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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **ORTHOPHENYLPHENOL** - Review of a carcinogenicity study in the rat, submitted under **Section 6(a)(2)** of FIFRA.

EPA DP Barcode D224668; EPA Submission No. S502942; EPA MRID# 43954301; EPA Pesticide Chemical Codes 064103(OPP)/064104 (SOPP), Caswell No.s 623AA(OPP)/787(SOPP); Reregistration Case# 2575.

TO: Kathryn Davis/Thomas D. Luminello, PM 52
SRRD (7508W)
and
Terri Stowe/Kathy Depukat, PM 22
Herbicide-Fungicide Branch/Registration Division (7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/26/96
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y M. Ioannou 7/26/96
Section Head, Review Section I
and
Stephanie R. Irene, Ph.D.
Acting Chief, Toxicology Branch II
Health Effects Division (7509C) Y M. Ioannou for 7/26/96

Action Requested: Review a carcinogenicity study in the rat with Orthophenylphenol, submitted under **Section 6(a)(2)** of FIFRA.

Recommendations: TBII reviewed the carcinogenicity study in the rat with Orthophenylphenol submitted by the registrant in support of reregistration (*Technical Grade ortho-PHENYLPHENOL: A Combined Chronic Toxicity/Oncogenicity Study In the Rat*, Bayer Corporation, Agriculture Division, Toxicology for Bayer Corporation, Organic Products Division, Laboratory Project Study ID 92-272-SC, February 23, 1996, EPA MRID# 43954301). This study was submitted under **Section 6(a)(2)** of FIFRA; there was an increase in tumor incidence (papilloma and transitional cell carcinomas) in the urinary bladder in male rats.

This study (MRID# 43954301) satisfies the guideline requirements of §83-1a and §82-2a (§83-5 combined chronic/oncogenicity study) for reregistration of 064103 (orthophenylphenol), 064104 (Na-orthophenylphenate) and 064108 (K-orthophenylphenate).

This study (MRID# 43954301) along with the mouse carcinogenicity study (MRID# 43545501) will be submitted to the HED RfD/QA Peer Review Committee and the HED Carcinogenicity Peer Review Committee for reconsideration.

The following is the summary from the review:

In a combined chronic toxicity/ carcinogenicity study (MRID# 43954301) CDF[F-344]/BR rats from SASCO, Inc., Madison WI received *ortho*-PHENYLPHENOL, Technical Grade (99.5-100% a.i.; Batch # - S-01-93, Mixture of Bayer AG, Leverkusen, Germany and Dow, Midland, Michigan) in the diet for 24 months at dose levels of 0, 800, 4000 and 8000 ppm in males and 0, 800, 4000, and 10000 ppm in females (39, 200, and 402 mg/kg/day for males for the 800, 4000, and 8000 ppm dose groups and 49, 248, and 647 mg/kg/day for females for the 800, 4000, and 10000 ppm dose groups). An interim sacrifice group of twenty animals/sex for control and high dose groups and ten animals/sex for the low and mid dose groups were sacrificed at 12 months. Systemic toxicity was noted as decreased body weights ($p < 0.05$) and body weight gains in both males and females of the mid and high dose groups during the first 13 weeks of the study (for the 2-year carcinogenicity group). At study termination, only the high dose groups had reduced body weights ($p < 0.05$) and body weight gains. Food consumption was slightly decreased in the 2-year carcinogenicity group in the high dose group at all time points measured and was decreased in the mid dose females at 13 weeks. Food efficiency determined for the first 13 weeks was slightly decreased in the mid dose group and greatly decreased in the high dose group.

There was an increase in observed masses in the urinary bladder of high dose males at 24 months. High dose females had an increased incidence of kidneys with pitted zones at 24 months. Mid and high dose females had an increase in wet/stained ventrum at 12 months and both high dose males and females had a similar observation at 24 months, this was attributed to the urine and red staining in the perigenital area noted in the clinical observation data.

Non-neoplastic observations noted an increase in incidence of calculus in the kidneys in high dose males at the 12 month sacrifice and the 24 month study termination. There was also increased hyperplasia of the urinary bladder at 12 and 24 months in high dose males (an high dose females at 24 months) along with

an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in high dose males. High dose males and females also had an increase in cysts of the kidney at 24 months. High dose females had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney.

At the 12 month sacrifice, the incidence of papilloma in the urinary bladder was 0/20, 0/10, 0/10, and 6/20 ($p < 0.05$) and the incidence of transitional cell carcinoma in the urinary bladder was 0/20, 0/10, 0/10, and 3/20 for the control, low, mid, and high dose groups, respectively. At the 24 month study termination the incidence of transitional cell carcinoma in the urinary bladder was 0/50, 0/50, 2/50, and 34/50 ($p < 0.05$) and the incidence of papilloma in the urinary bladder was 0/50, 1/50, 0/50, and 6/50 ($p < 0.05$), for the control, low, mid, and high dose groups, respectively. Females were not similarly affected.

The Systemic Toxicity NOEL is equal to 800 ppm (39 mg/kg/day for males and 49 mg/kg/day for females and the Systemic Toxicity LOEL is equal to 4000 ppm (200 mg/kg/day for males and 248 mg/kg/day for females) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity.

This study is classified as Acceptable and satisfies the guideline requirements (§83-5) for a combined chronic toxicity/carcinogenicity study in the rat.

I. Toxicology Profile for Orthophenylphenol and Sodium Orthophenylphenate (40 CFR 158.340)

Technical: Orthophenylphenol and Sodium Orthophenylphenate
Use Pattern: food use

This compound is an registered active ingredient; the following data are available for Orthophenylphenol or Sodium Orthophenylphenate technical. **This table does not necessarily indicate requirements for reregistration.**

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	Yes
§81-4 Primary eye irritation in rabbits	Yes	Yes
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization - guinea pig	Yes	Yes
§82-1(a) 90 day feeding study - rat	Yes	NO[1]
§82-1(b) 90 day feeding - dog	Yes	NO[1]
§82-2 21 day dermal - rabbit	Yes	Yes
§83-1(a) 2-year feeding - rodent	Yes	Yes[2]
§83-1(b) 1 year feeding - nonrodent	Yes	Yes
§83-2(a) Carcinogenicity - rat	Yes	Yes[2]
§83-2(b) Carcinogenicity - mouse	Yes	Yes
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	Yes	Yes
§83-4 Multigeneration reproduction-rat	Yes	Yes
§84-2(a) Mutagenicity - Gene Mutation	Yes	Yes
§84-2(b) Muta - Struct. Chromosome Aberr.	Yes	Yes
§84-4 Muta - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	**NO**

[1] = satisfied by a chronic toxicity study
[2] = study discussed in this memo

II. Data Gaps

The following are data gaps for the technical necessary for permanent food use registration:

§85-1 General metabolism - rat

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. Reference Dose

The Health Effects Division RfD/Peer Review Committee met on September 15, 1994 to discuss and evaluate the existing and recently submitted toxicology data in support of Ortho-phenylphenol registration and to assess the Reference Dose (RfD) for this chemical.

In the meeting of September 15, 1994, because of technical and other considerations, the RfD/Peer Review Committee's decision regarding the reassessment of the RfD was deferred pending OPP/HED's evaluation of the chronic toxicity study used in the JMPR evaluation.

