	>1,000,000	95.00	0.011491	13,053
30.00	0.000104	>1,000,000	97.50	0.021146	7,093
40.00	0.000214	702,166	99.00	0.035138	4,268
50.00	0.000373	402,376	99.50	0.049426	3,034
60.00	0.000594	252,701	99.75	0.067861	2,210
70.00	0.000949	158,105	99.90	0.098498	1,522
80.00	0.001741	86,148			

a/ Analysis based on all two-day participant records in CSFII 1994-98 survey.
2/ Margin of Exposure = NOEL/ Dietary Exposure.

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Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Western region

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.003141	0.003378
Standard Deviation	0.009706	0.010025
Standard Error of mean	0.000098	0.000107
Margin of Exposure	47,752	44,408

Percent of Person-Days that are User-Days = 93.00%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000028	>1,000,000	90.00	0.008437	17,778
20.00	0.000084	>1,000,000	95.00	0.017585	8,530
30.00	0.000187	803,062	97.50	0.030207	4,965
40.00	0.000333	450,543	99.00	0.046575	3,220
50.00	0.000523	286,919	99.50	0.061955	2,421
60.00	0.000797	188,186	99.75	0.083901	1,787
70.00	0.001247	120,327	99.90	0.116059	1,292
80.00	0.002605	57,586			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000009	>1,000,000	90.00	0.007481	20,052
20.00	0.000045	>1,000,000	95.00	0.016610	9,030
30.00	0.000127	>1,000,000	97.50	0.028856	5,198
40.00	0.000264	569,115	99.00	0.045037	3,330
50.00	0.000447	335,918	99.50	0.059249	2,531
60.00	0.000693	216,477	99.75	0.081419	1,842
70.00	0.001110	135,105	99.90	0.115550	1,298
80.00	0.002218	67,639			

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DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
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Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Hispanics

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.003129	0.003380
Standard Deviation	0.009944	0.010294
Standard Error of mean	0.000134	0.000147
Margin of Exposure	47,934	44,375

Percent of Person-Days that are User-Days = 92.57%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000034	>1,000,000	90.00	0.008092	18,536
20.00	0.000106	>1,000,000	95.00	0.017938	8,362
30.00	0.000202	742,526	97.50	0.030187	4,969
40.00	0.000338	444,332	99.00	0.044793	3,348
50.00	0.000520	288,233	99.50	0.065744	2,281
60.00	0.000801	187,359	99.75	0.091370	1,641
70.00	0.001276	117,581	99.90	0.123170	1,217
80.00	0.002497	60,065			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000009	>1,000,000	90.00	0.007255	20,675
20.00	0.000057	>1,000,000	95.00	0.017109	8,767
30.00	0.000141	>1,000,000	97.50	0.028371	5,287
40.00	0.000271	553,889	99.00	0.043083	3,481
50.00	0.000440	341,280	99.50	0.062143	2,413
60.00	0.000689	217,851	99.75	0.090524	1,657
70.00	0.001111	134,974	99.90	0.116430	1,288
80.00	0.002169	69,141			

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Non-hispanic whites

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.002316	0.002511
Standard Deviation	0.007363	0.007635
Standard Error of mean	0.000044	0.000047
Margin of Exposure	64,768	59,725

Percent of Person-Days that are User-Days = 92.21%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000023	>1,000,000	90.00	0.006180	24,270
20.00	0.000077	>1,000,000	95.00	0.011767	12,747
30.00	0.000160	935,288	97.50	0.020938	7,163
40.00	0.000290	517,866	99.00	0.033440	4,485
50.00	0.000457	328,324	99.50	0.049032	3,059
60.00	0.000697	215,096	99.75	0.066943	2,240
70.00	0.001083	138,524	99.90	0.093669	1,601
80.00	0.002026	74,026			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	>1,000,000	90.00	0.005565	26,954
20.00	0.000037	>1,000,000	95.00	0.011017	13,614
30.00	0.000107	>1,000,000	97.50	0.019725	7,604
40.00	0.000219	685,474	99.00	0.032251	4,651
50.00	0.000381	393,875	99.50	0.046420	3,231
60.00	0.000601	249,480	99.75	0.065127	2,303
70.00	0.000961	156,082	99.90	0.091698	1,635
80.00	0.001776	84,439			

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Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Non-hispanic blacks

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001750	0.001979
Standard Deviation	0.007107	0.007528
Standard Error of mean	0.000097	0.000110
Margin of Exposure	85,707	75,783

Percent of Person-Days that are User-Days = 88.42%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000017	>1,000,000	90.00	0.003461	43,343
20.00	0.000061	>1,000,000	95.00	0.008393	17,872
30.00	0.000121	>1,000,000	97.50	0.017782	8,435
40.00	0.000211	711,169	99.00	0.030538	4,911
50.00	0.000335	447,158	99.50	0.043242	3,468
60.00	0.000549	273,263	99.75	0.061153	2,452
70.00	0.000785	191,165	99.90	0.109923	1,364
80.00	0.001285	116,721			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.002901	51,700
20.00	0.000016	>1,000,000	95.00	0.007425	20,202
30.00	0.000067	>1,000,000	97.50	0.014538	10,317
40.00	0.000141	>1,000,000	99.00	0.028381	5,285
50.00	0.000248	605,270	99.50	0.041858	3,583
60.00	0.000423	354,581	99.75	0.057909	2,590
70.00	0.000659	227,535	99.90	0.096527	1,553
80.00	0.001157	129,610			

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Non-hisp/non-white/non-black

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.004156	0.004513
Standard Deviation	0.012749	0.013225
Standard Error of mean	0.000287	0.000317
Margin of Exposure	36,088	33,235

Percent of Person-Days that are User-Days = 92.10%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000033	>1,000,000	90.00	0.011864	12,643
20.00	0.000094	>1,000,000	95.00	0.027486	5,457
30.00	0.000208	721,419	97.50	0.036789	4,077
40.00	0.000373	402,205	99.00	0.053176	2,820
50.00	0.000602	248,973	99.50	0.067386	2,225
60.00	0.000929	161,491	99.75	0.126486	1,185
70.00	0.001510	99,351	99.90	0.174842	857
80.00	0.003958	37,898			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000007	>1,000,000	90.00	0.010781	13,912
20.00	0.000043	>1,000,000	95.00	0.024588	6,100
30.00	0.000138	>1,000,000	97.50	0.036372	4,124
40.00	0.000285	525,456	99.00	0.051592	2,907
50.00	0.000480	312,471	99.50	0.066132	2,268
60.00	0.000816	183,843	99.75	0.094644	1,584
70.00	0.001299	115,517	99.90	0.174554	859
80.00	0.002912	51,507			

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

All infants

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001909	0.003352
Standard Deviation	0.008156	0.010581
Standard Error of mean	0.000150	0.000259
Margin of Exposure	78,570	44,748

Percent of Person-Days that are User-Days = 56.95%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000142	>1,000,000	90.00	0.005331	28,139
20.00	0.000322	466,356	95.00	0.012643	11,863
30.00	0.000537	279,217	97.50	0.019480	7,700
40.00	0.000804	186,594	99.00	0.047038	3,188
50.00	0.001142	131,322	99.50	0.079674	1,882
60.00	0.001573	95,344	99.75	0.104829	1,430
70.00	0.002256	66,484	99.90	0.161061	931
80.00	0.003224	46,519			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.003627	41,355
20.00	0.000000	>1,000,000	95.00	0.006218	24,121
30.00	0.000000	>1,000,000	97.50	0.013901	10,790
40.00	0.000000	>1,000,000	99.00	0.024537	6,113
50.00	0.000174	861,314	99.50	0.053066	2,826
60.00	0.000532	282,066	99.75	0.080702	1,858
70.00	0.001054	142,369	99.90	0.115070	1,303
80.00	0.001897	79,088			

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Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Nursing infants (<1 yr old)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001399	0.003473
Standard Deviation	0.008429	0.013008
Standard Error of mean	0.000290	0.000703
Margin of Exposure	107,242	43,191

Percent of Person-Days that are User-Days = 40.27%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000093	>1,000,000	90.00	0.004410	34,014
20.00	0.000238	630,246	95.00	0.007982	18,793
30.00	0.000416	360,369	97.50	0.026363	5,689
40.00	0.000676	222,022	99.00	0.066778	2,246
50.00	0.001019	147,227	99.50	0.104436	1,436
60.00	0.001410	106,353	99.75	0.105252	1,425
70.00	0.001901	78,892	99.90	0.201392	744
80.00	0.002713	55,283			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.002214	67,747
20.00	0.000000	>1,000,000	95.00	0.003896	38,499
30.00	0.000000	>1,000,000	97.50	0.006718	22,327
40.00	0.000000	>1,000,000	99.00	0.026370	5,688
50.00	0.000000	>1,000,000	99.50	0.048665	3,082
60.00	0.000005	>1,000,000	99.75	0.081029	1,851
70.00	0.000352	426,223	99.90	0.105257	1,425
80.00	0.001029	145,723			

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Non-nursing infants (<1 yr old)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.002103	0.003323
Standard Deviation	0.008041	0.009906
Standard Error of mean	0.000174	0.000272
Margin of Exposure	71,330	45,141

Percent of Person-Days that are User-Days = 63.28%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000150	>1,000,000	90.00	0.005430	27,622
20.00	0.000340	441,276	95.00	0.012962	11,572
30.00	0.000564	265,777	97.50	0.018767	7,992
40.00	0.000845	177,535	99.00	0.046108	3,253
50.00	0.001161	129,194	99.50	0.077619	1,932
60.00	0.001651	90,839	99.75	0.103393	1,450
70.00	0.002334	64,274	99.90	0.154448	971
80.00	0.003360	44,646			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.004008	37,422
20.00	0.000000	>1,000,000	95.00	0.007315	20,505
30.00	0.000000	>1,000,000	97.50	0.015445	9,712
40.00	0.000064	>1,000,000	99.00	0.023943	6,264
50.00	0.000364	411,611	99.50	0.055209	2,716
60.00	0.000744	201,486	99.75	0.080308	1,867
70.00	0.001234	121,593	99.90	0.115561	1,298
80.00	0.002253	66,590			

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Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Children 1-2 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.007302	0.007682
Standard Deviation	0.021233	0.021712
Standard Error of mean	0.000328	0.000344
Margin of Exposure	20,542	19,525

Percent of Person-Days that are User-Days = 95.05%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000118	>1,000,000	90.00	0.021301	7,041
20.00	0.000335	448,320	95.00	0.040069	3,743
30.00	0.000597	251,062	97.50	0.070280	2,134
40.00	0.000899	166,856	99.00	0.117290	1,278
50.00	0.001271	118,052	99.50	0.150125	999
60.00	0.001780	84,280	99.75	0.172729	868
70.00	0.002595	57,813	99.90	0.220955	678
80.00	0.004458	33,647			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000054	>1,000,000	90.00	0.019342	7,755
20.00	0.000232	646,726	95.00	0.038354	3,910
30.00	0.000488	307,444	97.50	0.068373	2,193
40.00	0.000798	187,882	99.00	0.116604	1,286
50.00	0.001152	130,231	99.50	0.147224	1,018
60.00	0.001639	91,508	99.75	0.172226	870
70.00	0.002417	62,066	99.90	0.220557	680
80.00	0.004058	36,964			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Children 3-5 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.006182	0.006448
Standard Deviation	0.017335	0.017655
Standard Error of mean	0.000185	0.000193
Margin of Exposure	24,263	23,263

Percent of Person-Days that are User-Days = 95.88%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000079	>1,000,000	90.00	0.017644	8,501
20.00	0.000239	627,012	95.00	0.033191	4,519
30.00	0.000455	329,432	97.50	0.055818	2,687
40.00	0.000740	202,662	99.00	0.093840	1,598
50.00	0.001125	133,314	99.50	0.116424	1,288
60.00	0.001619	92,625	99.75	0.140724	1,065
70.00	0.002404	62,389	99.90	0.200704	747
80.00	0.004404	34,057			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000045	>1,000,000	90.00	0.016743	8,958
20.00	0.000176	852,718	95.00	0.032524	4,611
30.00	0.000379	395,605	97.50	0.053672	2,794
40.00	0.000657	228,359	99.00	0.090898	1,650
50.00	0.001028	145,851	99.50	0.115326	1,300
60.00	0.001529	98,076	99.75	0.133627	1,122
70.00	0.002278	65,841	99.90	0.200330	748
80.00	0.004114	36,458			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Children 6-12 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.003678	0.003882
Standard Deviation	0.010580	0.010833
Standard Error of mean	0.000164	0.000172
Margin of Exposure	40,781	38,637

Percent of Person-Days that are User-Days = 94.74%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000042	>1,000,000	90.00	0.009912	15,132
20.00	0.000120	>1,000,000	95.00	0.019871	7,548
30.00	0.000239	627,079	97.50	0.036606	4,097
40.00	0.000405	370,683	99.00	0.054553	2,749
50.00	0.000649	231,273	99.50	0.065764	2,280
60.00	0.001001	149,847	99.75	0.090643	1,654
70.00	0.001527	98,210	99.90	0.113235	1,324
80.00	0.002830	52,994			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000021	>1,000,000	90.00	0.009324	16,087
20.00	0.000078	>1,000,000	95.00	0.018764	7,993
30.00	0.000189	795,479	97.50	0.034622	4,332
40.00	0.000343	437,599	99.00	0.053298	2,814
50.00	0.000565	265,672	99.50	0.062880	2,385
60.00	0.000913	164,299	99.75	0.090454	1,658
70.00	0.001431	104,820	99.90	0.113075	1,326
80.00	0.002559	58,622			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Youth 13-19 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001553	0.001684
Standard Deviation	0.005052	0.005239
Standard Error of mean	0.000102	0.000110
Margin of Exposure	96,604	89,098

Percent of Person-Days that are User-Days = 92.23%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000020	>1,000,000	90.00	0.003055	49,095
20.00	0.000069	>1,000,000	95.00	0.007336	20,446
30.00	0.000130	>1,000,000	97.50	0.013111	11,440
40.00	0.000222	674,352	99.00	0.030587	4,903
50.00	0.000349	430,324	99.50	0.036139	4,150
60.00	0.000521	288,007	99.75	0.050662	2,960
70.00	0.000808	185,650	99.90	0.064764	2,316
80.00	0.001267	118,390			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000004	>1,000,000	90.00	0.002818	53,233
20.00	0.000032	>1,000,000	95.00	0.006655	22,540
30.00	0.000092	>1,000,000	97.50	0.012423	12,074
40.00	0.000170	884,815	99.00	0.028246	5,310
50.00	0.000290	516,489	99.50	0.034239	4,380
60.00	0.000452	331,592	99.75	0.050049	2,997
70.00	0.000729	205,821	99.90	0.064687	2,318
80.00	0.001145	131,017			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Adults 20-49 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001587	0.001737
Standard Deviation	0.004551	0.004733
Standard Error of mean	0.000047	0.000051
Margin of Exposure	94,490	86,379

Percent of Person-Days that are User-Days = 91.42%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000016	>1,000,000	90.00	0.004096	36,620
20.00	0.000061	>1,000,000	95.00	0.008596	17,449
30.00	0.000131	>1,000,000	97.50	0.014901	10,066
40.00	0.000230	652,656	99.00	0.024710	6,070
50.00	0.000372	403,428	99.50	0.032216	4,656
60.00	0.000567	264,693	99.75	0.040806	3,675
70.00	0.000837	179,156	99.90	0.049949	3,003
80.00	0.001390	107,950			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000002	>1,000,000	90.00	0.003454	43,424
20.00	0.000024	>1,000,000	95.00	0.007951	18,866
30.00	0.000082	>1,000,000	97.50	0.013716	10,935
40.00	0.000170	883,790	99.00	0.024070	6,231
50.00	0.000302	497,046	99.50	0.031461	4,767
60.00	0.000484	310,207	99.75	0.039408	3,806
70.00	0.000741	202,389	99.90	0.047906	3,131
80.00	0.001239	121,104			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Adults 50+ yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.002416	0.002631
Standard Deviation	0.005884	0.006093
Standard Error of mean	0.000061	0.000066
Margin of Exposure	62,074	57,010

Percent of Person-Days that are User-Days = 91.84%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000022	>1,000,000	90.00	0.007959	18,846
20.00	0.000077	>1,000,000	95.00	0.013485	11,123
30.00	0.000156	962,331	97.50	0.021891	6,852
40.00	0.000283	530,471	99.00	0.029857	5,023
50.00	0.000445	337,450	99.50	0.036859	4,069
60.00	0.000687	218,216	99.75	0.042208	3,553
70.00	0.001144	131,080	99.90	0.057111	2,626
80.00	0.002781	53,939			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000004	>1,000,000	90.00	0.007415	20,227
20.00	0.000033	>1,000,000	95.00	0.012375	12,120
30.00	0.000102	>1,000,000	97.50	0.020916	7,171
40.00	0.000207	723,136	99.00	0.029490	5,086
50.00	0.000371	404,458	99.50	0.036326	4,129
60.00	0.000588	254,906	99.75	0.041201	3,640
70.00	0.000962	155,870	99.90	0.056449	2,657
80.00	0.002213	67,788			

California Department of Pesticide Regulation Ver. 7.87
DEEM ACUTE Analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file: Acute Tier 2.RS7 Adjustment factor #2 NOT used.
Analysis Date: 02-07-2005/10:13:33 Residue file dated: 02-04-2005/15:43:01/14
NOEL (Acute) = 150.000000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: "Monitoring Data (High End Values) Tier 2 Analysis"

Females 13-49 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.001619	0.001777
Standard Deviation	0.004828	0.005030
Standard Error of mean	0.000063	0.000069
Margin of Exposure	92,658	84,424

Percent of Person-Days that are User-Days = 91.11%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000016	>1,000,000	90.00	0.004062	36,925
20.00	0.000058	>1,000,000	95.00	0.008997	16,673
30.00	0.000130	>1,000,000	97.50	0.014544	10,313
40.00	0.000226	662,615	99.00	0.027993	5,358
50.00	0.000359	417,530	99.50	0.035366	4,241
60.00	0.000545	275,061	99.75	0.044666	3,358
70.00	0.000825	181,925	99.90	0.052432	2,860
80.00	0.001364	109,951			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000002	>1,000,000	90.00	0.003508	42,764
20.00	0.000022	>1,000,000	95.00	0.008144	18,419
30.00	0.000078	>1,000,000	97.50	0.013482	11,125
40.00	0.000167	898,847	99.00	0.027439	5,466
50.00	0.000290	516,605	99.50	0.034467	4,351
60.00	0.000464	323,323	99.75	0.044248	3,389
70.00	0.000723	207,353	99.90	0.052182	2,874
80.00	0.001203	124,674			

C.3. CHRONIC DIETARY EXPOSURE: RESIDUES
Filename: H:\HAS-RCD\Ortho-Phenylphenol\DEEM\REVISED VERSION\Chronic Dietary OPP\Chronic Tier 2 (OEHHA Q1).RS7
Chemical: Ortho-Phenylphenol
RfD(Chronic): 0 mg/kg bw/day NOEL(Chronic): 39 mg/kg bw/day
RfD(Acute): 0 mg/kg bw/day NOEL(Acute): 0 mg/kg bw/day Q*= .002
Date created/last modified: 02-22-2007/14:55:29/14 Program ver. 7.87
Comment: Monitoring Data (Average Values)

Food Code	Crop Grp	Food Name	Def Res (ppm)	Adj.Factors #1	#2	Comment
52	11	Apples	0.012500	1.000	1.000	PDP Av
		Full comment: PDP Average				
53	11	Apples-dried	0.012500	8.000	1.000	
54	11	Apples-juice/cider	0.005200	1.000	1.000	AF=1 (
		Full comment: AF=1 (instead of 1.3) CA PDP Average				
377	11	Apples-juice-concentrate	0.005200	3.000	1.000	AF=3 (
		Full comment: AF=3 (adjust fr. 3.9/1.3) CA PDP J Ave.				
198	1AB	Carrots	0.005200	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
61	12	Cherries	0.005000	1.000	1.000	LOD CA
		Full comment: LOD CA PDP Average				
62	12	Cherries-dried	0.005000	4.000	1.000	
63	12	Cherries-juice	0.005000	1.500	1.000	
20	10	Citrus citron	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
148	9B	Cucumbers	0.044600	1.000	1.000	PDP Av
		Full comment: PDP Average				
23	10	Grapefruit-juice	0.005500	1.000	1.000	AF=1 (
		Full comment: AF=1 (instead of 2.1) CA OJ Surrogate				
441	10	Grapefruit-juice-concentrate	0.005500	4.000	1.000	AF=4 (
		Full comment: AF=4 (adjust fr. 8.26/2.1) CA OJ Surrogate				
448	10	Grapefruit peel	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
22	10	Grapefruit-peeled fruit	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
97	O	Kiwi fruit	10.000000	1.000	0.650	1/2 To
		Full comment: 1/2 Tolerance (PCT: 0.65)				
24	10	Kumquats	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
28	10	Lemons-juice	0.005500	2.000	1.000	AF=1 (
		Full comment: AF=1 (instead of 2) CA OJ Surrogate				
442	10	Lemons-juice-concentrate	0.005500	5.700	1.000	AF=5.7
		Full comment: AF=5.7 (adjust fr 11.4/2) CA OJ Surrogate				
27	10	Lemons-peel	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
26	10	Lemons-peeled fruit	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
32	10	Limes-juice	0.005500	1.000	1.000	AF=1 (
		Full comment: AF=1 (instead of 2) CA OJ Surrogate				
443	10	Limes-juice-concentrate	0.005500	3.000	1.000	AF=3 (
		Full comment: AF=3 (adjust fr. 6/2) CA OJ Surrogate				
31	10	Limes-peel	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				
30	10	Limes-peeled fruit	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP: Average)				

141	9A	Melons-cantaloupes-juice	0.006600	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
142	9A	Melons-cantaloupes-pulp	0.006600	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
64	12	Nectarines	0.005120	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
36	10	Oranges-juice	0.005500	1.000	1.000	AF=1 (
		Full comment: AF=1 (instead of 1.8) CA PDP Average				
33	10	Oranges-juice-concentrate	0.005500	3.720	1.000	AF=3.7
		Full comment: AF=3.72 (adjust fr. 6.7/1.8) CA PDP Av.				
35	10	Oranges-peel	0.006200	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
34	10	Oranges-peeled fruit	0.006200	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
65	12	Peaches				
		11-Uncooked	0.002900	1.000	1.000	
		12-Cooked: NFS	0.002900	1.000	1.000	
		13-Baked	0.002900	1.000	1.000	
		14-Boiled	0.002900	1.000	1.000	
		31-Canned: NFS	0.005400	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
		41-Frozen: NFS	0.005400	1.000	1.000	Canned
		Full comment: Canned Peaches Surrogate (CA Ave)				
66	12	Peaches-dried	0.002900	7.000	1.000	PDP Av
		Full comment: PDP Average				
402	12	Peaches-juice	0.005400	1.000	1.000	Canned
		Full comment: Canned Peaches Surrogate (CA Ave)				
56	11	Pears				
		11-Uncooked	0.261500	1.000	1.000	
		12-Cooked: NFS	0.261500	1.000	1.000	
		13-Baked	0.261500	1.000	1.000	
		14-Boiled	0.261500	1.000	1.000	
		31-Canned: NFS	0.005100	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
57	11	Pears-dried	0.261500	6.250	1.000	
404	11	Pears-juice	0.005100	1.000	1.000	Canned
		Full comment: Canned Pears Surrogate (CA Average)				
155	8	Peppers-sweet(garden)	0.005800	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
90	O	Pineapples-dried	0.005200	5.000	1.000	CA PDP
		Full comment: CA PDP Average				
91	O	Pineapples-juice	0.005200	1.700	1.000	CA PDP
		Full comment: CA PDP Average				
406	O	Pineapples-juice-concentrate	0.005200	6.300	1.000	CA PDP
		Full comment: CA PDP Average				
89	O	Pineapples-peeled fruit	0.005200	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
67	12	Plums (damsons)	0.002900	1.000	1.000	Peache
		Full comment: Peaches Surrogate (PDP Average)				
68	12	Plums-prunes (dried)	0.002900	5.000	1.000	Peache
		Full comment: Peaches Surrogate (PDP Average)				
69	12	Plums/prune-juice	0.005400	1.400	1.000	Canned
		Full comment: Canned Peaches Surrogate (CA Ave)				
218	1CD	Sweet potatoes (incl yams)	0.005200	1.000	1.000	CA PDP
		Full comment: CA PDP Average				
38	10	Tangerines	0.006200	1.000	1.000	Orange
		Full comment: Orange Surrogate (CA PDP Average)				

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 39  10  Tangerines-juice                    0.005500   1.000  1.000  AF=1 (
         Full comment: AF=1 (instead of 2.3) OJ Surrogate
420  10  Tangerines-juice-concentrate        0.005500   3.200  1.000  AF=3.2
         Full comment: AF=3.2 (adjust fr. 7.35/2.3)  OJ Surrogate
163  8   Tomatoes-catsup                     0.005500   2.500  1.000  Canned
         Full comment: Canned Tomato Surrogate (CA Ave.)
423  8   Tomatoes-dried                      0.006100  14.300  1.000
160  8   Tomatoes-juice                      0.005500   1.500  1.000  Canned
         Full comment: Canned Tomato Surrogate (CA Ave.)
162  8   Tomatoes-paste                      0.010600   1.000  1.000  AF=1 (
         Full comment: AF=1 (insteadof 5.4) PDP Average
161  8   Tomatoes-puree                      0.005500   3.300  1.000  Canned
         Full comment: Canned Tomato Surrogate (CA Ave.)
159 8    Tomatoes-whole
            11-Uncooked                      0.006100   1.000  1.000
            12-Cooked: NFS                   0.006100   1.000  1.000
            13-Baked                         0.006100   1.000  1.000
            14-Boiled                        0.006100   1.000  1.000
            15-Fried                         0.006100   1.000  1.000
            31-Canned: NFS                   0.005500   1.000  1.000  CA PDP
           Full comment: CA PDP Average
            32-Canned: Cooked                0.005500   1.000  1.000
            33-Canned: Baked                 0.005500   1.000  1.000
            34-Canned: Boiled                0.005500   1.000  1.000
            42-Frozen: Cooked                0.005500   1.000  1.000  Canned
           Full comment: Canned Tomato Surrogate (CA Ave.)
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C.4. CHRONIC DIETARY EXPOSURE: RESULTS
California Department of Pesticide Regulation Ver. 7.87
DEEM Chronic analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file name: H:\HAS-RCD\Ortho-Phenylphenol\DEEM\REVISED VERSION\Chronic Tier 2.RS7
Adjustment factor #2 NOT used.
Analysis Date 02-04-2005/15:49:38 Residue file dated: 02-04-2005/15:45:28/14
NOEL (Chronic) = 39 mg/kg bw/day
COMMENT 1: Monitoring Data (Average Values)

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Percent of NOEL	Total Exposure Margin of Exposr 1/
U.S. Population (total)	0.000121	0.00%	323,236
U.S. Population (spring season)	0.000131	0.00%	297,170
U.S. Population (summer season)	0.000126	0.00%	310,724
U.S. Population (autumn season)	0.000130	0.00%	299,030
U.S. Population (winter season)	0.000094	0.00%	416,650
Northeast region	0.000120	0.00%	325,326
Midwest region	0.000130	0.00%	300,376
Southern region	0.000094	0.00%	413,434
Western region	0.000153	0.00%	254,537
Hispanics	0.000117	0.00%	332,048
Non-hispanic whites	0.000126	0.00%	309,841
Non-hispanic blacks	0.000074	0.00%	527,686
Non-hisp/non-white/non-black	0.000176	0.00%	221,800
All infants (< 1 year)	0.000103	0.00%	377,096
Nursing infants	0.000051	0.00%	762,967
Non-nursing infants	0.000123	0.00%	316,380
Children 1-6 yrs	0.000325	0.00%	119,829
Children 7-12 yrs	0.000245	0.00%	159,375
Females 13-19 (not preg or nursing)	0.000120	0.00%	326,181
Females 20+ (not preg or nursing)	0.000093	0.00%	417,409
Females 13-50 yrs	0.000096	0.00%	404,802
Females 13+ (preg/not nursing)	0.000051	0.00%	760,557
Females 13+ (nursing)	0.000218	0.00%	178,716
Males 13-19 yrs	0.000083	0.00%	472,034
Males 20+ yrs	0.000067	0.00%	584,950
Seniors 55+	0.000087	0.00%	446,990
Children 1-2 yrs	0.000417	0.00%	93,595
Children 3-5 yrs	0.000335	0.00%	116,450
Children 6-12 yrs	0.000225	0.00%	173,425
Youth 13-19 yrs	0.000100	0.00%	388,390
Adults 20-49 yrs	0.000075	0.00%	518,812
Adults 50+ yrs	0.000091	0.00%	426,726
Females 13-49 yrs	0.000093	0.00%	420,912

California Department of Pesticide Regulation Ver. 7.87
DEEM Chronic analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file name: H:\HAS-RCD\Ortho-Phenylphenol\DEEM\REVISED VERSION\Chronic Tier 2.RS7
Adjustment factor #2 NOT used.
Analysis Date 02-04-2005/15:52:05 Residue file dated: 02-04-2005/15:45:28/14
NOEL (Chronic) = 4.9 mg/kg bw/day
COMMENT 1: Monitoring Data (Average Values) and Estimated NOEL

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Percent of NOEL	Margin of Exposr 1/
U.S. Population (total)	0.000121	0.00%	40,612
U.S. Population (spring season)	0.000131	0.00%	37,337
U.S. Population (summer season)	0.000126	0.00%	39,040
U.S. Population (autumn season)	0.000130	0.00%	37,570
U.S. Population (winter season)	0.000094	0.00%	52,348
Northeast region	0.000120	0.00%	40,874
Midwest region	0.000130	0.00%	37,740
Southern region	0.000094	0.00%	51,944
Western region	0.000153	0.00%	31,980
Hispanics	0.000117	0.00%	41,719
Non-hispanic whites	0.000126	0.00%	38,929
Non-hispanic blacks	0.000074	0.00%	66,299
Non-hisp/non-white/non-black	0.000176	0.00%	27,867
All infants (< 1 year)	0.000103	0.00%	47,379
Nursing infants	0.000051	0.00%	95,860
Non-nursing infants	0.000123	0.00%	39,750
Children 1-6 yrs	0.000325	0.01%	15,055
Children 7-12 yrs	0.000245	0.00%	20,024
Females 13-19 (not preg or nursing)	0.000120	0.00%	40,982
Females 20+ (not preg or nursing)	0.000093	0.00%	52,444
Females 13-50 yrs	0.000096	0.00%	50,860
Females 13+ (preg/not nursing)	0.000051	0.00%	95,557
Females 13+ (nursing)	0.000218	0.00%	22,454
Males 13-19 yrs	0.000083	0.00%	59,307
Males 20+ yrs	0.000067	0.00%	73,494
Seniors 55+	0.000087	0.00%	56,160
Children 1-2 yrs	0.000417	0.01%	11,759
Children 3-5 yrs	0.000335	0.01%	14,631
Children 6-12 yrs	0.000225	0.00%	21,789
Youth 13-19 yrs	0.000100	0.00%	48,798
Adults 20-49 yrs	0.000075	0.00%	65,184
Adults 50+ yrs	0.000091	0.00%	53,614
Females 13-49 yrs	0.000093	0.00%	52,884

California Department of Pesticide Regulation Ver. 7.87
DEEM Chronic analysis for ORTHO-PHENYLPHENOL (1994-98 data)
Residue file name: H:\HAS-RCD\Ortho-Phenylphenol\DEEM\REVISED VERSION\Chronic Dietary OPP\Chronic Tier 2 (OEHHA).RS7
Adjustment factor #2 NOT used.
Analysis Date 02-22-2007/15:18:34 Residue file dated: 12-28-2006/11:43:03/14
Q* = 0.002
COMMENT 1: Monitoring Data (Average Values)

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Lifetime risk (Q*= .002)
U.S. Population (total)	0.000119	2.38E-07
U.S. Population (spring season)	0.000128	2.57E-07
U.S. Population (summer season)	0.000124	2.48E-07
U.S. Population (autumn season)	0.000130	2.61E-07
U.S. Population (winter season)	0.000091	1.82E-07
Northeast region	0.000117	2.35E-07
Midwest region	0.000128	2.57E-07
Southern region	0.000092	1.85E-07
Western region	0.000152	3.05E-07
Hispanics	0.000114	2.27E-07
Non-hispanic whites	0.000125	2.49E-07
Non-hispanic blacks	0.000070	1.40E-07
Non-hisp/non-white/non-black	0.000178	3.56E-07
All infants (< 1 year)	0.000106	2.11E-07
Nursing infants	0.000055	1.10E-07
Non-nursing infants	0.000125	2.50E-07
Children 1-6 yrs	0.000326	6.52E-07
Children 7-12 yrs	0.000243	4.86E-07
Females 13-19 (not preg or nursing)	0.000115	2.29E-07
Females 20+ (not preg or nursing)	0.000092	1.84E-07
Females 13-50 yrs	0.000094	1.87E-07
Females 13+ (preg/not nursing)	0.000049	9.87E-08
Females 13+ (nursing)	0.000217	4.34E-07
Males 13-19 yrs	0.000076	1.52E-07
Males 20+ yrs	0.000065	1.29E-07
Seniors 55+	0.000088	1.76E-07
Children 1-2 yrs	0.000422	8.43E-07
Children 3-5 yrs	0.000334	6.68E-07
Children 6-12 yrs	0.000223	4.46E-07
Youth 13-19 yrs	0.000095	1.89E-07
Adults 20-49 yrs	0.000073	1.45E-07
Adults 50+ yrs	0.000092	1.83E-07
Females 13-49 yrs	0.000090	1.80E-07

APPENDIX D. COMMENTS AND RESPONSES TO COMMENTS FROM LAXNESS CORPORATION AND THE DOW CHEMICAL COMPANY

Lanxess Corporation
The Dow Chemical Company

Title:

Response to Department of Pesticide Regulation
Ortho-Phenylphenol (OPP) and Sodium _Ortho_-Phenylphenate (SOPP)
Risk Characterization Document (RCD) Dietary Exposure Draft

Date:

October 31, 2006

DPR Chemical Codes:

Ortho-Phenylphenol (OPP) DPR Chem. Code 448
Sodium Ortho-Phenylphenate (SOPP) DPR Chem. Code 248

Submitted by:

Lanxess Corporation
111 RIDC Park West Drive
Pittsburgh, PA 15275
&
The Dow Chemical Company
1803 Building
Midland, MI 48674

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Ortho-Phenylphenol and Sodium Ortho-Phenylphenate
DPR Draft Dietary Risk Characterization Document (RCD)

Lanxess/Dow Response
October 31, 2006

1. Purpose of Document

The Department of Pesticide Regulation (DPR) completed their Draft Risk Characterization Document for Dietary Exposure (Draft RCD) on ortho-phenylphenol (OPP) and sodium ortho-phenylphenate (SOPP) on June 6, 2006. This document provides the response to this draft RCD from Lanxess Corporation and The Dow Chemical Company, the data generators for these active ingredients. We appreciate the opportunity to respond to the comprehensive effort by DPR staff and hope that the following comments are found to be helpful and constructive. This document is organized to present our general responses to the major issues first, followed by a detailed page-by-page treatment of the Draft RCD. We appreciate the Department's attempts to put the uniquely complex database associated with these two antimicrobial ingredients into clear context. As this response details important differences that Lanxess and Dow have with some of the conclusions being drawn in the Draft RCD, particularly regarding the oncogenic potential of these compounds, we are confident that our continued efforts to address these differences as the RCD is further developed will be beneficial to your final product.

