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

OCT 19 2000

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: **Toxicity Review** of Subchronic Oral Toxicity Study [870.3100 (§82-1)] with Immunotoxicity [870.7800 (§85-5)], Neurotoxicity [870.6200 (§83-1, §82-7, §81-8)] and One-Generation Reproduction in the Rat with Glycolic Acid

EPA Reg. No.:071654-R
Glycolic Acid
DP BARCODE: D264010, D264011, D264012, D264014
SUBMISSION CODE: S576708
P.C. CODE: 000101
EPA ID. NO.: 071654-R

From: S. L. Malish, Ph.D., Toxicologist, RASSB/AD S. L. Malish 10/10/00

To: Robert Brennis, PM 32
PM Team Reviewer, Marianne Clark
Regulatory Management Branch
Antimicrobials Division (7510C)

Thru: Winston Dang, Team Leader, Team One,
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)[7510C]

and

Norman Cook 10.19.00

Norman Cook, Chief, RASSB/AD (7510C)

Applicant: E.I. Dupont de Nemours, Inc., Wilmington, DE

FORMULATION:

Active Ingredient:	% by weight
Glycolic Acid	≈70%

Laboratory: E.I. Dupont de Nemours, Inc.'s Haskell Laboratories for Toxicology and Industrial Medicine, Newark, DE

ACTION REQUESTED: **Toxicity Review** [MRID 449753-04]: Subchronic Oral Toxicity Study [870.3100 (§82-1)] with Immunotoxicity [870.7800 (§85-5)], Neurotoxicity [870.6200 (§83-1, §82-7, §81-8)] and a One-Generation Reproduction in the Rat with Glycolic Acid

CONCLUSIONS: All studies were considered **Acceptable/Guideline** except the Reproduction and Fertility Study which was considered **Acceptable/Non-guideline.**

The **EXECUTIVE SUMMARIES** for each sub-study are listed below -

A. Subchronic Toxicity [870.3100 (§82-1)]

Animals in all studies were included in the subchronic observations until removal for specific toxicity subsets. Eight deaths [3♂, 5♀] were attributed to gavage accidents and one death in a female was attributed to causes unrelated to treatment. The gavage accidents produced of lung noises and irregular respiration and were the result of the aspiration of the highly acidic test material into the respiratory tract. Microscopic examination of the lungs revealed inflammation, hyperplasia and hypertrophy which resulted from the irritant action of the test material during or after gavage administration.

Statistically significant decreases in body weights [and body weight gains] occurred by day 92: 8% [13%] and 13% [22%] in males in the 300 and 600 mg/kg/day dose groups, respectively, and 6% [14%] and 11% [22%] in females in the 300 and 600 mg/kg/day dose groups, respectively. The reduced body weights in the 300 and 600 mg/kg/day dose groups were attributable to statistically significantly reduced food intake and food efficiency.

In the high dose males, statistically significant changes indicative of renal insufficiency were noted: increased BUN on day 93, high urine volume and low urine osmolality in the mid-dose group on day 46 and the high dose group on day 46 and 93. Absolute kidney weights increased at 600 mg/kg/day. These weight changes correlated grossly with renal pelvis dilatation and microscopically with unilateral hydronephrosis, hyperplasia of the transitional epithelium of the renal pelvis in male rats and oxalate crystal formation at both the 300 (6/10) and 600 (10/10) mg/kg/day dose levels.

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Oxalate crystal formation was not present in female rats and hydronephrosis and hyperplasia did not occur in females in a dose-dependent manner.

Other changes clinical chemistry were largely unaffected by treatment and/or changes were not toxicologically significant. The moderately increased neutrophils in both males and females in the 300 and 600 mg/kg/day dose groups resulted from the inflammatory processes in the lungs and kidneys.

Ophthalmoscopic examinations were not remarkable compared to the control group.

The LOAEL for systemic toxicity is 300 mg/kg/day and is based on decreased body weight, and body weight gain as a result of decreased food consumption and food efficiency in both males and females. Renal insufficiency [high urine volume/low urine osmolality] occurred in the mid dose males [day 46] and the high dose males [days 47 and 94] and was correlated with hyperplasia of the renal pelvis transitional epithelium, unilateral hydronephrosis, and oxalate crystal nephrosis in only male rats. Females did not show compound related kidney pathology. The NOAEL for systemic toxicity is 150 mg/kg/day in male and female rats.

This study is considered to be **Acceptable/Guideline** and fulfills guideline [OPPTS 870.3100 (§82-1)] for a subchronic oral toxicity study in the rat.

B. Immunotoxicity [870.7800 (§85-5)]

In the immunotoxicity study, administration of the test material for 23 days followed by injection of sheep red blood cells (SRBC) had no effect on the following immune parameters: relative weight of the spleen, absolute and relative weights of the thymus, immune response as measured by SRBC-specific serum IgM titer and histopathology of the spleen and thymus.

The mean absolute spleen weight [except for the decreased spleen weight in males at 600 mg/kg/day] and the mean relative weight to mean body weight at all dose levels were not

remarkable. The mean spleen weight change was considered to be of little toxicological importance and appeared due to the decrease in the mean body weight of the animals.

A LOAEL for immunotoxicity was not attained. The NOAEL for immunotoxicity is ≥600 mg/kg/day [HDT] based on no effects of glycolic acid on the humoral immune response to SRBC at the highest dose tested.