The Committee recommended deletion of the existing RfD or regulatory value for this chemical from the HED files. The existing regulatory value for this chemical was generated by an Ad Hoc Committee, HED/OPP, on March 22, 1994 under special circumstances to support existing tolerances. No data or data evaluation records were available for review by the Ad Hoc Committee in the assessment of this regulatory value. The Ad Hoc Committee used the toxicology one-liner summaries to derive this value. The regulatory value was based on a developmental toxicity study in rabbits with a NOEL of 25.0 mg/kg/day (note: in a recent reevaluation by the RfD/Peer Review Committee this NOEL was raised to 100 mg/kg/day). An uncertainty factor (UF) of 100 was applied to account for inter-species extrapolation and intra-species variability. For RfD reassessment purposes, and based on technical and regulatory reasons, the chemical should now be considered under review.

It should be noted that this chemical has been reviewed by the FAO/WHO joint committee on pesticide residue (JMPR) in 1990 and an acceptable daily intake (ADI) of 0.02 mg/kg/day was established based on a chronic toxicity study in rats with a NOEL of 40 ppm (2.0 mg/kg/day). A safety factor (SF) of 100 was used to account for the inter-species extrapolation and intra-species variability.

V. Pending Regulatory Actions

None.

VI: Toxicological Issues Pertinent to this Request

A. New toxicology Data on Orthophenylphenol and Sodium Orthophenylphenate

The new study has been discussed above.

B. Carcinogenicity

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on January 5, 1994 to discuss and evaluate the weight-of-the-evidence on Orthophenylphenol (OPP) and Sodium Orthophenylphenate (SOPP) with particular reference to its carcinogenic potential. The CPRC concluded that under the existing Carcinogen Risk Assessment guidelines, the evidence for OPP & SOPP is sufficient for classification as **Group B2 - probable human carcinogen**, based on evidence of multiple tumor types in multiple sites.

However, in consideration of what is known about the metabolism of these compounds and the anticipated human exposure, the CPRC felt that it was inappropriate to apply a low-dose extrapolation methodology (Q*) to the animal data. Therefore, the CPRC recommended the use of the **Margin of Exposure (M.O.E.)** methodology to be applied for the estimation of human risk, for the time being. Review of recently submitted 6(a)(2) data from the mouse carcinogenicity study may lead to a reconsideration of this interim decision.

C12002

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/25/96
Senior Pharmacologist, Review Section I, TBII (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. J.M. Ioannou 7/26/96
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: Chronic Oral (Feeding) Toxicity/Carcinogenicity
Species: Rat Guideline: §83-5

EPA Numbers: EPA MRID# 43954301
EPA DP Barcode D224668
EPA Submission Barcode S502942
EPA Pesticide Chemical Code 064103
Toxicology Chemical No. 623AA
EPA Reregistration Case # 2575

Test Material: *ortho*-PHENYLPHENOL, Technical Grade
Synonyms: 2-Biphenyl; *ortho*-Biphenyl; *ortho*-Diphenyl; *ortho*-Hydroxybiphenyl; 2 Hydroxydiphenyl; 2-Phenylphenol; Dowcide 1; Preventrol O Extra; Remol TRF; Tetrosin OE; Tumescal OPE

Title of Report: Technical Grade *ortho*-PHENYLPHENOL: A Combined Chronic Toxicity/Oncogenicity Study In the Rat

Sponsor: Bayer Corporation, Organic Products Division, Mobay Road, Pittsburgh, PA 15205
and
The Dow Chemical Company, Specialty Chemicals/Performance Products, Midland, Michigan 48674

Testing Facility: Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stillwell, KS 66085-9104

Study Number: Laboratory Project Study ID 92-272-SC

Author(s): B.S. Wahle, W.R. Christenson

Report Issued: February 23, 1996

Executive Summary: In a combined chronic toxicity/carcinogenicity study (MRID# 43954301) CDF[F-344]/BR rats from SASCO, Inc., Madison WI received *ortho*-PHENYLPHENOL, Technical Grade (99.5-100% a.i.; Batch # - S-01-93, Mixture of Bayer AG, Leverkusen, Germany and Dow, Midland, Michigan) in the diet for 24 months at dose levels of 0, 800, 4000 and 8000 ppm in males and 0, 800, 4000, and 10000 ppm in females (39, 200, and 402 mg/kg/day for males for the 800, 4000, and 8000 ppm dose groups and 49, 248, and 647 mg/kg/day for females for the 800, 4000, and 10000 ppm dose groups). An interim sacrifice group of twenty animals/sex for control and high dose groups and ten animals/sex for the low

and mid dose groups were sacrificed at 12 months. Systemic toxicity was noted as decreased body weights ($p < 0.05$) and body weight gains in both males and females of the mid and high dose groups during the first 13 weeks of the study (for the 2-year carcinogenicity group). At study termination, only the high dose groups had reduced body weights ($p < 0.05$) and body weight gains. Food consumption was slightly decreased in the 2-year carcinogenicity group in the high dose group at all time points measured and was decreased in the mid dose females at 13 weeks. Food efficiency determined for the first 13 weeks was slightly decreased in the mid dose group and greatly decreased in the high dose group.

There was an increase in observed masses in the urinary bladder of high dose males at 24 months. High dose females had an increased incidence of kidneys with pitted zones at 24 months. Mid and high dose females had an increase in wet/stained ventrum at 12 months and both high dose males and females had a similar observation at 24 months, this was attributed to the urine and red staining in the perigenital area noted in the clinical observation data.

Non-neoplastic observations noted an increase in incidence of calculus in the kidneys in high dose males at the 12 month sacrifice and the 24 month study termination. There was also increased hyperplasia of the urinary bladder at 12 and 24 months in high dose males (an high dose females at 24 months) along with an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in high dose males. High dose males and females also had an increase in cysts in the kidney at 24 months. High dose females had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney.

At the 12 month sacrifice, the incidence of papilloma in the urinary bladder was 0/20, 0/10, 0/10, and 6/20 ($p < 0.05$) and the incidence of transitional cell carcinomas in the urinary bladder was 0/20, 0/10, 0/10, and 3/20 for the control, low, mid, and high dose groups, respectively. At the 24 month study termination the incidence of transitional cell carcinoma in the urinary bladder was 0/50, 0/50, 2/50, and 34/50 ($p < 0.05$) and the incidence of papilloma in the urinary bladder was 0/50, 1/50, 0/50, and 6/50 ($p < 0.05$), for the control, low, mid, and high dose groups, respectively. Females were not similarly affected.

The Systemic Toxicity NOEL is equal to 800 ppm (39 mg/kg/day for males and 49 mg/kg/day for females and the Systemic Toxicity LOEL is equal to 4000 ppm (200 mg/kg/day for males and 248 mg/kg/day for females) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity.

This study is classified as Acceptable and satisfies the guideline requirements (§83-5) for a combined chronic toxicity/carcinogenicity study in the rat.

Compliance

A signed and dated STATEMENT OF DATA CONFIDENTIALITY (no claim of confidentiality was made), GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT, FLAGGING STATEMENT for potential adverse effects (the study meets or exceeds the criteria numbered 1, 2 and 3 in 40 CFR 158.34), and Quality Assurance Statement were provided.

A. Materials and Methods: A scanned copy of the Materials and Methods section from the investigators report is attached, the protocol used was acceptable for a combined chronic feeding/carcinogenicity study in rats.

1. Test compound: *ortho*-PHENYLPHENOL, Technical Grade
Description - light tan and white flakes
Batch # - S-01-93 (Mixture of Bayer AG, Leverkusen, Germany and Dow, Midland, Michigan)
Purity - 99.5-100%
CAS# 90-43-7

2. Vehicle(s): An acetone/corn oil mixture was used as a vehicle to dissolve the test substance prior to mixing with the dietary carrier.