We request that the Department reconsider the conclusions of the Draft RCD on OPP and SOPP in light of the following response. Due to the unique complexity of the database on these ingredients, we believe it would be beneficial to have a meeting with the Department on the issues that are addressed in this document, as a follow-up to your review of our comments and concerns.

2. Summary of Lanxess/Dow Response

A substantial amount of scientific data is available to characterize the toxicity of OPP and SOPP. DPR has done a thorough job of compiling this large summary of information in an effort to characterize the toxicity and potential risk from estimated exposure. It is clear that DPR invested significant time and effort in their review of OPP and SOPP toxicity, and in creating the Draft RCD for dietary exposure. However, we believe it is unfortunate that the Draft RCD does not integrate the available information using the well accepted "mode of action" framework for the evaluation of carcinogenic data. We are hopeful that this response will provide the rationale and guidance that will enable DPR to incorporate these conclusions into subsequent drafts of the dietary RCD.

Section 3. Other Regulatory Evaluations. A number of other expert panels and regulatory bodies have also reviewed the toxicity of OPP and SOPP and characterized the potential risks. Though we recognize that the Department has the right and responsibility of independently characterizing pesticide products, to the extent that the conclusions that are currently being drawn by the Department differ significantly from those of these other panels and regulatory bodies, we believe it would be useful to take these other evaluations of OPP and SOPP into account. These evaluations were apparently not taken into account by DPR staff when they considered the likely mode of action and potential risk from exposure to OPP and SOPP, or at least the Draft RCD does not indicate that such a cross-checking was performed. We suggest that this information be considered as part of the DPR process. A short summary of some of these evaluations is presented in Section 3 of this response document.

Sections 4-6. General Comments. Critical issues in the Draft RCD on OPP and SOPP are the genotoxicity, metabolism, and developmental toxicity of these compounds. In preparing this response, we have asked three experts to review the Draft RCD and prepare general responses for these issues as presented in the RCD. The expert reviews are presented in this response document as:

- Section 4: Genetic Toxicity, written by Dr. David Brusick;
- Section 5: Metabolism, written by Dr. Michael Bartels; and
- Section 6: Developmental Toxicity, written by Dr. Edward Carney and C.L Zablotny.

Section 7. Specific Comments. The Department's Draft Dietary RCD on OPP and SOPP is clearly a document of professional quality. However, as you would expect, we have numerous comments and concerns regarding the details presented in the Draft RCD. These are summarized on a page-by-page basis in Section 7 of this response document. We are concerned that selected details within the toxicity descriptions are overemphasized while other information more

relevant to the mode of action are not discussed or are discussed in only a cursory fashion. This occurs throughout the document in a pattern that, ultimately, results in a characterization of the risk of OPP and SOPP that is at odds with the characterization that Lanxess and Dow and other authoritative bodies that have evaluated the same evidence have come to. Specific instances of disagreement that we have with the document are noted in Section 7 of this Response document.

3. Expert and Regulatory Evaluations of OPP / SOPP

This section summarizes the recent evaluations of OPP and SOPP that have been performed by other regulatory bodies. It is important to note that both Lanxess and Dow recognize that DPR has the responsibility and, in fact, a proud tradition of independently assessing pesticide ingredients and their risks. We respect this right. However, since the recent evaluations by other regulatory bodies have so fundamentally differed with the conclusions that the Draft RCD appears to be leading DPR to make regarding OPP and SOPP, we thought it important to ensure that these analogous examinations be considered. We believe that this is important for several reasons. First, it is increasingly important to have the international regulatory community aligned on their characterizations and regulation of chemicals, given the worldwide marketplace. A fundamental difference in characterization by any jurisdiction is significant. Second, the different conclusions drawn in the Draft RCD concerning the cancer potential of OPP and SOPP would ultimate have a significant (and we believe unnecessary) adverse impact on the ability of Californians to use these products in a manner consistent with other jurisdictions. Third, since both OPP and SOPP are currently listed as carcinogens under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), and DPR is taking the lead in assessing the chemicals for OEHHA, the primary agency for Proposition 65, we are concerned that the "safe harbor" No Significant Risk Levels (NSRL) for these compounds under Proposition 65 would not be established at scientifically reasonable levels by OEHHA, resulting in burdensome and unnecessary litigation under the Act for the users of these chemicals. For these reasons, we summarize the relevant regulatory actions of other agencies below and discuss the "weight of evidence" procedure that we believe DPR must utilize in order to appropriately characterize the toxicity and risks of OPP and SOPP.

a. U.S. EPA concluded that OPP is a threshold carcinogen unlikely to represent a carcinogenic risk to humans.

The U.S. EPA Office of Pesticide Programs Cancer Assessment Review Committee (CARC) evaluated OPP and SOPP using the EPA Guidelines for Carcinogen Risk Assessment (2005). This evaluation was part of the extensive reevaluation of these compounds by the U.S. EPA leading to their Reregistration Eligibility Decision (RED) on these compounds. Following their evaluation of all the relevant scientific data on these compounds, the CARC made came to the following conclusion:

> "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."

The CARC cited much of the same data on OPP and SOPP including chronic toxicity and oncogenicity studies, genetic toxicity data, and mechanistic and metabolism literature to support their conclusions. These same data were summarized in an extensive summaries provided to the Department by Lanxess and Dow on August 2, 2001 and June 10, 2004. The CARC further noted the following regarding the oncogenic consequences of exposures to higher levels of OPP and SOPP:

> "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."

The CARC further noted (as described below) that:

> "the chronic Reference Dose selected for assessing non-cancer risk would also address the concerns for the precursor events leading to development of bladder and liver tumors."

With respect to the mode of action and the potential for a genotoxic mechanism, the CARC made the following conclusion:

> "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 toxicity 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."

We have enclosed a copy of the 2005 CARC report. We understand that the Department has copies of the 2006 Toxicology Disciplinary Chapter April 17, 2006) and Occupational and Residential Exposure Chapter (April 4, 2006) on OPP and SOPP, which contribute to an understanding on the methods and rationale used by the Agency in coming to their conclusions on OPP and SOPP.

b. JMPR concluded that OPP is a threshold carcinogen unlikely to represent a carcinogenic risk to humans.

The Joint FAO / WHO Meeting on Pesticide Residues (JMPR) evaluated OPP and SOPP in the late 1990s. At their meeting in 1999 they came to the conclusion

> "that the urinary bladder tumours observed in male rats and the liver tumours observed in male mice exposed to 2-phenylphenol are threshold phenomena that are species- and sex-specific, and that 2-phenylphenol is therefore unlikely to represent a carcinogenic risk to humans. In coming to this conclusion, the Meeting was aware that a working group convened by IARC had classified 2-phenylphenol, sodium salt, in Group 2B (possibly carcinogenic to humans) and 2-phenylphenol in Group 3 (not classifiable as to its carcinogenicity to humans). The Meeting noted, however, that the IARC classification is based on hazard identification, not on risk assessment, and is furthermore limited to published literature, with the exclusion of unpublished studies on toxicity and carcinogenicity."

We have previously submitted a copy of the JMPR / WHO / FAO evaluation of OPP and SOPP (WHO 2000).

c. Weight of Evidence process is appropriate and required part of RCD process

The EPA Guidelines for Carcinogen Risk Assessment (2005) established an approach to evaluate the weight of evidence for carcinogenicity and mode of action of potential carcinogenic agents based on the Hill criteria that were originally developed to evaluate epidemiological associations. We are concerned that this approach as outlined in the EPA Guidelines was not followed in the development of DPR's Draft RCD of OPP and SOPP, and no weight of the evidence narrative was presented in the RCD, though this is a key part of the carcinogen review as described in the Guidelines.

DPR has long worked under the policy established by the Director, that the evaluation of studies and the characterization of pesticides by the Department shall be performed following the same guidelines used by the EPA. The Guidelines and Standard Evaluation Procedures established by the Agency provide a broad guidance to ensuring that consistent conclusions are drawn by the two most influential agencies in the United States for pesticides.

The 2005 EPA Guidelines for Carcinogenic Risk Assessment state that the modified Hill criteria serve as the framework for an analytical approach to evaluate the hypothesized mode(s) of action (MOA). According to the

Guidelines, the modified Hill criteria are useful for organizing thoughts about aspects of causation, and are consistent with the scientific method of developing hypotheses and testing those hypotheses experimentally. The modified Hill criteria as described in the EPA Guidelines for Carcinogen Risk Assessment are:

1) Strength, consistency and specificity of associations;
2) Dose-response concordance of events and lesions;
3) Temporal relationship between events and toxicological process; and
4) Biological plausibility and coherence of the hypothesized modes of action.

A number of questions that may be relevant in evaluating each criterion are presented in the Guidelines.

Under the EPA Guidelines, the results of this evaluation based on the modified Hill criteria are summarized in a weight of the evidence narrative. The weight of evidence narrative is described as a one to two page summary that explains an agent's human carcinogenic potential and the conditions that characterize its expression. The narrative highlights the key issues and decisions that were the basis for the evaluation of the agent’s potential hazard. Often these key issues are described in greater detail elsewhere in the assessment. The narrative is intended to be clear, transparent, and useful to risk managers and non-expert readers.

Multiple weight of the evidence evaluations of the potential modes of action for OPP and SOPP have been performed by other regulatory and international bodies. These bodies have concluded that a nonlinear mode of action is responsible for the bladder tumors in rats.

We believe that it is important that a true weight of the evidence evaluation on the mode of action for OPP and SOPP should be performed by DPR as outlined in the EPA Guidelines for Carcinogen Risk Assessment. Furthermore, the EPA Guidelines reference and use the weight of evidence process and procedures developed by an International Life Sciences Institute (ILSI) workgroup This workgroup included the USEPA and, Accordingly, the product of the workgroup (Meek et al., 2003) is a recognized approach within the EPA Guidelines for Carcinogen Risk Assessment. The ILSI document describes the evaluation process for determining the human relevance framework for using mode of action information to assess the relevance of animals tumors (Meek et al., 2003).

4. Genetic Toxicity

Dr. David Brusick, Author. Dr. Brusick is a widely recognized expert in genetic toxicity. He has been with Covance Laboratories for many years and is the author of over 100 scientific publications, including a textbook, Principles of Genetic Toxicology (Second Edition, 1987); was the editor of In Vitro Toxicology (1988-1993), a journal of cellular and molecular toxicology; and edited a volume entitled Method for Genetic Risk Assessment. Dr. Brusick has served on numerous committees including the USEPA's Scientific Advisory Panel.

a. Genotoxic "Potential" vs. Genotoxic Risk

The Draft RCD consistently described the positive findings within the database as indications that OPP, SOPP, PHQ (2-phenylhydroquinone) or PBQ (2-phenylbenzylquinone) had genotoxic potential. While the term genotoxic potential was used frequently in the Draft RCD to evaluate study responses, it was not clear what the phrase "genotoxic potential" meant in the context of the overall Draft RCD.

A large investigation of appropriate methods for the assessment of genetic testing conducted by the International Commission for the Protection Against Environmental Mutagens and Carcinogens concluded that almost all chemicals have genotoxic "potential" if subjected to a sufficiently large battery of *in vitro* and *in vivo* assays (Mendelsohn et al., 1992). With more than 80 published genetic toxicology studies for OPP/SOPP and their metabolites, these compounds can be classified as sufficiently tested and some of the OPP/SOPP studies reported positive results. Following an assessment methodology that focuses on the subset of positive findings is likely to assume that the compound in question is genotoxic and therefore poses a genetic hazard or risk when, in fact, the identification of genotoxic potential should not automatically imply a risk to the genomic integrity of an individual organism. Many other factors must be considered in extrapolating from routine test results to how "potential" may be expressed in a real world setting. The Draft RCD provided a comprehensive compilation of the full data set for OPP and SOPP, but it is difficult to know whether the authors intended to imply that OPP and SOPP should be considered genotoxic carcinogens or not.

b. General Comments on the Treatment of Genotoxicity in the RCD

The Draft RCD section on genetic toxicology did not contain a summary conclusion statement and did not attempt to extrapolate the genetic testing responses to a mechanism of tumor induction. The overall Summary section of the Draft RCD stated that:

> "*The overall data indicate that OPP and SOPP have genotoxic potential, and their metabolites may contribute to their genotoxicity in vivo*."

This summary statement (conclusion) is consistent with the general conclusions found in other reviews of the genotoxicity of OPP and SOPP, only if genotoxicity is understood to include chromosomal damage observed at cytotoxic concentrations (Reitz et al., 1983; Bomhard et al., 2002; Brusick, 2005). The primary source of reactivity from OPP and SOPP toward DNA and chromosomes appears to be associated with the intrinsic potential of the highly reactive PHQ and PBQ species to react with macromolecules including DNA. The Draft RCD; however, did not discuss the mechanisms involved in the reactions of OPP/SOPP or their metabolites with DNA in light of a carcinogenic mode of action.

The genetic toxicology section of the RCD appears to be primarily a tabulation of the genotoxicity publications with the type of assessment primarily based on the presence of positive responses. Reference was made in several places to a weight-of-evidence assessment, but no specific process regarding the weight-of-evidence approach was defined. In one of the recent reviews of the genetic toxicology of OPP and SOPP (Brusick, 2005), a semi quantitative weight-of-evidence method published in 1994 was used on the set of responses for OPP. The method provides a single value for all of the *in vitro* and *in vivo* data ranging from -100 (all negative) to +100 for all positive. OPP's score was -10.6 and similar to the genotoxicity of some other nongenotoxic carcinogens such as malathione, amitrole and aniline.

c. Specific Concerns on the Genotoxicity Treatment in the RCD

- **Ability of OPP/SOPP or their metabolites to induce gene mutation**- In the Draft RCD, the author(s) conclude on page 110 that: "Taken altogether, the weight of evidence on gene mutation suggests that OPP has mutagenic potential." This conclusion was based on the results of only five studies showing some evidence of positive responses to OPP (three Ames studies, a mouse lymphoma study (Harbell, 1989) and a study of ouabain resistance induced in human RSa cells (Suzuki et al., 1985)). Reaching such a conclusion with the data set evaluated is likely flawed for the following reasons:

  (1) The Ames study by Haworth et al. (1983) was a borderline response with strain TA1535 that was not confirmed in any of thirteen other independent studies using the same strain at equal or higher concentrations of OPP.

(2) The other two Ames tests (Hanada, 1977; Nishioka and Ogasawara, 1979) were only abstracts with no actual data shown to substantiate the classification and they reported responses in two different and unrelated strains (TA1536 and TA98).

(3) The mouse lymphoma positive is probably the result of chromosomal damage as this assay is known to produce positive responses from clastogens and OPP is clearly clastogenic *in vitro.*

(4) The ouabain system in RSa repair deficient cells has not been subjected to even modest validation with a range of mutagens and nonmutagens. Without such data, a positive in this test must be viewed with caution. The results of this assay are suspicious for several other reasons, including a very high,100-fold increase in mutants at only relatively mild levels of cytotoxicity.

One cannot eliminate these studies solely on the suspicion of technical flaws, but these arguments combined with the substantial amount of negative results in well-conducted gene mutation assays in microbial and mammalian cells (~20 negative reports) for SOPP, PHQ and PBQ, provide strong argument for concluding that the five studies reported as positive do not constitute a sufficient weight-of–evidence to classify OPP a gene mutagen. The types of *in vitro* methods used in genetic testing are known to have intrinsic rates of false positive responses and spurious positive responses would be expected to be found among studies in a large data set such as that for OPP (Tennant et al., 1987; Brusick, 1998).

- **Formation of relevant DNA adducts from OPP and SOPP *in vivo*-** The Draft RCD suggested that the negative DNA adduct studies for OPP and SOPP lacked a level of sensitivity to detect adducts in the rat urinary bladder and thus these studies were of limited value in defining the possible risk of OPP and SOPP. Studies by Pathak and Roy (1993) reported adducts with the ^{32}P-postlabelling method in mouse skin treated with 10-20 mg/animal of SOPP even though this compound is not carcinogenic in mice. Therefore, an initial concern is whether adducts, if produced by OPP or its metabolites, are even relevant to any risk concerns. If adducts are produced, they do not seem to be found in the urinary bladder of rats exposed to oral or dietary OPP.

  In five studies with OPP or SOPP no adducts were found in urinary bladder or liver cells using ^{32}P-postlabelling (Grether et al., 1989; Smith et al., 1998), ^{14}C-labelled compound (Reitz et al., 1983) or accelerated mass spectroscopy (AMS) methods (Kwok et al., 1999) even though some of these studies used relatively high dose levels. Based on the reports of Pathak and Roy, these studies should have been able to find adducts if present. Ushiyama et al.,

(1992) using ^{32}P-post labeling did report finding one spot not in the control. This finding was at a 2% dietary level of SOPP which produces other neoplastic endpoints. The Ushiyama et al. data conflict with other studies using equivalent doses (Smith et al., 1998) and the spot might be a spurious intrinsic adduct known to occur with this methodology (Phillips et al., 2000). This interpretation difference cannot be resolved with the existing data; however, it appears that the weight-of-evidence supports a lack of adduct formation in rat urinary bladder DNA from oral exposure to OPP or SOPP.

d. Summary and Conclusions related to Genotoxicity of OPP/SOPP

The Draft RCD defines a very mixed set of responses for OPP and SOPP in tests ranging from effects (oxidative reactions, binding and breakage) in purified DNA to micronuclei and DNA breaks in the rat urinary bladder at tumorigenic levels of both OPP and SOPP. The findings support a widely held view that OPP/SOPP or their metabolites have properties that at sufficiently toxic concentrations can interact with and damage DNA, principally through breakage (Bomhard et al., 2002; Brusick, 2005; Balakrishnan and Eastmond (2006). What is not found in this section of the Draft RCD is an attempt to interpret the results in the database in a manner that can be used to assess the role that these data might have in the tumorigenic process. Without including an integrative process, readers may presume that OPP and SOPP should be treated as genotoxic carcinogens when most other assessments of the data set summarized in the Draft RCD have concluded that OPP and SOPP do not induce tumors primarily through genotoxic processes.

We proposed that the Department reevaluate the genetic toxicity data, considering their integral role in assessing the oncogenic potential of OPP and SOPP in humans using a weight of evidence approach for these extensive data.

5. Metabolism -- Summary of dose-dependent metabolism in the Male F344 Rat

Dr. Michael Bartels, Author. Dr. Bartels is the Technical Leader for the Analytical Chemistry and Biotransformation Groups with the Toxicology Laboratory of Dow Chemical. He is an author of 57 peer-reviewed publications and/or book chapters and has served on numerous grant review Study Sections for NIH, NCI, EPA and NSF. Michael's primary research interests are determination of reactive metabolites at target sites *in vivo* and extrapolation of *in vitro* results to *in vivo* exposure assessments.

a. Introduction

OPP is a widely used fungicide and disinfectant. This compound has been shown to have selective effects in producing tumors in the bladder of the male rat, at dose levels above 200 mg/kg bw. The U.S. EPA has classified this material as "Not Likely to be Carcinogenic to Humans" below 200 mg/kg/day, based on "convincing evidence of a non-linear mode of action for development of bladder tumors in carcinogenicity studies in rats" (U.S. EPA 2006). The authors also state that "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".

The metabolite quantitation results from a variety of subchronic toxicity and acute adsorption, distribution, metabolism and excretion (ADME) studies in rats have been collated and are presented in Table 1. These results, for free and/or total (free + conjugated) metabolites of OPP are listed in order of administered dose. The metabolite levels are expressed as the amount excreted in urine per 24 hr/kg body weight. Three of the five studies evaluated both free and conjugated OPP metabolites (Bartels 1998, Nakao 1983, Smith 1998). Two studies report levels of free metabolites only (Hasegawa et al. 1991, Morimoto et al. 1989). In general, virtually all of an administered single dose of OPP has been found to be excreted in the urine of the male rat (90%), within 24 hr of administration of the test material via oral gavage (Bartels 1998, Reitz 1983).

As shown in Table 1, the major metabolite of OPP is the sulfate conjugate of the parent compound (OPP-S). The corresponding glucuronide conjugate (OPP-G) was found at lower levels, except at the high dose of 924 mg/kg (via diet). Lower levels of the hydroxylated metabolite 2-phenylhydroquinone (PHQ) were observed as the glucuronide and sulfate conjugates (PHQ-G and PHQ-S, respectively). Low levels of another ring-hydroxylated product (2,4'-dihydroxybiphenyl) were seen as the sulfate conjugate (DHB-S). A scheme of the overall metabolic fate of OPP in the rat is shown in Figure 1.

Figure 1. Metabolic scheme for OPP in the rat.

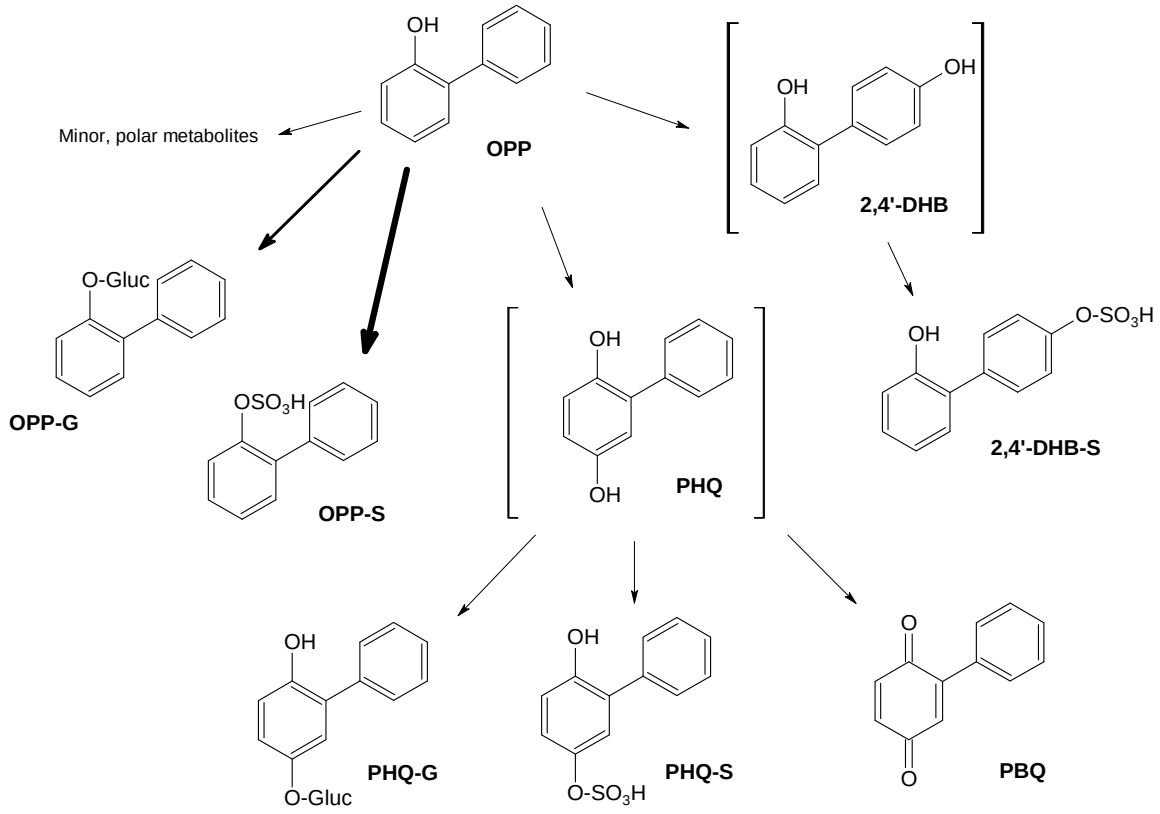


Table 1. Free and conjugated metabolites of OPP (as mg OPP equivalents /kg bw/day) from a variety of acute or subchronic ADME or toxicity studies in the rat with OPP (data from Nakao 1983, Reitz 1983, Morimoto 1989, Hasegawa 1991, Smith 1998, Bartels 1998).