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C. Neurotoxicity [870.7800 (§85-5)]

In a neurotoxicity study, no differences in the functional observational battery observations or motor activity were observed among the control and treated groups. No treatment-related clinical

signs indicating systemic toxicity were observed. No treatment-related lesions were observed in the brain or nerve tissue selected for neuropathological evaluation.

A LOAEL for neurotoxicity was not attained. Based on the absence of clinical signs related to neurotoxicity, effects on behavior and activity, and lesions in neurological tissue, the NOAEL for neurotoxicity is ≥600 mg/kg/day [HDT].

This study is considered Acceptable/Guideline and fulfills [OPPTS 870.6200 (§81-1, §82-7, §83-8)] guidelines for a neurotoxicity study in the rat.

D. One Generation Reproduction and Fertility Effects [§870.3550]

The reproduction study was initiated with reduced body weights in both treated males and females on day 92 following the 90-day subchronic study; lower body weights continued for most groups through the mating, gestation, and lactation period, with treated females improving slightly by study termination and treated males declining further compared with the controls. The same kidney lesions with oxalate crystal formation observed in the subchronic study were observed in the kidneys of males in the reproduction study. Dose-dependent increased relative testes weights were attributed to dose-dependent reduced body weights. Although body weights of P_1 males and females were reduced, there were no effects on reproduction indices of percent mating, fecundity, gestation length, number of implantation sites or implantation efficiency in any treated group. There were no differences attributable to treatment in litter size, pup viability, pup weight, or gross lesions among pups from litters of control and treated dams.

Parental NOAEL/LOAEL: Maternal/Paternal - see subchronic toxicity.
Reproductive Effects: A LOAEL for reproductive effects was not attained. Based on the absence of effects on reproduction indices and on pup litter size, survival and weight, the NOAEL for reproductive toxicity is ≥600 mg/kg/day [HDT].

This study is considered **unacceptable/upgradable**. The study was intended as a screening study to measure potential reproductive effects from oral administration of glycolic acid. While the data showed no significant reproductive effects, data on histopathological examination of the ovaries, testes, and epididymides was not provided per guideline requirements and is needed for a complete screening evaluation. The study can be upgraded to acceptable if detailed histopathological information is provided for the ovaries, testes, and epididymides of the high dose and control parental animals as detailed in the guideline [§870.3550].

EPA Reviewer: Steven L. Malish, Ph.D.
Team 1 RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Tim McMahon, Senior Scientist
RASSB/Antimicrobials Division (7510C)

S L Malish 10/5/00
10/12/00

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study [870.3100 (§82-1)] with Immunotoxicity [870.7800 (§85-5)] , Neurotoxicity [870.6200 (§83-1, §82-7, §81-8)] and One-Generation Reproduction [Non-Guideline] /Rat

DP BARCODE: D264010, D264011, D264012, D264014
P.C. CODE: 000101
SUBMISSION CODE: S576708
EPA ID. NO.: 071654-R

TEST MATERIAL (PURITY): Glycolic Acid 70% Solution (70.58% a.i.)

SYNONYMS: Hydroxyethanoic acid 70% solution; acetic acid, hydroxy- 70% solution

CITATIONS: Kreckmann, K.H. (1999). Glycolic acid 70% solution: Subchronic Toxicity 90-Day Gavage Study in Rats with Immunotoxicity, Neurotoxicity, and One-Generation Reproduction Evaluations. E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. Laboratory Study No. 1597, August 13, 1999. MRID 44975304. Unpublished.

SPONSOR: DuPont Specialty Chemicals, E.I. du Pont de Nemours and Company, Wilmington, DE 19898.

EXECUTIVE SUMMARY: Glycolic acid (70.58% a.i.) was administered, by gavage [10 ml/kg] to a total of 320 Crl:CD®(SD) IGS BR rats [divided into 4 groups of 10 animals/sex/ at doses of 0, 150, 300 or 600 mg/kg/day. The study consisted of 90 day subchronic and neurotoxicity subparts, a 29 day immunotoxicity subpart, and a reproduction subpart, consisting of mating, gestation and lactation phases initiated after the subchronic portion of the study was completed. In the reproduction subpart, the test compound in P_1 males was administered up to the day 131 sacrifice, while in P_1 females the test compound was administered up to the sacrifice on day 21 of lactation.

A. Subchronic Toxicity [870.3100 (§82-1)]

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Animals in all studies were included in the subchronic observations until removal for specific toxicity subsets. Eight deaths [3♂, 5♀] were attributed to gavage accidents and one death in a female was attributed to causes unrelated to treatment. The gavage accidents produced of lung

noises and irregular respiration and were the result of the aspiration of the highly acidic test material into the respiratory tract. Microscopic examination of the lungs revealed inflammation, hyperplasia and hypertrophy which resulted from the irritant action of the test material during or after gavage administration.

Statistically significant decreases in body weights [and body weight gains] occurred by day 92: 8% [13%] and 13% [22%] in males in the 300 and 600 mg/kg/day dose groups, respectively, and 6% [14%] and 11% [22%] in females in the 300 and 600 mg/kg/day dose groups, respectively. The reduced body weights in the 300 and 600 mg/kg/day dose groups were attributable to statistically significantly reduced food intake and food efficiency.

In the high dose males, statistically significant changes indicative of renal insufficiency were noted: increased BUN on day 93, high urine volume and low urine osmolality in the mid-dose group on day 46 and the high dose group on day 46 and 93. Absolute kidney weights increased at 600 mg/kg/day. These weight changes correlated grossly with renal pelvis dilatation and microscopically with unilateral hydronephrosis, hyperplasia of the transitional epithelium of the renal pelvis in male rats and oxalate crystal formation at both the 300 (6/10) and 600 (10/10) mg/kg/day dose levels.