3. Test animals: Species: rat
Strain: CDF[F-344]/BR
Age: 8 weeks
Weight: 107.5-108.9 g for males; 96.3-96.8 g for females
Source: SASCO, Inc., Madison WI

4. Animal husbandry (scanned from page 19 of the investigators report)

For additional reading regarding the care afforded the animals both prior to and during the in-life portion of this study, which was conducted at an American Association for Accreditation of Laboratory Animal Care-accredited facility, see *The Animal Care Report* which has been included in *Appendix VII* of this general toxicology report. Upon receipt from the vendor, animals were examined and subsequently killed (CO_2 asphyxiation) if deviations in general appearance and/or behavior were observed. Those animals considered acceptable were individually housed in suspended stainless steel wire-mesh cages, each containing a feeder, a source of water (pressure-activated water nipples), and deotized cage board in the bedding tray.

In addition, with their arrival at the test facility (1) all animals were given free and continuous access to food (Purina Mills Rodent Lab Chow 5001-4 in "etts" form) and tap water (municipal water supply of the city of Kansas City, MO); (2) all animals were observed at least once daily for moribundity and mortality; (3) all activities, planned or unplanned, associated with the animals and/or their room was documented; and (4) prior to being released for study by a veterinarian, all animals were acclimatized to their ambient laboratory conditions (room temperature 18 to 26°C, relative humidity 40 to 70%, and a daily photoperiod of 12 hr of light [7:00 a.m. to 7:00 p.m.] alternating with 12 hr of darkness) for at least 6 days.

Analysis of feed, water, corn oil, and ambient room conditions. Animal room conditions were monitored and recorded continuously. Feed, water, and corn oil (used in diet preparation to facilitate mixing of the test substance in the feed) were periodically sampled and analyzed for a variety of potential impurities. No evidence of a confounding effect on the study objectives was suggested from the results of these analyses, which have been archived.

5. Animal assignment (scanned from pages 17-18 of the investigators report)

Distribution/grouping of animals. Following the pre-exposure acclimation period, a weight stratification-based computer program, obtained from INSTEM Computer Systems (Stone, Staffordshire, U.K.), was used to allocate the animals to one of 4 dose groups within 3 main groupings of animals (see below) on Thursday, February 25, 1993, just preceding the experimental start of the study. A summary listing of the mean post-randomization weights for each dose group is presented in *Appendix I, Table 2.*

1) ***The 1-year sacrifice group.*** This group consisted of 40 animals (20 males and 20 females) in both the control and high-dose groups and 20 animals (10 males and 10 females) in both the low and intermediate dose levels for a total of 120 animals.

2) ***The 2-year sacrifice group.*** This group consisted of 100 animals (50 males and 50 females) in all 4 dose groups for a total of 400 animals.

3) ***The replacement group.*** For approximately the first month of treatment, an additional 5 rats/dose/sex were placed on study as part of the 1- and 2-yr Sacrifice *Groups.* The purpose of these animals was to serve as potential "replacements" for any animals that unexpectedly died or developed noncompound-related problems, such as behavioral or physical abnormalities, at a very early stage in the study. When replacement occurred, the animal retained its original number and all data collected previously and in the future on the replacement animal was incorporated into the database of the dose group in which the replaced animal was a member. At the end of the replacement period, all remaining replacement animals which were not utilized for replacement purposes were sacrificed in a timely manner. In this study, 0, 2, 1, and 3 animals were replaced in the control-, low-, mid-, and high-dose groups of the 1-yr sacrifice group, and 1, 0, 2, and 3 animals were replaced in the control-, low-, mid-, and high-dose groups of the 2-yr sacrifice group.

Doses selected and rationale. In this combined chronic toxicity/oncogenicity testing study in the rat, OPP was administered as an admix to the rodent's diet at concentrations of 0, 800, 4,000, or 8,000/10,000 ppm (males/females). These doses, which were approved by the EPA, were selected based upon a review of the literature [4-6] as well as upon the data generated from a A *4-week range-finding toxicity study in the rat.* A summary of the results of this study is presented in *Appendix VI* of this general toxicology report.

6. Diet preparation (scanned from pages 15-17 of the investigators report)

Preparation of the test diets. An acetone/corn oil mixture was used as a vehicle to dissolve the test substance prior to mixing with the dietary carrier. The control diet (including the acetone/corn oil mixture) was

prepared the same as the treated diet, excluding only the test chemical. A sample of each batch of feed mixed was taken and retained in the freezer until the study was complete and the analytical data deemed satisfactory. Replacement admixtures for each treatment group were prepared weekly and stored under freezer conditions until presented to the animals the following week.

Source, Storage, Physical/Chemical Properties, and Nomenclature

In this combined chronic toxicity/oncogenicity testing study, animals were administered the technical grade of *ortho*-phenylphenol (OPP) as a dietary admixture, based on the analytically determined percentage of purity of the chemical. At all times prior to, during, and subsequent to the conclusion of the exposure portion of this study, the test batch of OPP was stored under freezer conditions (~-23°C). A general overview of the test article as well as specific information regarding the background and analytical characterization of the particular test batch of OPP which was used in this study is presented in *Appendix I, Table 1.*

Analysis of the OPP Test Material

For additional reading, beyond the overview presented below, regarding all the confirmational analytical chemistry conducted with OPP as it pertains to the study reported herein, see *The Analytical Chemistry Report* which has been included in *Appendix V* of this general toxicology report. On March 16, 1993, just following the experimental start (March 3, 1993), and then again during (at approximately 6-month intervals) as well as subsequent to termination of the inlife portion of this study the concentration/stability of the AI of the test chemical, while maintained under storage conditions at the test facility, was reconfirmed. The AI analyses were conducted in principle according to methodology described previously [2].

From the study report: *(Appendix V, Table 1).* Four (4) separate analyses of batch No. S-01-93, conducted 3/93, 11/93, 8/94, and 9/95, revealed average chemical purities of 99.7, 99.9, 100.0, and 99.5%, respectively. These data confirm that the AI of OPP was stable under storage conditions at the test facility for the duration of this 2-yr combined chronic toxicity/oncogenicity study in the rat.

Analysis of the Concentration of OPP Admixed in Rodent Feed

The concentration of the AI of OPP in the various test diets was analytically verified at 9 separate times during the in-life phase of this 2-yr study. All dietary analyses for this study (i.e., homogeneity, stability, and concentration verifications) for this study were conducted in principle according to methodology described previously [3].

From the study report: *(Appendix V, Table* 6). Mean analytical concentrations for each dose group were 732 ppm (CV = 6%), 3730 ppm (CV = 8%), 7385 ppm (CV = 8%), and 9510 ppm (CV = 6%) with all values remaining within 10% of the corresponding nominal concentrations of 800, 4,000, 8,000, and 10,000 ppm, respectively.

Analysis of the Homogeneity of OPP Admixed in Rodent Feed

The homogeneity of the AI of OPP was determined for feed containing nominal concentrations of 800 and 10,000 ppm of the test material. Briefly, the homogeneity determination was carried out as follows: 3 samples were taken for analysis from each of 3 distinct layers (top, middle, bottom; 9 samples total) in a Hobart mixing bowl. A homogeneous distribution of the AI in the feed was defined in terms of a coefficient of variation (CV), derived from the 9 samples taken, which was ≤ 10%.