Study	Dose of OPP equiv. (mg/kg)	Route	Metabolites (mg OPP equiv. in urine /kg bw)											Percent total mets as PHQ eq.	Percent dose as free PHQ+PBQ
			Unk #1	Unk #2	PHQ-G	PHQ-S	DHB-S	PHQ	OPP-S	DHB	PBQ	OPP-G	OPP		
Smith [a]	0	diet-13 wk	ND	ND	ND(.05)	0.040	ND(.04)	ND(.04)	0.12	ND	ND	0.093	ND(.04)	NA	
Reitz [b]	5	oral gavage	ND	ND	ND(.07)	ND	ND	ND(.07)	3.9	ND	ND	0.63	ND(.09)	3%	ND(1.4%)
Bartels [c]	28	oral gavage	0.75	ND(.03)	0.78	0.45	0.75	ND(.03)	21	ND(.03)	ND(.03)	1.7	ND(.03)	5%	ND(0.1%)
Reitz	50	oral gavage	ND	ND	ND(.68)	ND	ND	ND(.68)	38.7	ND	ND	6.3	ND(.90)	3%	ND(1.4%)
Smith	56	diet-13 wk	ND	ND	0.61	0.49	0.51	0.27	14.6	ND	ND	1.2	0.39	8%	0.48%
Smith	282	diet-13 wk	ND	ND	4.7	3.4	1.4	1.3	65	ND	ND	5.8	1.36	11%	0.44%
Morimoto [d]	327	diet- 5 mo	ND	ND	ND	ND	ND	3.3	ND	ND	0.24	ND	0.35		1.1%
Reitz	500	oral gavage	ND	ND	118	ND	ND	ND(9.56)	294	ND	ND	66	ND(9.56)	26%	ND(1.9%)
Smith	556	diet-13 wk	ND	ND	13	13	1.3	1.7	89	ND	ND	17.1	2.3	20%	0.30%
Morimoto	655	diet- 5 mo	ND	ND	ND	ND	ND	11.0	ND	ND	0.25	ND	7.0		1.7%
Nakao [e]	885	diet- 19.4 wk	ND	ND	27	ND	ND	ND	ND	ND	ND	74	ND	26%	<1.0%
Smith	924	diet-13 wk	ND	ND	45	33	0.59	1.3	76	ND	ND	71	1.8	35%	0.14%
Hasagawa [f]	924	diet-8 wk	ND	ND	ND	ND	ND	5.7	ND	ND	0.19	ND	1.8		0.64%
Morimoto	1309	diet- 5 mo	ND	ND	ND	ND	ND	28.8	ND	ND	0.34	ND	28.4		2.2%

[a] All urinary metabolite amounts corrected from 18 hr to 24 hr collections;
Urine samples collected in metal metabolism cages, with wire-mesh bottom, over dry ice

[b] Urine concentrations calculated from metabolite profile percentages x estimated % of administered dose in 0-24hr urine
Percent recovery in 0-24 hr urine for 5 and 50 mg/kg groups estimated from results of Bartels (1998) as 89.6% at 28 mg/kg
Urine samples collected in glass metabolism cages, over dry ice;
Metabolite profile percentages estimated from figures in publication, except for 24.6% value for Peak III at 500 mg/kg

[c] All urinary metabolite concentations were given as amounts in 24hr urine by authors;
Urine samples collected in glass metabolism cages, over dry ice

[d] All urinary metabolite concentations adjusted to daily amounts using listed avg. body wts urine volume estimates (conc x 28ml urine/day for males & 20ml urine/day for females / 272g bw for males & 170g bw for females;
Urine samples collected in metabolism cages (assumed metal with wire-mesh base and at ambient temp.) for a 24hr period
Dose level of 0.5%, 1.0% or 2.0% Na-OPP assumed to be equimolar equivalent to 1.25% OPP diet of Smith (1998) of 924 mg/kg/day

[e] All urinary metabolite concentations were given as amounts in 24hr urine by authors;
Urine samples collected in metabolism cages (assumed metal with wire-mesh base and at ambient temp.) from 7am-1pm x 3 consecutive days;
Dose level of 2% OPP-Na calculated from author's statement of daily test material intake (280 mg/day for males & 180 mg/day for females) / average body wt at day 120 (280g for males & 170g for females)

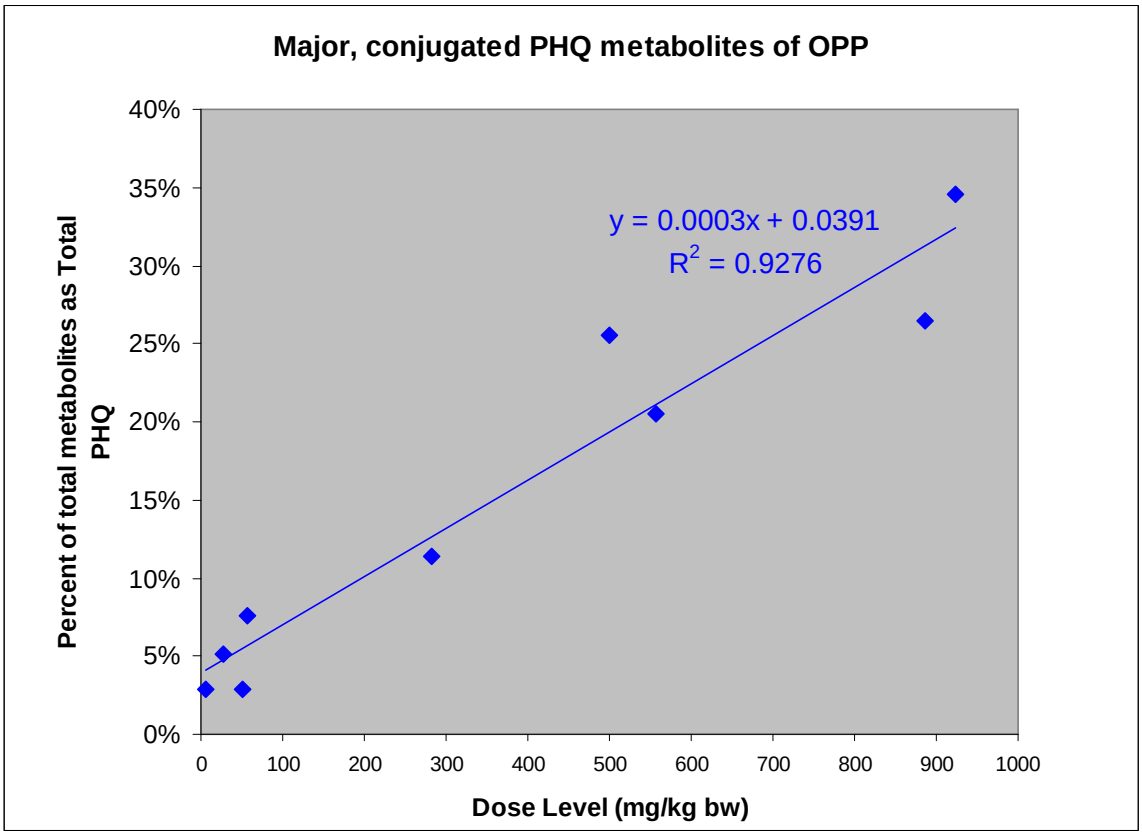
[f] All urinary metabolite concentations adjusted to daily amounts (conc. x 28ml urine/day for males & 20ml urine/day for females;
Urine samples collected in metal metabolism cages (assumed with wire-mesh base and at ambient temp.) from 7am-1pm x 3 consecutive days;
Dose level of 1.25% OPP in diet equal to high dose of Smith (1998) (924 mg/kg/day)

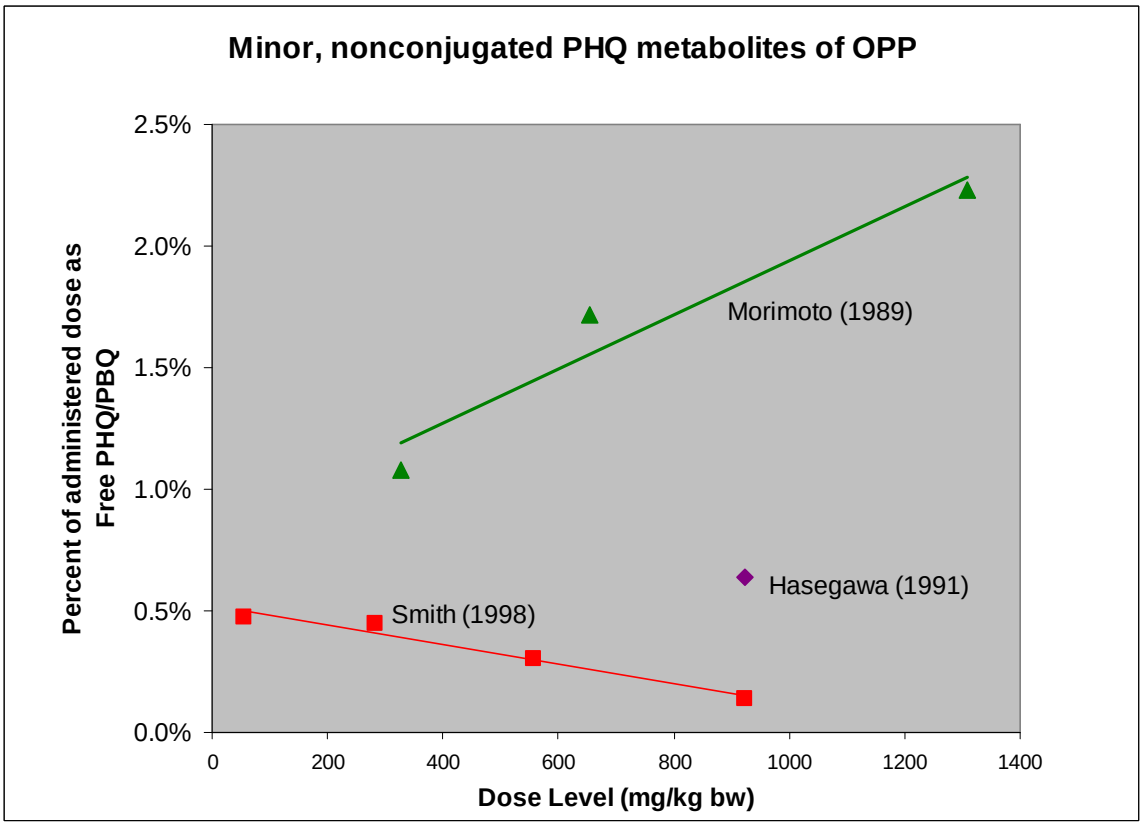
b. Comparison of Conjugated vs. Free Metabolites.

The formation of the PHQ metabolite, or the oxidized version (2-phenylbenzoquinone; PBQ), has been reported as correlating with the bladder tumors seen in the male rat. As shown in Table 1 and Figure 2, there is a good correlation of total PHQ (PHQ-G + PHQ-S + PHQ + PBQ) with increasing dose, and across various acute and subchronic studies. Below 200 mg/kg, approximately 3-8% of the total metabolites were found as PHQ (primarily conjugated). Above 200 mg/kg, the percent of total metabolites as PHQ equivalents increased up to 35%. The relationship of total PHQ correlates well with dose level (r^2=0.9276; Fig. 2).

In contrast, the levels of free PHQ+PBQ do not correlate well with increasing dose between various studies (Fig. 2). Morimoto et al. (1989) report increasing percentages of PHQ+PBQ across the dose levels of 327, 655 and 1309 mg/kg (1.1 to 2.2%, OPP equivalents)(Fig. 2). In contrast, Smith et al. (1998) report decreasing percentages of the dose excreted as free PHQ/PBQ (0.48% at 56 mg/kg, down to 0.14% at 924 mg/kg OPP). In fact, these authors found lower absolute amounts of free PHQ at 924 mg/kg than at 556 mg/kg (1.3 vs. 1.7 mg/kg/day urine, respectively). At this same high dose of 924 mg/kg OPP, Hasegawa et al. (1991) report four-fold higher levels of free PHQ+PBQ than those of Smith et al (5.7 mg/kg/day urine, or 0.64% of admin. dose). This variation in measured levels of nonconjugated metabolites indicates possible systematic errors in some of these data.

Figure 2. **Top:** Correlation of Total (free + conjugated) PHQ metabolites with administered dose from a variety of acute or subchronic ADME or toxicity studies with OPP (data from Nakao 1983, Reitz 1983, Morimoto 1989, Hasegawa 1991, Smith 1998, Bartels 1998); **Bottom:** Correlation of Free PHQ/PBQ metabolites with dose.





c. Free OPP and PHQ Possibly Arising from Sample Degradation

Since there is no significant dose-dependence in the formation of these minor, nonconjugated metabolites, it may be that they are formed as artifacts of sample collection and/or storage, vs. being eliminated in the non-conjugated form. The collection of urine samples for analysis from these various studies was all performed in metabolism cages. However, for the single-dose ADME studies, the authors Bartels et al. (1998) and Reitz et al. (1983) utilized glass metabolism cages with separators for urine and feces, to minimize cross-contamination of these two sample types. Urine samples for these two studies, as well as the study of Smith et al., were collected frozen, on dry ice. No detectable PHQ or PBQ was seen by Bartels et al. at the dose level of 28 mg/kg (LOD 0.1% of dose). Free PHQ+PBQ levels at the high dose range of 924-1309 mg/kg were also found to be lowest by Smith et al (vs. Hasegawa 1991 or Morimoto 1989). Nakao et al. (1983) reported that rat urine contains significant glucuridase activity. It is also generally known that collection of urine samples at room temperature will facilitate bacterial production, and increases in urinary beta-glucuronidase (Kirk 1977). It would also be expected that collection of urine in metal metabolism cages, with wire-mesh bottoms, would also allow for some fecal contamination of the collected urine samples. Since the highest levels of free PHQ and OPP were reported in studies employing wire-mesh caging and urine collection at ambient temperature, the actual levels of these unconjugated metabolites may have been increased by partial (~1%) degradation of conjugated OPP and PHQ.

In addition, the measurement of free OPP or PHQ could be in error if the conjugated forms of these metabolites degraded during sample preparation or analysis. Smith et al. verified that no significant degradation of these conjugates occurred in the GC/MS analysis of OPP and PHQ in used in that study (1998) or the study of Bartels et al (1998). No corresponding verification of glucuronide or sulfate conjugate stability was conducted by Hasegawa or Morimoto, who report the highest levels of free OPP and PHQ at the high dose range of 924-1309 mg/kg (Hasegawa 1991, Morimoto 1989).

Based on this evaluation of the variability of the trace levels of free PHQ and PBQ levels across studies, it becomes clear that there is a possibility that some of the reported results may be erroneously high, due to sample degradation during collection or analysis. Further investigation of this technical issue should be conducted prior to utilization of this data in any risk assessments for OPP.

d. Similarity of Single-Dose vs. Repeat Administration Pharmacokinetics- Impact on Reported Negative DNA Alkylation Results.

Reitz et al. (1983) examined the effect of repeat administration on the pharmacokinetics of OPP in the male rat. Comparable percentages of the test material were found to be absorbed in the single vs. repeat-dose groups (95.6±10% vs. 88.1±9%, respectively). These authors also report urinary excretion nearly complete within 24 hr for both groups. No direct comparison of the metabolic fate of OPP has been conducted in any published studies with this compound. However, as shown in Table 1, quite comparable metabolite profiles were observed between single-dose and repeat administration studies. At the dose level of 500 mg/kg, Reitz reported 26% of an oral gavage dose of OPP as PHQ metabolites (1983). In the subchronic study of Smith et al. (1998), the authors report 20% of the metabolites as total PHQ at the dose level of 556 mg/kg/day. These data show comparable kinetics and metabolism of OPP, following single- vs. repeated administration.

Several studies have examined the potential for OPP and/or its reactive metabolites to bind to DNA of the bladder epithelium. The most sensitive and direct assay employed in these studies was the accelerator mass spectrometry technique (AMS)(Kwok et al. 1999). These authors showed no detectable adduction of OPP-derived radioactivity to the DNA of the urinary bladder (LOD = 1 adduct / 10^{12} nucleotides) across the dose range of 15-1,000 mg/kg (single oral gavage)(Fig. 3). Results of a 13-week study with OPP, across the dose range of 0.08-1.25% OPP (56-924 mg/kg) were consistent with the AMS results, showing no detectable DNA adducts in the bladder epithelium via ^{32}P-postlabelling assay (Smith 1998). Some evidence of OPP-induced DNA adducts have been reported with this ^{32}P-technique (Ushiyama 1992), however these results were with whole bladder tissue and only at the very high dose of 2.0% OPP-Na (1.77% OPP equivalent).

Based on the similarity in the metabolic fate of OPP between single and repeat administration, and the reported lack of DNA adducts below 1.77% OPP in the diet, it would appropriate to conclude that no adducts of OPP with the DNA of bladder epithelium should form during repeated exposure to this test material at dose levels up to 1,000 mg/kg.

Figure 3. Figure showing lack of binding of 14C-OPP equivalents to DNA in the urinary bladder of the male F344 rat, across the dose range of 15-1000 mg/kg bw. (Figure from Kwok 1999).

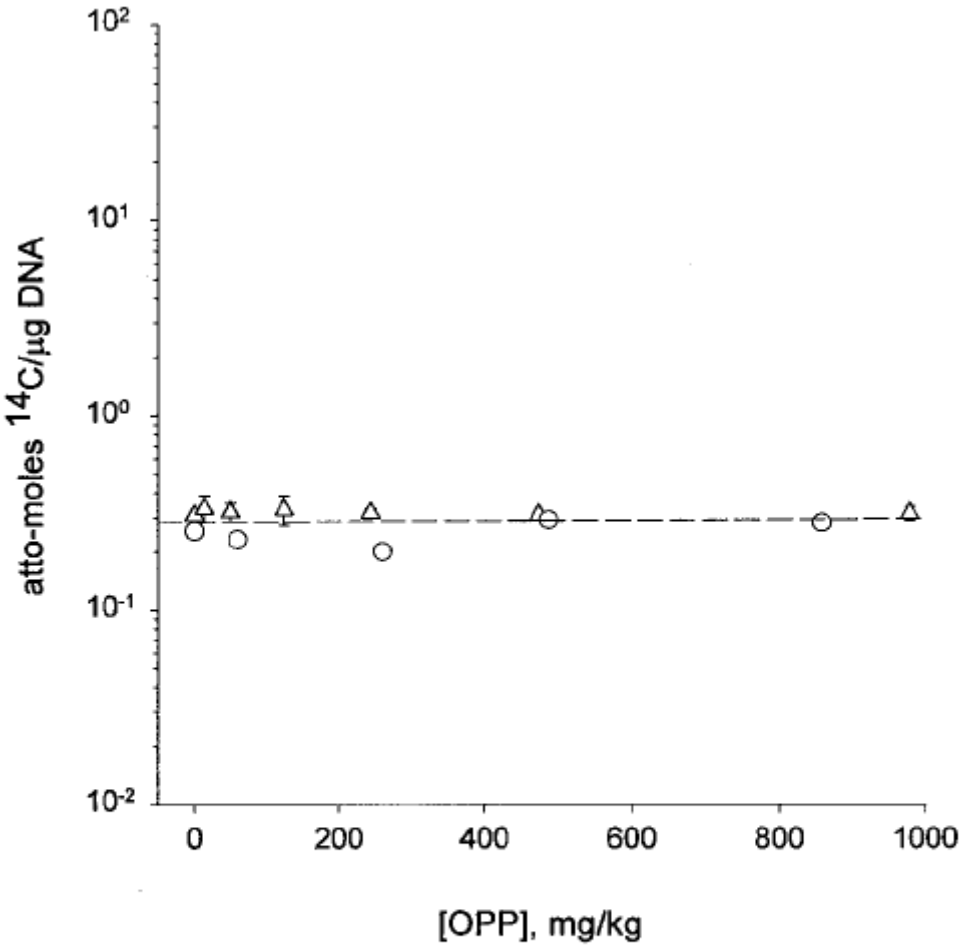


FIG. 3. Radiocarbon content of DNA isolated from the urinary bladder of rats: (△) mean of 4 rats with 1 μCi/animal; (○) pooled samples of 4 rats with 5 μCi/animal. No significant difference compared to the control was found in the group mean data (△) analyzed by one-way analysis of variance (ANOVA) ($p > 0.05$).

e. Reactive Metabolite Involvement in Threshold-based Effects of OPP.

Numerous authors have discussed the mode of action of OPP-induced bladder tumors in the male rat as being related to the increase in reactive metabolite formation. The purported reactive metabolites are the semiquinone or quinone form of OPP, arising from ring hydroxylation. Similar reactive metabolite formation has been reported for a variety of aromatic compounds and is often verified by identification of adducts of a test material with glutathione (GSH)(Evans 2004).

One example of threshold-based toxicity is with the analgesic compound acetaminophen. Slikker et al. provide a comprehensive overview of the quinone-based toxicity seen for this compound. This toxicity only occurs above saturation levels of Phase II conjugation (2004). Mitchell et al. showed that covalent adducts of acetaminophen occur only above dose levels of 375 mg/kg in the mouse (1973)(Fig. 4). Depletion of GSH or pretreatment with sulfhydryl compounds was shown by these authors to enhance or protect, respectively, from the test material-induced effects. Hasegawa et al. have shown that the reactive iminoquinone metabolite of acetaminophen (NAPQI) binds well with tissue proteins, but not with DNA *in vivo* (1988). This NAPQI metabolite of acetaminophen has also been shown to react with glutathione and be eliminated in the bile (Chen 2003).

A similar threshold-based correlation between dose and endogenous nucleophile-OPP adducts has been reported by Reitz (1984) and Kwok (1999)(Fig. 5). Nakagawa has reported finding a GSH adduct of OPP in the bile of rats administered 1000 mg OPP/kg (1989). Other groups have shown an increase in OPP-induced tissue damage with GSH depletion (Nakagawa 1988) or an inhibition of OPP-related effects with sulfhydyl compound coadminstration (Tayama 1991). Only above a threshold of GSH or protein adduct formation (>200 mg/kg; Fig. 5-6) are effects such as bladder lesions, DNA damage and tumors seen (Kwok 1999, Morimoto 1989).

Figure 4. Acetaminophen-induced depletion of glutathione and threshold-based covalent binding of acetaminophen to protein above 375 mg /kg bw) (Figure from Mitchell 1973).

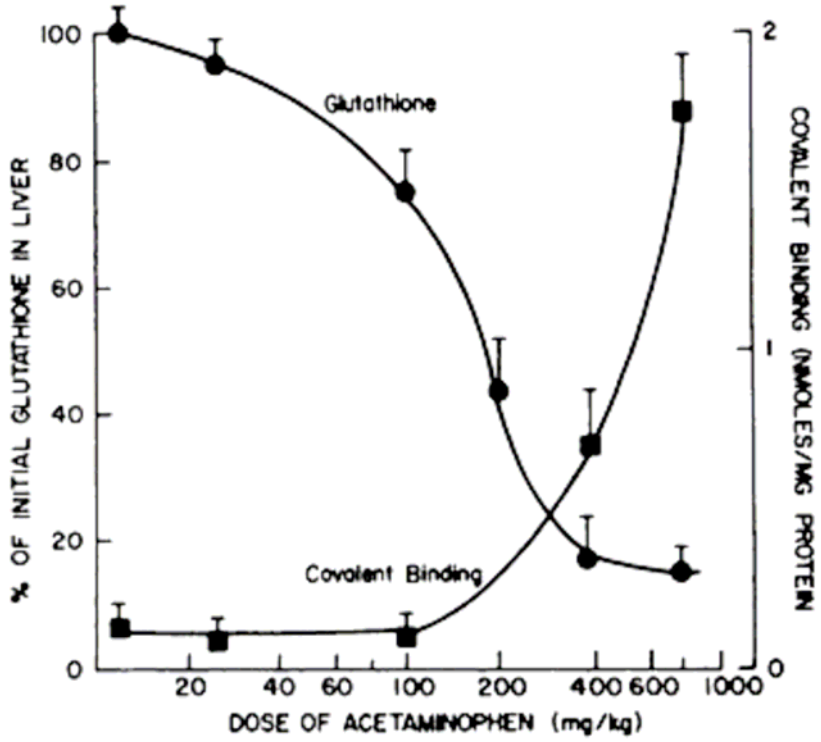


FIG. 4. Hepatic glutathione and covalent binding to hepatic protein after increasing doses of acetaminophen. Glutathione (●——●) concentration and covalent binding (■——■) of ^{3}H-acetaminophen were determined 120 minutes after administration as described in "Methods." Points are means ± S.E.M. of at least three mice.

Figure 5. Threshold-based covalent binding of ^{14}C-OPP equivalents (top) and bladder lesion incidences (bottom), both above 200 mg/kg bw in the male F344 rat. (Figures from Kwok, 1999).

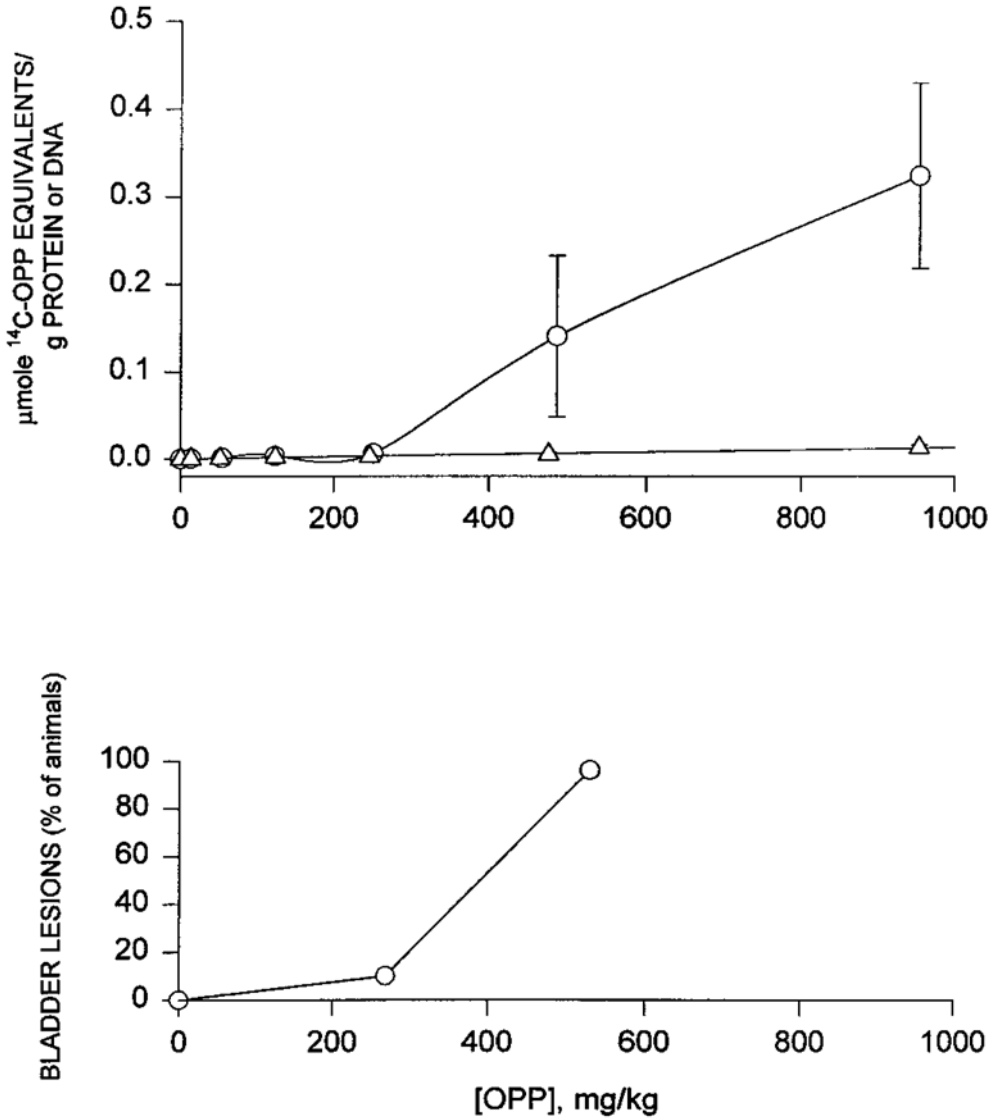


FIG. 5. (A) Correlation between the dose responses of *in vivo* macromolecular binding (△, DNA; ○, protein). The mean (±SEM) of 6–8 animals combined from 2 experiments are shown. (B) The total bladder lesions (combined PN hyperplasia, papillomas, and carcinomas) at OPP doses of 0, 269, and 531 mg/kg/day reported by Hiraga and Fujii (1984) are shown.

Figure 6. Threshold-based response in bladder tumor incidence and DNA damage above 0.5% Na-OPP (324 mg OPP equiv./kg bw) (Figure from Morimoto 1989).

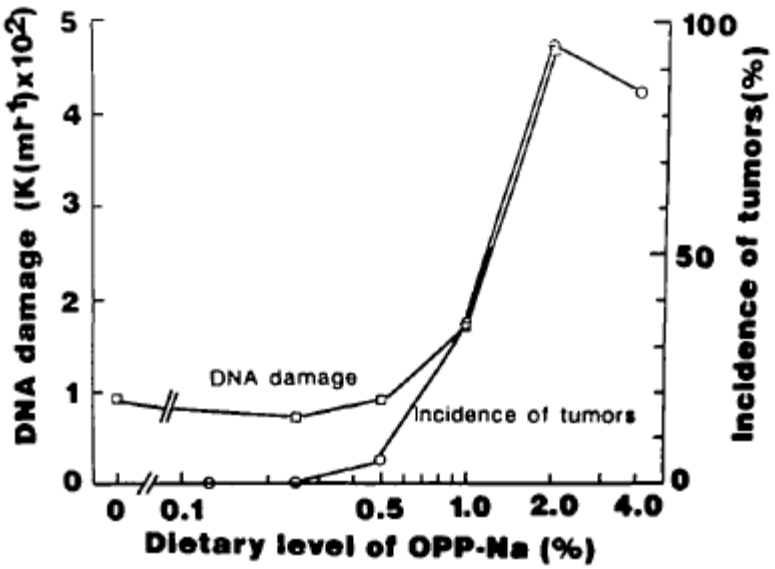


Fig. 3. Dose–response relations of OPP-Na dietary levels to DNA damage and carcinogenicity. Three-month feeding studies with OPP-Na were performed in male rats. Two rats were used throughout all the experiments. The dose–response curves of OPP-Na dietary levels to DNA damage were in good agreement with the incidence of tumors of the bladder reported by Hiraga and Fujii (1981).

f. Summary of dose-dependant metabolism.

As discussed above, virtually all of the absorbed OPP is excreted as Phase II conjugates of the parent compound or the hydroxylated metabolite PHQ. These conjugated metabolites would not be expected to be reactive to endogenous macromolecules. The trace levels of free PHQ or PBQ, or other forms of reactive metabolites arising from OPP, would be expected to be detoxified efficiently with GSH and other protein nucleophiles at low doses. While there has been much discussion on the specific form of the reactive metabolite(s) involved in OPP-induced bladder tumor formation, numerous datasets from several groups have shown that, regardless of the specific biochemistry involved, the observed bladder effects are seen only above a threshold of approximately 200 mg/kg bw. This threshold-based mechanism of toxicity has also been seen for other aromatic compounds such as acetaminophen. Based on these observations, it is appropriate to conclude that the induction of bladder tumors in the male F344 rat is a threshold-based phenomenon and not an effect linear with administered dose.

6. Developmental Toxicity Endpoint

Drs. Edward Carney & Carol Zablotny, Authors. Dr. Zablotny is the principal author of the rabbit developmental toxicity study that is the subject of this section. Dr. Carney is the lead scientist for developmental effects at the Dow Chemical Company and Adjunct Professor of Environmental Health Sciences at the University of Michigan School of Public Health.

a. OPP Rabbit Developmental Toxicity Study: Review of Resorption Rate Data.

The Draft RCD concludes that the NOAEL for developmental toxicity for OPP/SOPP is 25 mg/kg/day, based on an increase in the frequency of litters with resorptions in the rabbit developmental toxicity study. This section addresses the issues of concern regarding this conclusion and requests reconsideration by the Department.

Study. Zablotny, C.L., Breslin, W.J. and Kociba, R.J. (1991): Ortho-phenyl-phenol (OPP): Gavage teratology study in New Zealand White rabbits.

Dose levels. 0, 25, 100 or 250 mg/kg/day given on gestation days (GD) 7-19

Background. The original study authors concluded that there were no developmental effects at any dose level, with a maternal toxicity NOAEL of 100 mg/kg/day and a developmental NOAEL of 250 mg/kg/day. However, DPR asserted that there was “an increase in the frequency of litters with resorptions in the 100 and 250 mg/kg/day groups”, and therefore considered the developmental NOAEL to be 25 mg/kg/day. Written rebuttals concerning the statistical analysis of the resorption rate data were exchanged between Dow and DPR. The Department ultimately retaining their conclusion that there was a treatment-related effect on resorption rate at 100 and 250 mg/kg/day. In contrast to the position taken by DPR, the U.S. EPA concurred with Dow’s interpretation of the data, as indicated in the Registration Eligibility Document (RED) for OPP.

i. Summary of data review

The alleged increase in the percentage of litters with resorptions in the 100 and 250 mg/kg/day groups represented a borderline change which hovered between statistical significance and lack thereof depending on the statistical methods employed. Dow utilized a censored Wilcoxon test with a Bonferroni correction for multiple comparisons, resulting in P-values of 0.10 and 0.13 for the 100 and 250 mg/kg/day groups, respectively, which is above the cut-off for statistical significance of 0.05. The censored Wilcoxon test was developed by a well regarded U.S. government agency statistician (J. Haseman) and the Bonferroni

correction is a standard method used across many areas of statistical analysis. Both methods have been and continue to be accepted by global regulatory agencies.

ii. Data review objective

The study data as well as correspondence between Dow and DPR were reviewed to determine whether or not the original study conclusions remain valid according to current standards.

b. Statistical methodology

In its review of the study, DPR questioned the use of the Bonferroni correction. Without such a correction, the significance values would be divided by three and therefore fell just slightly below the 0.05 cut-off. DPR also used a Fisher's exact test to analyze the percentage of litters with resorptions, which resulted in statistically significant P-values of 0.0157 and 0.0237 for the middle- and high-dose groups, respectively. Although the Fisher's test is also well accepted, it is a generic type of test for percentage data, whereas the censored Wilcoxon test is particularly well suited for litter data such as resorption rate.