Oxalate crystal formation was not present in female rats and hydronephrosis and hyperplasia did not occur in females in a dose-dependent manner.

Other changes clinical chemistry were largely unaffected by treatment and/or changes were not toxicologically significant. The moderately increased neutrophils in both males and females in the 300 and 600 mg/kg/day dose groups resulted from the inflammatory processes in the lungs and kidneys.

Ophthalmoscopic examinations were not remarkable compared to the control group.

The LOAEL for systemic toxicity is 300 mg/kg/day and is based on decreased body weight, and body weight gain as a result of decreased food consumption and food efficiency in both males and females. Renal insufficiency [high urine volume/low urine osmolality] occurred in the mid dose males [day 46] and the high dose males [days 47 and 94] and was correlated with hyperplasia of the renal pelvis transitional epithelium, unilateral hydronephrosis, and oxalate crystal nephrosis in only male rats. Females did not show compound related kidney pathology. The NOAEL for systemic toxicity is 150 mg/kg/day in male and female rats.

This study is considered to be **Acceptable/Guideline** and fulfills guideline [OPPTS 870.3100 (§82-1)] for a subchronic oral toxicity study in the rat.

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B. Immunotoxicity [870.7800 (§85-5)]

In the immunotoxicity study, administration of the test material for 23 days followed by injection of sheep red blood cells (SRBC) had no effect on the following immune parameters: relative weight of the spleen, absolute and relative weights of the thymus, immune response as measured by SRBC-specific serum IgM titer and histopathology of the spleen and thymus.

The mean absolute spleen weight [except for the decreased spleen weight in males at 600 mg/kg/day] and the mean relative weight to mean body weight at all dose levels were not remarkable. The mean spleen weight change was considered to be of little toxicological importance and appeared due to the decrease in the mean body weight of the animals.

A LOAEL for immunotoxicity was not attained. The NOAEL for immunotoxicity is ≥600 mg/kg/day [HDT] based on no effects of glycolic acid on the humoral immune response to SRBC at the highest dose tested.

This study is considered **Acceptable/Guideline** and fulfills the guidelines [OPPTS 870.7800 (§85-7)] for a immunotoxicity study the rat.

C. Neurotoxicity [870.6200 (§83-1, §82-7, §81-8)]

In a neurotoxicity study, no differences in the functional observational battery observations or motor activity were observed among the control and treated groups. No treatment-related clinical signs indicating systemic toxicity were observed. No treatment-related lesions were observed in the brain or nerve tissue selected for neuropathological evaluation.

A LOAEL for neurotoxicity was not attained. Based on the absence of clinical signs related to neurotoxicity, effects on behavior and activity, and lesions in neurological tissue, the NOAEL for neurotoxicity is ≥600 mg/kg/day [HDT].

This study is considered **Acceptable/Guideline** and fulfills [OPPTS 870.6200 (§81-1, §82-7, §83-8)] guidelines for a neurotoxicity study in the rat.

D. One Generation Reproduction and Fertility Effects [§870.3550]

The reproduction screening study was initiated with reduced body weights in both treated males and females on day 92 following the 90-day subchronic study; lower body weights continued for most groups through the mating, gestation, and lactation period, with treated females improving slightly by study termination and treated males declining further compared with the controls. The same kidney lesions with oxalate crystal formation observed in the subchronic study were observed in the kidneys of males in the reproduction study. Dose-dependent increased relative testes weights were attributed to dose-dependent reduced body weights. Although body weights of P_1 males and females were reduced, there were no effects on reproduction indices of percent

mating, fecundity, gestation length, number of implantation sites or implantation efficiency in any treated group. There were no differences attributable to treatment in litter size, pup viability, pup weight, or gross lesions among pups from litters of control and treated dams.

Parental NOAEL/LOAEL: Maternal/Paternal - see subchronic toxicity.
Reproductive Effects: A LOAEL for reproductive effects was not attained. Based on the absence of effects on reproduction indices and on pup litter size, survival and weight, the NOAEL for reproductive toxicity is ≥600 mg/kg/day [HDT].

This study is considered **unacceptable/upgradable**. The study was intended as a screening study to measure potential reproductive effects from oral administration of glycolic acid. While the data showed no significant reproductive effects, data on histopathological examination of the ovaries, testes, and epididymides was not provided per guideline requirements and is needed for a complete screening evaluation. The study can be upgraded to acceptable if detailed histopathological information is provided for the ovaries, testes, and epididymides of the high dose and control parental animals as detailed in the guideline [§870.3550].

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glycolic acid [70.58% a.i.]

   Description: pale-yellow liquid
   Lot/Batch #: Not provided
   Purity: 70.58% (total acid)
   Stability of compound: Stable in commercially supplied water for 5 hours at room temperature and 14 days under refrigeration.

2. Vehicle and/or positive control

   Dosing solutions were prepared in commercially supplied water.