From the study report: *(Appendix V, Table 3)*. The mean concentrations of OPP in feed, sampled from 3 distinct layers in a mixing bowl and containing a nominal concentration of either 800 or 1000 ppm, were determined to be 744 ppm (CV = 5%) and 9,175 ppm (CV = 7%), respectively. Based on a CV ≤ to 10%, OPP was judged to be homogeneously distributed in the feed over a concentration range of 800-10,000 ppm.

Analysis of the Stability of OPP Admixed in Rodent Feed

The stability of the AI of OPP as a dietary admixture was determined for feed containing nominal concentrations of 800 and 10,000 ppm of the test material. Stability in the feed was assessed following 1, 3, 7, 10, and 14 days of room temperature storage (~22°C) and 7, 14, 21, and 28 days of freezer storage (~-23°C). Briefly, the stability analysis was carried out as follows: An initial sample was taken immediately after the test substance was mixed with the ration. Each batch of ration, mixed at the 2 concentrations, was then divided into 2 portions which were both placed into the freezer for 7 days. After 7 days of freezer storage, one portion was removed for room temperature testing. The 800- and 10,000-ppm feed samples which remained in the freezer were sampled for analysis on Days 7, 14, 21, and 28; the 800- and 10,000-ppm samples which were returned to room temperature were sampled for analysis following 0, 1, 3, 7, 10, and 14 days of room temperature storage. Chemical stability in the ration was defined in terms of that period of time that 80% of the chemical, relative to the initial concentration, was recoverable.

From the study report: *(Appendix V, Tables* 4 & 5). Following 14 days of room temperature storage, the mean analytically determined concentrations of OPP in the 800- and 10,000-ppm admixtures were determined to be 743 and 9,822 ppm, respectively. Following 28 days of freezer storage, the mean analytically determined concentrations of OPP in the 800- and 10,000-ppm admixtures were determined to be 671 and 9,321 ppm, respectively. Based on the criteria of at least 80% recovery of the initial chemical concentration, OPP mixed in rodent ration was judged to be stable following both room temperature and freezer storage for a minimum of 14 and 28 days, respectively, over a concentration range of 800-10,000 ppm.

7. Observations (scanned from page 19 of the investigators report)

Body Weight and Food Consumption Determinations

Individual body weight and food consumption determinations were scheduled to be performed once each week on all animals. Body weights were also measured immediately prior to all necropsies to allow for calculation of organ to body weight ratios. In addition, using specifically defined criteria, food consumption data were corrected, as conditions dictated, to account for misleading indications of food intake (i.e., excessive spillage, clogged feeders, etc.).

Clinical observations and Mortality

General observations for moribundity and mortality were scheduled to be conducted twice daily (once on weekends and holidays). Detailed physical examinations for clinical signs of toxicity were scheduled to be performed once each week on all animals. Clinical examinations included evaluation of external surface areas, orifices, posture, general behavior, respiration, and excretory products.

8. Ophthalmological examinations (scanned from page 20 of the investigators report)

Following acclimatization, pre-exposure ophthalmic exams were conducted on the entire pool of potential study animals. To the extent possible, only those rats free of ocular abnormalities and thus clinically "normal" were distributed to dose groups and subsequently placed on study. With conclusion of the 1- and 2-yr exposure periods, ophthalmic exams were conducted again on all surviving animals comprising the two sacrifice groups. The defining conditions for the 5-level grading system (minimal, mild or slight, moderate, marked, and severe) in use at this facility for evaluating ophthalmologic lesions/observations are presented in *Appendix I, Table 4*.

9. Hematology and clinical chemistry (scanned from page 20 of the investigators report)

Clinical Pathology

Blood and urine were nominally collected at 3, 6, 12, 18, and 24 months into the study from the first 20 surviving rats/sex/dose of the *2-yr Sacrifice Group*. Blood was drawn via the orbital sinus following an overnight fast; to the extent possible, urine was collected on the same non-fasted animals the week prior to the week of bleeding. To execute the collection process, the animal's remain in their wire-mesh cages and a "metabolism tray" (to reduce fecal and food contamination of the urine) is attached to the bottom of their cage. A petri dish is placed beneath the tray to catch the urine, which is collected during the night. Generally, urine collection containers are set up during the afternoon and collected the following morning for analysis. A listing of the specific clinical pathologic parameters evaluated, their method of evaluation, as well as abbreviations and units used for each parameter is presented in *Appendix* I, *Table* 5.

a. Hematology

The investigators determined levels of sodium, potassium, chloride, urea nitrogen, fasting glucose, creatinine, uric acid, triglyceride, cholesterol, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, total bilirubin, direct bilirubin, total protein, albumin, phosphorus, calcium, and globulin.

b. Clinical chemistry

The investigators determined platelet count, leucocyte count, erythrocyte count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, methemoglobin, leucocyte differential including atypical lymphocytes, band neutrophils, basophils, blasts, hemotrak comments, eosinophils, lymphocytes, metamyelocytes, monocytes, myelocytes, nucleated RBC, plasma cells, promyelocytes, and segmented neutrophils, erythrocyte morphology including anisocytosis, basophilic stippling, hypersegmented neutrophils, hypochromasia, macrocytosis, microcytosis, poikilocytosis, polychromasia, spherocytosis, target cells, and toxic granulation, and special stains including reticulocyte counts, Heinz Bodies.

c. Urinalysis

The investigators determined urine appearance, urine clarity, urine color, specific gravity, a reagent strip measuring pH, urine protein, urine glucose, ketones, urine bilirubin, urine blood, urobilinogen, and nitrite, and urine microscopic.

10. Sacrifice and Pathology (scanned from page 20 of the investigators report)

Gross Pathology

All animals placed on study were subject to a postmortem examination which included (1) documenting and saving all gross lesions, (2) weighing designated organs, and (3) collecting representative tissue specimens for histopathologic evaluation. A listing of the standard tissue specimens that were preserved (nominally in buffered 10% formalin) and organs that were weighed during each autopsy is presented in *Appendix I, Table 6*.

The following organs were collected (bolded organs were weighed also): **adrenals**, aorta, bone including femur, rib/cc jct, and sternum, bone marrow, **brain** including cerebellum, cerebrum-midbrain and medulla/pons, cecum, cervix, clitoral gland, colon, epididymis, esophagus, exorbital/lacrimal gland, eyes, gross lesions, harderian gland, **heart**, joint (the femur/tibial), **kidneys**, larynx, **liver**, **lungs**, lymph nodes (cervical and mesenteric), mammary gland, muscle, optic nerve, sciatic nerve, **ovaries**, pancreas, parathyroid, physical identifier, pituitary, preputial gland, prostate, rectum, salivary gland, seminal vesicles, skin, skull, small intestine including duodenum, ileum and jejumen, spinal cord including cervical, lumbar and thoracic regions, **spleen**, stomach, **testicles**, thymus, **thyroid**, trachea, ureters, urinary bladder, uterus and vagina.

Micropathology

With the exception of the physical identifier (tail tattoo), the vagina, and the exorbital/lacrimal, clitoral, and preputial glands, representative sections of all tissues collected were processed, embedded in paraffin, sectioned, mounted, stained with hematoxylin and eosin (H&E), and examined under a light microscope by a veterinary pathologist. The defining conditions for the 5-level grading system (minimal, mild or slight, moderate, marked, and severe) in use at this facility for evaluating non-neoplastic histopathologic lesions is presented in *Appendix I, Table 4*.