As the statistical methodology has been debated in the past, this review took a broader weight of evidence approach to the toxicological interpretation of the data that considered statistical analyses along with other factors, such as dose-response relationships, biological plausibility, consistency across end points, and historical control values.

c. Resorption rate parameters

First, it is important to realize that the original study reported four different resorption rate parameters which look at resorption rate from slightly different perspectives (Table 2). Misleading conclusions can be drawn if any of these individual values are interpreted in isolation. In particular, the percentage of litters with resorptions can often be problematic, as it tends to be quite variable (e.g., the historical control range was 11.1 – 66.7%) and it does not discriminate between litters with one resorption vs. those with many resorptions. To address this problem, several years ago Dow laboratories replaced the percentage of litters with resorptions, as well as the percentage of implantations resorbed, with the parameter "percent post-implantation loss", which is the mean of the responses in each litter. The latter parameter is considered the best overall indicator of resorption rate, and is the measure preferred by the U.S. EPA. For the purposes of this review, percent post-implantation loss was calculated and analyzed statistically (Table 2).

Table 2. Resorption rate and related litter parameters.

	Dose level (mg/kg/day)				Historical control	
	0	**25**	**100**	**250**	**Range**	**Mean**
No. resorptions/litter	0.9	0.9	1.4	1.1	0.1 – 1.1	0.55
% implantations resorbed	12.8	11.7	20.2	14.6	1.8 – 13.9	7.0
% litters with resorptions	33.3	57.1	76.9	72.2	11.1 – 66.7	36.2
No. resorptions/litter with resorption(s)	2.8	1.5	1.8	1.5	1.0 – 2.8	1.5
% Post-implantation loss	12.2	16.7	19.2	18.3	NA	NA
No. viable fetuses/litter	6.3	6.5	5.5	6.5	4.7 – 9.6	7.2
Gravid uterus weight (g)	420	401	365	412	323 – 530	423

[1]Not reported in the original study, but shown here as per current practice. Historical control ranges not available for the time frame of the original study.

The values for the first two resorption rate parameters for the high-dose OPP group (Table 2) were just slightly above those of the concurrent control, and within or just slightly above the historical control range. However, the concurrent control group also was at the high end of the historical control range. The conclusions of DPR appeared not to be focused on the latter changes, but on the third parameter, the percentage of litters with resorptions, which was clearly elevated relative to controls. On the other hand, the mean number of resorptions per litter with resorptions in the high-dose group was actually lower than in the control group. Overall, this indicates that the increase in the percentage of litters with resorptions was mainly the result of the resorptions in the high-dose group being distributed across more litters, whereas the control group resorptions tended to be concentrated in fewer litters. The mean percentage post-implantation loss, which was analyzed as per current practice, was slightly higher than controls and had a P-value of 0.20, which was well above the 0.05 level for statistical significance.

d. Weight of evidence evaluation for increased resorptions

Pursuing the weight of evidence evaluation further, the values for number of viable fetuses per litter and gravid uterus weight were also considered based on the fact that decreases in viable fetuses per litter and gravid uterus weight would be expected in the event of a meaningful increase in resorptions. However, there was no such effect in the high-dose group. In fact, the number of viable fetuses per litter was slightly higher than control.

In the middle-dose group, resorption rate parameters as well as viable fetuses per litter and gravid uterus weight were actually altered to a greater degree than in the high-dose group. However, statistical analysis of percent post-implantation loss indicated that the middle-dose value was not significantly different from control (P=0.17). In addition, the lack of a dose-response relationship is not consistent with a treatment-related effect of OPP on resorption rate. Another important piece of evidence comes from the rabbit probe developmental toxicity study which preceded the main study, as there was no increase in resorption rate despite the fact that higher dose levels were evaluated.

OPP has also been evaluated for developmental toxicity in two studies in rats. In one such study (John *et al.,* 1981), doses as high as 700 mg/kg/day in Sprague-Dawley rats had no effect on resorption rate. A similar study in pregnant Wistar rats showed an increase in resorptions, but only at dose levels ≥ 600 mg/kg/day which were associated with significant maternal toxicity (e.g., ataxia) (Kaneda *et al.,* 1978). There was no increase in resorptions at 300 mg/kg/day, despite the presence of significant maternal toxicity. In the present rabbit study, significant maternal toxicity (e.g., 13% mortality) was present at the high dose level of 250 mg/kg/day, but there was no evidence of maternal toxicity at lower dose levels.

e. Conclusions for data evaluation of developmental endpoint

The alleged effect of OPP on resorption rate is, at most, a borderline effect. The combined weight of evidence considered in this review favors the interpretation of the original authors, who concluded that OPP did not increase resorption rate in rabbits. This conclusion is based on the lack of corresponding effects on viable fetuses per litter and gravid uterine weight, the lack of a clear dose-response relationship, the absence of a resorption rate effect at higher dose levels evaluated in the probe study, and the lack of such an effect in rats at doses which did not cause maternal toxicity. We request that the Department reconsider your evaluation and conclusions regarding this study and its relevance to the Draft Dietary RCD.

7. Page-by Page Detailed Consideration of RCD

This section provides comments on various issues in the Draft Dietary RCD on OPP and SOPP. The comments are provided on a page-by-page basis.

RCD Section I. Executive Summary

Page 13 paragraph 2

The organization of this paragraph creates the illusion that OPP pharmacokinetics in humans exposed to low doses is associated with the toxicological effects that occur in rats at the LD50 dose. This illusion is created because the first sentence: "*Studies have indicated that the pharmacokinetics of OPP in rats is similar to humans*," is followed immediately in the paragraph by details of the toxic effects observed in rats exposed to the lethal dose of OPP. This juxtaposition of an "introductory" statement about the pharmacokinetics of OPP in humans that was actually measured at low doses, and the description of severe toxic effects observed in rats at the lethal dose is an introduction of bias in the executive summary that is pervasive throughout the Draft RCD. Since the collective database on OPP and SOPP illustrate a threshold effect for most toxicological endpoints, including carcinogenicity, we believe that it is crucial to carefully identify and differentiate the dosing levels and their implications while characterizing these ingredients.

Page 13 paragraph 3

In the first sentence the Draft RCD should state the fact that only rats exposed to high doses of SOPP in the diet (greater than 1,300 mg/kg/day, which is greater than the LD50) developed bladder tumors as early as 13 weeks from the beginning of exposure. The dose-response relationship with respect to the bladder tumor development should be mentioned in the executive summary.

Page 14 paragraph 2

In this summary of genotoxicity there is no mention of any negative studies and no discussion of the weight of the evidence for genotoxicity. As noted in Section 4 of this document, the summary should reflect the total body of data available and the weight of the evidence for and against a genotoxic mode of action for OPP and SOPP.

Page 15 paragraph 1

The statement that there was a dose-related increase in papilloma and carcinoma of the urinary bladder in **both sexes** in multiple rat bioassays is not accurate. Female rats are significantly less sensitive to the effects of OPP / SOPP on the urinary bladder than male rats. Although a single

chronic study shows a slight increase in combined papilloma and carcinoma in female rats exposed to SOPP, the incidence is statistically significant only by a trend test conducted by DPR and not by pair-wise comparison (Hiraga, 1983, also described in Fuji and Hiraga, 1985). Only one single carcinoma has been detected in the urinary bladders of female rats exposed to OPP or SOPP.

This paragraph states that the low dose extrapolation model is the most appropriate for characterizing human health risk associated with OPP exposure because "*mutagenic activity of OPP and its metabolites is the most plausible mode of carcinogenic action currently available.*" It is unclear how this conclusion was reached. No weight of evidence evaluation for either the genotoxic or threshold modes of action was presented. We recommend that a weight of evidence evaluation for each hypothesized mode of action be performed and presented before a "most plausible" mode of action is identified.

Page 15 paragraph 4

This paragraph indicates that "*DPR selected acute NOELs from multiple-day oral developmental toxicity studies due to the lack of a suitable single-day toxicity study.*" This sentence as written suggests that DPR is lacking studies they would like to use as a basis for risk assessment of acute exposure. Actually the appropriate (and suitable) studies have been submitted to DPR but there was no acute endpoint of concern in the study results. Though, as discussed in Section 6 of this response document, we do not agree with the finding of developmental toxicity, we recommend deleting the last part of the sentence beginning with the word "due."

Page 16 paragraph 1

This paragraph suggests that there is uncertainty in the chronic NOEL that is based in part on histopathology, because a study using scanning electron microscopy (SEM) showed more lesions than were identified by light microscopy. The SEM revealed necrosis or exfoliation occurring in the urinary bladder epithelia of both control and treated rats. The differences between the control group animals and the animals at the low and mid-dose levels were small and of uncertain biological and statistical significance. No increase in BrdU-labeling, a sensitive indicator or cell replication, was observed at the low and mid-dose levels. The lesions observed at the low and mid-dose levels should not be classified as either adverse or treatment-related.

RCD Section II. Introduction

Page 18 paragraph 1

The EPA Carcinogenicity Peer Review Committee citation is indicating OPP is a B2 carcinogen is out of date. The Peer Review Committee has

been replaced by the Carcinogen Assessment Review Committee (CARC), which produced a Cancer Assessment Document on OPP and SOPP dated September 27, 2005. See Section 3 of this document for details of the CARC review.

RCD Section II.B. Chemical Identification

Page 19 paragraph 1

In apparent support of the statement: "*A clear understanding of the biocidic mechanism of OPP and SOPP is not available,*" an experimental study is described, indicating that a direct toxic effect of OPP was induced on hepatocytes *in vitro*. The text continues to say that "*the investigators* ***speculated*** *[emphasis added] that the effect was attributable to the disturbance of mitochondrial respiration and the depletion of protein and nonprotein thiols.*" No indication is given by the authors of the RCD as to the weight that they give this "speculation", or whether the speculation is supported by sufficient data to be considered seriously. We suggest that the RCD authors provide some indication as to whether and why they believe this is the biocidic mechanism or whether other mechanisms have been proposed and/or considered.

RCD Section III. Toxicology Profile
RCD Section III.A. Pharmacokinetics

Page 22, paragraph 1

The authors of the RCD have organized this paragraph in a way that biases the document by stating the facts in what we believe is a misleading order, such that the minor metabolic pathways operative only at high doses are described first, while the detoxification pathways operative at low doses that result in nontoxic metabolites are barely mentioned.

Sentence 7, "*The pathways…*" begins the discussion of OPP metabolism, and it is organized to mention the pathways operative at high doses that lead to quinone production and redox recycling first, rather than beginning with the primary metabolic pathway, sulfation. It is five sentences later in the paragraph before the "major conjugation reaction was sulfation at the low dose" receives a mention as a part of a sentence. The major metabolite of OPP is the sulfate conjugate (Section 5, Table 1, this Response document). The sulfation pathway is operative both at low and high doses, but at high doses may be saturated, allowing other metabolic pathways, described first in the RCD, to operate. We recommend that this "summary" paragraph be rewritten to summarize the major and minor metabolic pathways of OPP as they are understood.

Also in the same sentence is the statement that "The pathways mediated *via* rat liver microsomes or hepatocytes included…adduction with macromolecules." Metabolic pathways are not usually described as including adduction.

Page 22 paragraph 2, last sentence

"*There appeared to be a dose-dependent increase in OPP oxidation, and sulfation was the dominant detoxification pathway of OPP at the low dose."*

OPP oxidation was not measured or estimated in the human study that this sentence apparently refers to (Cnubben et al, 2002). This sentence should therefore be deleted.

Page 26, paragraph 2, sentence 2

"*Several patterns of these unconjugated metabolites were noticeable: (1) they were reported only in the repeated dosing studies (Hasegawa et al., 1991; Bartels et al., 1998; Smith et al., 1998); and (2) they amounted up to 5% of total dose recovered in the urine (Smith et al., 1988*);"

There are several errors in this sentence. There is no Smith et al., 1988 citation listed in the RCD. We assume the intended citation is the Smith et al., 1998 reference. The unconjugated metabolites PHQ and PBQ are measured combined and reported as free PHQ. The maximum free PHQ reported in the urine of rats in the Smith 1998 study was 1.5% of total metabolites measured, not 5%. Thus only trace amounts of free PHQ were observed in all dose groups.

Page 26, paragraph 2 end of sentence 2, sentence 3

"…*and (3) and their mode of formation appeared to follow a linear kinetics. That is, the relative concentration of OPP- to-PHQ appeared to be independent of dose (Levy, 1968)*."

While it is true that there was not a dose-dependent increase in the occurrence of free OPP and PHQ in the urine of rats after 13-weeks of exposure to 282, 556, and 924 mg/kg/day of OPP in the diet, the levels of OPP and free PHQ were significantly lower in rats exposed to the low dose of 56 mg/kg/day (Smith et al., 1998). This is consistent with a difference in OPP metabolic pathways favored at low and high doses and nonlinear kinetics of free PHQ formation at low doses. [see comments regarding metabolism in Section 5 of this Response, and response to RCD page 167 for additional details]

RCD Section III.A.2.b. Dermal-- Human

Page 32, first complete paragraph, penultimate sentence

"*…indicating that other metabolic pathway(s) (e.g. oxidation) may be important at high dose levels"*.

Although this conclusion supports the use of a nonlinear approach to risk assessment. the report by Cnubben et al., (2002) did not measure the proportion of oxidative metabolites and thus does not support this statement.

RCD Section III.B. Acute Toxicity

Page 35, first paragraph, sentence 1

In reference to 17 acute lethality studies available: "…*however none of them has information for establishing a No-Observed-Effect-Level (NOEL)."*

The purpose of an acute lethality study is not to identify a NOEL. We suggest that this sentence be modified to acknowledge that the lace of NOEL information is typical of these tests.

RCD Section III.C. Subchronic Toxicity

Page 43, paragraph 1, mid-paragraph

"*In OPP and SOPP-exposed males, the highest tumor incidences did not occur at the highest tested dose. The "umbrella-shape" bladder tumor dose-responses suggest an inverse relationship between bladder tumorigenesis and the nephritis, which occurred only at very high dose levels and in association with a decrease in urinary pH.*"

The author did mention that the dose levels were very high (1,493 mg/kg), but did not note that in addition, male rats at the high OPP dose level exhibited a mean body weight decrease relative to the controls of 26 to 35% throughout the 13 week study (Hiraga and Fuji, 1984). This extreme decrease in body weight is an indication that the maximum tolerated dose (MTD) was exceeded and the data should not be used for risk assessment. Body weight data were not presented in the 13-week SOPP dietary study publication, but the doses again were very high (2,487 mg/kg), such that toxicity that could cause significant weight loss and excedence of the MTD likely occurred (Hiraga and Fuji, 1981). Effects observed in these animals exposed to OPP or SOPP at these very high doses are not relevant for risk assessment and mode of action considerations, given the dramatic decreases in body weight that occurred during the exposures.

Page 43, paragraph 3, last part of the only sentence

"*..DPR uses the subchronic toxicity data for developing the toxic mode of action instead of selecting the subchronic critical NOEL.*"

The studies summarized in the subchronic toxicity section are not brought together in any way to describe a mode of action as this sentence would indicate. We suggest that a summary of the weight of the evidence for a mode of action be included following the sentence above.

RCD Section III.C.1. *ortho*-Phenylphenol

Page 45, paragraph 4 last three sentences

"Throughout most of the study, reduced body weights occurred in both sexes at 12500 and 25000 ppm. However, these reductions (up to 13% and 35% for the males; 9% and 32% for the females, respectively) appeared to be related to decreases in food consumption (g/animal/day)."

As noted in our comment for page 43, paragraph 1, Hiraga and Fuji describe these body weight reductions in the males that received OPP as 2.5% of the diet as a decrease in body weight of 26-35%, occurring throughout the study (1984). This is a significant decrease in body weight that should not be dismissed as inconsequential simply because it is accompanied by decreased food consumption. The effects observed at the high dose should not be considered for risk assessment.

Page 50, paragraph 4, penultimate sentence

"*Because of these observations, the investigators speculated that OPP-induced simple hyperplasia was reversible.*"

The data from the 4-week "recovery" group of rats treated with OPP demonstrate a significant decrease in severity of urinary bladder epithelial hyperplasia compared to the hyperplasia observed after 13 weeks of continuous exposure. This does show reversibility of hyperplasia even though the urinary bladder epithelia had not completely returned to control appearance during the time course of 4 weeks. We believe that this documented recovery is not speculation.

Page 54, paragraph 1, last sentence

"*The following describes the results of the histopathology and III.E. GENOTOXICITY details the results of DNA adduct formation.*"

There was no adduct formation in the study referred to, as noted in Table 33 of the RCD. It could be noted briefly on page 54 that DNA binding in the study was negative.

RCD Section III.C. 3. Special Subchronic Toxicity Studies
RCD Section III.C. 3.a. Mode of Action

Page 61, paragraph 3, last sentence

"*…(3) the difference in potency between OPP and SOPP as bladder carcinogens may be related to the difference in urinary pH.*"

The weight of evidence to support or refute this statement should be presented in detail.

RCD Section III.D. Chronic Toxicity/Oncogenicity

Page 66, paragraph 2, last sentence

"*…OPP and SOPP promoted the carcinogenesis in urinary bladder initiated by a known rat bladder carcinogen, N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), in vivo.*"

We recommend that the authors provide a brief explanation of the statement above. The effect of promotion in this case is not unexpected. High doses of OPP and SOPP cause regenerative hyperplasia as shown by SEM. Regenerative hyperplasia is a recognized mechanism whereby an increase cellular and DNA replication occurs and thus may increase the response to a genotoxic carcinogen such as BBN.

RCD Section III.D.1 *ortho*-Phenylphenol

Page 74, first paragraph, third sentence.

"*Consistent with the proposal that P/N hyperplasia, papilloma, and carcinoma constitute a morphological continuum of urinary bladder neoplasia (Jokinen, 1990), the terminal sacrifice males at 8000 ppm exhibited a markedly increased incidence of carcinoma (p<0.01) in association with decreased incidences of P/N hyperplasia and papilloma*."

P/N hyperplasia is pre-neoplastic and is reversible (Oyasu, 1995). The increase in carcinoma and decrease in other lesions at terminal sacrifice is due to the fact that carcinoma is an endstage lesion and other lesions may progress to it upon continued exposure.

Page 74, paragraph 3 second sentence

"*At ophthalmology, increased (p<0.05) incidence of cataract occurred in males at 8000 ppm (61% incidence) and increased (p<0.05) incidences of cataract, uveitis, and corneal vascularization occurred in females at 4000 ppm (incidences of 27%, 22% and 22% respectively).*"

The incidences of these effects in controls should be reported for comparison.

Page 82. First paragraph.

This discussion does not follow the generally accepted approach to the analysis of the hepatocellular tumors. The combined incidence of hepatocellular adenomas and carcinomas (including hepatoblastomas) should be combined and compared with the incidence of combined tumors in the control group. When analyzed properly there is no increase in hepatocellular tumors at the low dose level.

RCD Section III.D.2. Sodium *ortho*-Phenylphenate

Page 95, First complete paragraph, third sentence from end of paragraph.

"*Whether the lesions in the 24-week exposed group that were present at the end of the OPP-exposed period completely regressed is important. If indeed there were complete regressions, the simple hyperplasia that reappeared, even though there was no apparent stimulus provoking the response, would need to be considered effectively as a preneoplastic lesion.*"

Transitional cell hyperplasia is well understood and is clearly reversible. There is no biological basis to believe that hyperplasia can spontaneously reappear. Given that the normal turnover rate for a bladder stem cell is 200 days (Oyasu, 1995), the regression of hyperplasia is gradual. Complete regression of bladder epithelial cell hyperplasia would not be expected during the time course of this study.

RCD Section III.E. Genotoxicity
RCD Section III.E.1. *ortho*-Phenylphenol
RCD Section III.E.1.a. Gene Mutation

Page 110, Paragraph 2

This paragraph describes results and focuses on the limitations of the bacterial reverse mutation assays conducted on OPP that are presented in table 32 of the RCD. The paragraph does not summarize the weight of evidence that supports or refutes whether point mutations occurred in the majority of tests. Briefly, there were approximately 15 reports of Ames Assays presented in table 32, and 50 tests were run on 11 bacterial strains with and without S9 metabolic activation. Forty-six of the tests were negative with and without activation and 4 were positive. One of the positives occurred at a cytotoxic dose, and no dose data were reported for the other 3 positive results. The combined results suggest that OPP does not cause addition or deletion of DNA base pairs in bacterial systems. For further discussions of this issue, refer to Section 4 of this response document.

Page 110 paragraph 3

This paragraph describes results of 3 gene mutation studies in mammalian cells *in vitro* and 1 in fruit flies, and concludes that OPP has mutagenic potential. Table 32 indicates that 2 of these studies were positive and 2 were negative for gene mutation. One of the positive studies, conducted by Suzuki et al., used ouabain-resistant human embryonic cells (Rsa) that are not among the cells accepted by either EPA OPPTS (870.5300) or OECD (476) guidelines for evaluation of gene mutations in mammalian cells, and has not been validated (see Section 4 of this Response document for additional details). The fact that 2 of the four gene mutation studies were negative and one of the two positive studies is a non-standard study reporting significant cytotoxicity suggests that the evidence for mutagenicity from these studies is equivocal.

RCD Section III.E.1.b. Chromosomal Damage

Page 110 paragraph 4

This paragraph begins the discussion of studies conducted to evaluate chromosome aberrations in mammalian cells *in vitro* and in whole rats treated with OPP, summarized in RCD table 33. Seven studies are reported and the table indicates that four were positive and three were negative. Three of the positive studies were from a single laboratory (Tayama) and one cell type (CHO-K1). The first study reported a dose-dependent decrease in cell survival, and was conducted in the absence of metabolic activation (Tayama-Nawai et al., 1984). In this study, the lowest OPP dose that caused a weakly positive response for chromosome aberration (588 µM) also cause cytotoxicity reflected in a 50% decrease in cell survival. This weak response was less than half the lowest response elicited by the positive control MNNG.

The Tayama et al.,1989 study reported a positive response at lower OPP doses with the addition of S9 metabolic activation. The third study by Tayama et al., (1991) reported weakly positive chromosome aberration responses at 588 µM in the presence of S9 metabolic activation that was reduced in the presence of GSH or cysteine. In the same study in the absence of S9, cysteine did not protect the cells from OPP at a dose previously shown to cause significant toxicity (reflected as 35% survival; Tayama-Nawai et al., 1984), and an increase in chromosome aberrations was reported. The Tayama studies generally reported that OPP is a weak inducer of chromosome aberrations *in vitro*, which occur in the presence of cytotoxicity.

The fourth study that reported chromosome aberrations was an abstract that reported chromosome aberrations in human fibroblasts without S9 activation at OPP concentrations up to 5.9 µM, significantly lower than the

other studies. This report of chromosome aberrations at a very low dose without supporting data may or may not be reliable.

Taken as a whole, both the positive and negative studies summarized in table 33 indicate that OPP may be a weak inducer of chromosome aberrations *in vitro* in sensitive cell systems at cytotoxic concentrations. It should be noted that chromosomal aberration studies are prone to spurious positive findings due to a variety of phenomena observed above threshold concentrations (Kirkland and Muller, 2000). Increases in chromosomal aberrations observed under such conditions may not be toxicologically relevant.

Page 111 paragraph 3

This paragraph mentions the 3 miscellaneous studies listed at the end of the Draft RCD Table 33, a dominant lethal study, a micronucleus study and a hyperdiploidy study, but does not characterize the contribution of these studies to the weight of the evidence for potential genotoxicity of OPP. The dominant lethal and hyperdiploidy studies were negative. The micronucleus study (Balakrishnan et al., 1999) reported an increase in micronuclei in the urothelial cells of rats fed a diet containing a very high dose of OPP (20,000 ppm) for two weeks. A new study by the same authors (Balakrishnan and Eastmond, 2006) report no increase in micronuclei in the urothelial cells of rats fed lower doses of OPP for two weeks (i.e., 2,000 and 4, 000 ppm OPP in the diet, corresponding to 148 and 320 mg/kg/day). Micronuclei were observed in the urothelial cells of rats exposed to 644 or 1114 mg/kg/day of OPP via the diet in this new study. This new study demonstrates that OPP does not cause an increase in micronuclei in the bladder urothelial cells of rats exposed to low doses of OPP for 2 weeks, and supports the observation that a threshold exists for development of adverse effects on the urinary bladder of rats. The micronucleus study in RCD table 33 should be updated to reflect the negative result for micronucleus formation in rat urothelium in rats exposed to low doses of OPP. These studies do not support a genotoxic mode of action for OPP on the rat bladder at low doses.

RCD Section III.E.1.c. DNA Damage (a) DNA Binding

Page 111 paragraph 5

This paragraph begins the discussion of studies summarized in the Draft RCD Table 34 that reports results of studies to evaluate DNA binding by OPP. Seven studies are presented, three of them negative for DNA binding of OPP in the urinary bladder of rats, and four of them positive. Two of these positive studies were performed on rat liver, one in calf thymus and one in herring sperm DNA.

As noted in the Draft RCD, the first two positive studies listed in Table 34 for rat liver DNA binding with OPP, Pathak & Roy, 1992a, and Ushiyama et al., 1992 were positive only with the addition of a microsomal activation system, and negative without activation. The OPP concentration in the noncellular assay by Pathak and Roy was 1,000 µM, a very high level given that cellular toxicity was observed in other *in vitro* studies at concentrations nearly an order of magnitude lower. The DNA binding that did occur during the 2-hour incubation of OPP and DNA with microsomal activation was only 15 to 22% of the DNA binding that occurred with PHQ incubation in the same study, and the adducts appeared to be identical. This indicates that OPP itself does not bind to DNA, but at high concentrations and with metabolic activation, reactive OPP metabolites may interact with DNA.

Page 112, paragraph 3

This paragraph addresses the negative results from 3 *in vivo* studies conducted to evaluate the potential binding of OPP to the rat urinary bladder, basically concluding that none of the studies is sensitive enough to detect DNA binding if it occurred. The Rietz and Kwok studies are criticized because the rats received only a single dose of OPP, which could limit the formation of DNA adducts. The Smith et al., study (1998) is criticized because the sample size of 12 rats per dose group and 5 dose levels was considered too small for evaluation of urothelium, and the method of ^{32}P postlabeling was used to detect adducts instead of radiolabeled OPP.

In answer to this, it is not clear how the Draft RCD authors determined that a sample size of 12 rats per dose group was not sufficient to detect an effect. It seems that an n of 12 per group would be sufficiently large to detect DNA binding to OPP, particularly since enough DNA for each analysis was obtained from half an individual rat bladder.

The final criticisms of the Smith et al. study in the Draft RCD were that: 1) the researchers did not enhance the ^{32}P-postlabelled adduct detection, and, 2) they did not determine the fate of DNA adducts during processing.

In answer to this, Smith et al. describe in detail their reasoning for not using a p1–mediated enhancement, which could have resulted in a partial loss of certain adducts. In addition, a positive control was included in the adduct assay to ensure that ^{32}P postlabeled nucleotides could be detected. The weight of the evidence suggests that OPP does not bind to DNA in the rat urothelium in significant quantities following short- or long-term *in vivo* exposure to rats.

RCD Section III.E.1.c. DNA Damage (b) Nonspecific DNA Damage

Page 112 paragraph 4

This paragraph addresses the results from 5 studies conducted to evaluate non-specific DNA damage following OPP exposure to *in vitro* systems. As noted in the Draft RCD, the first positive study listed is based on an abstract of a study and is difficult to evaluate due to a lack of detail. The second positive test, by Hirayama et al (1981), reports inhibition of 4 strains of *E. coli* culture growth following application of OPP to a disc placed in the center of the culture plate. The agar plate was streaked from a center point outward prior to application of the disc and sample, and the length of inhibition of growth in the streaks was the measure used to evaluate the potential mutagenicity in this system. One set of plates was treated with kanamycin, a non-mutagen, which inhibited growth in all cultures to the same degree (7 mm of inhibition in each plate). In contrast, the genotoxic positive controls in this study inhibited growth of each strain to a different degree (e.g., AF-2 inhibited between 0 and 9 mm depending on the E. coli strain). The results of tests on OPP do not appear to be significantly different from kanamycin, though a number of dose levels were used. The greatest range for OPP at a single dose level was 5 to 9 mm. This is a non-standard study that is at best weakly positive for DNA damage in a bacterial system.

Three nonstandard studies were conducted using *B. subtilis* as the test species. Two of these were positive, one was negative. These studies date back to 1977 and 1978 and are of limited value given that a sufficient number of studies conducted in bacterial strains accepted by OECD and EPA Guidelines are available to characterize the potential for point mutations.

RCD Section III.E.1.c. DNA Damage (c) DNA Breakage and Oxidation

Page 112 paragraph 5

This paragraph discusses three studies that reported negative results for DNA breakage and (or) oxidation in the presence of OPP. The Draft RCD authors state: “In one study that also tested DNA breakage in the presence of metabolic activation, the result was positive (Nagai et al., 1990).”

In answer to this, Nagai et al. state: “By using supercoiled pUC18 DNA as substrate, it was proved that strand scission occurred by PHQ, but not by OPP or PBQ.” This study should be considered negative for DNA breakage by OPP. The Draft RCD has separate sections for consideration of potential genotoxicity of PHQ and PBQ, and positive studies for these metabolites should be considered under these headings (III.E.3 and III.E.4).

Page 113 paragraph 1

This paragraph discusses the results of three studies listed in the Draft RCD Table 34 that were conducted to evaluate the potential for OPP to cause DNA breaks *in vivo*. Two of these studies were negative, the other one was reported to be positive (Sasaki et al., 1997). The study by Sasaki and coworkers evaluated the fragmentation of DNA in 5 organs of mice 3 to 24 hours after oral administration of 2,000 mg/kg OPP. This is a very high dose, given that the oral LD50 for male mice as reported in the Draft RCD is between 1,200 and 3,499 mg/kg. Adverse effects noted in the necropsies from the acute studies, as described in the Draft RCD, included: bleeding from the digestive system (i.e., stomach and duodenum) and hemorrhage in the lungs. Sasaki and coworkers note that cell death leads to DNA fragmentation, and so positive responses in their assay should be considered in conjunction with cytotoxicity data. Unfortunately, in their assay Sasaki et al. are unable to determine whether observed DNA damage is due to effects directly on DNA or secondary to cytotoxicity. Given the high dose of OPP that the mice received and the inability of the researchers to assess cell viability during the time frame of the experiment, the reported effect of DNA fragmentation by OPP cannot be attributed to genotoxicity. Thus none of the studies conducted in whole animals to evaluate the potential for OPP to cause DNA breaks can be considered positive.