3. Test animals

   Species: Albino rat
   Strain: Crl:CD® (SD) IGS BR
   Age and weight at study initiation: 48 days on first day of dosing; males: 231.7 g; females: 164.5 g
   Source: Charles River Laboratories, Inc., Raleigh, NC
   Housing: One animal/cage except during mating; stainless steel, wire-mesh cages suspended above cage boards. Beginning on gestation day 20 and during lactation,

adult females were housed with their litters in polycarbonate pans with bedding; following a cohabitation period, females without evidence of copulation were also individually housed in polycarbonate pans with bedding.
Diet: Animals were fed Certified Rodent Checkers LabDiet® 5002 (PMI Nutrition International, Inc.) *ad libitum.* Drinking water (tap) was available *ad libitum.*
Environmental conditions:
Temperature: 23 ± 1°C
Humidity: 50 ± 10%
Air changes: Not reported
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 7 days following 6-day quarantine

B. STUDY DESIGN

1. In life dates

Subchronic: Start: October 5, 1998; end: January 7, 1999
Immunotoxicity: Start: October 5, 1998; end: November 2, 1998
Neurotoxicity: Start: October 5, 1998; end: January 8, 1999
Reproduction: Start: October 5, 1998; end: March 6, 1999

2. Animal assignment

Animals were assigned to the test groups in Table 1 by means of computerized, stratified randomization following selection for adequate body weight (20% of the mean within a sex) and freedom from clinical signs. Animals in the subchronic, and neurotoxicity parts of the study were dosed for 90 days. Animals in the immunotoxicity part of the study were dosed for 29 days while animals in the reproduction part were dosed for 131 days [male] and through lactation in the female. Subchronic, immunotoxicity, neurotoxicity and reproductive toxicity endpoints were based on 10 animals/sex/ group, but for parameters such as body weight during the first 90 days of dosing, all animals not yet sacrificed in the other subparts were included. Animals received the test material daily by gavage.

TABLE 1. Study design^

Dose group	Dose Level (mg/kg)	Number of animals							
		Subchronic Toxicity		Immunotoxicity		Neurotoxicity		Reproduction	
		Male	Female	Male	Female	Male	Female	Male	Female
1 (Control)	0	10	10	10	10	10	10	10	10
2	150	10	10	10	10	10	10	10	10
3	300	10	10	10	10	10	10	10	10
4	600	10	10	10	10	10	10	10	10

3. Dose selection rationale

Doses were selected based on results of a developmental toxicity study (Haskell Laboratory Report No. 191-96) [MRID 445895-01] in which glycolic acid was administered to groups of 25 Crl:CD® BR female rats by gavage on days 7-21 of gestation at daily doses of 0, 75, 150, 300, or 600 mg/kg/day. In that study, adverse effects occurred in dams administered 600 mg/kg/day. These effects included statistically significantly lower body weight gain and food consumption and clinical signs of abnormal gait/staggering, lung noise, irregular respiration, and lethargy. Mean fetal weight was significantly reduced and there was a significant increase in the incidence of skeletal fetal malformations (fused and absent ribs, fused vertebra, hemivertebra, and abnormally fused and cleft/nonfused sternebra) and variations. Lung noise was also present in 2/25 dams in the 300 mg/kg/day group and there was an increase (not statistically significant) in the incidence of skeletal malformations. No effects were observed in the groups receiving 150 or 75 mg/kg/day.

4. Test material preparation and analysis

Dosing solutions were prepared at concentrations of 15, 30, and 60 mg/mL by diluting the glycolic acid with commercially supplied water. The solutions were stored under refrigeration for up to 14 days. The solutions were administered by oral gavage at a dose volume of 10 mL/kg to achieve dose levels of 150, 300, and 600 mg/kg/day, based on the most recently recorded weights of the animals. For pregnant rats from gestation day 18 until delivery, doses were based on the GD 18 body weights.

Three samples of the control dosing solution (water) and seven samples/ concentration of the 15, 30, and 60 mg/mL solutions were taken on the first day of dosing (October 5, 1998). Two samples at each level and one control sample were analyzed to determine concentrations. One sample at each level was held at room temperature for 5 hours and analyzed to determine stability. The remaining four samples of each dosing solution were refrigerated for 7 or 14 days prior to analyses for stability (two samples on each day). Samples were also taken for concentration verification throughout the study (November 10 and December 29, 1998 and January 11 and February 23, 1999). The undiluted sample of glycolic acid supplied by the sponsor was also tested for stability by analyzing samples taken on test day 2 and test day 143.

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Homogeneity analysis: Dosing solutions were not tested for homogeneity.

Stability analysis: The percent active ingredient of the glycolic acid 70% solution was 69.5% on test day 2 and 68.1% on test day 143.

After 5 hours of storage at room temperature, the concentrations of the test material in the single samples of the 15, 30, and 60 mg/mL dosing solutions were 100.0, 97.7, and 106.6% of nominal concentrations. Following 7 days of refrigeration, average concentrations in the 15, 30, and 60 mg/mL dosing solutions were 102.7, 105.3, and 109.3% of nominal. Following 14 days of refrigeration, the respective average values were 103.3, 98.7, and 107.0% of nominal.

Concentration analysis: Mean concentrations of the 15, 30, and 60 mg/mL dosing solutions sampled on test day 1 were 98.7, 98.3, and 107.5%, respectively of nominal. Mean concentrations of the dosing solutions taken on test days 37, 86, 99, and 142 ranged between 92.0 and 98.7% of nominal for the 15 mg/mL dosing solution, between 97.0 and 100.3% of nominal for the 30 mg/mL dosing solution, and between 97.0 and 102.7% of nominal for the 60 mg/mL dosing solution.

The analytical data indicated that the test substance was stable for 5 hours at room temperature and 14 days under refrigeration and that the actual doses to the animals were within an acceptable range.