11. Statistics (scanned from page 21 of the investigators report)

Continuous data that were examined statistically were evaluated initially for equality or homogeneity of variance using Bartlett's test [8]. Group means were further analyzed by a one-way variance analysis (ANOVA) [8] followed by Dunnett's test [9,10]. In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA [11] followed by the Mann-Whitney-U test for between-group comparisons. Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the chi-square, Fisher exact, or chi-square and Fisher exact tests. On a case by case basis, and at the discretion of the study director, data were subject to additional statistical procedures other than those mentioned above. For the Bartlett test, a probability (p) value $\leq$ 0.001 was considered significant; for all other statistical tests, differences with p values $\leq$ 0.05 were considered statistically significant. All statistical evaluations were performed using software obtained from either INSTEM Computer Systems or SAS Institute, Inc. (Cary, NC).

B. Results

1. Observations

a. Mortality

The investigators provided group summary and individual animal data. The following table presents the Group Mean Death Report (from Table MORT1-SUM, pages 278-279):

	Control	LDT	MDT	HDT
Total number of animals	50/50[1]	50/50	50/50	50/50
Mean time to death (days)	701/658	689/705	698/673	656/697
Total found dead on study	4/6	5/1	2/3	4/5
Total unscheduled killed	15/11	12/9	16/13	20/13
Total scheduled killed	31/33	33/40	32/34	26/32

[1] = male/female

There was a slight increase in unscheduled killed animals in the male high dose group with a slight decrease in mean time to death; however, the females had a slight increase in the mean time to death.

b. Clinical observations

The investigators provided group summary and individual animal data. There was an increase in abnormal color urination in high dose males, urine staining in high dose males and all treated females, red stains in high dose males and brown stain in mid and high dose females.

c. Ophthalmological examinations

No treatment related ophthalmological observations were reported.

3. Body weight

The investigators provided group summary and individual animal data. The following table presents mean body weights and mean body weight gains (calculated by the reviewer from group summary data) data (in grams) for selected intervals in the study:

Week	Group:	Control	Low	Mid	High
			1-Year Interim Sacrifice		
1	M	134.6±15.0	134.2±11.0	132.6±10.6	131.7±12.7
	F	109.3±6.5	109.9±8.3	107.8±7.4	106.7±6.4
13	M	286.7±19.9	276.0±20.8	272.5±18.3	266.8*±19.4
	F	162.5±7.0	169.5*±7.2	163.4±9.8	151.7*±4.9
1-13	M	152.1	141.8(93.2)[1]	139.9(92.0)	135.1(88.8)
	F	53.2	59.6(112.0)	55.6(104.5)	45.0(84.6)
52	M	373.8±28.0	363.8±35.4	359.7±26.9	340.3*±24.5
	F	202.0±10.8	208.7±12.2	201.3±12.9	186.3*±7.8
1-52	M	239.2	229.6(96.0)	227.1(94.9)	208.6(87.2)
	F	92.7	98.8(106.6)	93.5(100.9)	79.6(85.9)
			2-Year Oncogenicity Group		
1	M	135.6±13.8	133.8±14.8	132.8±13.5	129.2±13.8
	F	109.3±7,3	108.9±8.1	107.3±7.4	107.3±8.3
13	M	288.7±17.1	281.3±18.0	275.0*±18.3	260.3*±18.4
	F	169.5±8.0	166.3±10.4	160.9*±7.1	154.2*±6.5
1-13	M	153.1	147.5(96.3)	142.2(92.9)	131.1(85.6)
	F	60.2	57.4(95.4)	53.6(89.0)	46.9(77.9)
52	M	370.7±23.0	371.0±23.3	356.2*±24.0	334.8*±21.5
	F	206.2±15.3	203.0±13.2	199.1*±13.1	186.0*±9.2
1-52	M	235.1	237.2(100.9)	223.4(95.0)	205.6(87.5)
	F	96.9	94.1(97.1)	91.8(94.7)	78.7(81.2)
78	M	381.8±22.5	382.0±21.6	361.7*±24.4	337.5*±18.0
	F	235.0±15.3	231.3±20.3	224.1*±19.5	201.8*±11.3
1-78	M	246.2	248.2(100.8)	228.9(93.0)	208.3(84.6)
	F	125.7	122.4(97.4)	116.8(92.9)	94.5(75.2)
104	M	346.2±33.2	353.3±18.8	342.2±24.3	309.8*±17.8
	F	247.7±17.1	240.1±21.8	236.4±25.1	210.2*±17.3
1-104	M	210.6	219.5(104.2)	209.4(99.4)	180.6(85.8)
	F	138.4	131.2(94.8)	129.1(93.3)	102.9(74.4)

[1] = percent of control; * = $p < 0.05$; Data extracted from Study ID 92-272-SC, Table BW-MEAN, pages 44-121 of the investigators report.

During the first 13 weeks of the study (for the 2-year oncogenicity group), there were decreased body weights ($p < 0.05$) and body weight gains in both males and females of the mid and high dose groups. At 1 year and 78 weeks, there were decreased body weight gains in both males and females of the high dose groups. At study termination, only the high dose groups had reduced body weights ($p < 0.05$) and body weight gains.

4. Food consumption, food efficiency and compound intake

The investigators provided group summary and individual animal data. The following table presents selected food consumption data (in grams/animal/day) and food efficiency (in percent):

Week	Group:	Control	Low	Mid	High
		1-Year Interim Sacrifice			
2	M	14.32±1.47	14.40±0.91	14.40±1.00	14.05±1.29
	F	11.43±0.59	10.93±1.54	11.59±0.60	10.94±0.65
13	M	16.68±1.12	16.57±1.08	16.65±1.25	16.32±0.85
	F	11.25±0.73	11.76±0.58	11.56±0.51	11.29±0.63
FE[1]	M	10.0	9.4(95)[2]	9.2(92)	9.1(91)
	F	5.2	5.6(>)	5.3(>)	4.4(85)
52	M	17.25±1.29	17.29±1.37	16.49±1.40	15.86*±1.29
	F	11.69±0.62	12.47*±0.85	11.70±0.65	11.54±0.60
		2-Year Oncogenicity Group			
2	M	14.66±1.47	14.20±1.36	14.40±1.24	13.61*±1.25
	F	11.44±0.70	10.90*±0.63	11.21±0.85	10.82*±0.76
13	M	16.77±0.95	16.57±0.94	16.65±1.05	15.86*±0.92
	F	11.81±0.85	11.68±0.79	11.24*±0.54	11.14*±0.66
FE	M	10.0	9.8(98)	9.4(94)	9.1(91)
	F	5.6	5.4(96.4)	5.2(92.9)	4.6(82.1)
52	M	17.37±1.11	17.16±1.16	16.64*±1.29	15.98*±1.34
	F	12.61±1.10	12.40±1.02	11.80*±0.99	11.22*±0.82
78	M	19.03±1.27	19.72*±1.12	18.90±1.12	18.30*±0.99
	F	14.71±1.02	14.73±1.20	14.50±1.04	13.82*±0.95
104	M	16.79±2.47	17.56±1.54	17.01±1.63	15.88±1.11
	F	14.66±1.39	14.41±1.76	13.52*±1.61	13.13*±1.46

* = < 0.05; [1] = Food Efficiency for first 13 weeks in percent, calculated by the reviewer from mean food consumption data; [2] = percent of control; Data extracted from Study ID 92-272-SC, Table FC-MEAN, pages 132-209 of the investigators report.