RCD Section III.E.1.c. DNA Damage (d) Sister Chromatid Exchange (SCE)

Page 113 paragraph 2

The first sentence: "*In the absence of metabolic activation, results of all available in vitro tests for SCE were positive in CHO cells (NTP, 1986) and CHO-K1 cells (Tayama et al., 1983b; Tayama-Nawai et al., 1984).*" is not correct, and leaves out at least one report of a negative study by the same author. The Tayama-Nawai paper presents the results of two assays for SCE in the absence of S9, one allowing 27 hours for expression, and the other allowing 42 hours for expression. The study reported negative SCE results for OPP in the whole range of doses tested after an expression time of 42 hours (concentrations 0-150 µg/ml or 0 to 882 µM), and weak positives after an expression time of 27 hours. The maximum positive response reported in CHO-K1 cells treated with 735 µM OPP after the 27 hour expression was 9.4 SCEs/cell compared to the control 5.3 SCEs/cell, while the positive control substance, MNNG caused 37.6 SCEs/cell. Tayama-Nawai et al. state that these results suggest that the DNA damage leading to formation of SCEs caused by OPP is "slight and rather temporary." Not mentioned in this paragraph of the RCD is the study by Tayama et al., published in 1989 that reported no increase in SCE in CHO-K1 cells in the absence of S9 and presence of 588 µM OPP (publication table 2). Thus, two studies of OPP in the absence of

metabolic activation were negative for SCE, and others were only weakly positive.

Page 113 paragraph 3

This paragraph discusses some of the data that surrounding the interpretation of the mode of action of OPP in initiating SCE. The last two sentences state: "*These results support the notion that two different mechanisms may be involved in the SCE induction: a direct effect of OPP in the absence of metabolic activation and an electrophilic reaction of OPP metabolite(s) in the presence of metabolic activation (Tayama and Nakagawa, 1991). Also, the investigators concluded that the involvement of reactive oxygen radicals including* H_2O_2 *and* $O_2^{\cdot -}$ *in the latter process was minor (Tayama and Nakagawa, 1994).*"

Tayama and Nakagawy (1994) actually concluded: "The reactive oxidation product(s) of PHQ, produced enzymatically or nonenzymatically, may be the ultimate agent responsible for the cell damage."

RCD Section III.E.2. Sodium *ortho*-Phenylphenate
RCD Section III.E.2.c. Nonspecific DNA Damage

Page 114, paragraph 5

"...*these DNA effects included nonspecific damage, binding, breakage, oxidation, and cell transformation.*"

It is not clear why "nonspecific damage" is included in this sentence because all studies under that heading in the Draft RCD were described as negative. In addition, this sentence relating specifically to SOPP is a bit misleading because all of the DNA binding reported was associated with oxidative metabolism of SOPP rather than unmetabolized or conjugated SOPP.

RCD Section III.E.2.c. Nonspecific DNA Damage (b) DNA Binding

Page 115 paragraph 1,

"*Results of covalent binding of SOPP to DNA in vivo are available in the mouse skin (Pathak and Roy, 1993) and the rat urinary bladder (Reitz et al., 1983; Ushiyama et al., 1992)*."

This sentence from the Draft RCD indicates that the Reitz study was positive for SOPP binding to DNA in the rat urinary bladder, but the Reitz study was negative for DNA binding of SOPP in the urinary bladder.

"*After a topical application of SOPP to mouse skin, Pathak and Roy (1993) found four major adducts by 32P postlabeling. These adducts were*

identical to those obtained by reacting OPP with purified DNA in vitro (Pathak and Roy, 1993)."

We suggest that it is more relevant to the mode of action of SOPP that the DNA adducts identified in the mouse skin following dermal SOPP exposure were identical to those obtained following application of PHQ to mouse skin, and that formation of the DNA adducts induced by SOPP was inhibited by inhibition of cytochromes P450 in the mice. This suggests that metabolism of SOPP occurred in the mouse skin resulting in the formation of oxidative metabolites of SOPP that interacted with DNA.

"*Ushiyama et al. (1992) detected a single predominant adduct of DNA by 32P postlabeling after the repeated dosing for 13 weeks. This DNA adduct had the chromatographic mobility which matched with adducts from PHQ-DNA and PBQ-dGMP reactions.*"

This again suggests that SOPP does not adduct DNA, but that oxidative metabolites of SOPP may bind to DNA. In the Ushiyama study, the rats were exposed to a very high dose of 2% SOPP in the diet, a level that would saturate the sulfation and glucuronidation metabolic pathways that detoxify SOPP, and result in an increase in oxidative metabolites.

RCD Section III.E.2.c. Nonspecific DNA Damage (c) DNA Breaks and Cell Transformation

Page 115 paragraph 2

"*Results of in vivo DNA break formation are available in two single dosing studies in different organs in the mouse (Sasaki et al., 2002) and the rat (Sekihashi et al., 2002) and a repeated dosing study in the rat urinary bladder (Morimoto et al., 1989).*"

The biological significance of the comet assays performed in the lab of Sasaki and Sekihashi, reported as positive in multiple organs, is questionable. The significance is questionable given that the reported DNA breaks occurred after a single dose in organs that did not develop cancer following chronic exposure in rats and mice, and a number of food additive compounds that have been studied extensively also tested positive in the reported assays. Some of these food additives include: the preservatives, butylated hydroxyanisole (BHA; 21CFR 172.110, among other uses), butylated hydroxytoluene (BHT; 21CFR 173.115 among other uses), saccharin (21CFR 180.37) and sucralose (21CFR 172.831).

The reference to the Morimoto article neglects to mention that no DNA damage was observed by these researchers in the urinary bladders of rats exposed to as much as 0.5% SOPP in the diet, and the authors report "slight damage" following dietary exposure to 1 or 2% SOPP in the diet for

3 to 5 months. This reinforces the observation that there is a threshold below which no adverse effect of SOPP exposure is observed in rats.

Page 115 paragraph 3

"*In an in vivo study, Honma et al. (1983) reported positive concanavalin A agglutination [footnote deleted] in rat bladder epithelial cells after 1 week of SOPP dietary exposure at the dose range that resulted in tumor induction after a longer dosing period (e.g., 13 weeks [Hiraga and Fujii, 1981])*".

This study did not measure DNA strand breaks or specific cell transformation and so does not belong in the consideration of genotoxicity or this section of the Draft RCD. The assay measures the agglutinability of bladder cells isolated from a mutant strain of rats deficient in albumin after exposure to SOPP in the presence of concanavalin A. Concanavalin A is a lectin protein that binds specifically to certain structures found in sugars. It is used to characterize glycoproteins and other sugar-containing entities. Concanavalin A is a lymphocyte mitogen that can aggregate cancer cells, and also aggregates red blood cells and immunoglobulins. Normal cells aggregate in the presence of concanavalin A after proteolytic treatment. It is possible that cytotoxicity could result in an increase in agglutination in this assay. The utility of this study to characterize potential genotoxicity of SOPP is very limited.

RCD Section III.F. Reproductive Toxicity

Page 141, paragraph 5, second sentence

"*Based on the morphometric data for the transitional epithelium in the urinary bladder, the systemic parental NOEL was 40 mg/kg/day.*"

The biological significance of the morphometric effects observed in the transitional epithelium at the mid dose of 140 mg/kg/day (the next higher dose above 40 mg/kg/day) is not clear, given that the effect is not consistent among groups. For example the average number of cells per layer and thickness is increased compared to controls in F0 females in the 140 mg/kg group, while the number of cells per layer and thickness is decreased compared to controls in F1 females (though the decrease in thickness is not statistically significant), and there is no difference between F1 males and controls in either morphometric measure at 140 mg/kg/day. Hyperplasia of the transitional epithelium was increased compared to controls only in the high dose groups (490 mg/kg/day). We suggest that the biologically significant parental systemic NOEL for this study is 140 mg/kg/day.

RCD Section III.G. Developmental Toxicity
RCD Section III.G.1. *ortho*-Phenylphenol

Page 154, paragraph 2

The Zablotny study summarized here is evaluated in detail in Section 6 of this response document.

Page 160, first full paragraph, last sentence

"*Without the individual data, the only statistical comparison that is available is a Fisher exact test using litter incidences; the resulting p-value is 0.06 (1/17 vs 6/19), which becomes 0.01 if the data from the olive-oil and water negative control groups are combined (2/37 vs 6/19).*"

This comparison is inappropriate. It is not acceptable to combine control groups with different vehicles to achieve statistical significance.

RCD Section IV. Risk Assessment for Dietary Exposure
RCD Section IV.A. Hazard Identification

Page 161, paragraph 1, last sentence

"*In the latter, DPR employs the principle in cancer risk assessment guideline of the United States Environmental Protection Agency (USEPA) for selecting the endpoint and method needed.*"

This sentence refers to oncogenic risk mentioned in the first sentence of the paragraph. We suggest that DPR follow the EPA Guidelines for Carcinogen Risk Assessment (2005) more closely as noted in Section 3 of this document.

RCD Section IV.A.2.Selection of Toxicity Endpoints

Page 161 paragraph 2, sentences 3 & 6

"*The major toxicity for OPP and SOPP in experimental animals was developmental and urinary tract effects" and "Also, the use of NOELs for these endpoints in risk characterization would protect against effects at higher doses.*"

We agree that use of a NOEL for urinary tract effects in the risk assessment and characterization will protect against other effects that were observed at higher doses, including those proliferative effects in the urinary bladder that occur only at high doses.

Page 161 paragraph 3, second sentence

"*In Wistar rats, the effects were decreased fetal body weight and increased incidence of resorptions (Kaneda et al., 1978)*"

This statement is not supported by the summary of the study presented on pages147-149 of the Draft RCD, which states that: "…it would appear that the fetus (and not the litter) was the experimental unit for the statistical analysis of resorptions" and: "DPR considered this study unacceptable because of inadequate reporting, including the lack of individual data." Further the possibility of increased resorptions was included in the study conclusion section only in parentheses. We recommend that mention of this "effect" of increased incidence of resorptions in rats be removed from the developmental effects hazard identification section.

Page 161 paragraph 3 sentence 4

"*Fetuses from Jcl:ICR mice exposed to SOPP exhibited reduced mean body weight and an increased incidence of cleft palate (Ogata et al., 1978).*"

Fetal mice exposed to the low dose of SOPP (100 mg/kg/day) exhibited an increase in cleft palate compared to controls, but fetal mice exposed to the mid or high doses (200 or 400 mg/kg/day) did not exhibit an increase in cleft palate. The lack of a dose response calls into question the biological significance of this effect. The endpoint of cleft palate from this study should not be used or considered for risk assessment.

Page 163 paragraph 1, sentence 3

"*As discussed in the III TOXICOLOGY PROFILE, the increased water intake may have been due to the effect of OPP on water metabolism.*"

Throughout the Draft RCD the authors focus on the OPP-induced polydipsia and polyuria. The Draft RCD notes that rats exposed to high doses of OPP in the diet drink more water than the controls, urinate a larger volume, and the urine is more dilute. As in the sentence above, they suggest that there may be an effect on water metabolism, or alternatively kidney damage. Ultimately this speculation about polydipsia and polyuria plays very little role in the risk assessment that we can identify. We suggest that since this endpoint is not relevant for risk assessment, the focus on this endpoint be reduced in this document. For example, are Tables 6, 10, and 18, which describe urine parameters, necessary and relevant to the risk assessment?

RCD Section IV.A.3.Selection of Critical NOELs

Page 163, paragraph 3

The discussion in this paragraph centers on the justification for using a NOEL of 25 mg/kg/day from the Zablotny study (1991) as the basis for the acute exposure risk assessment, supported by the results from the Ogata study (1978).

We have noted our disagreements regarding the use of the Zablotny (1991) study as an endpoint for risk assessment in Section 6 of this document. We have also noted that the Ogata et al., study (1978) is inappropriate for risk assessment, given that the effect that is the basis for the LOEL was observed only at the low dose.

Page 164, full paragraph 2, sentence 2

"*The characterizing features of the tumor dose response relationship were zero occurrences at relatively low doses and a steep increase in the tumor incidence at higher doses, a pattern commonly described as "nonlinear" or "hockey-stick" (Cohen and Ellwein, 1989).*"

This characteristic dose-response curve was observed repeatedly in study after study, and provides significant evidence to support the position that OPP and SOPP act by a nonlinear mechanism to cause tumors in the rat urinary bladder only at high doses.

Page 164 full paragraph 2, sentence 3

"*Also typical of the bladder carcinogenicity of OPP and SOPP is the apparent shortened time-to-tumor with increasing dose, such that detection of tumors occurred as early as 13 weeks of exposure at relatively higher dose levels (Hiraga and Fuji, 1981, 1984).*"

Decreased time-to-tumor was only seen at excessive dose levels and is therefore not relevant in the weight of the evidence analysis.

RCD Section IV.A.4.Oncogenicity of OPP and SOPP

Page 167 paragraph 3, sentence 1

"*The biotransformation pathway of OPP involves an initial oxidation to PHQ.*"

This is not true. The primary metabolite, particularly at low doses is OPP sulfoxide. OPP sulfoxide and OPP glucuronide are not metabolites derived from PHQ.

Page 167 paragraph 3 sentences 3 and 4, and entire paragraph 4

"*Smith et al., (1998) reported the sulfate and glucuronide conjugates of OPP in the urine of male rats after a repeated dosing (56-294 mg/kg/day) decreased from 87% of total OPP at the low dose to 64% of total OPP at the high dose. However, this detoxification saturation MOA is inadequate to address two key observations: the presence of unconjugated PHQ and PBQ and their role in the bladder tumor formation.*"

As noted in section 5 of this response document, the levels of free PHQ+PBQ do not correlate well with increasing dose between various

studies. As shown in Figure 2 of this Response, the amount of nonconjugated metabolites had a negative correlation with OPP dose level in the 13- week subchronic study of Smith 1998. In contrast, the results of Morimoto et al. (1989) afford a positive correlation with dose. Morimoto et al. (1989) report increasing percentages of PHQ+PBQ across the dose levels of 327, 655 and 1309 mg/kg. In contrast, Smith et al. (1998) report decreasing percentages of the dose excreted as free PHQ/PBQ (0.48% at 56 mg/kg, down to 0.14% at 924 mg/kg OPP). At this same high dose of 924 mg/kg OPP, Hasegawa et al. (1991) report four-fold higher levels of free PHQ+PBQ than those of Smith et al (5.7 mg/kg/day urine, or 0.64% of admin. dose). This variation in measured levels of nonconjugated metabolites indicates possible systematic errors in some of these data. Since there is no significant dose-dependence in the formation of these minor, nonconjugated metabolites, it may be that they are formed as artifacts of sample collection and/or storage, and not eliminated in the non-conjugated form. Based on the evaluation of the variability of the trace levels of free PHQ and PBQ levels across studies presented in section 5, it is clear that there is a possibility that some of the reported results may be erroneously high, due to sample degradation during collection or analysis.

These data do not appear to be reliable for use in risk assessments for OPP, and should not be the primary basis for supporting, refuting or discarding a proposed mode of action. The published threshold-based results for covalent adduction of test material to endogenous macromolecules (Fig. 5) is a more accurate means of assessing reactive metabolite formation vs. dose levels.

Page 168 second full paragraph, last sentence

"*In addition, Niho et al. (2002) found evidence for the involvement of DNA change in the cell proliferation event induced by SOPP.*"

Niho et al., (2002) presented no DNA data at all. We suggest that this sentence be removed.

Page 168 third full paragraph, sentences 2 and 3

"*In the context of the biological based two-event model as proposed by Greenfield et al., (1984), reactive species derived from PHQ autoxidation may have induced genetic damage in the urinary bladder of rats at lower doses. At higher doses, the proliferation of bladder epithelial cells magnified the genetic damage induced, an effect that may have been due to the cytotoxic effects of PHQ and other metabolites (e.g., PBQ), which also were found in the urine of rats treated with OPP or SOPP*"

There is no evidence of genetic damage occurring in the urinary bladders of rats exposed to OPP or SOPP at low doses. To the contrary, data

available suggest that no genetic damage is induced in the urinary bladders of rats exposed to OPP at low doses (e.g., Reitz et al., 1983, Smith et al., 1998, Balakrishnan & Eastmond, 2006). If genetic damage occurred at low doses it is likely that tumors would be observed at these low doses, particularly given the large number of bioassays that have been conducted. There is no indication that genetic damage is a precursor of the proliferative effects observed in the urothelium. In fact these hyperplastic precursor lesions exhibit reversibility, which would not be the case if genetic damage were the underlying cause of the lesions. As noted in Section 3, the 2006 U.S. EPA Toxicology Chapter for the RED states: "Evidence suggests that there are not sufficient oxidative metabolites generated *in vivo* to result in genotoxic MOA, but that a non-genotoxic MOA is operative."

Page 170 paragraph 1, sentence 1

"*Other sites that OPP and SOPP induced tumors were kidneys (carcinoma in renal papilla and pelvis [Hiraga and Fuji, 1981; Hiraga, 1983]) liver (adenoma and hepatoblastoma [Quast and McGuirk, 1995]), and circulatory system (hemangiomas [Ito, 1983; Quast and McGuirk, 1995]).*"

Kidney tumors were observed in rats exposed to a very high dose of 2,000 mg/kg/day SOPP for 91 weeks, a dose that very likely exceeded the MTD (Hiraga and Fuji, 1981). Any kidney tumors identified in rats exposed above the MTD should not be considered for risk assessment purposes. There was not a significant increase in kidney tumors in the Hiraga, 1983 study. The statement regarding kidney tumors should be deleted.

Significantly higher incidences of hepatocellular adenomas were observed in male mice exposed to dietary OPP at dose levels of 500 and 1000 mg/kg bw/day for 18 months. The hepatic tumor incidence in females and the incidence of hepatocellular carcinoma in males were not increased (Quast and McGurk, 1995, Quast et al., 1997). A slight increase in hepatoblastomas was observed at the mid-dose level in males but a dose-response was not apparent. Hepatocellular adenoma is very common in sensitive strains of mice such as the $B6C3F_1$ strain used in this study. This tumor type occurs often secondary to prolonged cell proliferation or oxidative stress (Parke and Ioannides, 1990; Grasso and Hinton, 1991; Klaunig et al., 1995). In the case of OPP, reactive oxygen species (ROS) associated with the quinone metabolites are produced at the high dose levels. The mouse has a low capacity to repair damage by ROS in the liver and is highly susceptible to liver tumors associated with chemicals that produce ROS (Klaunig et al., 1998). Liver tumors were not induced in other species, even at high dose levels exceeding the limit dose. The finding of hepatocellular tumors in the mouse (Quast and McGuirk, 1995) should be assigned little weight in the assessment of the carcinogenic potential of OPP.

The circulatory tumors mentioned were identified only in a low dose group of male mice, were not statistically significant, and did not show a dose-response pattern. The Draft RCD authors suggest that a reduction in body weight in mice at the mid and high doses was responsible for the lack of a dose response pattern in tumor development, but in fact the body weights of the mid dose male mice were not significantly lower than controls at the 2 year point (according to the Draft RCD summary). Thus there is no reason that circulatory tumors should not have been identified in the mid-dose group if, in fact, this was a compound related effect. Reference to circulatory tumors should be removed from the oncogenicity summary.

The data from a number of oncogenicity studies indicate that test article-related tumors appropriate for cancer risk assessment are urinary bladder tumors in male rats. Thus relevant tumors occurred in a single species and were sex-specific.

Page 170 paragraph 2

"*Based on evidence of multiple tumor types in multiple studies, USEPA published a review in 1994 and classified the carcinogenic potentials of OPP and SOPP according to the 1986 Guidelines for Carcinogenic Risk Assessment Guideline (USEPA, 1986) as Group B2 (i.e., probable human carcinogen) (Rinde and Dapson, 1994). However, in the review, the USEPA considered that the linear extrapolation model for projecting human health risk was inappropriate, based on the available information on OPP metabolism and carcinogenesis up to 1994. At that time, the information indicated that formation of PHQ, a penultimate carcinogen, occurred only at ≥0.5% (i.e., 5000 ppm) dietary OPP levels and the tumorigenic response found in rats occurred only at doses at or above 0.5% dietary dose level of OPP. Hence, the USEPA considered the tumorigenic effect of OPP as being irrelevant to anticipated human exposure and recommended a risk assessment approach that would allow the concept of apparent threshold be incorporated for estimating the risk (Rinde and Dapson, 1994).*"

This paragraph cites and describes a risk assessment performed by the US EPA that was released in 1994 that has been superseded by a revised risk assessment released in 2006. The revised assessment is based on consideration of additional data not available or considered in 1994 and uses the new EPA Guidelines for Carcinogen Risk Assessment (2005). This paragraph in the Draft RCD is outdated and should be revised based on the current evaluation of OPP by the EPA. The EPA Health Effect Division's Carcinogenicity Assessment Review Committee (CARC) classified OPP and SOPP as "**Not Likely to be Carcinogenic to Humans**" below a defined dose range based on convincing evidence that

the carcinogenic effects are not likely below 200 mg/kg/day in experimental animal studies.

Page 170 paragraph 3

"*As described previously (III.A. PHARMACOKINETICS), the formation of PHQ occurred in rats that received diets containing ≥800 ppm OPP (the lowest dose tested) (Smith et al., 1998). Hence, this recent finding refutes the previous conception that the formation of PHQ occurred only at ≥5000 ppm dietary OPP levels. Also, using a biological based two-event model (Greenfield et al., 1984), genotoxic and cytotoxic effects of PHQ and other OPP metabolites provide a reasonable explanation to the nonlinear bladder tumor dose-response of OPP. In the light of this new information, together with no evidence that the tumor induction is not relevant to humans, DPR considers that the low-dose extrapolation model is the most appropriate for cancer risk characterization associated with human exposures to OPP or SOPP, as opposed to the threshold model*."

From this paragraph, it appears that the determination to evaluate OPP and SOPP as a genotoxic carcinogen is based primarily on a single piece of evidence, that trace amounts of PHQ and or PBQ were detected in the urine of rats exposed to 56 mg/kg/day of OPP for 13 weeks. If, in fact, this is the case, this opinion would be rectified by the performance of a weight of evidence assessment of the data,

8. References

References Note: * Copies of references marked with an asterisk are included in this submission. All other references have either been provided to DPR in previous submissions, were cited by DPR in the Draft RCD and are presumed to be available to DPR, or were issued by the U.S EPA and are publically available on-line.

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M E M O R A N D U M

TO: Gary T. Patterson, Ph.D.
Supervising Toxicologist
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VIA: Joyce Gee, Ph.D.
Senior Toxicologist
Medical Toxicology Branch

FROM: Eric S.C. Kwok, Ph.D., D.A.B.T.

DATE: April 9, 2007

SUBJECT: RESPONSE TO COMMENTS FROM LAXNESS CORPORATION AND THE DOW CHEMICAL COMPANY ON DRAFT OPP AND SOPP RISK CHARACTERIZATION DOCUMENT – DIETARY EXPOSURE

This memorandum addresses comments from the Registrants (the Lanxess Corporation and the Dow Chemical Company) on the Department's draft Risk Characterization Document (RCD) for the active ingredients ortho-phenylphenol (OPP) and sodium ortho-phenylphenate (SOPP) (dated June 6, 2006) (Lanxess and Dow, 2006). We would like to thank the Registrants for their comments. The Registrant opposed DPR's position on the potential genotoxicity of OPP and SOPP, the oncogenicity dose-response assessment using a default linear approach, and the developmental effects of OPP and SOPP. However, in response to a DPR special request for reviewing the draft OPP RCD (dated December 8, 2006) in these three areas, the Reproductive and Cancer Hazard Assessment Branch (RCHAB) of OEHHA concurred with DPR's determinations. That is, in the response (dated January 18, 2007 [Appendix F]), OEHHA concluded that current data do not support the position that OPP is not a genotoxic carcinogen and the use of a threshold model for cancer risk assessment. Also, OEHHA concludes that the available scientific data support concerns regarding developmental toxicity of OPP. We have made several changes to the draft RCD to clarify and address the points raised by the Registrants. Our responses to these comments are as follows (the page number noted refers to the final draft RCD):

General Comments

Comment #1: Page 6 under (a) USEPA concluded that OPP is a threshold carcinogen unlikely to represent a carcinogenic risk to humans*: "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."* Also*, " 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."

Response: We have expressed our concerns with USEPA threshold mode-of-action (MOA) determination of OPP and SOPP in rats and mice *via* two separate submissions to Docket ID Number EPA-HQ-OPP-2006-0154 (these submissions are available online at http://www.regulations.gov) during the public comment periods for "2-Phenylphenol and Salts Risk Assessment" and "Reregistration Eligibility Decision of 2-Phenylphenol and Salts." Briefly, for the urinary bladder tumors in rats, the overall data indicated that there is insufficient evidence to conclude that non-genotoxic (i.e., threshold) MOA is the only probable pathway for the oncogenicity of OPP and SOPP. In fact, our analysis indicates that the oncogenic effects in the rat urinary bladder may involve a genotoxic mechanism (i.e., non-threshold MOA). Regarding the mouse liver tumors, the threshold risk characterization approach adopted by USEPA is inconsistent with the Agency's determination that the MOA is unknown. Hence, in the Docket submission, we urged USEPA to consider following the Agency's latest Guideline for Carcinogen Risk Assessment (USEPA, 2005). That is, "*…linear extrapolation is used as a default approach for characterizing the cancer risk when the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data.*" Also, "*…in the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data: animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity.*"

Comment #2: Page 8 Under (c) Weight of Evidence process is appropriate and required part of RCD process: "*We believe that it is important that a true weight of the evidence evaluation on the mode of action for OPP and SOPP should be performed by DPR as outlined in the EPA Guidelines for Carcinogen Risk Assessment. Furthermore, the EPA Guidelines reference and use the weight of evidence process and procedures developed by an International Life Sciences Institute (ILSI) workgroup This workgroup included the USEPA and, Accordingly, the product of the workgroup (Meek et al., 2003) is a recognized approach within the EPA Guidelines for Carcinogen Risk Assessment."*

Response: We considered the USEPA Guideline for Carcinogen Risk Assessment (USEPA, 2005) and the reference by Meek *et al.* (2003) in writing the draft RCD. We have revised the IV.A.5. Oncogenicity Weight of Evidence of OPP and SOPP (page 164) to enhance clarity and added the citation by Meek *et al.* (2003) that inadvertently was missed in the draft RCD.

The comments on genetic toxicity were prepared by Dr. D. Brusick.

Comment #3: Page 10 Under 4(a) Genotoxic "Potential" vs. Genotoxic Risk: "*While the term genotoxic potential was used frequently in the draft RCD to evaluate study responses, it was not clear what the phrase "genotoxic potential" meant in the context of the overall draft RCD."* Also, under 4(b) *General Comment on the Treatment of Genotoxicity in the RCD: "The draft RCD did not discuss the mechanisms involved in the reactions of OPP and SOPP or their metabolites with DNA in the light of a carcinogenic mode of action" (page 10-11).*

Response: As stated in the draft RCD, the current data are not sufficient for drawing a conclusion of an exclusive mode of action for causing urinary bladder cancer in rats. Dr. Brusick (a contributing author who wrote comments on genetic toxicity) in the rebuttal stated: "*The primary source of reactivity from OPP and SOPP toward DNA and chromosomes appears to be associated with the intrinsic potential of the highly reactive PHQ and PBQ species to reactive with macromolecule including DNA.*" DPR believed that Dr. Brusick's analysis is consistent with our interpretation that OPP and SOPP have genotoxic potential, as their reactive metabolites are capable of interacting with macromolecules including DNA (see also DPR response to issue #5).

Comment #4: Page 10-11 Under 4(b): *"The genetic toxicity section of the RCD appears to be primarily a tabulation of the genotoxicity publications with the type of assessment primarily based on the presence of positive responses. Reference was made in several places to a weight-of-evidence assessment, but no specific process regarding the weight-of-evidence approach was defined. In one of the recent reviews of the genetic toxicology of OPP and SOPP (Brusick, 2005), a semi quantitative weight-of-evidence method published in 1994 was used on the set of responses for OPP. The method provides a single value for all of the in vitro and in vivo data ranging from -100 (all negative) to +100 for all positive. OPP's score was -10.6 and similar to the genotoxicity of some other nongenotoxic carcinogens such as malathione, amitrole and aniline."*

Response: As detailed in the draft RCD, we realize that many of the Registrant-submitted studies showed weak or negative genotoxicity. However, we cannot ignore the *in vivo* and *in vitro* studies published in the open literature that supported the genotoxic potential of OPP and SOPP and their metabolites.

Regarding the weight-of-evidence analysis, we recognize Dr. Brusick's expertise in genetic toxicology and his recent review of genotoxicity and carcinogenic mode of action for OPP in the open literature published under the auspices of the Registrants (Brusick, 2005). However, we do not agree with Dr. Brusick's quantitative genotoxicity analysis that chemicals can be arbitrarily grouped and labeled based on the same or similar scores. For example, the agent score of aniline (-8.9) that Dr. Brusick identified as a non-genotoxic carcinogen has a similar value to benzene (a known human carcinogen; agent score = -7.1 [Mendelsohn *et al.*, 1992]). Furthermore, the approach that Dr Brusick used to generate the score gives no considerations to results from

studies investigating DNA binding, breakage, oxidation, and damage/repair of OPP and that the method is unable to account for the genotoxicity data of reactive OPP metabolites (i.e., PHQ and PBQ) (Brusick, 2005). These metabolites may contribute to the genotoxicity of OPP and SOPP *in vivo.*

Comment#5: Page 12 Under 4(c) Specific Concerns on the Genotoxicity Treatment in the RCD: *"One cannot eliminate these* (five positive gene mutation) *studies solely on the suspicion of technical flaws, but these arguments combined with the substantial amount of negative results in well-conducted gene mutation assays in microbial and mammalian cells (~20 negative reports) for SOPP, PHQ, and PBQ, provide strong argument for concluding that the five studies reported as positive do not constitute a sufficient weight-of-evidence to classify OPP a gene mutagen."*

Response: We agree with Dr. Brusick's assessment that positive evidence for the genotoxic potential of OPP and SOPP is not overwhelming. However, we believe that because different assays provided different information for the genotoxicity, it is not appropriate to dismiss all the positive studies based simply on the relative number of negative *versus* positive reports. Hence, we maintain that evidence exists to indicate that OPP, SOPP and their reactive metabolites (i.e., PHQ and PBQ) have genotoxic potential.

Comment #6: Page 12 Under 4(c): "*Studies by Pathak and Roy (1993) reported adducts with the ^{32}P-postlabelling method in mouse skin treated with 10-20 mg/animal of SOPP even though this compound is not carcinogenic in mice. Therefore, an initial concern is whether adducts, if produced by OPP or its metabolites, are even relevant to any risk concerns. If adducts are produced, they do not seem to be found in the urinary bladder of rats exposed to oral or dietary OPP*."