5. Statistics

A one-way analysis of variance (ANOVA) followed by Dunnett's test for comparison with the mean was performed for body weight, body weight gain, food consumption, food efficiency, and gestation length. Clinical pathology and immunotoxicology parameters were examined with Levene's test for homogeneity and the Shapiro-Wilk test for normality. If these tests were not significant, an ANOVA followed with Dunnett's test was applied; if the results of the preliminary tests were significant, the Kruskal-Wallis test was followed by Dunn's test. If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. Depending on the data a sequential application of the Jonckheere-Terpstra trend test was alternately applied to immunotoxicology parameters.

Data for motor activity were examined for homogeneity and normality by Levene's test and Shapiro-Wilk tests; if the results of these tests were not significant, an ANOVA followed with linear contrasts was applied; if the tests for homogeneity and normality were significant, the Kruskal-Wallis test was followed with a modified Dunn's test. Organ weight, grip strength, and foot splay were tested for homogeneity of variances with Bartlett's test. Significant results were tested with the Kruskal-Wallis test followed by Dunn's test; nonsignificant results were examined with an ANOVA followed with Dunnett's test.

Incidences of clinical observations, FOB parameters, mating index, gestation index and litter survival were analyzed by sequential application of the Cochran-Armitage test for trend. Implantation site numbers, implantation efficiency, sex ratio, percent

born alive, viability index, and lactation index were analyzed by the Jonckheere-Terpstra trend test. Mean pup weights with covariates of litter size and sex ratio were analyzed with a linear contrast of the least square means.

Except for Bartlett's test ($p<0.005$), significance was judged at the $p<0.05$ level.

C. METHODS

1. Observations

All animals were observed once daily for clinical signs of abnormal behavior and appearance, morbidity, and mortality. At weighing, each rat was individually handled and examined for abnormal behavior and appearance. [Rats in the neurotoxicity subgroup were evaluated at approximately one to two hours after dosing on test day 1 and approximately one to two hours after dosing on one day during the weeks that the functional observational battery was conducted].

2. Body weight

All rats in the subchronic toxicity, immunotoxicity, neurotoxicity, and reproductive toxicity substudies were weighed weekly during the 90-day gavage part of the study. In addition, the neurotoxicity subgroup rats were weighed on the day of neurotoxicity evaluations. During the reproduction part of the study (following the 90-day gavage administration), male rats were weighed weekly and female rats were weighed on gestation days 0, 7, 14, 18, and 21 and during lactation on days 0, 7, 14, and 21. Females that did not deliver were weighed weekly. Pups were weighed on days 0 (birth), 4, 7, 14, and 21.

3. Food consumption and food efficiency

Food consumption for all animals was determined over each weighing period and for each female in the reproduction study on gestation days 0, 7, 14, and 21. Mean daily food consumption and mean food efficiency were calculated by the study authors.

Weekly mean food efficiencies for all treatment groups (all studies combined, males and females separately) were calculated by the study authors for the subchronic/ pre-mating period using the formula [g body weight gain/g food consumed]. Food efficiencies were also calculated for days 1-29 and 1-92.

4. Ophthalmoscopic examination 12

Ophthalmoscopic examinations were conducted by a veterinary ophthalmologist on all rats prior to study initiation (test day -9) and on rats in the subchronic study on test

day 86 prior to scheduled sacrifice. Examinations were conducted under subdued lighting following induction of mydriasis with a 1% tropicamide solution.

5. Blood was collected from the orbital sinus of each rat in the subchronic study, under light anesthesia with carbon dioxide, for hematology and clinical chemistry examinations. Samples were taken on test days 46 and 93 for male rats and on test days 47 and 94 for female rats. Rats were fasted for 16 hours prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		Heinz body determination
	(Thromboplastin time)		RBC morphology
	(Clotting time)		Methemoglobin
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Albumin/globulin ratio
	Magnesium	X	Serum creatinine*
X	Phosphorus*	X	Serum urea nitrogen* (urea)
X	Potassium*	X	Total cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Sorbitol dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

6. Urinalysis*

Urinalysis was conducted on samples taken from animals in the subchronic study (one day prior to the blood sampling). The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrites
X	Protein	X	Urobilinogen
X	Osmolality		

*Not required for subchronic studies by Subdivision F Guidelines.

7. Sacrifice and pathology

A complete necropsy was conducted on all animals that died prematurely or were sacrificed moribund. All animals in the subchronic toxicity groups surviving the treatment period were sacrificed on test day 93 (males) or test day 94 (females) by exsanguination under carbon dioxide anesthesia. Gross morphological examination was conducted on all rats and major tissues and organs. The CHECKED (X) tissues from all subchronic dose groups were examined microscopically. The DOUBLE-CHECKED (XX) tissues were also weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)^{T}
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum* (sm. intest.)	X	Spleen*	X	Eyes (optic n.)^{T}
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal glands*
X	Colon* (lg. intest.)	XX	Kidneys*$^{+}$	X	Lacrimal gland^{T}
X	Rectum*	X	Urinary bladder*	X	Mammary gland^{T}
XX	Liver*$^{+}$	XX	Testes*$^{+}$	X	Parathyroids*
	Gall bladder*	X	Epididymides	X	Thyroids*
X	Pancreas*	X	Prostate		Zymbal gland
		X	Seminal vesicle	X	Harderian gland
	RESPIRATORY	XX	Ovaries		
X	Trachea*	X	Uterus*		OTHER
X	Lung*	X	Vagina	X	Bone (femur with joint)
X	Nose			X	Skeletal muscle
X	Pharynx			X	Skin
X	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
$^{+}$ Organ weight required in subchronic and chronic studies.
^{T} Required only when toxicity or target organ

8. Immunological evaluations

On test day 23, rats in the immunotoxicity study were injected intravenously in the lateral tail vein with 0.5 mL of 4 x 10^8 Sheep Red Blood Cells (SRBC)/mL. Six days later, on test day 29, the rats were euthanized by carbon dioxide anesthesia and exsanguination. The spleen and thymus were removed and weighed (these organs were not weighed in the subchronic study). Serum was collected and analyzed for SRBC-specific IgM antibody using the enzyme-linked immunosorbent assay (ELISA). As a positive control, sera previously collected from rats injected with SRBC and dosed with the known immunosuppressive agent cyclophosphamide were analyzed concurrently.