Food consumption was slightly decreased in the 2-year oncogenicity group in the male and female high dose animals at all time points measured and in the mid dose females at 13 weeks, mid dose males and females at 52 weeks, and mid dose females at 104 weeks. Food efficiency determined for the first 13 weeks in males and females was slightly decreased (6-7%) in the mid dose group and greatly decreased (9-18%) in the high dose group.

Compound intake for the entire study period was determined to be 39, 200, and 402 mg/kg/day for males for the 800, 4000, and 8000 ppm dose groups and 49, 248, and 647 mg/kg/day for females for the 800, 4000, and 10000 ppm dose groups (from Table AI-MEAN, page 228 of the study report).

5. Hematology and clinical chemistry

The investigators provided group summary and individual animal data.

a. Hematology

No treatment related effects were noted in all treated males or the low and mid dose females. There were some slight decreases in hemoglobin, hematocrit, MCV and MCH in high dose females at 24 months; however, these changes were only about 3-6% from control and according to the investigators, the changes were within historical control ranges (not provided).

b. Clinical chemistry

No treatment related effects were noted in measured parameters in treated males and females.

c. Urinalysis

No treatment related effects were noted in all treated females and low and mid dose males. There was an increase in evidence of blood in the urine in high dose males at 18 and 24 months, this may be due to bladder neoplasia that was observed in these males (see neoplastic observations following).

6. Pathology

a. Gross pathological observations

Gross lesions were noted as follows (from Appendix II, Tables GP1-SUM-INT and GP1-SUM, pages 394-426 and 428-494):

		Control	Low	Mid	High
		One-Year Animals			
# animals examined		20	10	10	20
Urinary bladder					
mass	M	0/20	0/10	0/10	2/20
Ventrum	wet/stained				
	M	0/1	0/0	0/1	2/5
	F	0/16	1/6	3*/9	13*/19
		Two-Year Animals			
# animals examined		50	50	50	50
Urinary bladder					
mass	M	0/50	0/50	2/50	37*/50
Ventrum	wet/stained				
	M	1/31	1/28	3/33	18*/41
	F	3/40	7/41	10/40	19*/43
Kidneys					
pitted zone	F	0/50	0/50	0/50	7*/50

* = p < 0.05

There was an increase in observed masses in the urinary bladder of high dose males at 24 months. High dose females had an increased incidence of kidneys with pitted zones at 24 months. Mid and high dose females had an increase in wet/stained ventrum at 12 months and high dose male and female animals had a similar observation at 24 months; this was attributed to the urine and red staining in the perigenital area noted in the clinical observation data.

b. Organ weight

The investigators provided group summary and individual animal data for both 1 year termination animals and 2 year animals. The following table presents selected organs (from Appendix II, Tables OW1K-SUM and OW2K-SUM, pages 499 and 500 and 505 and 506):

Organ	Group:	Control	Low	Mid	High
		2 Year Males			
Kidneys	absolute	4.011	3.981	3.816	3.502*
	relative	1.183	1.156	1.144	1.136
Liver	absolute	14.903	16.573*	13.923	12.142*
	relative	4.373	4.817	4.178	3.939*
Lungs	absolute	1.924	1.965	1.868*	1.678*
	relative	0.571	0.573	0.563	0.545*
Spleen	absolute	1.096	1.699*	1.675	0.862*
	relative	0.322	0.498*	0.516	0.279*
Testes	absolute	4.989	5.043	5.650	6.689*
	relative	1.474	1.478	1.693	2.153*
		2 Year Females			
Spleen	absolute	0.968	0.878	0.642	1.164*
	relative	0.392	0.366	0.268	0.539

* = P < 0.05

Except for the lesions noted in the kidneys of high dose males (calculus), no related pathology was noted in the organs to account for the differences in organ weights. It is interesting to note that the spleen weights were reduced in males and increased in females.

c. Microscopic pathology

i. Non-neoplastic observations

The investigators provided group mean and individual animal data. The following table presents selected non-neoplastic observations (from Tables MP 1-SUM-INT and MP 1-SUM, pages 508-551 and 553-655):

Observation Group:	Control	Low	Mid	High
	12 months			
(# males/females examined)	20/20	10/10	10/10	20/20
Kidneys				
Calculus	8/11	4/3	2/6	16*/14
Urinary Bladder				
Hyperplasia				
nodular/papillary	0/0	0/0	0/0	20*/0
simple	0/0	0/0	0/0	20*/0
	24 months			
(# males/females examined)	50/50	50/50	50/50	50/50
Kidneys				
Calculus	38/16	25/27*	21/33*	29/21
Chronic nephropathy	49/27	48/28	46/22	40/32
Cyst	4/14	7/8	5/5	17*/37*
Hyperplasia	4/3	3/0	3/3	7/30*
Hyperplasia, epithelium, pelvis	31/24	22/19	16/20	26/21
Infarct	2/3	0/0	0/3	7/29*
Inflammation, acute	7/2	11/0	3/0	5/11*
Inflammation, chronic	0/0	3/2	1/1	0/0
Mineralization, papilla	0/0	0/0	0/2	0/12*
Pituitary				
Hyperplasia	14/5	7/7	13/8	13/2
Testes				
Hyperplasia, Interstitial cell	1	2	2	0
Thyroids				
(# males/females examined)	50/50	49/50	50/50	50/50
Hyperplasia, follicular cell	0/0	2/0	1/1	0/0
Hyperplasia, c-cell	15/11	13/8	11/14	12/11
Urinary Bladder				
(# males/females examined)	50/50	50/49	50/50	50/50
Calculus	3/0	1/0	1/0	21*/0
Congestion	1/0	0/0	1/0	16*/0
Hemorrhage	0/0	0/1	0/0	9*/1
Hyperplasia,				
nodular/papillary	1/0	0/0	0/0	43*/1
simple	2/0	2/0	6/0	42*/6*
Inflammation, acute	0/0	0/0	1/0	1/0
Inflammation, chronic	2/0	4/0	2/0	7/4
Mineralization	3/0	6/0	5/2	18*/1
Necrosis	0/0	0/0	0/0	20*/2

* = $p < 0.05$

ii. Neoplastic observations

The investigators provided group mean and individual animal data. The following table presents selected neoplastic observations (from Tables MP 1-SUM-INT and MP 1-SUM, pages 508-551 and 553-655):

Observation Group:	Control	Low	Mid	High
	12 months			
(# males/females examined)	20/20	10/10	10/10	20/20
Urinary Bladder				
Papilloma	0/0	0/0	0/0	6*/0
Carcinoma, transitional cell	0/0	0/0	0/0	3/0
	24 months			
(# males/females examined)	50/50	50/50	50/50	50/50
Adrenals				
Pheochromocytoma	9/0	7/1	5/0	5/3
Pheochromocytoma, malignant	0/0	0/0	3/0	1/0
Adenoma, cortical	1/0	0/1	2/1	0/0
Adenocarcinoma	0/0	1/0	0/0	0/0
Liver				
Carcinoma, hepatocellular	0/0	1/1	2/1	0/0
Lungs				
Carcinoma, alveolar/bronchiolar	1/0	0/0	0/0	0/0
Pancreas				
(# males/females examined)	50/49	50/50	49/50	50/50
Adenoma	0/0	2/0	0/0	0/0
Adenoma, Islet cell	3/0	1/0	3/0	1/0
Carcinoma, Islet cell	0/0	0/0	1/0	0/0
(# males/females examined)	50/50	50/50	50/50	50/50
Pituitary				
Adenoma	26/25	25/25	20/27	16/20
Carcinoma	0/1	0/0	0/0	1/0
Adenocarcinoma	0/0	0/0	0/0	0/1
Testes				
Interstitial cell tumor	47	43	45	46
Thyroids				
(# males/females examined)	50/50	49/50	50/50	50/50
Adenoma, c-cell	7/2	8/3	4/2	7/5
Carcinoma, c-cell	4/2	2/1	0/1	0/0
Carcinoma, follicular	0/0	0/1	0/0	0/0
Urinary Bladder				
(# males/females examined)	50/50	50/49	50/50	50/50
Carcinoma, transitional cell	0/0	0/0	2/0	34*/0
Mesothelioma	0/0	0/0	2/0	0/0
Papilloma	0/0	1/0	0/0	6*/0