Response: We disagree with Dr. Brusick's assessment that the speculative interpretation of results of DNA adduction *via* a particular route can be extrapolated to another route. Also, in contrast to Dr. Brusick's comment on the carcinogenic effect in mice, male mice that received 5000-20000 SOPP ppm in diet exhibited a dose-dependent increase in the incidences of carcinoma in the liver (Ito, 1983). Furthermore, both *in vivo* DNA adduction studies of SOPP in mouse skin (Pathak and Roy, 1993) and rat urinary bladder (Ushiyama *et al.*, 1992) reported that major DNA adducts exhibited chromatographic mobility matched closely with adducts from PHQ-DNA reaction *in vitro*.

Comment #7: Page 12-13 Under 4(c): "*Ushiyama et al., (1992) using ^{32}P-post labeling did report finding one spot not in the control. This finding was at a 2% dietary level of SOPP, which produces other neoplastic endpoints. The Ushiyama et al. data conflict with other studies using equivalent doses (Smith et al., 1998) and the spot might be a spurious intrinsic adduct known to occur with this methodology (Phillips et al., 2000). This interpretation difference cannot be*

resolved with the existing data; however, it appears that the weight-of-evidence supports a lack of adduct formation in rat urinary bladder DNA from oral exposure to OPP or SOPP."

Response: SOPP at 2% dietary level induced cancer in the rat urinary bladder. Hence, it is not clear to DPR the relevance of referring to "other neoplastic endpoints" and attempting to relate it to the identification of DNA adducts in the rat urinary bladder. Also, the draft RCD discussed the different method employed in the studies by Ushiyama *et al.*, (1992) and Smith *et al.* (1998) (whole bladder *vs* urothelium) with respect to different detection sensitivity (enhanced *vs* non-enhanced ^{32}P-postlabeling). Hence, we considered the results from these studies as mixed, not "conflicting" as stated in Dr. Brusick comments. This issue has been discussed extensively in the draft RCD.

Regarding the interpretational difference, we agree with Dr. Brusick's assessment that we do not have enough information to definitively conclude that OPP and SOPP are not genotoxic. Hence, based on the available data, we concluded that OPP and SOPP have genotoxic potential.

The comments on metabolism were prepared by Dr. M. Bartels of Dow Chemical.

Comment #8: Page 17 Under 5(b) Comparison of Conjugated vs. Free Metabolites: *"the levels of free PHQ+PBQ do not correlate well with increasing dose between various studies (Fig. 2). Morimoto et al. (1989) report increasing percentages of PHQ+PBQ across the dose levels of 327, 655 and 1309 mg/kg (1.1 to 2.2%, OPP equivalents)(Fig. 2). In contrast, Smith et al. (1998) report decreasing percentages of the dose excreted as free PHQ/PBQ (0.48% at 56 mg/kg, down to 0.14% at 924 mg/kg OPP). In fact, these authors found lower absolute amounts of free PHQ at 924 mg/kg than at 556 mg/kg (1.3 vs. 1.7 mg/kg/day urine, respectively). At this same high dose of 924 mg/kg OPP, Hasegawa et al. (1991) report four-fold higher levels of free PHQ+PBQ than those of Smith et al (5.7 mg/kg/day urine, or 0.64% of admin. dose). This variation in measured levels of nonconjugated metabolites indicates possible systematic errors in some of these data."*

Response: The draft RCD provided a detailed discussion that both Morimoto *et al.* (1989) and Smith *et al.* (1998) studies that showed a dose-related increase in the urinary concentrations of PHQ plus PBQ. The percents of PHQ plus PBQ, calculated by Dr. Bartels, were based on the measured values in the studies by Moriomoto *et al.* (1989) using five rats per dose (i.e., 5 samples per dose) and Smith *et al.* (1998) using a pooled sample from 11 rats per dose (i.e., 1 sample per dose). Because of the lack of standard derivation data presented by Smith *et al.* (1998), any further comparisons of these data are not possible. Hence, we considered that the results from these two studies are, at most, "mixed" rather than "in contrast." Even if they were in clear disagreement, there is no further evidence to support the dismissal of one over the other.

Also, the speculation of systemic error by Dr. Bartels is not supported by the data presented given that Hasegawa *et al.* (1991) measured the urinary PHQ and PBQ concentrations using 3-5 rats per dose *versus* Smith *et al.* (1998) study using only one sample per dose. As discussed above, the lack of standard derivation data presented by Smith *et al.* (1998) makes any further comparisons of these data impossible.

Comment #9: Page 19 Under 5(c) Free OPP and PHQ Possibly Arising from Sample Degradation: *"Since the highest levels of free PHQ and OPP were reported in studies employing wire-mesh caging and urine collection at ambient temperature, the actual levels of these unconjugated metabolites may have been increased by partial (~1%) degradation of conjugated OPP and PHQ." … "Based on this evaluation of the variability of the trace levels of free PHQ and PBQ levels across studies, it becomes clear that there is a possibility that some of the reported results may be erroneously high, due to sample degradation during collection or analysis. Further investigation of this technical issue should be conducted prior to utilization of this data in any risk assessments for OPP."*

Responses: Even though problems that Dr. Bartels described may have existed in the studies by Morimoto *et al.* (1989) and Hasegawa *et al.* (1991), the study conducted by Dr. Bartels (Bartels *et al*., 1998 and Smith *et al.*, 1998 [Table 1, page 16 of the rebuttal]) still demonstrated the occurrence of free OPP and PHQ in the urine of OPP-treated rats. Also, it is unclear to us how Dr. Bartels derived the ~1% value.

We conduct risk assessments based on the data available. Further investigation as suggested by Dr. Bartels may provide additional information for our understanding of the carcinogenic mode of action of OPP and SOPP. We cannot mandate such an investigative study but would be delighted to review this information when it becomes available.

Comment #10: Page 22 Under Section 5(d) Reactive Metabolite Involvement in Threshold-based Effects of OPP: *"Only above a threshold of GSH or protein adduct formation (>200 mg/kg; Fig. 5-6* [pages 24-25]*) are effects such as bladder lesions, DNA damage and tumors seen (Kwok et al., 1999, Morimoto et al., 1989)* (Page 22)*."*

Response: The apparent association between the adduct formation and histologic lesions that may present a possible threshold should not be considered as a direct proof of causality of GSH and (or) protein involvement in the carcinogenic effect of OPP.

The comments on developmental effects were prepared by Drs. E. Carney and C. Zablotny, both associated with Dow Chemical.

Comment #11: Page 27 Under 6(a) OPP Rabbit Developmental Toxicity Study: Review of Resorption Rate Data: *"Dow utilized a censored Wilcoxon test* (developed by Dr. J. Haseman [a

well regarded US government agency statistician]) *with a Bonferroni correction for multiple comparisons, resulting in P-values of 0.10 and 0.13 for the 100 and 250 mg/kg/day groups, respectively, which is above the cut-off for statistical significance of 0.05."* Also, page 28 under 6(b) Statistical Methodology: *"In its review of the study, DPR questioned the use of the Bonferroni correction. Without such a correction, the significance values would be divided by three and therefore fell just slightly below the 0.05 cut-off."*

Response: We recognize Dr. Joseph K Haseman's expertise in biostatistics and his censored Wilcoxon test (Haseman and Hoel, 1974). We are also aware that Dr. Haseman raised specific concerns regarding the application of Bonferroni correction to the p-values when making pairwise comparisons (Haseman *et al.*, 2001). That is, "it (i.e., Bonferroni correction) is a rather conservative approach and would have a relatively high false-negative rate; "such an approach (i.e., Bonferroni correction) would be unnecessary if an investigator uses multiple comparison procedures." By following Dr. Haseman's recommendation, we found that OPP-treated rabbits exhibited increases ($p \leq 0.05$) in resorptions (expressed in proportion of fetuses affected per litter) at 100 and 250 mg/kg/day using a non-parametric multiple comparison test (i.e., Shirley's test [Shirley, 1977]).

Comment #12: Page 29 Under 6(c) Resorption Rate Parameters: *"Overall, this indicates that the increase in the percentage of litters with resorptions was mainly the result of the resorptions in the high-dose group being distributed across more litters, whereas the control group resorptions tended to be concentrated in fewer litters."*

Response: The draft RCD has extensive discussion on this issue and actually agree with Drs. Carney and Zablotny assessment that OPP-treated rabbits exhibited increase in the number of litters with resorptions.

Comment # 13: Page 30 Under Section 6(d) Weight of Evidence Evaluation for Increased Resorptions: "…*the lack of a dose-response relationship is not consistent with a treatment-related effect of OPP on resorption rate. Another important piece of evidence comes from the rabbit probe developmental toxicity study which preceded the main study, as there was no increase in resorption rate despite the fact that higher dose levels were evaluated."*

Response: We disagree with this statement as data in Table 2 of the rebuttal showed that a clear dose-related response in both percent litters with resorptions (33.3%, 57.1%, 76.9%, and 72.2% for the control low-, mid-, and high-dose groups) and percent post-implantation loss (12.2%, 16.7%, 19.2%, and 18.3% for the control, low-, mid-, and high-dose groups). The latter parameter is considered a better parameter by Dow Chemical to represent the resorption data.

Regarding the supporting evidence, negative results from a limited probe study cannot supercede the positive evidence from a full study. Also, in contrast to Drs. Carney and Zablotny

assessment, the draft RCD has extensive discussion on the evidence of increased resorptions in the probe study.

Comment #14: Page 30 Under Section 6(d): *"OPP has also been evaluated for developmental toxicity in two studies in rats. In one such study (John et al., 1981), doses as high as 700 mg/kg/day in Sprague-Dawley rats had no effect on resorption rate. A similar study in pregnant Wistar rats showed an increase in resorptions, but only at dose levels ≥ 600 mg/kg/day which were associated with significant maternal toxicity (e.g., ataxia) (Kaneda et al., 1978). There was no increase in resorptions at 300 mg/kg/day, despite the presence of significant maternal toxicity. In the present rabbit study, significant maternal toxicity (e.g., 13% mortality) was present at the high dose level of 250 mg/kg/day, but there was no evidence of maternal toxicity at lower dose levels."*

Response: We disagree with Drs. Carney and Zablotny assessment that the evidence of developmental toxicity of OPP in rabbits somehow needed to be validated by a similar result in rats. This hard lesson was learned years ago from the detrimental outcome of thalidomide on development that showed in rabbits but not in rats. Information is not available for further elucidation of any species specificity for the fetal loss by OPP. The USEPA guideline for developmental toxicity risk assessment stated: "*the evidence necessary to judge that a potential hazard exists generally would be data demonstrating an adverse developmental effect in a single, appropriate, well-conducted study in a single experimental animal species* (USEPA, 1991)."

Page-by-page Details Consideration of RCD and DPR Responses

Comment #15 (Page 13 paragraph 2): We disagree with Registrants' comments that "*since the collective database on OPP and SOPP illustrate a threshold effect for most toxicological endpoints, including carcinogenicity, we believe that it is crucial to carefully identify and differentiate the dosing levels and their implications while characterizing these ingredients*." We do not believe the current data support the Registrants' assertion that the carcinogenic effects of OPP and SOPP operate only *via* a threshold mechanism of action (see also response to comment #1).

Comment #16 (Page 13 paragraph 3): We disagree with the Registrants' comment that "*…high doses of SOPP in the diet (greater than 1,300 mg/kg/day, which is greater than the LD50) developed bladder tumors as early as 13 weeks…."* In contrast to the Registrants' "excessive dosing" assertion, all animals in this 13-week dietary exposure study survived. Hence, we made no change to the text.

Comment #17 (Page 14 paragraph 2): We have added text to describe the negative genotoxicity studies and their relationship to the positive studies in evaluating the genotoxic potential of OPP and SOPP (page 2) as suggested.

Comment #18 (Page 15 paragraph 1): We disagree with the Registrants' comment that "*The statement that there was a dose-related increase in papilloma and carcinoma of the urinary bladder in* ***both sexes*** *in multiple rat bioassays is not accurate*." Studies by Hiraga and Fujii (1981), Hiraga (1983), and Eigenberg (1989b) reported the occurrence of OPP/SOPP-induced urinary bladder tumors in the females. Also, we disagree with the Registrant analysis that *"Although a single chronic study shows a slight increase in combined papilloma and carcinoma in female rats exposed to SOPP, the incidence is statistically significant only by a trend test conducted by DPR and not by pair-wise comparison (Hiraga, 1983, also described in Fuji and Hiraga, 1985)."* While urinary bladder tumors were not statistically significant in terms of incidence in these studies, we considered that these were treatment-related findings because transitional cell carcinomas of the urinary bladder are rare spontaneous neoplasms in female F344 rats (0.2% incidence in the historical controls of NTP bioassay [Haseman *et al*., 1990]).

In response to the comment that "*No weight of evidence evaluation for either the genotoxic or threshold modes of action was presented*." We have revised the weight of evidence evaluation and added text to clarify the rationale of using the low dose extrapolation model in cancer risk characterization (pages 3-4) as suggested.

Comment #19 (Page 15 paragraph 4): We disagree with the Registrant comment that "*Actually the appropriate (and suitable) studies have been submitted to DPR but there was no acute endpoint of concern in the study results."* Instead of no acute endpoint of concern, we believe that the information available in single-day (i.e., acute) toxicity studies is not sufficient for the acute toxicity endpoint selection. Hence, as stated in the RCD, we selected acute NOELs from multiple-day oral developmental toxicity studies due to the lack of suitable single-day toxicity study.

Comment #20 (Page 16 paragraph 1): We disagree with the Registrants' assertion that the urinary bladder lesions at the low and mid dose were not treatment-related as OPP did not induce unique type of lesion in the study by Christenson *et al.* (1996a). That is, " *SEM revealed necrosis or exfoliation occurring in the urinary bladder epithelia of both control and treated rats*". Also, our analysis indicated significant ($p<0.05$) increases in lesion-severity score of the number of rats in the low- and mid-dose (page 43). Based on these results, the Registrants' conclusion that "*The differences between the control group animals and the animals at the low and mid-dose levels were small and of uncertain biological and statistical significance"* is not supported by the data. Hence, we made no change to the text.

Comment #21 (Page 18 paragraph 1): We have revised the text (page 7) to include the new USEPA carcinogenicity designation of OPP and SOPP as recommended.

Comment #22 (Page 19 paragraph 1): Studies by Nakagawa *et al.* (1992a,b; 1993) (page 8) are examples of investigating the biocidic mechanism of OPP. In contrast to the Registrant's

suggestion, we did not give any "weight" to these proposals, as a clear understanding of the biocidic mechanism of OPP and SOPP is not available. Hence, we made no change in the text.

Comment #23 (Page 22 paragraph 1): The intent of summary paragraph of III.A. PHARMACOKINETICS (page 11) is to provide an overall view on the types of OPP reaction and product observed under different experimental conditions: *in vitro* followed by *in vivo*. Hence, we disagree with the Registrants' comment that "*…authors of the RCD have organized this paragraph in a way that biases the document by stating the facts in what we believe is a misleading order, such that the minor metabolic pathways operative only at high doses are described first, while the detoxification pathways operative at low doses that result in nontoxic metabolites are barely mentioned.*" Also, Figure 1 (page 14) of the RCD has information on the major and minor transformation pathways of OPP. Regarding the other comment on the low dose detoxification pathways, the Registrants stated: "*the sulfation pathway is operative both at low and high doses, but at high doses may be saturated, allowing other metabolic pathways to operate.*" Results of Smith *et al.* (1998) showed the occurrence of PHQ (oxidation product of OPP) and its sulfate and glucuronide conjugates (PHQ-S and PHQ-G) started at the lowest dose tested whereat sulfation pathway was not saturated. Hence, the Registrants' conclusion that other reaction pathways occurred only after the saturation of sulfate pathway is not supported by the data.

Comment #24 (Page 22 paragraph 2, last sentence): We have revised the text (page 12) to indicate that products from other reaction pathways (e.g., oxidation) in addition to OPP conjugates may have occurred at higher doses.

Comment #25 (Page 26 paragraph 2, sentence 2): We have corrected the typographical errors by replacing the Smith *et al.* (1988) with Smith *et al.* (1998) and 5% with 2% (page 15) as noted.

Comment #26 (Page 26 paragraph 2 end of sentence 2, sentence 3): We disagree with the Registrants' comment that "*the levels of OPP and free PHQ were significantly lower in rats exposed to the low dose of 56 mg/kg/day (Smith et al., 1998). This is consistent with a difference in OPP metabolic pathways favored at low and high doses and nonlinear kinetics of free PHQ formation at low doses.*" As detailed in response to comment #8, the percents PHQ calculated by Dr. Bartels were based on the measured values in the studies by Smith *et al.* (1998) using a pooled sample from 11 rats per dose (i.e., 1 sample per dose). Because the lack of standard derivation data presented by Smith *et al.* (1998), any further interpretation of these data are not possible. Hence, the Registrants' conclusion that "*…the levels of OPP and free PHQ were significantly lower in rats*" is not supported by the data presented.

Comment #27 (Page 32 first complete paragraph, penultimate sentence): We have revised the text (page 22) to indicate that products from other reaction pathways (e.g., oxidation) in addition to OPP conjugates may have occurred at higher doses.

Comment #28 (Page 35 first paragraph, sentence 1): The Registrants' comments stated: "*the purpose of an acute lethality study is not to identify a NOEL*" and *"..the lace of NOEL information is typical of these tests."* We believe the Registrants correctly point out that an acute toxicity study may contain information for NOEL selection despite the intent purpose of this type of study is for LD_{50} determination.

Comment #29 (Page 43 paragraph 1): We disagree with the Registrants' comment that "*…male rats at the high OPP dose level exhibited a mean body weight decrease relative to the controls of 26 to 35% throughout the 13 week study (Hiraga and Fuji, 1984). This extreme decrease in body weight is an indication that the maximum tolerated dose (MTD) was exceeded and the data should not be used for risk assessment."* We believe that adverse effects observed at dose levels should not be dismissed arbitrarily based only on the observation that the animals exhibited a significant body weight reduction (i.e., exceedance of maximum tolerated dose [MTD]). Current state of science considers that the body weight reduction alone can be used as an indication of adequate dosing for studying the carcinogenic effects of a chemical; however, consensus within the scientific community has not been reached regarding how the same criterion alone can be used for dismissing of chemical-induced adverse effects. Furthermore, the Registrants did not consider that the "extreme" body weight reduction appeared to be related to decrease in feed consumption that the investigators reported over the same period.

The Registrants stated:*" body weight data were not presented in the 13-week SOPP dietary study publication, but the doses again were very high (2,487 mg/kg), such that toxicity that could cause significant weight loss and exceedance of the MTD likely occurred (Hiraga and Fuji, 1981). Effects observed in these animals exposed to OPP or SOPP at these very high doses are not relevant for risk assessment and mode of action considerations, given the dramatic decreases in body weight that occurred during the exposures*." We believe that the Registrants' comment on the body weight reduction of SOPP-treated rats and the extrapolation of this speculation to disqualify the study results are not supported by the information presented.

Comment #30 (Page 43 paragraph 3, last part of the only sentence): The comment by the Registrant that "*the studies summarized in the subchronic toxicity section are not brought together in any way to describe a mode of action as this sentence would indicate*" is noted.

Comment #31 (Page 45 paragraph 4 last three sentences): As we noted in our response to comment #29, we disagree with the Registrants' comment that "*…Hiraga and Fuji describe these body weight reductions in the males that received OPP as 2.5% of the diet as a decrease in body weight of 26-35%, occurring throughout the study (1984). This is a significant decrease in body weight that should not be dismissed as inconsequential simply because it is accompanied by decreased food consumption. The effects observed at the high dose should not be considered for risk assessment."* We believe that adverse effects observed at dose levels should not be

dismissed arbitrarily based only on the observation that the animals exhibited a significant body weight reduction (i.e., exceedance of maximum tolerated dose [MTD]), although the effect on weight is noted.

Comments #32 (Page 50 paragraph 4, penultimate sentence): We disagree with the Registrant analysis that "*This does show reversibility of hyperplasia even though the urinary bladder epithelia had not completely returned to control appearance during the time course of 4 weeks.*" Since the urinary bladder epithelia had not completely returned to control appearance during the time course of 4 weeks, the data do not support the "reversibility" claim. Hence, unless additional data are available, the Registrant's claim about the (complete) recovery of tissue after the cessation of OPP exposure is speculative.

Comment #33 (Page 54 paragraph 1, last sentence): The comment by the Registrant that *"there was no adduct formation in the study referred to, as noted in Table 33 of the RCD and it could be noted briefly on page 54 that DNA binding in the study was negative"* is noted.

Comment #34 (Page 61 paragraph 3, last sentence): The RCD has a detailed description of the study by Fujii *et al.* (1978). Hence, we made no change in the text regarding the Registrant's request that "*the weight of evidence to support or refute this statement* [*i.e. "the difference in potency between OPP and SOPP as bladder carcinogens may be related to the difference in urinary pH*"] *should be presented in detail."*

Comment #35 (Page 66 paragraph 2): The RCD presented a detailed description of the studies (Fukushima *et al.*, 1983; 1985) pertinent to the statement "OPP and SOPP promoted the carcinogenesis in urinary bladder initiated by a known rat bladder carcinogen, N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), *in vivo*" (page that appeared in the section summary was presented in the text [pages 90-91]). We believe that a brief explanation of the statement above, as suggested by the Registrants, is not necessary.

Comment #36 (Page 74 first paragraph, third sentence): The Registrant stated: "*P/N hyperplasia is pre-neoplastic and is reversible (Oyasu, 1995).*" Since the assessment by Oyasu (1995) is not specific to OPP, unless additional data are available, our health protective assumption is that the pre-neoplastic lesion will progress into neoplastic lesions.

Comment #37 (Page 74 paragraph 3, second sentence): We have added the control incidences of the eye effects (page 64) as recommended.

Comment #38 (Page 82 first paragraph): We disagree with the Registrants' comment that *"This discussion does not follow the generally accepted approach to the analysis of the hepatocellular tumors. The combined incidence of hepatocellular adenomas and carcinomas (including hepatoblastomas) should be combined and compared with the incidence of combined tumors in*

the control group. When analyzed properly there is no increase in hepatocellular tumors at the low dose level." Statistical significance of the observed tumor increase is an indication of the tumorigenic effect of a chemical; however, we believe that the non-significant increase in the tumor incidence, especially for rare tumor, should also be considered for evaluating the carcinogenic effect of a chemical. Tumor multiplicity and rare tumor occurrence are two of the important parameters employed by the United States (e.g., National Toxicology Program [NTP, 2000]) and other international organizations for cancer risk identification (e.g., International Agency of Research on Cancer [IARC, 2006]). Using these nationally and internationally accepted criteria, we determined that OPP-treated mice exhibited hepatoblastoma, a rarely observed variant of hepatocellular carcinoma, in the males and an increase in the average number of liver tumors per mouse (i.e., tumor multiplicity) in both sexes at the lowest tested dose (i.e., 250 mg/kg/day).

Comment #39 (Page 95 first complete paragraph, third sentence from end of paragraph): We do not believe a generic statement that "*transitional cell hyperplasia is well understood and is clearly reversible*" can be applied specifically to OPP (see also response to comments #32 and #36). Regarding the comment that "*there is no biological basis to believe that hyperplasia can spontaneously reappear,"* the RCD has a detailed discussion on the biological basis for reappearance of hyperplasia after the cessation of exposure to a known genotoxic bladder carcinogen. Lastly, it is difficult to understand the rationale for applying "*normal turnover rate for stem cell*" to evaluate the regression of hyperplasia (i.e., "not normal" cells) in the urinary bladder of OPP-treated rats. Also, the labeling index of rat urothelium that the Registrant adopted from the study by Oyasu (1995) for calculating "the stem cell turnover rate" has no time period specified. Even if the time data are available, there is no single standard time for calculating the cell turnover rate.

Comments #40 and 41(Page 110, paragraphs 2 and 3): We disagree with the Registrant assessment that weight of evidence is simply an exercise of the relative number of negative *versus* positive reports (see also response to Comment #5). We have added a conclusion regarding the results of gene mutation studies in bacterial and mammalian cell systems *in vitro* (page 101) as requested.

Comment #42 (Page 110 paragraph 4): We agree with the Registrants' assessment that OPP has clastogenic potential. The Registrant also commented that technical flaws may have potentially occurred in these chromosomal aberrations studies (i.e., "…*chromosomal aberration studies are prone to spurious positive findings due to a variety of phenomena observed above threshold concentrations (Kirkland and Muller, 2000).*" However, Dr. Brusick, when evaluating the results of genotoxic effects in mammalian cells, stated that suspicion of technical flaw should not be the sole reason for dismissing the study results (i.e., "…*one cannot eliminate these studies solely on the suspicion of technical flaw*"). Hence, DPR maintained the results of OPP chromosomal aberrations studies in mammalian cells *in vitro* to be a valid finding.

Comment #43 (Page 111 paragraph 3): We have added the results of hyperdiploidy and micronucleus studies into our discussion of the chromosomal damage effects of OPP *in vivo* (page 102-103). Also, we have updated Table 33 (page 118) to include the study by Balakrishnan and Eastmond (2006).

Comment #44 (Page 11 paragraph 5): We agree with the Registrants' conclusion that OPP metabolites can interact with DNA. As detailed in the RCD (page 103), evidence also indicates that OPP itself may intercalate or form a complex with DNA.

Comment #45 (Page 112 paragraph 3): We have modified the text (page 104) to clarify our concern that the amount of DNA (instead of sample size) used for ^{32}P-postlabeling was very small.

We believe that the disregard of appropriate sensitive adduct enrichment method (namely both nuclease P_1 and butanol extraction) that based on *a priori* assumptions of no added advantage for detecting ^{32}P-postlabeled adducts and partial loss of certain adducts is unjustified; especially, the investigators did not show any data to indicate that the latter assumption is true for OPP. Also, this decision of not using an enrichment procedure makes the results difficult to compare with others and to evaluate for the conclusion reached by the Registrants.

Regarding the lack of appropriate control experiment, while Smith *et al.* (1998) included PBQ-deoxyguanosine-3-monophosphate adduct for ensuring that the ^{32}P-postlabeled nucleotides could be detected, the investigators did not include appropriate controls for evaluating the fate of adducts during extraction and enrichment procedures of DNA from the urinary bladder. To our knowledge, Smith *et al.* (1998) were the first to apply ^{32}P-postlabeling on isolated urothelium in the urinary bladder of OPP-treated rats. However, the investigators conducted no study to indicate the same ^{32}P-postlabeling procedure with no sensitive adduct enrichment method applied has the equal or better sensitivity, compared to the method with enhancement procedures, for detecting DNA adducts in the urinary bladder of rats treated with genotoxic carcinogens (preferably the one that is structurally similar to OPP).

Comment #46 (Page 112 paragraph 4): The comment by the Registrants regarding the number of positive *versus* negative studies for non-specific DNA damage is noted (see also response to comment # 41, 42, and 43).

Comment #47 (Page 112 paragraph 5): The RCD has text (page 104) to describe the meaning of the positive result of the study by Nagai *et al.* (1990) on DNA breakage induced by OPP in the presence of metabolic activation. Hence, we made no change to the text.

Comment #48 (Page 113 paragraph 1): The Registrants dismissed the positive results reported by Sasaki *et al.* (1997) on OPP-induced DNA breakage by stating: "*Sasaki and coworkers note that cell death leads to DNA fragmentation, and so positive responses in their assay should be considered in conjunction with cytotoxicity data. Unfortunately, in their assay Sasaki et al. are unable to determine whether observed DNA damage is due to effects directly on DNA or secondary to cytotoxicity. Given the high dose of OPP that the mice received and the inability of the researchers to assess cell viability during the time frame of the experiment, the reported effect of DNA fragmentation by OPP cannot be attributed to genotoxicity*." While the Registrant argument may be true, the genotoxic effect may also have occurred independent of cytotoxicity. Since OPP is an established carcinogen, the use of high dose for testing by Sasaki *et al.* (1997) is justifiable and may be relevant to its carcinogenic mode of action (MOA). Hence, we believe that the results of Sakai *et al.* (1997) have merit in contributing to our understanding of the genotoxic effects of OPP.

Comment #49 (Page 113 paragraph 2): The Registrants identified that Tayama-Nawai *et al.* (1984) conducted SCE induction in CHO-K1 cells using 2 expression times: 27 and 42 hrs. However, the Registrant's interpretation of the study results is inconsistent with the intent of the study, i.e., establishment of expression time in mammalian cell culture mutagenicity assay. The purpose of this type of expression time study is to demonstrate phenotypic lag and to indicate the time after treatment that must elapse before the stable mutation is expressed and can be scored. In fact, in all subsequent studies conducted by this research group, the expression time of CHO-K1 cells was 27 hr. Hence, we disagree with the Registrant's assessment that "*the draft RCD leaves out at least one report of a negative study by the same author*" and therefore, made no change to the text.

We disagree with the other comment by the Registrants regarding "… *the study by Tayama et al., published in 1989 that reported no increase in SCE in CHO-K1 cells in the absence of S9 and presence of 588 μM OPP (publication table 2*)." Table 2 described the SCE induction in the presence of S9 with or without OPP. With 10-50% S9, 588 μM OPP caused an increase ($p<0.05$) in SCE frequencies. The "negative result" that the Registrants referred to is the experiment with S9 but without OPP added.

Comment #50 (Page 113 paragraph 3): The comment by Registrants that "*the reactive oxidation product(s) of PHQ, produced enzymatically or nonenzymatically, may be the ultimate agent responsible for the cell damage*" is noted.

Comment #51 (Page 114 paragraph 5): We have corrected the typographical errors by replacing non-specific DNA damage by DNA damage (page 106) as noted.

Comment #52 (Page 115 paragraph 1): The Registrants' comments that "*This sentence from the Draft RCD indicates that the Reitz study was positive for SOPP binding to DNA in the rat*

urinary bladder, but the Reitz study was negative for DNA binding of SOPP in the urinary bladder' and *"We suggest that it is more relevant to the mode of action of SOPP that the DNA adducts identified in the mouse skin following dermal SOPP exposure were identical to those obtained following application of PHQ to mouse skin, and that formation of the DNA adducts induced by SOPP was inhibited by inhibition of cytochromes P450 in the mice. This suggests that metabolism of SOPP occurred in the mouse skin resulting in the formation of oxidative metabolites of SOPP that interacted with DNA*" are noted. We have added text to indicate that reactive metabolites (e.g., PBQ) of SOPP are capable of binding to DNA *in vivo* as suggested (page 107).

Comment #53 (Page 115 paragraph 2): We disagree with the Registrants' argument for dismissing the DNA breakage results of Sasaki *et al.* (2002) and Sekihashi *et al.* (2002) in OPP-treated rats and mice based on the logic that DNA damage in carcinogenesis is a simple matter of DNA damage = mutation = carcinogenesis. We have modified the text (page 124) to indicate increased DNA breakage occurred at 10000 and 20000 ppm.