9. Neurotoxicity evaluations

Acute Toxicity - The 10 males and 10 females in the neurotoxicity study were evaluated at approximately one to two hours after dosing on day 1 and approximately one to two hours after dosing during the weeks that the functional observational battery was conducted.

Functional observational battery (FOB) - The 10 males and 10 females in the neurotoxicity study were evaluated by the FOB. The rats were divided into four subgroups which were tested over a two-day period. Baseline FOB parameters were established on test days -3 and -4. The FOB was performed prior to dosing three times during treatment: on test days 9 and 10, 45 and 46, and 86 and 87.
Home cage observations were made of the presence of palpebral closure and abnormal activity (writhing, circling, biting), gait, or body posture. During handling (removal from the home cage), rats were assessed for fur appearance, ease of removal, ease of handling, and muscle tone. Presence of vocalizations, piloerection, bite marks, palpebral closure, lacrimation, exophthalmus, and salivation were recorded. In the open field, rats were evaluated for activity level, grooming, posture, ease of respiration, rate of respiration, coordination, locomotion, gait, and righting reflex. Rats were also observed/evaluated for convulsions/ tremors, defecation, diarrhea, urination, palpebral closure, and vocalizations. Responses to approach/touch, noise, and tail pinch were recorded. Pupillary constriction in response to a beam of light was observed. Forelimb and hindlimb grip strength and hindlimb splay were measured.

Motor activity sessions were conducted following the FOB and prior to dosing on the same day as the FOB assessments. Duration and number of movements were automatically assessed in a Coulbourn® activity monitor. Groups and gender were balanced as to time of day. Each 60-minute session was composed of six successive 10-minute blocks. The data from the 10-minute blocks were analyzed separately.

Pathology examinations were performed on six randomly selected rats/sex/group after approximately 13 weeks of administration of the test substance. Six untreated rats/sex served as controls. On test days 94 and 95, three rats/sex/group (on each day) were euthanized by pentobarbital anesthesia followed by exsanguination and whole body perfusion fixation. Animals were examined grossly during this process. Tissue samples from the nervous system (six regions of the brain; two levels of the spinal cord; sciatic, tibial and sural nerves; gasserian ganglion; cervical and lumbar dorsal root fibers and ganglia; and cervical and lumbar ventral root fibers) and skeletal muscle (gastrocnemius) were taken. Tissues from the control and high-dose group were processed for histopathology.

10. Reproductive assessment

The test compound in P_1 males was administered up to the day 131 sacrifice, while in P_1 females the test compound was administered up to the sacrifice on day 21 of lactation [Conversation with study director, Kim H. Kreckmann on October 5, 2000].

Treatment with glycolic acid was discontinued during the reproductive assessment. On test day 97, females were housed individually with a randomly selected male of the same dose group until evidence of copulation was observed or after two weeks when there was no evidence of copulation. Females were transferred back to their cages until day 20 of gestation at which time they were transferred to polycarbonate pans. Pregnant females were observed twice daily for signs of delivery and pups. At delivery (day 0 postpartum) live and dead pups in each litter were counted and live pups were individually weighed. Pups were handled and observed for abnormal behavior on each of the following observation days. On day 4 postpartum, litters were culled randomly to eight pups (four/sex when possible); litters of less than eight pups were not culled. Litter counts and individual pup weights were determined prior to and after culling. On days 7, 14, and 21 postpartum, pups in each litter were counted by sex and individually weighted. On day 21 postpartum, F_1 offspring (weanlings) were sacrificed by carbon dioxide asphyxiation and given gross pathological evaluations. Gross lesions from weanlings showing gross kidney pathology were evaluated microscopically. Offspring that were found dead during the lactation period also underwent gross pathological evaluation.

For reproductive assessment, the proportion of affected fetuses per litter or the litter mean was used as the experimental unit:

Mating index (%) = (number copulating/number cohabited) x 100
Fecundity index (%) = (number bearing litters/number copulating) x 100
Gestation index (%) = (number of litters with at least one live pup/number of litters) x 100
Implantation efficiency (%) = (number of pups born/number of implantation sites) x 100
Pups born alive (%) = (number of pups born alive/number of pups born) x 100
Viability index (%) = (number of pups alive [day 4 preculling]/number of pups born alive) x 100

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Lactation index (%) = (number pups alive at weaning/number pups alive day 4 preculling) x 100
Litter survival (%) = (number of litters weaned/number of viable litters delivered) x 100

All P_1 parental rats, including rats that had died or were sacrificed *in extremis*, and females that had not successfully mated were sacrificed by carbon dioxide asphyxiation and exsanguination and subjected to gross pathological examination. P_1 male rats were sacrificed following production of litters (study day 131), and P_1 females were sacrificed on lactation day 21 (study days 142 to 153). The testes of each male were weighed and the uteri of all cohabited female rats were grossly examined for the presence and number of implantation sites. All gross lesions were preserved. [Testes of the P_1 males were not examined microscopically in the reproduction study, but were examined in animals terminated in the subchronic study].