* = p < 0.05

For non-neoplastic effects there was an increase in incidence of calculus in the kidneys in high dose males at the 12 month sacrifice and the 24 month study termination. There was also increased hyperplasia of the urinary bladder at 12 and 24 months in high dose males and high dose females at 24 months along with an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in high dose males. High dose males and females also had an increase in cysts in the kidney at 24 months. High dose females had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney. Relevant neoplastic observations were confined to the urinary bladder with an increased incidence of papilloma and transitional cell carcinomas of high dose males noted as early as the 12 month sacrifice and observed again at the 24 month study termination in both the mid and high dose males (transitional cell carcinoma). Females were not similarly affected.

C. Discussion/Conclusions

Systemic toxicity was noted as decreased body weights ($p < 0.05$) and body weight gains in both males and females of the mid and high dose groups during the first 13 weeks of the study (for the 2-year oncogenicity group). At 1 year and 78 weeks, there were decreased body weights ($p < 0.05$) in both males and females of the mid and high dose groups, while only the males had reduced body weight gains at 52 weeks. At 78 weeks only the high dose animals had reduced body weight gains. At study termination, only the high dose groups had reduced body weights ($p < 0.05$) and body weight gains. Food consumption was slightly decreased in the 2-year oncogenicity group in the high dose group at all time points measured and was decreased in the mid dose females at 13 weeks, mid dose males and females at 52 weeks, and mid dose females at 104 weeks. Food efficiency determined for the first 13 weeks was slightly decreased in the mid dose group and greatly decreased in the high dose group.

There was an increase in observed masses in the urinary bladder of high dose males at 24 months. High dose females has an increased incidence of kidneys with pitted zones at 24 months. Mid and high dose females had an increase in wet/stained ventrum at 12 months and both high dose males and females had a similar observation at 24 months, this was attributed to the urine and red staining in the perigenital area noted in the clinical observation data.

Non-neoplastic observations noted an increase in incidence of calculus in the kidneys in high dose males at the 12 month sacrifice and the 24 month study termination. There was also

increased hyperplasia of the urinary bladder at 12 and 24 months in high dose males (and high dose females at 24 months) along with an increase in congestion, hemorrhage, mineralization and necrosis of the urinary bladder at 24 months in high dose males. High dose males and females also had an increase in cysts in the kidney at 24 months. High dose females had an increase in hyperplasia of the kidney along with increased infarct, acute inflammation and mineralization of the kidney.

There was an increase in tumor incidence in the urinary bladder of papilloma and transitional cell carcinomas in the high dose males noted as early as the 12 month sacrifice and observed again at the 24 month study termination in both the mid and high dose males (transitional cell carcinoma). Females were not similarly affected.

Systemic Toxicity NOEL = 800 ppm (39 mg/kg/day for males and 49 mg/kg/day for females

Systemic Toxicity LOEL = 4000 ppm (200 mg/kg/day for males and 248 mg/kg/day for females) based on decreased body weight gains, decreased food consumption and reduced food efficiency, and increased clinical and gross pathological signs of toxicity.

MATERIALS AND METHODS

Mode of Administration, Doses, and Basis for their Selection

Route of administration and rationale. The probable routes of human exposure to OPP are via ingestion of foodstuffs that might contain low residues of the chemical, accidental ingestion, or dermal contact during manufacture or use. Thus, formulation with feed was the desired method of delivery to develop the chronic toxicological profile and evaluate the oncogenic potential of the test substance in the rat.

Doses selected and rationale. In this combined chronic toxicity/oncogenicity testing study in the rat, OPP was administered as an admix to the rodent's diet at concentrations of 0, 800, 4,000, or 8,000/10,000 ppm (males/females). These doses, which were approved by the EPA, were selected based upon a review of the literature [4-6] as well as upon the data generated from a *A 4-week range-finding toxicity study in the rat*. A summary of the results of this study is presented in *Appendix VI* of this general toxicology report.

Preparation, Administration, & Disposal of the Dietary Admixtures of OPP

Preparation of the test diets. An acetone/corn oil mixture was used as a vehicle to dissolve the test substance prior to mixing with the dietary carrier. The control diet (including the acetone/corn oil mixture) was prepared the same as the treated diet, excluding only the test chemical. A sample of each batch of feed mixed was taken and retained in the freezer until the study was complete and the analytical data deemed satisfactory. Replacement admixtures for each treatment group were prepared weekly and stored under freezer conditions until presented to the animals the following week.

Administration and disposal of the test diets. OPP was administered at a constant concentration in the feed for the duration of the study. Up to the day that an animal was sacrificed, its particular test diet remained continuously available for ad libitum consumption. Each week, when a fresh batch of feed was presented to the animals, any uneaten feed was collected and disposed of by incineration. Due to time constraints, males and females were not placed on study on the same day. For example, male and female animals were first administered their test diet on Wednesday and Thursday, respectively, of the same week. Thus, initiation of dosing was staggered over 2 days with "Day 0" for each sex representing the day that group of animals was first exposed to their particular test diet. Additionally, it should also be pointed out that as a result of the stagger-start all time references in the data tables to a particular day of the study are relative to the day that particular sex was first placed on study. For example, Day 7 data represents the 7th day of exposure to the test substance relative to the day that particular sex was first administered their test diet. Only in the case of male animals, do time references is the data tables represent the actual elapsed time since the experimental start (March 3, 1993) of the overall study.

Source, Number, Distribution to Dose Groups, and Age of the Animals

Source, number, and age. Male and female (nulliparous and non-pregnant) rats (CDF[F-344]/BR) were obtained from SASCO, Inc. (Madison, WI). The experimental design called for a total of 600 animals (300 males & 300 females) to be placed on study. All animals were approximately 8 weeks old at the experimental start of the study (March 3, 1993).

Distribution/grouping of animals. Following the pre-exposure acclimation period, a weight stratification-based computer program, obtained from INSTEM Computer Systems (Stone, Staffordshire, U.K.), was used to allocate the animals to one of 4 dose groups within 3 main groupings of animals (see below) on Thursday, February 25, 1993, just preceding the experimental start of the study. A summary listing of the mean post-randomization weights for each dose group is presented in *Appendix I, Table 2.*

1) ***The 1-year sacrifice group.*** This group consisted of 40 animals (20 males and 20 females) in both the control and high-dose groups and 20 animals (10 males and 10 females) in both the low and intermediate dose levels for a total of 120 animals.

2) ***The 2-year sacrifice group.*** This group consisted of 100 animals (50 males and 50 females) in all 4 dose groups for a total of 400 animals.