Comment #54 (Page 115 paragraph 3): We agree with the Registrants' comment that the utility of the study by Honma *et al.* (1983) to characterize potential genotoxicity of SOPP is very limited. However, studies that contribute to the understanding of the genotoxic potential of SOPP cannot be ignored.

Comment #55 (Page 141 paragraph 5): The RCD has detailed the rationale for establishing a systemic parental NOEL of 40 mg/kg/day (page 135). The same NOEL also was chosen by the study author (Eigenberg, 1989b).

Comment #56 (Page 154 paragraph 2): See responses to comments #11-14 for the study by Zablotny *et al*. (1991a,b).

Comment #57 (Page 160 first full paragraph, last sentence): We disagree with the Registrants' interpretation of our intent is to "… *to combine control groups with different vehicles to achieve statistical significance*." Also, even without combining the control data, the resulting p-value indicates that the chance of committing false positive is only 6% (i.e., almost the same as the p=0.05) (page 154).

Comment #58 (Page 161, paragraph 1, last sentence): We have added the citation of Cancer Risk Assessment Guideline of USEPA (2005) into the text (page 155) as suggested.

Comment #59 (Page 161 paragraph 2, sentences 3 & 6): The Registrant comment that "*we agree that use of a NOEL for urinary tract effects in the risk assessment and characterization will protect against other effects that were observed at higher doses, including those proliferative effects in the urinary bladder that occur only at high doses"* is noted.

Comment #60 (Page 161 paragraph 3, second sentence): We disagree with the following Registrants' comments. That is, "*"In Wistar rats, the effects were decreased fetal body weight and increased incidence of resorptions (Kaneda et al., 1978)" This statement is not supported by the summary of the study presented on pages147-149 of the Draft RCD, which states that: "…it would appear that the fetus (and not the litter) was the experimental unit for the statistical analysis of resorptions"* and*: "DPR considered this study unacceptable because of inadequate reporting, including the lack of individual data." Further the possibility of increased resorptions was included in the study conclusion section only in parentheses. We recommend that mention of this "effect" of increased incidence of resorptions in rats be removed from the developmental effects hazard identification section.* For the purpose of risk assessment, the study unacceptable status that is based mainly on the adherence of the FIFRA guideline will not necessarily affect its usefulness in identifying an adverse effect. Regarding the statistical analysis of resorption, litter is preferable to fetuses as the experimental unit; however, in the absence of the litter incidence data, we maintained that the increased incidence of resorptions in rats reported by Kandea *et al.* (1978) is a valid finding.

Comment #61 (Page 161 paragraph 3, sentence 4): We disagree with the Registrants' comment that "*the lack of a dose response calls into question the biological significance of this* [cleft palate] *effect."* The RCD has a detailed description of the events that may associate with the lack of increased cleft palate incidence with dose: increased early post-implantation loss in the mid- and high dose groups and increased mortality at the high dose (page 152).

Comment #62 (Page 163 paragraph 1): We disagree with the Registrants' comment that *"throughout the Draft RCD the authors focus on the OPP-induced polydipsia and polyuria… draft RCD notes that rats exposed to high doses of OPP in the diet drink more water than the controls, urinate a larger volume, and the urine is more dilute… We suggest that since this endpoint is not relevant for risk assessment, the focus on this endpoint be reduced in this document*." The urinalysis parameters that are described in Table 6, 10, and 18 provide a clear indication of the effects of polydipsia and polyuria. Among these parameters, we have chosen the most sensitive ones (i.e., decreased protein concentration and specific gravity) for risk assessment, among other endpoints considered.

Comment #63 (Page 163, paragraph 3): Please see response to comments #12-14 and #61 on the studies by Zablotny *et. al*, (1991a,b) and Ogata *et al.* (1978).

Comment #64 (Page 164 full paragraph 2, sentence 2): We disagree with the Registrants' comment that "*this characteristic dose-response curve was observed repeatedly in study after study, and provides significant evidence to support the position that OPP and SOPP act by a nonlinear mechanism to cause tumors in the rat urinary bladder only at high doses."* For chemical-induced tumorigenesis in the urinary bladder, the shape of a dose-response should not

be considered as proof of a carcinogenic mechanism. This is substantiated by the nonlinear urinary bladder tumor dose-response exhibited by carcinogens that act *via* a non-genotoxic MOA (e.g., saccharin) or a genotoxic MOA (e.g., *o*-anisidine and 2-acetylaminofulorene) in rodents including rats (Gold and Zeiger, 1997).

Comment #65 (Page 164, full paragraph 2, sentence 3): We disagree with the Registrant that *"decreased time-to-tumor was only seen at excessive dose levels."* For example, Hiraga and Fujii (1981) showed that the time the carcinoma first appeared in SOPP treated male F344 rats was week 84 at 500 mg/kg/day and week 55 at 1000 mg/kg/day. Also, results of Christenson *et al.* (1996a) and Whale and Christenson (1996) reported markedly increased incidences of cell proliferation and tumors and shortened latency of tumor development in male rats from 4000 to 8000 ppm OPP in the diet.

Comment #66 (Page 167 paragraph 3, sentence 1): We have revised IV.A.4. Mode of Action for Urinary Bladder Tumors (page 158) including the sentences that described the biotransformation pathway of OPP.

Comment #67 (Page 167 paragraph 3 sentences 3 and 4, and entire paragraph 4): We disagree with the Registrants' comment that "*…the levels of free PHQ+PBQ do not correlate well with increasing dose between various studies. As shown in Figure 2 of this Response, the amount of nonconjugated metabolites had a negative correlation with OPP dose level in the 13- week subchronic study of Smith 1998… , Smith et al. (1998) report decreasing percentages of the dose excreted as free PHQ/PBQ (0.48% at 56 mg/kg, down to 0.14% at 924 mg/kg OPP). At this same high dose of 924 mg/kg OPP, Hasegawa et al. (1991) report four-fold higher levels of free PHQ+PBQ than those of Smith et al (5.7 mg/kg/day urine, or 0.64% of admin. dose). This variation in measured levels of nonconjugated metabolites indicates possible systematic errors in some of these data."* We have presented our responses to these issues previously (see response to comments #8 and 9).

Comment #68 (Page 168 second paragraph, last sentence): We have revised the text (page 164) to clarify the results presented by Niho *et al.* (2002).

Comment #69 (Page 168 third full paragraph, sentences 2 and 3): We disagree with the Registrants' comments that "*there is no evidence of genetic damage occurring in the urinary bladders of rats exposed to OPP or SOPP at low doses."* We believe that currently available information is insufficient to clearly identify the mutagenic events at lower doses *in vivo*. However, a genotoxic MOA is consistent with the concerns that genotoxic PHQ increased with dose and that its enzymatic and non-enzymatic transformations resulted in multiple reactive species (e.g., PBQ and ROS) that can interact with cellular macromolecules including DNA.

The conclusion of Registrant comment that "*if genetic damage occurred at low doses it is likely that tumors would be observed at these low doses, particularly given the large number of bioassays that have been conducted"* is flawed. As described previously, both genotoxic and non-genotoxic carcinogens in the urinary bladder exhibited nonlinear tumor dose response (see response to comment #64).

The Registrants' comment that "*there is no indication that genetic damage is a precursor of the proliferative effects observed in the urothelium. In fact these hyperplastic precursor lesions exhibit reversibility, which would not be the case if genetic damage were the underlying cause of the lesions*." The RCD has a detailed description that the reversibility claimed by the Registrants is not supported by its own data (see response to comment #32) and the results of Niho *et al.* (2002) (page 164). Regarding the Registrants' comments that " *the 2006 U.S. EPA Toxicology Chapter for the RED states…not sufficient oxidative metabolites generated in vivo to result in genotoxic MOA, but that a non-genotoxic MOA is operative*," we have raised concerns that USEPA does not appear to follow its latest Guidelines for Carcinogen Risk Assessment (see response to comment #1).

Comment #70 (Page 170 paragraph 1, sentence 1): We disagree with the Registrants' comments that "*Kidney tumors were observed in rats exposed to a very high dose of 2,000 mg/kg/day SOPP for 91 weeks, a dose that very likely exceeded the MTD (Hiraga and Fuji, 1981).*" As detailed in the RCD (page 77), the study by Hiraga and Fujii (1981) showed that low incidences of the kidney tumor also occurred in the renal pelvis at 250-2000 mg/kg/day and the papilla at 250-1000 mg/kg/day. We considered that these tumors were treatment-related findings because transitional cell carcinomas of the kidneys are rare spontaneous neoplasms in male F344 rats (0.2% incidence in the historical controls of NTP bioassay [Haseman *et al*., 1990]).

Also, the Registrants' statement:" *the finding of hepatocellular tumors in the mouse (Quast and McGuirk, 1995) should be assigned little weight in the assessment of the carcinogenic potential of OPP"* is not supported by the information available. USEPA determined that mode-of-action information on the liver tumors of mice treated with OPP or SOPP is lacking. The latest Guideline for Carcinogens Risk Assessment (USEPA, 2005) stated: " *In the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data: animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity*."

We disagree with the Registrants' comment that "*… there is no reason that circulatory tumors should not have been identified in the mid-dose group if, in fact, this was a compound related effect."* The true circulatory tumor (e.g., hemangiosarcoma) incidence cannot be determined because not all mice on test in the mid-dose (also the low-dose) group had all of the pertinent

organs examined microscopically. For example, the number of spleens examined microscopically in the mid-dose male group was only 13.

Based on the reasons presented above, the current data do not support the Registrants' conclusion that *"The data from a number of oncogenicity studies indicate that test article-related tumors appropriate for cancer risk assessment are urinary bladder tumors in male rats. Thus relevant tumors occurred in a single species and were sex-specific."*

Comment #71 (Page 170 paragraph 2): We have updated VI.A.6 Carcinogenic Dose response Assessment (page 167) as suggested.

Comment #72 (Page 170 paragraph 3): We disagree with the Registrants' comment that *"From this paragraph, it appears that the determination to evaluate OPP and SOPP as a genotoxic carcinogen is based primarily on a single piece of evidence, that trace amounts of PHQ and or PBQ were detected in the urine of rats exposed to 56 mg/kg/day of OPP for 13 weeks."* As detailed in the RCD, multiple studies showed the occurrence of unconjugated PHQ and PBQ in the urine of OPP- or SOPP-treated rats and the concentration of urinary PHQ was similar to that reported to induce DNA breakage, nucleotide oxidation, and (or) chromosomal aberrations in vitro. Also, our weight of evidence assessment of the data indicated that OPP belongs to the category of Likely to be Carcinogenic in Humans (page 164).

APPENDIX E. COMMENTS AND RESPONSES TO COMMENTS FROM PESTICIDE AND ENVIRONMENTAL TOXICOLOGY BRANCH OF THE OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

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MEMORANDUM

TO: Gary T. Patterson, Ph.D., Chief
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DATE: August 28, 2006

SUBJECT: COMMENTS AND RECOMMENDATIONS REGARDING THE DRAFT DIETARY RISK CHARACTERIZATION DOCUMENT FOR THE ACTIVE INGREDIENTS *ORTHO*-PHENYLPHENOL AND SODIUM *ORTHO*-PHENYLPHENATE

Thank you for the opportunity to review the Risk Characterization Document (RCD) for dietary exposures to ortho-phenylphenol and sodium ortho-phenylphenate, dated June 6, 2006. These fungicides are used in the post-harvest treatment of a variety of fruits and vegetables. This document quantifies the exposures to these chemicals through the food supply, and compares the exposures to toxicologic screening levels determined in animal studies.

The Office of Environmental Health Hazard Assessment (OEHHA) reviews risk assessments prepared by the Department of Pesticide Regulation (DPR) under the general authority of the Health and Safety Code, section 59004, and also under the Food and Agricultural Code, section 13129, which gives OEHHA the authority to provide advice, consultation, and recommendations to DPR concerning the risks to human health associated with exposure to pesticide active ingredients.

The document is comprehensive, well reasoned and clear. We appreciate and concur with the conclusions and the detailed description of the data indicating that ortho-phenylphenol is a genotoxic carcinogen, warranting calculation of its slope factor in humans via a linear low-dose extrapolation model (slope factor = 2.2×10^{-3} [mg/kg/day OPP]$^{-1}$). Our comments are presented

below, divided into a few general comments followed by a longer list of additional comments. Please feel free to contact us if you have any questions.

General Comments

1) In the chronic feeding study by Wahle and Christenson (1996), female rats exhibited cardiomyopathy at the low (49 mg/kg/day) and mid (248 mg/kg/day) dose levels (both $p<0.05$). It is clear from effects in hearts, kidneys and bladders that male and female rats react differently to OPP. Female mice also reacted differently than male mice, exhibiting increased absolute heart weights in a chronic feeding study (Ito, 1983). Thus, it is unjustified to assume that since male rats exhibited no cardiomyopathy at 39 mg/kg/day (Wahle and Christenson, 1996) the same would be true for female rats. However, selection of the male NOEL of 39 mg/kg/day as the study NOEL makes this assumption. We recommend using the NOEL of 39 mg/kg/day for quantifying the risk to the male human population, and a NOEL of 5.0 mg/kg/day (49 mg/kg/day divided by an uncertainty factor of 10 for LOEL to NOEL extrapolation of a serious toxic effect) for quantifying the risk to the female human population. It should be noted that the DPR toxicology summary of Wahle and Christenson (1996) concluded that 39 mg/kg/day in male rats was a LOEL (Appendix A). We recommend that this discrepancy be addressed.

2) In Tables 51 and 53, and at various places in the text, the 95th, 97.5th and 99th percentile groups for dietary exposure are presented. If we understand the data correctly, the groups are based on three different levels of food consumption, with the same OPP residue level used for all three. We recommend making this clear. Also with regard to these levels of food consumption, it is not clear why these high ends of the food consumption spectrum were taken into account only in the acute exposure assessment. A range of low to high food eaters would also be expected over the long term. We recommend discussing why these high percentile food consumers were not used to calculate dietary exposures for the chronic exposure assessment and also for calculating the lifetime cancer risk.

3) There are a number of tables (17, 19, 24, 31) where incidences of different tumors or different nonneoplastic lesions were combined. It appears that the different tumors or different nonneoplastic lesions never occurred in the same animal. For example, in Table 17 at the mid dose level, the papilloma incidence was 3/24, the carcinoma incidence was 20/24, and the combined tumor incidence was 23/24. This was probably an intentional assumption made by either the study authors or DPR in calculating the combined incidences. If so, this should be stated in the legend of each table.

4) We recommend something be said about the conversion of SOPP to OPP. Does this risk assessment consider that all SOPP becomes converted to OPP following ingestion, and what about following application to the various fruits and vegetables?

Additional Comments

Page 23, Absorption. We recommend stating how the radioactive OPP and SOPP were administered.

Page 23, "the oral absorptions of OPP and SOPP from the gastrointestinal tract." This phrase does not make sense. We recommend correcting.

Page 23 and various places in the Metabolism section. When discussing in vivo studies, recommend including by what route and method the animals were dosed (in food, water, gavage, injection, etc.).

Page 27 and various places in the Absorption section. The phrase "oral absorption" is unclear. We recommend explaining what is meant.

Table 6. We recommend indicating in the table that these measurements were made at the completion of the 13 week period.

Table 7. We recommend stating in the footnote what P/N means.

Table 8. The weeks in footnote [a] do not match the weeks in the table. We recommend correcting.

Page 50, "Although bromodeoxyuridine (BrdU)-labeling index, an indicator of cell proliferation, appeared to decrease after 4 weeks, the index increased ($p<0.05$) with dose at both weeks 4 and 13 (Table 8)." The index increased with dose at week 4, but not at week 13, where only the high dose exceeded the control. We recommend correcting.

Tables 9 and 11. It is not clear why some values for numbers of animals (all 10) have asterisks. We recommend explaining.

Page 50, "At the end of the 4-week recovery period, 684 mg/kg/day group still exhibited some urinary-tract effects: one animal exhibited renal tubular proliferation, two animals had renal tubular dilation, and one animal had mild hyperplasia in the bladder (Table 7)." Table 7 does not show renal tubular proliferation or renal tubular dilation. We recommend correcting the text. It would be helpful to the reader if "(data not shown)" were used where appropriate.

Table 10. In footnote [b], we recommend changing protein to creatinine.

Page 54, "(Table 10); correspondingly, there were reductions in urinary specific gravity and creatinine concentrations." Table 10 shows osmolality, which is not the same as specific gravity. We recommend correcting the text.

Table 12. We recommend adding when these animals were sacrificed.

Table 13. We recommend correcting 10,000 ppm in the table.

Page 57, "The observation that the high-dose males exhibited no bladder tumors but nephritis ..." Table 12 shows that 1/10 high dose males exhibited a urinary bladder transitional carcinoma. We recommend correcting the text.

Page 61, "as the nephritis progressed, it disturbed the acid-base balance of the urine, which in turn affected the transformation and proliferation of the bladder epithelium." We recommend being more specific and stating that as the nephritis progressed, the urine became more acidic, thereby inhibiting both transformation and proliferation of the bladder epithelium.

Page 63, "TBZ appeared to synergize the increase in papillomas and carcinomas." Using the data in Table 15, it is not possible to determine whether there is a true synergistic interaction between SOPP and TBZ. We recommend dropping the word "synergize" and instead stating that TBZ appeared to increase papillomas and carcinomas.

Table 15. According to the data, no males had both papillomas and carcinomas. We recommend checking to be sure this is correct.

Table 16. We recommend adding when the measurements were made.

Table 17. The data indicate that no animal had both pyelonephritis and interstitial nephritis. The data also indicate that no animal had both papillomas and carcinomas. We recommend checking to be sure this is correct.

Table 18. A few of the standard errors are 0. We recommend checking to be sure they are correct.

Table 18. The controls exhibited six to ten-fold increases in the protein concentration of their urine during the course of the study. OPP had the opposite effect on urine protein concentration, presumably due to increased water intake. We recommend discussing the possible reason(s) for the increases in the controls.

Table 19, footnote [6]. We recommend adding that the 0.49% value refers to "historical" controls.

Table 19. The data indicate that in the high dose group, no animal had both carcinomas and papillomas. We recommend checking to be sure this is correct.

Page 74, third paragraph. For the eye alterations, we recommend stating when the examinations were performed and adding the incidences in the controls.

Page 87, "transitional cell carcinomas of the kidneys are rare spontaneous neoplasms in male F344 rats." We recommend providing a historical control value if available.

Tables 24 and 25. We recommend indicating when the animals were sacrificed, perhaps in a footnote.

Table 24, 56 week recovery study. Two animals had papillomas and 21 had carcinomas. Twenty-three animals had "combined tumors." This implies that of the animals with carcinomas (21/25), none had any papillomas. We recommend checking to be sure this is correct.

Page 93, "but the reverse is true for the kidney effects (neoplastic lesions only)." We recommend changing neoplastic to nonneoplastic.

Page 95, last paragraph, "For the animals whose exposure was extended to 52 weeks (therefore, a recovery period of 68 weeks)." However, Table 27 shows that the 52 week exposure period was followed by a 62 week recovery period. We recommend correcting.

Page 97, top paragraph. The data are consistent with the conclusions of the first sentence, but they do not prove them. No data were presented concerning DNA changes. We recommend modifying the paragraph accordingly.

Page 97, second paragraph. We recommend adding in what tissue alkaline phosphatase was measured.

Page 97, "However, DPR considered that the occurrence of hepatic hemangiomas in each of the SOPP-exposed male groups was treatment-related because of their rare spontaneous occurrence in this strain of mice. For example, Haseman et al. (1999) reported that in the NTP database of feeding studies, only 3 hemangiomas occurred among 1350 livers from untreated male B6C3F1 mice (0.2% incidence)." We recommend dropping the reference to the low historical control value since the concurrent control gave an incidence of 2%. Thus, it may be necessary to reconsider the argument that the hemangiomas in livers of dosed males were treatment-related.

Page 99, third paragraph. Should read SOPP rather than OPP.

Page 103, first paragraph. As mentioned above, recommend not using the word "synergistic" since the data in Table 31 do not allow this conclusion.

Bottom of page 141. No reproductive NOEL is given, while parental and pup NOELs are listed. We recommend including the reproductive NOEL or the specific reason(s) why not.

Page 143, last paragraph. We recommend checking whether the definition of fertility index is correct.

Page 144, second paragraph, "In each of these periods, the increased incidence of lactation day-21 weanlings with stunted growth occurred at 500 mg/kg/day (Table 42)." We recommend qualifying this statement by adding that these were the only statistically significant ($p<0.05$) increases in pup stunting (smaller increases occurred at 100 mg/kg/day).

Table 43. We recommend adding that the units are grams. Also recommend adding the litter incidences of resorptions and whether they were significantly elevated compared to control.

Table 46 and text. We recommend checking whether the dates in the study citations are correct.

Page 158, "Second, there is no reason to expect that if OPP and/or SOPP were developmental toxicants, they would induce a type of malformation that does not occur "spontaneously" in fetuses from control animals." We fully agree. By their argument, most if not all chemically-induced malformations would be disregarded since most if not all malformations induced by chemicals also occur spontaneously in untreated animals.

Page 160, "Reduced body-weight gain could be the basis for the maternal LOEL." We recommend stating the maternal LOEL.

Page 160, "In conclusion, this study documented that SOPP affected the fetuses and that the increased toxicity of fetuses occurred at the same or lower doses as those causing parental toxicity." We recommend showing the maternal body-weight gain data (if need be, read the data from the graph) and discussing whether the decreased maternal body-weight gain is of sufficient magnitude to cause malformations in mice, and cleft palate in particular.

Page 163. Specific gravity is not the same as osmolality. We recommend correcting.

Page 163, section IV.A.3. We recommend adding the citations when specific studies are discussed.

Pages 162-3. Polydipsia and dilution of urinary constituents are discussed as possible toxicological endpoints. These occurred at some quite low dose levels in the subchronic rat studies (54 mg/kg/day in Christenson et al., 1996a). We recommend checking to determine whether any of these changes occurred in the first week of the subchronic study. If so, they could be used to set the critical acute NOEL by the same reasoning used to select the maternal NOEL from the developmental rat study (Kaneda et al., 1978) as the NOEL for assessing acute risks to the general population.

Page 168, third paragraph. We recommend correcting the citation to read 1996b instead of 1996a.

Page 174, PDP section. For the fruit rinds, such as for oranges and lemons, we recommend that DPR clarify whether the residue samples came from only the edible part of the fruit or whether rind samples were included.

Page 174, PDP section, "For OPP- and SOPP-treated commodities, PDP reported only residue of OPP." Is it assumed that all SOPP was converted to OPP prior to sampling and analysis? We recommend discussing this and the data, if any, that show this to be true.

Page 174, PDP section. If the residue data have geographical locations associated with them, recommend reviewing them to determine whether California samples are significantly different from the average values. If the California values are significantly higher, recommend using the California-specific residue data together with the Western Region food consumption data, to calculate California-specific exposures.

Table 50. We recommend checking the "detected residues" range for nectarines to be sure the values are correct.

Page 180, "Based on the classification of related raw agricultural commodities into crop groups (as established in 40 CFR 180.40) and the agricultural practice specified in the product labels, suitable surrogate for tangerine, lime, lemon, kumquat, and citrus citron was orange (all belong to Citrus Fruit Group 10) and surrogate for plum was peach (both belong to Stone Fruit Group 12)." We recommend explaining how the surrogate data were used to do this. It is not obvious.

Page 180, discussion of adjustment factors. In Table 50, the acute point estimate residue values do not appear to be influenced at all by the adjustment factors. We recommend explaining this. Also recommend discussing how these factors affected the chronic residue values.

Page 178, discussion of Table 51. We recommend stating that the different percentile categories shown in Tables 51 and 53 are based on different RAC consumption estimates, not different residue concentrations.

Page 181, last paragraph. We recommend stating what assumption was made regarding the amount of each RAC consumed each day. In other words, for the exposures in Table 52, were the average daily consumptions of each RAC used?

Bottom of page 183 to top of page 185, discussion of Table 53, "Since the lowest MOEs were $\geq$ 10-fold greater than the acceptable MOE (i.e., 100)." This is not true, since 893 is less than 1000.

Page 185, third paragraph. Does this paragraph mean that KOPP is not used for post-harvest application to foods, has no established tolerances, or some other possibility? We recommend explaining.

Page 191, fifth paragraph, "Nevertheless, this analysis produced MOEs ranged from 10^3-10^4 at the 95th, 97.5th and 99th percentiles." The MOE was 893 for females 13-49 years (Table 53). We recommend correcting.

Page 192, last paragraph. Given that this RCD identified a developmental NOEL in a rabbit study that was lower than the maternal NOEL, we recommend presenting justification in this section for not including an additional FQPA safety factor in the calculations of risks to fetuses and children.

Table 56. We recommend adding the units for the Tolerance column.

Page 196, "Table 56 summarizes the ranges of the exposure and MOE values at the 95th percentile for each of the evaluated commodity at its tolerance level in the background of the chronic dietary exposure." We recommend stating in the text what the 95th percentile represents, and also adding this information to the table.

Page 198. Should read 10^{-6} rather than 10^6.

Again, thank you for the opportunity to review this document and we hope that you find our comments useful. Should you have any questions regarding OEHHA's review of this RCD, please contact Dr. Charles Vidair at (510) 622-2070 (primary reviewer), Mr. Robert Schlag at (916) 323-2624, or me at (510) 622-3165.

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MEMORANDUM

TO: Gary T. Patterson, Ph.D.
Supervising Toxicologist
Medical Toxicology Branch

VIA: Joyce Gee, Ph.D.
Senior Toxicologist
Medical Toxicology Branch

FROM: Eric S.C. Kwok, Ph.D., D.A.B.T.

DATE: April 9, 2007

SUBJECT: RESPONSE TO COMMENTS FROM PESTICIDE AND ENVIRONMENTAL TOXICOLOGY BRANCH OF THE OFFICE OF ENVIRONMENTAL HEALTH AND HAZARD ASSESSMENT ON DRAFT OPP AND SOPP RISK CHARACTERIZATION DOCUMENT – DIETARY EXPOSURE

This memorandum addresses comments from the Pesticide and Environmental Toxicology Branch (PETB) of Office of Environmental Health Hazard Assessment (OEHHA) on the Department's draft Risk Characterization Document (RCD) for the active ingredients ortho-phenylphenol (OPP) and sodium ortho-phenylphenate (SOPP) (dated August 28, 2006). We would like to thank the reviewers for their constructive comments. In the comment, OEHHA concurred with DPR's position about the potential genotoxicity of OPP and SOPP, the oncogenicity dose-response assessment using default linear approach, and the concerns regarding the developmental effects of OPP and SOPP. Also, OEHHA supported the selected critical endpoints (urinary tract effects) and No-Observed-Effect Levels (NOEL) for evaluating the chronic toxicity of OPP in the male human population but recommended a separate NOEL for the females to account for the higher sensitivity of females than males to different endpoints noted in the critical study of OPP. We have made several changes to the draft RCD to clarify and address the points raised by OEHHA. Our responses to these comments are as follows (the page number noted in the response refer to the final draft RCD):

General Comments

Comment #1: In the chronic feeding study by Wahle and Christenson (1 996), female rats exhibited cardiomyopathy at the low (49 mgkglday) and mid (248 mgkglday) dose levels (both $p<0.05$). It is clear from effects in hearts, kidneys and bladders that male and female rats react differently to OPP. Female mice also reacted differently than male mice, exhibiting increased absolute heart weights in a chronic feeding study (Ito, 1983). Thus, it is unjustified to assume that since male rats exhibited no cardiomyopathy at 39 mgkg/day (Wahle and Christenson, 1996) the same would be true for female rats. However, selection of the male NOEL of 39 mg/kg/day as the study NOEL makes this assumption. We recommend using the NOEL of 39 mgkg/day for quantifying the risk to the male human population, and a NOEL of 5.0 mgkglday (49 mgkg/day

divided by an uncertainty factor of 10 for LOEL to NOEL extrapolation of a serious toxic effect) for quantifying the risk to the female human population. It should be noted that the DPR toxicology summary of Wahle and Christenson (1996) concluded that 39 mg/kg/day in male rats was a LOEL (Appendix A). We recommend that this discrepancy be addressed.

Response: We have modified the IV.A.2. SELECTION OF TOXICITY ENDPOINTS (page 155) and SELECTION OF CRITICAL NOELs (page 157) as recommended. Also, we have modified the related sections in IV.C. RISK CHARACTERIZATION (page 180) to reflect the changes.

Comments #2: In Tables 5 1 and 53, and at various places in the text, the 95th, 97.5th and 99th percentile groups for dietary exposure are presented. If we understand the data correctly, the groups are based on three different levels of food consumption, with the same OPP residue level used for all three. We recommend making this clear. Also with regard to these levels of food consumption, it is not clear why these high ends of the food consumption spectrum were taken into account only in the acute exposure assessment. A range of low to high food eaters would also be expected over the long term. We recommend discussing why these high percentile food consumers were not used to calculate dietary exposures for the chronic exposure assessment and also for calculating the lifetime cancer risk.

Response: We have revised the text in IV.B.4. ACUTE EXPOSURE (page 174) to indicate the point estimation exposure calculation is based on the distribution of consumption rates and a fixed residue level for each commodity as requested by the reviewers.

We agree with the reviewer's comment on that "a range of low to high food eaters would also be expected over the long term." However, in contrast to the reviewer's suggestion, the goal of chronic exposure assessment is to estimate how much of a given pesticide residue might be consumed on a daily basis over the course of a lifetime. Hence, DPR use average residue values and average consumption values in assessing repeated exposure of OPP over time (DPR-MT3, 2006). We have modified the text in IV.B.5. CHRONIC EXPOSURE (page 178) to clarify this point.

Comment #3: There are a number of tables (17, 19, 24, 31) where incidences of different tumors or different nonneoplastic lesions were combined. It appears that the different tumors or different nonneoplastic lesions never occurred in the same animal. For example, in Table 17 at the mid dose level, the papilloma incidence was 3/24, the carcinoma incidence was 20/24, and the combined tumor incidence was 23/24. This was probably an intentional assumption made by either the study authors or DPR in calculating the combined incidences. If so, this should be stated in the legend of each table.

Response: We agree with the reviewer's observation that some tumor incidence data presented implied that "the different tumors or different nonneoplastic lesions never occurred in the same animal." However, all of these data are from the studies wherein the study authors provided no individual data or information on their criteria in quantifying the tumor data. We, therefore, modified the legend of relevant tables to indicate that DPR reported the data "as is" from the open literature.

Comment #4: We recommend something be said about the conversion of SOPP to OPP. Does this risk assessment consider that all SOPP becomes converted to OPP following ingestion, and what about following application to the various fruits and vegetables?

Response: To justify the assumption that OPP may likely be the chemical form that human exposed in SOPP-treated raw agricultural commodities (RAC), we have added the text (page 175) to the IV.B.4 ACUTE EXPOSURE to explain why SOPP deposited on the surface of treated RAC may likely be hydrolyzed into OPP (i.e., its free acid).

Additional Comments and DPR Responses

Comment #1 (Page 23, Absorption): We have added text (page 12) to indicate how the radioactive OPP and SOPP were administered as requested.