II. RESULTS

A. SUBCHRONIC TOXICITY

1. Mortality and clinical signs

Survival was based on 30 animals/sex/group (the 10 animals/sex/group in the immunotoxicity study were sacrificed on day 29). At 92 days, survivals in the 0, 150, 300, and 600 mg/kg/day treatment groups were 29, 30, 29 and 28 animals, respectively, for males and 30, 30, 28 and 26 animals, respectively, for females. The day of death of each animal in each subgroup was listed below.

Deaths during the Study^

Sex	0 mg/kg/day	150 mg/kg/day	300 mg/kg/day	600 mg/kg/day*
Males	1 at day 75 (N)(GA)	———	1 at day 90 (N)(GA)	1 at day 73 (R)(GA) 1 at day 46 (S)(CR)
Females	———	———	1 at day 26 (N)(GA) 1 at day 82 (S)(NCR)	1 each at days 13, 35, 39 (N)(GA) 1 at day 78 (N)(GA)

^Adapted from Table, p. 47 and Table 134, p. 106 (MRID 44975304).
N = Neurotoxicity subset, R = Reproduction toxicity subset, S= Subchronic toxicity subset, NCR = Non-compound related death, CR = Compound related death, GA = Gavage accident (trauma related death)

There were no clinical signs related to systemic toxicity in any treated group. Clinical signs of irregular respiration and lung noise, primarily in male and female rats in the 300 and 600 mg/kg/day groups appeared to be related to treatment with dosing solutions of a low pH. These signs resulted from the occasional aspiration of very small amounts of the dosing solutions during the dosing procedure and subsequent irritation of the respiratory tract. Incidences of lung noise in male rats in the 0, 150, 300, and 600 mg/kg/day dose groups were 1/40, 7/40, 23/40, and 28/40, respectively. Incidences of lung noise in female rats in the respective dose groups were 0/40, 1/40,

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9/40, and 21/40, respectively. Differences between the middle and high dose groups and controls were judged by the authors to be the result of the difference in pH of the dosing solutions (pH values of 6.41, 1.95, 1.77, and 1.64 in the 0, 150, 300, and 600 mg/kg/day groups, respectively) and therefore differences in respiratory tract irritation.

2. Body weight and body weight gain

For the first 29 days of the study, body weights, body weight changes, and food consumption were based on the total number of treated animals (40/sex/group). Following sacrifice of the animals in the immunotoxicity subgroup on test day 29, these parameters were based on 30 animals/sex/group (or fewer when animals died). Administration of glycolic acid caused lower body weight gains in males and females in the 300 and 600 mg/kg/day dose groups compared with the control groups, resulting in lower final body weights (Table 2). For males in the 300 and 600 mg/kg/day groups, body weights were statistically significantly lower by test days 43 and 22, respectively. Similar depressions were observed in the respective female groups on test days 78 and 36. At study termination (test day 92), mean body weights of males in the 300 and 600 mg/kg/day groups were 92% and 87% of controls values, respectively ($p<0.05$ for both). For females, final mean body weights in the 300 and 600 mg/kg/day groups were 94% and 89% of the control weight, respectively ($p<0.05$ for both). Final body weights for males and females in the groups administered 150 mg/kg/day were only slightly lower than the controls, 98% and 96%, respectively. Body weight gains followed similar trends, but with greater differences from control values. For males, final mean body weight gains in the 300 and 600 mg/kg/day groups were 87% and 78% of the control weight, respectively ($p<0.05$ for both). For females, final mean body weight gains in the 300 and 600 mg/kg/day groups were 86% and 78% of the control weight, respectively ($p<0.05$ for both) [Table 2]..

3. Food consumption and food efficiency

Mean daily food consumption for days 1-92 for males in the 300 and 600 mg/kg/day groups were statistically significantly lower than that of controls (94% and 90% of the control value, respectively; $p<0.05$ for both) (Table 2). For females in the 300 and 600 mg/kg/day groups, values were 95% (not significant) and 91% of the control value ($p<0.05$). Mean daily food consumption for males and females in the 150 mg/kg/day treatment was similar to that of the respective control groups. Week to week food consumption was similar for the treated and untreated animals in each group over the course of the study.

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Mean food efficiency for male and female rats for days 1-92 was reduced in the 300 and 600 mg/kg/day groups. Respective percentages of control values were 92% and 87% for males and 91% and 86% for females (all $p<0.05$). Mean food efficiencies

were lower than control values for both males and females in the 150 mg/kg/day groups, but the differences were not statistically significant.

4. Compound consumption

The test compound was administered by gavage at the stated doses.

TABLE 2. Group mean body weights (g) on selected days, total body weight gains (g) food consumption (g), and overall food efficiency of rats gavaged with glycolic acid for 92 days[a,b] ^