3) ***The replacement group.*** For approximately the first month of treatment, an additional 5 rats/dose/sex were placed on study as part of the 1- and 2-yr Sacrifice *Groups.* The purpose of these animals was to serve as potential "replacements" for any animals that unexpectedly died or developed noncompound-related problems, such as behavioral or physical abnormalities, at a very early stage in the study. When replacement occurred, the animal retained its original number and all data collected previously and in the future on the replacement animal was incorporated into the database of the dose group in which the replaced animal was a member. At the end of the replacement period, all remaining replacement animals which were not utilized for replacement purposes were sacrificed in a timely manner. In this study, 0, 2, 1, and 3 animals were replaced in the control-, low-, mid-, and high-dose groups of the 1-yr sacrifice group, and 1, 0, 2, and 3 animals were replaced in the control-, low-, mid-, and high-dose groups of the 2-yr sacrifice group.

Identification of Animals

Following distribution of the animals, each rat was tattooed on the tail with a unique number, specifying the animal's sex, dose group, cage number, and study affiliation. The specific numbers assigned to the animals for a given dose group within a major grouping are shown in *Appendix I, Table 3.*

Care and Acclimation of Animals

For additional reading regarding the care afforded the animals both prior to and during the in-life portion of this study, which was conducted at an American Association for Accreditation of Laboratory Animal Care-accredited facility, see *The Animal Care Report* which has been included in *Appendix VII* of this general toxicology report. Upon receipt from the vendor, animals were examined and subsequently killed (CO2 asphyxiation) if deviations in general appearance and/or behavior were observed. Those animals considered acceptable were individually housed in suspended stainless steel wire-mesh cages, each containing a feeder, a source of water (pressure-activated water nipples), and deotized cage board in the bedding tray.

In addition, with their arrival at the test facility (1) all animals were given free and continuous access to food (Purina Mills Rodent Lab Chow 5001-4 in "etts" form) and tap water (municipal water supply of the city of Kansas City, MO); (2) all animals were observed at least once daily for moribundity and mortality; (3) all activities, planned or unplanned, associated with the animals and/or their room was documented; and (4) prior to being released for study by a veterinarian, all animals were acclimatized to their ambient laboratory conditions (room temperature 18 to 26°C, relative humidity 40 to 70%, and a daily photoperiod of 12 hr of light [7:00 a.m. to 7:00 p.m.] alternating with 12 hr of darkness) for at least 6 days.

Analysis of feed, water, corn oil, and ambient room conditions. Animal room conditions were monitored and recorded continuously. Feed, water, and corn oil (used in diet preparation to facilitate mixing of the test substance in the feed) were periodically sampled and analyzed for a variety of potential impurities. No evidence of a confounding effect on the study objectives was suggested from the results of these analyses, which have been archived.

Body Weight and Food Consumption Determinations

Individual body weight and food consumption determinations were scheduled to be performed once each week on all animals. Body weights were also measured immediately prior to all necropsies to allow for calculation of organ to body weight ratios. In addition, using specifically defined criteria, food consumption data were corrected, as conditions dictated, to account for misleading indications of food intake (i.e., excessive spillage, clogged feeders, etc.).

Clinical observations and Mortality

General observations for moribundity and mortality were scheduled to be conducted twice daily (once on weekends and holidays). Detailed physical examinations for clinical signs of toxicity were scheduled to be performed once each week on all animals. Clinical examinations included evaluation of external surface areas, orifices, posture, general behavior, respiration, and excretory products.

Ophthalmology

Following acclimatization, pre-exposure ophthalmic exams were conducted on the entire pool of potential study animals. To the extent possible, only those rats free of ocular abnormalities and thus clinically "normal" were distributed to dose groups and subsequently placed on study. With conclusion of the 1- and 2-yr exposure periods, ophthalmic exams were conducted again on all surviving animals comprising the two sacrifice groups. The defining conditions for the 5-level grading system (minimal, mild or slight, moderate, marked, and severe) in use at this facility for evaluating ophthalmologic lesions/observations are presented in *Appendix I, Table 4.*

Clinical Pathology

Blood and urine were nominally collected at 3, 6, 12, 18, and 24 months into the study from the first 20 surviving rats/sex/dose of the *2-yr Sacrifice Group.* Blood was drawn via the orbital sinus following an overnight fast; to the extent possible, urine was collected on the same non-fasted animals the week prior to the week of bleeding. To execute the collection process, the animal's remain in their wire-mesh cages and a "metabolism tray" (to reduce fecal and food contamination of the urine) is attached to the bottom of their cage. A petri dish is placed beneath the tray to catch the urine, which is collected during the night. Generally, urine collection containers are set up during the afternoon and collected the following morning for analysis. A listing of the specific clinical pathologic parameters evaluated, their method of evaluation, as well as abbreviations and units used for each parameter is presented in *Appendix* I, *Table 5.*

Gross Pathology

All animals placed on study were subject to a postmortem examination which included (1) documenting and saving all gross lesions, (2) weighing designated organs, and (3) collecting representative tissue specimens for histopathologic evaluation. A listing of the standard tissue specimens that were preserved (nominally in buffered 10% formalin) and organs that were weighed during each autopsy is presented in *Appendix I, Table 6.*

Micropathology

With the exception of the physical identifier (tail tattoo), the vagina, and the exorbital/lacrimal, clitoral, and preputial glands, representative sections of all tissues collected were processed, embedded in paraffin, sectioned, mounted, stained with hematoxylin and eosin (H&E), and examined under a light microscope by a veterinary pathologist. The defining conditions for the 5-level grading system (minimal, mild or slight, moderate, marked, and severe) in use at this facility for evaluating non-neoplastic histopathologic lesions is presented in *Appendix I, Table 4.*

Peer review. The initial micropathologic evaluation of this study was reviewed by a pathologist not associated with the Bayer Corporation. Slides from a random selection of 10% of the animals/sex/dose group, as well as all

tumors and potential target organs, were examined in the review. The results of this secondary evaluation are presented in *Appendix IX* of this general toxicology report.

Special Tissue Processing

At necropsy, urinary bladders were gently inflated with formalin and tied off (at the urethra) to maintain a distended form while being fixed. Following fixation, each bladder was bisected longitudinally along the bladder neck. Each bladder half was longitudinally cut up to three additional times to give a maximum of four distinct sections for each tissue block. Both tissue blocks were then step sectioned to give a total of four slides for each bladder (two slides per half bladder). Generally, 12-16 longitudinal sections were evaluated for each bladder. Conditions for grading urinary bladder lesions observed in this study were previously defined [7]. In addition, Dr. S.M. Cohen, Chairman of the Department of Pathology and Microbiology at the University of Nebraska Medical Center, Omaha, Nebraska, was consulted regarding the classification and grading of the urinary bladder lesions.

Statistics

Continuous data that were examined statistically were evaluated initially for equality or homogeneity of variance using Bartlett's test [8]. Group means were further analyzed by a one-way variance analysis (ANOVA) [8] followed by Dunnett's test [9,10]. In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA [11] followed by the Mann-Whitney-U test for between-group comparisons. Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the chi-square, Fisher exact, or chi-square and Fisher exact tests. On a case by case basis, and at the discretion of the study director, data were subject to additional statistical procedures other than those mentioned above. For the Bartlett test, a probability (p) value $\leq$ 0.001 was considered significant; for all other statistical tests, differences with p values $\leq$ 0.05 were considered statistically significant. All statistical evaluations were performed using software obtained from either INSTEM Computer Systems or SAS Institute, Inc. (Cary, NC).

Archiving of Raw Data and Related Material

The final report, protocol, raw data, wet tissues, tissue blocks and slides, and a sample from the test batch of OPP (No. S-01-93) have been archived at locations specified by the Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104.

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