Comment #2 (Page 23, oral absorptions of OPP and SOPP from gastrointestinal tract): We have modified the phase (page 12) for clarity.

Comment #3 (Page 23 and various places in Metabolism section): We have modified various places in the III.A. Pharmacokinetics to include the dosing route and method (e.g., food or gavage) as recommended.

Comment #4 (Page 27 and various places in the Absorption section): We have replaced the phase "oral absorption' (page 17 and various places) by the absorption of OPP *via* oral route.

Comment #5 (Table 6): We have added the text to the legend of Table 6 (page 41) to indicate the urinalysis measurements were made at the completion of 13-week period.

Comment #6 (Table 7): We have replaced the abbreviation P/N by papillary or nodular in the legend of Table 7 (page 42) as noted by the reviewer.

Comment #7 (Table 8): We agree with the comment on "the weeks in footnote do not match the weeks in the table (Table 8, page 42)." However, the study report provided no information for this discrepancy. Hence, we made no change in the text.

Comment #8 (Page 50): We have corrected the sentence (page 40) to indicate that the bromodeoxyuridine labeling index increased significantly ($p<0.05$) at both weeks 4 and 13 only at the highest dose as recommended.

Comment #9 (Tables 9 and 11): We have expanded the legend of Table 9 for describing the meaning of asterisks.

Comment #10 (Page 50): We have modified the text (page 40) to indicate that Table 7 presented no data on renal tubular proliferation and renal tubular dilation as recommended.

Comment #11 (Table 10): We have corrected typographical error by replacing the word protein with creatinine in footnote "b" of Table 10 (page 45).

Comment #12 (Table 10): We have corrected the typographical error by replacing the word specific gravity with osmolality in the text (page 44).

Comment #13 (Table 12): We have added the text to the legend of Table 12 (page 48) to indicate all animals were sacrificed at the end of the study as recommended.

Comment #14 (Table 13): We have corrected typographical error by replacing the dietary OPP level of 1000 ppm with 10,000 ppm in Table 13 (page 49).

Comment #15 (Page 57): We have modified the sentence (page 47) to indicate that the high-dose males exhibited mainly nephritis as recommended.

Comment #16 (Page 61): We have modified the sentence (page 51) by stating that as the nephritis progressed, the urine became more acidic, thereby inhibiting both transformation and proliferation of the bladder epithelium as recommended.

Comment #17 (Page 63): We replaced the word "synergize" (page 54) with "increase" in describing the papillomas and carcinomas incidences as recommended.

Comment #18 (Table 15): We added text in the legend of Table 15 (page 55) to indicate that the study authors reported the papilloma and carcinoma incidences (see also response to General Comment #3).

Comment #19 (Table 16): We have added the text to the legend of Table 16 (page 59) to indicate the urinalysis measurements were made at week 90.

Comment #20 (Table 17): We have added text in the legend of Table 17 (page 60) to indicate that the study authors reported the lesion incidences (see also response to General Comment #3)

Comment #21 and #22 (Table 18): We agree with the reviewer's observations that a few of the standard errors in urinary protein measurements are zero and that the controls exhibited a six- to ten-fold increases in the protein concentration of their urine during the course of the study (Table 18, page 62). For the results of urinary protein analysis, in contrast to the reviewer's suggestion that these data may have been erroneous, the individual data indicate that the values are not continuous (i.e., protein values were either 0, 15, 30, 100, or 300 mg/dL). Therefore, we made no correction to the data in Table 18 but adding a table legend to indicate that the urinary protein concentrations are categorical data.

Regarding the urinary protein concentrations, the reviewer recommended discussing the possible reason(s) for the increases in the controls. Unfortunately, we found no literature information on the increase of urinary protein concentration with age in rats. Since the reviewer did not indicate that how this information would enhance the understanding of OPP toxicity, we made no change in the final draft RCD with regard to this point.

Comments #23 and #24 (Table 19): We have modified the legend in Table 19 (page 65) to indicate that 0.49% value refers to historical control incidence of urinary bladder tumor as requested by the reviewer. Also, we added text in the Table legend to indicate that the study authors reported the summarized papilloma and carcinoma incidences (see also response to General Comment #3).

Comment #25 (Page 74): We have modified the text to indicate that the eye examinations were conducted in the terminal sacrifice animals and added the control incidences of the eye effects (page 64) as recommended.

Comment #26 (Page 87): We have added the historical control incidence of transitional cell carcinomas in kidneys (page 77) as recommended.

Comments #27 and #28 (Tables 24 and 25): We believe that title of Tables 24 and 25 (pages 81 and 82) have sufficient information to allow the readers to determine when the animals were sacrificed. Also, all incidences of urinary bladder tumors including the combined incidences in the 56-week recovery study (Table 24) were reported by the investigators. Hence, we made no changes in text (see also response to General Comment #3).

Comment #29 (Page 93): We have corrected the typographical error by replacing the word neoplastic with nonneoplastic in the text (page 83).

Comment #30 (Page 95, last paragraph): We have corrected the typographical error by replacing "68 weeks" with "60 weeks" in Tables 26 and 27 (pages 85 and 86). Also, we have replaced "62 weeks" in the text (page 87) with "60 weeks" as noted by the reviewer.

Comment #31 (Page 97, top paragraph): We have modified the study conclusion in page 87 as recommended.

Comment #32 (Page 97, second paragraph): We clarify the tissue wherein the alkaline phosphatase activity was measured by replacing alkaline phosphatase with serum alkaline phosphatase (page 87) as suggested.

Comment #33 (Page 97): We agree with the reviewer's comment that the incidence of rare spontaneous tumor in the controls is zero. However, we believe that one occurrence of hemangiomas (2% incidence) in the 50 livers from untreated B6C3F1 male mice in the study by Ito (1983) is not in conflict with the rare spontaneous occurrence of this tumor type in this sex reported by the NTP. That is, NTP historical control data reported three occurrences of hemangiomas among 1350 livers of untreated male B6C3F1mice in multiple studies. If we assume that the three incidences occurred in three different NTP studies, the per-study incidence of hemangiomas in the control males would be the same as that reported by Ito (1983) (i.e., 2% incidence, assuming that 50 animals were used per study). Hence, we made no change to the text.

Comment #34 (Page 99): We have corrected the typographical error by replacing OPP with SOPP (page 89).

Comment #35 (Page 103, first paragraph): We replaced the word "synergize" (page 93) with "increase" in describing the papillomas and carcinomas incidences as recommended

Comment #36 (Bottom of page 141): We have added text (page 135) to explain why DPR derived no reproductive NOEL as recommended.

Comment #37 (Page 143, last paragraph): We have modified the definition of fertility index (page 137) as recommended.

Comment #38 (Page 144, second paragraph): We have modified the text (page 138) by stating that the increased ($p<0.05$) incidence of lactation day-21 weanlings with stunted growth occurred at 500 mg/kg/day and the smaller increases also occurred at 100 mg/kg/day as recommended.

Comment #39 (Table 43): We have added the units (gram) that were inadvertently missed into Table 43 (page 142). We agree with the reviewer's suggestion that litter incidences of resorptions should be added into Table 43. However, the report provided no such information and therefore, we made no change in Table 43.

Comment #40 (Table 46 and text): We have checked the study citations in Table 46 (page 151) and text for correctness as recommended.

Comment #41 (Page 158): We are delighted that the reviewer's concurred with our data interpretation in page 152. That is *"*"Second, there is no reason to expect that if OPP and/or SOPP were developmental toxicants, they would induce a type of malformation that does not occur "spontaneously" in fetuses from control animals. We fully agree. By their argument, most if not all chemically induced malformations would be disregarded since most if not all malformations induced by chemicals also occur spontaneously in untreated animals."*

Comments #42 and 43 (Page 160): The reviewer sated in the first comment that*" Reduced body-weight gain could be the basis for the maternal LOEL."* We do not believe that the data available are sufficient for establishing a maternal LOEL albeit the reviewer's recommendation. The reviewer stated in the second comment that "*we recommend showing the maternal body-weight gain data (if need be, read the data from the graph) and discussing whether the decreased maternal body-weight gain is of sufficient magnitude to cause malformations in mice, and cleft palate in particular*." We agree with the reviewer's interpretation that decreased maternal body-weight gain may be of sufficient magnitude to be associated with malformations in mice. However, as stated in the draft RCD, we do not believe that there are sufficient data in the report for the reduced maternal body weight to be distinguished unambiguously from that associated with 15-21% fetal body weight reduction which also occurred at the same dose. Hence, we made no change in the text.

Comment #44 (Page 163): We have corrected the typographical error by replacing the phase " specific gravity (osmolality)" with "specific gravity (or osmolality)" in the text (page 157).

Comment #45 (Page 163, section IV.A.3.): We have added the citation when discussing the mice study as requested (page 157).

Comment #46 (Page 162-163): We agree with the reviewer's comment on the use of polydipsia and dilution of urinary constituents as possible toxicological endpoints for characterizing the acute dietary risk to the general population. Unfortunately, the subchronic studies available have no information on whether these effects occurred within a few days after the treatment initiation.

Comment #47 (Page 168, third paragraph): We have corrected the typographical error by replacing the citation Christenson *et al.* (1996a) with Christenson *et al.* (1996b) (page 164).

Comment #48 (Page 174, PDP section): We have added text (page 170) to indicate that PDP prepares samples similar to the typical consumer practices to more closely represent actual exposure to residues (e.g., oranges are peeled), as requested by the reviewer.

Comment #49 (Page 174, PDP section): See response to general comment #4.

Comment #50 (Page 174, PDP section): We have added text (page 170) to highlight the major difference in residue levels between samples analyzed in California *versus* those analyzed by all 10 participating states in the PDP as requested by the reviewer. These differences, however,

have no impact on the results of acute dietary exposure as the analysis employed the highest residue reported including those samples collected in California. Also, we have added text in IV.B.5. CHRONIC EXPOSURE (page 178) to indicate that the average exposures estimated from residues data reported by all the 10 participating states under chronic condition are essentially identical to those from samples analyzed in California.

Comment #51 (Table 50): We have corrected the typographical error of nectarine residue values (page 172) in Table 50.

Comment #52 (Page 180): We have added text (page 175) to explain how DPR use the surrogate data in estimating residual levels of RAC with no monitoring data available.

Comment #53 (Page 180): We have added text (page 177) to explain how the Acute and Chronic Modules of Dietary Exposure Evaluation Model (DEEM) use the residue values of RAC and adjustment factors to calculate the values of dehydrated foods.

Comment #54 (Page 178, discussion of Table 51): We have added text (page 175) to explain the exposure estimates were based on different RAC consumption estimates as recommended.

Comment #55 (Page 181, last paragraph): We have modified the text in IV.B.5. CHRONIC EXPOSURE (page 178) by stating the assumption made regarding the amount of each RAC consumed each day as recommended (see also response to general comment #2).

Comment #56 (Bottom of page 183 to top of page 185, discussion of Table 53): We have replaced the symbol ≥ by ~ in the text (page 180) to indicate that the lowest MOEs were approximately 10-fold greater than the acceptable MOE (i.e., 100).

Comment #57 (Page 185, third paragraph): We have added text (page 182) to indicate that KOPP has no post-harvest application based on labels of registered products in California.

Comment #58 (Page 191, fifth paragraph): We have corrected the typographical error by replacing 10^3 by 10^2 (page 189) as noted by the reviewer.

Comment #59 (Page 192, last paragraph): The USEPA has determined an FQPA factor of 1x. We have added text (page 190) to indicate that even if DPR take an additional FQPA safety factor into consideration, the risk calculation shows no significant health concerns for the Female (13-49 yrs) population subgroup.

Comment #60 (Table 56): We have added the unit for the Tolerance column in Table 56 (page 194) as recommended.

Comment #61 (Page 196): We have modified the text under VI.B. ACUTE EXPOSURE (page 191) to indicated that the exposure estimate was the sum of the estimated 95th percentile exposure in each of the population subgroups for the commodity of concern at the tolerance exposure. Also, we have added the information to the legend of Table 56 (page 194) as recommended.

Comment #62 (Page 198): We have corrected the typographical error by replacing 10^{6} by 10^{-6} (page 195) as noted by the reviewer.

APPENDIX F. COMMENTS AND RESPONSES TO COMMENTS FROM REPRODUCTIVE AND CANCER HAZARD ASSESSMENT BRANCH OF THE OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Office of Environmental Health Hazard Assessment

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Linda S. Adams
Secretary for Environmental Protection

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MEMORANDUM

TO: Gary T. Patterson
Department of Pesticide Regulation (DPR)
Medical Toxicology
P.O. Box 4015
Sacramento, California 95812-4015

FROM: Lauren Zeise, Chief
Reproductive and Cancer Hazard Assessment Branch
1515 Clay Street, 16th Floor
Oakland, California 94612

DATE: January 18, 2007

SUBJECT: COMMENT ON THE DPR OPP AND SOPP RISK CHARACTERIZATION DOCUMENT

Thank you for the opportunity to comment on the draft Risk Characterization Document (RCD) for *ortho*-phenylphenol (OPP) and sodium *ortho*-phenylphenol (SOPP), and specifically, on the sections of the document addressing the genotoxicity potential, oncogenicity, dose-response assessment, and developmental effects. You identified the key issues for our review to be: 1) the genotoxicity and oncogenicity of OPP and SOP and metabolites phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ); and 2) the developmental toxicity of OPP and SOPP. You asked three specific questions related to these issues:

1: Do the current data support the position that OPP is not a genotoxic carcinogen?
2: Do the current data support using a threshold model to characterize the cancer risk of OPP?
3: Do the current data support concerns regarding the developmental toxicity of OPP?

Let me say at the start that overall the document is clear and well written, with conclusions supported by the current scientific information. Below we will first address the three specific questions, followed by more detailed, mostly minor or editorial comments on each of the sections of the draft RCD that were identified as being relevant to these key issues.

Question 1: Do the current data support the position that OPP is not a genotoxic carcinogen?

OEHHA concludes that the current data do not support the position that OPP is not a genotoxic carcinogen. This conclusion is based on the observations of positive results in gene mutation studies in mammalian cell systems, positive results in clastogenicity studies, and DNA adduct formation by reactive metabolites of OPP and SOPP, as discussed in the RCD. The available data indicate that OPP and SOPP may act by a genotoxic mechanism.

Question 2: Do the current data support using a threshold model to characterize the cancer risk of OPP?

OEHHA concludes that the current data do not support the use of a threshold model for cancer risk assessment. Based on the evidence of the genotoxic potential of OPP and SOPP discussed in the RCD, a genotoxic mode of action is plausible for OPP and SOPP, and a linear extrapolation approach is deemed the most appropriate for quantitative cancer dose-response assessment.

Question 3: Do the current data support concerns regarding the developmental toxicity of OPP?

OEHHA conclude that the available scientific data support concerns regarding developmental toxicity. The developmental toxicity study in rabbits exhibited an increase in the incidence of resorptions which was not statistically analyzed by the registrants. However, the analyses provided by CDPR adequately demonstrate the statistical and biological difference from the control group for this endpoint. Maternal effects were also not noted at this dose level, further supporting the concern for developmental toxicity.

<u>Comments on specific sections of the draft RCD</u>

<u>Genotoxicity [Pages 109-137]</u>

Main suggestion: Expand and clarify explanations where the studies have bearing on the evidence of genotoxicity.

Other comments:

- A summary paragraph could be added to each subsection (OPP, SOPP, PHQ and PBQ) which provides a synthesis of the studies in the subsection and shows how they provide evidence for the genotoxic potential of OPP.

- The Summary (p.109) is organized in terms of evidence of genotoxicity of OPP/SOPP and PHQ/PBQ. The PHQ/PBQ paragraph provides key points that support the genotoxic potential of these species. The OPP/SOPP paragraph does as well but is not as strong. One idea would be to organize by *in vitro* results followed by a summary of *in vivo* results (including the comments about study design). Also this paragraph doesn't include any comments on possible effects of OPP without metabolic activation.

- We suggest clarifying why it is important in evaluating the adequacy of study design to consider whether the dose range employed in a particular genotoxicity study encompassed doses that demonstrated cytotoxicity:

    - page 111. Regarding the discussion of the studies of Tayama-Nawai et al. and Tayama et al., the relationship between doses of OPP that induced chromosomal aberrations and doses that showed cytotoxicity could be made clearer. For example, it could be clarified whether or not chromosomal aberrations were found at lower doses (than caused cytotoxicity) in the presence of metabolic activation. Also, chromosomal aberrations were not mentioned in the summary (p.109) as providing evidence of genotoxic potential; we suggest considering doing so.
    - In the section on PHQ, it states that PHQ was cytotoxic without metabolic activation but did not cause chromosomal aberrations. With metabolic activation, PHQ was no longer cytotoxic but caused chromosomal aberrations. A clarification regarding how these studies may relate to OPP results would be helpful.

- page 111: Direct effect of OPP in the absence of metabolic activation needs to be explained and expanded. There is a separate small paragraph on Gottesfeld et al. (1971), which is referred to later.

- page 111: Consider adding a sentence discussing the meaning of Tayama and Nakagawa's results regarding the suppression of effects by Cyst and GSH.

- page 113: If Brendler-Schwaab method is inconsistent with normal methods, consider a clearer reference to this in the text. Although the explanation is footnoted, the text suggests the two studies are of equal value, especially the last sentence (1st paragraph, p. 113).

- PHQ: Expanding discussion of Cyst, GSH effects and PHQ autoxidation would make clearer. (p. 118). Perhaps a sentence could be added to clarify the implications of

results by Tayama and Nakagawa (1991,1994), e.g., that inhibition of SCE by catalase, Cyst, GSH and ascorbate indicate that these scavengers inhibit PHQ autoxidation.

- Minor points and typos:

1. p. 111 footnote 42 states OPP was negative for chromosomal aberrations in CHO cells in the presence of metabolic activation. This should be referenced. Also, the flow of the writing may be improved by including the information in the footnote in the main text itself.

2. p. 111, under DNA binding, Pathak and Roy is referenced as 1992a; in the reference section, the date is 1992.

3. Gottesfield (pp.112, 116) should be Gottesfeld.

4. Bottom of p.111, last line, under DNA binding: Consider "damage **to** biomacromolecules." Same comment for the last line of p.112.

5. p. 113, Sasaki et al. and Brendler-Schwaab are cited in Table 34 not Table 35.

6. p. 118-119. 1st paragraph under chromosomal damage refers to Table 39 as does 1st paragraph under DNA breakage and oxidation. Instead it should refer to Table 38.

7. p. 118 bottom states that "chromatographic analyses of the cell mixtures detected sulfhydryl-compound adducts of PBQ (e.g. PHQ-GSH). Please double check that the GSH adduct was PHQ and not PBQ.

Pharmacokinetics and Metabolism [Pages 22-27]

- We suggest that the summary to this section state key points about the metabolism of OPP to a reactive species (e.g., that there was a dose-dependent conversion of OPP to PHQ; that PHQ was found at all doses tested; that in *in vivo* studies, unconjugated PHQ was reported only in the repeated dosing studies; that unconjugated PHQ was markedly higher in males than females).

- Metabolism section: In terms of clarity it might be helpful to separate the sections by headings, *In vitro studies* and *In vivo studies* and then have a summary paragraph about what the results for each section shows or a paragraph summing up both *in vivo* and *in vitro studies*.

Oncogenicity: Urinary bladder [Pages 164-170]

IV.A.4a. Urinary bladder: Excellent section with strong arguments.

- page 164: We recommend wording change to something like the Registrants argue that OPP is not genotoxic and that "the high dose effect..."

- p. 167: It is stated that "Kwok and Eastmond (1997) showed a striking linear correlation between amount of reactive species generated through **a** pH-dependent PHQ autoxidation and the preneoplastic and neoplastic lesion incidences." Consider including this information in the Pharmocokinetics and Metabolism section with some more detail and then again refer to it here. Also, the sentence would be clearer if read "generated through pH-dependent PHQ autoxidation..." (remove "a").

- Regarding potential relevance to humans, Kadlubar et al. (1977) contains a summary table of pH range of normal human urine. (11% of individuals had urinary pH of 7.) [*Cancer Research* 37:805-814]

- p. 168 "Niho et al. (2002) found evidence for involvement of DNA change in cell proliferation event induced by SOPP." This sentence needs to be clarified.

Oncogenicity: Cancer Risk Assessment (dose-response assessment) [p.170-172]
IV.A.4c.1.EPA's 1986 Cancer Risk Guideline (p. 170)

- Section should be updated to refer to U.S. EPA's 2005 Cancer Risk Guidelines

- In the 2nd paragraph in this section, reference to findings of PHQ formation in diets containing >800 ppm OPP (lowest dose tested). "In the light of this new information" it is not clear if referring to lower dose (800 ppm OPP) or model by Greenfield. Also, the sentence should read "In light of.." (remove "the").

- Minor/insignificant points and typos:
  p. 170, paragraph that starts with "As described previously...." Also, using a biological....cytotoxic effects of PHQ and other OPP metabolites provide a reasonable explanation of [instead of to] the nonlinear bladder...

Developmental toxicity of OPP and SOPP [p.147-160]

- CDPR's draft RCD provided an extensive discussion on the developmental toxicity of OPP and SOPP. If the statistical tests employed in the analyses by CDPR are included in

the text portion of the document, it will aid in supporting the conclusions. Both the U.S. EPA and the Registrants dismissed the developmental toxic effects of OPP and SOPP identified by CDPR; OEHHA concurs with CDPR's conclusion. The fact that statistical analyses of the incidence of resorptions (early or late or both) were not conducted by the registrant or U.S. EPA should be mentioned.

- Minor/insignificant points and typos:
  1. Page 147, 3rd paragraph – Wistar (spelling)
  2. Page 157, Table 46 - Ogata et al., 1978b not 1987b

cc: Martha Sandy, Ph.D., Chief
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M E M O R A N D U M

TO: Gary T. Patterson, Ph.D.
Supervising Toxicologist
Medical Toxicology Branch

VIA: Joyce Gee, Ph.D.
Senior Toxicologist
Medical Toxicology Branch

FROM: Eric S.C. Kwok, Ph.D., D.A.B.T.

DATE: April 9, 2007

SUBJECT: RESPONSE TO COMMENTS FROM REPRODUCTIVE AND CANCER HAZARD ASSESSMENT BRANCH ON SPECIAL REVIEW REQUEST FOR DRAFT OPP AND SOPP RISK CHARACTERIZATION DOCUMENT – DIETARY EXPOSURE

This memorandum addresses comments from the Reproductive and Cancer Hazard Assessment Branch (RCHAB) of Office of Environmental Health Hazard Assessment (OEHHA) on the Department's special request in three specific areas of the draft Risk Characterization Document (RCD) for the active ingredients *ortho*-phenylphenol (OPP) and sodium *ortho*-phenylphenate (SOPP) (dated December 8, 2006). These three areas were (1) potential genotoxicity of OPP, (2) the oncogenicity dose-response assessment using a default linear approach, and (3) the concerns regarding the developmental effects of OPP. We would like to thank the reviewers for their constructive comments. In the comment, OEHHA concurred with DPR's position on all these issues. We have made several changes to the draft RCD to clarify and address the points raised by OEHHA. Our responses to these comments are as follows (the page number noted refer to the final draft RCD):

Genotoxicity

Major Comments

Comment #1: Expand and clarify explanations where the studies have bearing on the evidence of genotoxicity.

Response: We have expanded GENOTOXICITY section (page 99-113) as recommended.

Comment #2: A summary paragraph could be added to each subsection (OPP, SOPP, PHQ and PBQ) which provides a synthesis of the studies in the subsection and shows how they provide evidence for the genotoxic potential of OPP.

Response: We have modified the texts (pages 105, 108, 111, and 113) as recommended.

Comment #3: The Summary (p. 109) is organized in terms of evidence of genotoxicity of OPP/SOPP and PHQ/PBQ. The PHQ/PBQ paragraph provides key points that support the genotoxic potential of these species. The OPP/SOPP paragraph does as well but is not as strong. One idea would be to organize by *in vitro* results followed by a summary of *in vivo* results (including the comments about study design). Also this paragraph doesn't include any comments on possible effects of OPP without metabolic activation.

Response: We have reorganized the summary (page 99) to the genotoxicity section and added comments on the genotoxic effects of OPP without metabolic activation as suggested.

Comment #4: We suggest clarifying why it is important in evaluating the adequacy of study design to consider whether the dose range employed in a particular genotoxicity study encompassed doses that demonstrated cytotoxicity:

- page 111. Regarding the discussion of the studies of Tayama-Nawai *et al.* (1984) and Tayama *et al.* (1991) the relationship between doses of OPP that induced chromosomal aberrations and doses that showed cytotoxicity could be made clearer. For example, it could be clarified whether or not chromosomal aberrations were found at lower doses (than caused cytotoxicity) in the presence of metabolic activation. Also, chromosomal aberrations were not mentioned in the summary (p. 109) as providing evidence of genotoxic potential; we suggest considering doing so.
- In the section on PHQ, it states that PHQ was cytotoxic without metabolic activation but did not cause chromosomal aberrations. With metabolic activation, PHQ was no longer cytotoxic but caused chromosomal aberrations. A clarification regarding how these studies may relate to OPP results would be helpful.

Response: Genotoxic effects that occurred in the absence or with minimal cytotoxicity provide a stronger support of the chemical mutagenic potential. We have revised the text to indicate the relationship between genotoxic and cytotoxic effects of OPP in the absence and the presence of metabolic activation (page 102) and the possible contribution of PHQ and PBQ to the enhanced cytotoxic and genotoxic effects of OPP in the presence of metabolic activation (page 108).

Comment #5: page 111: Direct effect of OPP in the absence of metabolic activation needs to be explained and expanded. There is a separate small paragraph on Gottesfeld et al. (1971), which is referred to later.

Response: We have modified the texts (page 102-105) to describe the direct effect of OPP in the absence of metabolic activation.

Comment #6: page 111: Consider adding a sentence discussing the meaning of Tayama and Nakagawa's results regarding the suppression of effects by Cyst and GSH.

Response: We added text (page 102) to discuss the meaning of Tayama and Nakagawa's results regarding the suppression of chromosomal effects of OPP by Cyst and GSH.

Comment #7: page 113: If Brendler-Schwaab method is inconsistent with normal methods, consider a clearer reference to this in the text. Although the explanation is footnoted, the text suggests the two studies are of equal value, especially the last sentence (1st paragraph, p. 113).

Response: We have revised the text (page 104) as suggested.

Comment #8: PHQ: Expanding discussion of Cyst, GSH effects and PHQ autoxidation would make clearer. (p.118). Perhaps a sentence could be added to clarify the implications of results by Tayama and Nakagawa (1991, 1994), e.g., that inhibition of SCE by catalase, Cyst, GSH and ascorbate indicate that these scavengers inhibit PHQ autoxidation.

Response: We have revised the text (page 110) as suggested.

Minor Comments

Comment #9: We have added the reference to the footnote statement that OPP was negative for chromosomal aberrations in CHO cells in the presence of metabolic activation. Also, we incorporated the footnote into the main text (page 102) as suggested.

Comment #10: We have corrected the typographical error by replacing Pathak and Roy (1992a) with Pathak and Roy (1992) (page 103).

Comment #11: We have corrected the typographical error by replacing Gottesfield with Gottesfeld (page 103).

Comment #12: We have modified the sentence, i.e., damage to biomacromolecules (page 103 and 104), as suggested

Comment #13: We have corrected the typographical error by replacing Table 35 with Table 34 (page 104).

Comment #14: We have corrected the typographical error by replacing Table 39 with Table 38 (pages 111 and 112).

Comment #15: We have checked the citation stating that sulfhydryl-compound adducts of PBQ was PHQ-GSH as suggested.

Pharmacokinetics and Metabolism

Comment #16: We suggest that the summary to this section state key points about the metabolism of OPP to a reactive species (e.g., that there was a dose-dependent conversion of OPP to PHQ; that PHQ was found at all doses tested; that in *in vivo* studies, unconjugated PHQ was reported only in the repeated dosing studies; that unconjugated PHQ was markedly higher in males than females).

Response: We have modified the text (page 11) as suggested.

Comment #17: Metabolism section: In terms of clarity it might be helpful to separate the sections by headings, *In vitro studies* and *In vivo studies* and then have a summary paragraph about what the results for each section shows or a paragraph summing up both *in vivo* and *in vitro studies.*

Response: We have modified the text (page 12-16) as suggested.

Oncogenicity: Urinary bladder

Comment #18: page 164: We recommend wording change to something like the Registrants argue that OPP is not genotoxic and that "the high dose effect.. ."

Response: We have revised extensively the IV.A.4. Mode of Action for Urinary Bladder Tumors (page 158).

Comment #19: page 167: It is stated that "Kwok and Eastmond (1997) showed a striking linear correlation between amount of reactive species generated through a pH-dependent PHQ autoxidation and the preneoplastic and neoplastic lesion incidences." Consider including this information in the Pharmacokinetics and Metabolism section with some more detail and then again refer to it here. Also, the sentence would be clearer if read, "generated through pH-dependent PHQ autoxidation..."(remove "a").

Response: We have modified the text in metabolism section (page 12-16) as suggested.

Comment #20: Regarding potential relevance to humans, Kadlubar et al. (1977) contains a summary table of pH range of normal human urine. (11% of individuals had urinary pH of 7) [Cancer Research 37:805-8141]

Response: We have modified the text (page 166) as suggested.

Comment #21: p. 168: "Niho et al. (2002) found evidence for involvement of DNA change in cell proliferation event induced by SOPP." This sentence needs to be clarified.

Response: We have revised the text (page 164) as suggested.

Oncogenicity: Cancer Risk Assessment (Dose-Response Assessment)

Comment #22: IV.A.4c. 1.EPA 's 1986 Cancer Risk Guideline (p. 170); Section should be updated to refer to U.S. EPA's 2005 Cancer Risk Guidelines.

Response: We have updated the text (page 166) as suggested.

Comment #23: In the 2nd paragraph in this section, reference to findings of PHQ formation in diets containing >800 ppm OPP (lowest dose tested). "In the light of this new information" it is not clear if referring to lower dose (800 ppm OPP) or model by Greenfield. Also, the sentence should read "In light of…"(remove "the").

Response: We have revised extensively the IV.A.5. Oncogenicity Weight of Evidence of OPP and SOPP text (page 164) and the above sentences have been deleted.

Developmental Toxicity of OPP and SOPP

Major Comments

Comment #24: CDPR's draft RCD provided an extensive discussion on the developmental toxicity of OPP and SOPP. If the statistical tests employed in the analyses by CDPR are included in the text portion of the document, it will aid in supporting the conclusions. Both the U.S. EPA and the Registrants dismissed the developmental toxic effects of OPP and SOPP identified by CDPR; OEHHA concurs with CDPR's conclusion. The fact that statistical analyses of the incidence of resorptions (early or late or both) were not conducted by the registrant or U.S. EPA should be mentioned.

Response: We have modified the text (page 148) as suggested.

Minor Comments

Comment #24: We have corrected the typographical error by replacing Wister by Wistar (page 141).

Comment #25: We have corrected the typographical error by replacing Ogata *et al.* (1987b) by Ogata *et al.* (1978b) (page 151).