Day of study	Exposure concentration (mg/kg/day)			
	0	150	300	600
Males				
1	232.3	232.6	232.5	229.5
15	338.5	338.3	331.0	327.5
29	421.1	416.6	406.6	394.4*
64	508.0	495.8	471.4*	449.6*
92	553.3	539.6 (98)	510.6 (92)*	481.0 (87)*
Total weight gain (day 1 to 92)	323.1	308.3 (95)	280.4 (87)*	252.5 (78)*
Mean daily food consumption (day 1 to 92)	28.1	27.8 (99)	26.5 (94)*	25.2 (90)*
Mean food efficiency+ (day 1 to 92)	0.126	0.121 (96)	0.116 (92)*	0.110 (87)*
Females				
1	164.3	165.0	165.0	163.7
15	207.9	212.3	215.1	210.7
29	249.5	251.4	251.0	237.9
64	292.7	288.5	280.2	268.5*
92	318.1	306.2 (96)	298.4 (94)*	284.1 (89)*
Total weight gain (day 1 to 92)	153.7	140.0 (91)	131.6 (86)*	119.8 (78)*
Mean daily food consumption (day 1 to 92)	20.6	20.0 (97)	19.5 (95)	18.7 (91)*
Mean food efficiency+ (day 1 to 92)	0.081	0.077 (95)	0.074 (91)*	0.070 (86)*

^Data taken from Tables 2-9, pp. 85-100, MRID 44975304.
*Significantly different from control, $p < 0.05$.
[a] 40 animals/sex/group through day 29; 30 animals/sex/group thereafter.
[b] Values in parenthesis are percent of control values.
+ () gm weight gain/gm food consumed.

5. Ophthalmoscopic examination

Ophthalmoscopic examinations revealed no treatment-related abnormalities.

6. Hematology

Toxicologically significant changes in hematology parameters were limited to male rats in the mid- and high-dose groups. Neutrophils were increased in male rats to a similar degree at mid study in the 300 and 600 mg/kg/day dose groups (Table 3). At study termination on day 93, an increase was observed only in the 600 mg/kg/day group, but, compared to the control group, the difference was not statistically significant; at this time, the mean value in the 300 mg/kg/day group was similar to, but lower than the control mean, indicating a transient change in this group. In females, the mean number of neutrophils in the 300 and 600 mg/kg/day dose groups were slightly, but not statistically significantly elevated at both time points. Hemoglobin was slightly lower at mid study in males in the 300 mg/kg/day group and in females in the 600 mg/kg/day group, but there was no clear dose-response relationship for either sex and the differences were insignificant at study termination. All other hematology changes were either not dose-related (greatest changes occurred in the low-dose group) or judged toxicologically insignificant, i.e., they were within the control group or historical control group ranges.

TABLE 3. Hematology parameters of rats gavaged with glycolic acid for 90 days^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
Males				
Hemoglobin (g/dL)				
day 46	15.5	15.3	14.4*	15.0
day 93	15.2	15.0	15.6	15.3
Neutrophils (/μL)				
day 46	932	1117	1663*	1749*
day 93	1692	1483	1436	2783
Females				
Hemoglobin (g/dL)				
day 47	15.4	14.8	14.9	14.8*
day 94	16.1	15.5*	15.6	15.4
Neutrophils (/μL)				
day 47	1032	949	1309	1310
day 94	1205	910	1316	1635

^Data were taken from Tables 16 and 17, pp. 109-114, MRID 44975304.
*Statistically significant, $p<0.05$.

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7. Clinical chemistry

Toxicologically significant [$p<0.05$] changes in clinical chemistry parameters were observed in male rats in the mid- and high-dose groups (Table 4). These included decreases in total protein and albumin (mid study and study termination) and an increase in blood urea nitrogen [high-dose group, study termination [(13 vs 22 ($C_{control}$)]. Decreases in serum globulin were mild. An non-statistically significant increase in BUN was seen on day 93 [16 vs. 13 C]. Potassium was statistically but mildly decreased [5.9 vs. 6.3 C] in male rats in the 600 mg/kg/day group (study termination). Total protein, albumin, and globulin were affected to a lesser degree in females and the blood urea nitrogen was decreased rather than increased, although not significantly by study termination. Females also had decreased sorbitol dehydrogenase activity [high dose group, mid-study (14.9 vs. 10.9 C) and study termination (16.5 vs. 21.8 C)], lower potassium levels [high-dose group, study termination (5.3 vs. 5.9 C)] and an increased glucose [high-dose group, study termination (124 vs. 105 C)]. Other statistically significant changes were considered to be minor fluctuations and of no toxicological importance.

TABLE 4. Clinical chemistry parameters in rats gavaged with glycolic acid for 90 days^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
Males				
Total protein (g/dL)				
day 46	7.0	6.7	6.4#	6.4#
day 93	7.0	6.6*	6.5*	6.4*
Albumin (g/dL)				
day 46	4.8	4.7	4.5*	4.5*
day 93	4.7	4.6	4.4*	4.3*
Globulin (g/dL)				
day 46	2.1	2.0	1.9*	1.9
day 93	2.3	2.0	2.1	2.1
Blood urea nitrogen (mg/dL)				
day 46	15	16	15	15
day 93	13	13	16	22#
Females				
Total protein (g/dL)				
day 47	7.6	7.1	7.0*	6.7*
day 94	7.7	7.5	7.3	7.1*
Albumin (g/dL)				
day 47	5.7	5.3	5.1*	5.2
day 94	5.7	5.7	5.4	5.3
Globulin (g/dL)				
day 47	1.9	1.8	1.9	1.5*
day 94	2.0	1.8	1.9	1.8
Blood urea nitrogen (mg/dL)				
day 47	19	16	16	14*
day 94	15	15	15	13

^Data were taken from Tables 18 and 19, pp. 115-120, MRID 44975304.
*Statistically significant, Dunnett's test, $p<0.05$.
#Statistically significant, Dunn's multiple comparisons, $p<0.05$

8. Urinalysis

On day 46, male rats produced a statistically significant [$p<0.05$] increase in urine at the high dose [13.2 vs. 5.8 C], a decreased osmolality at the mid [1017 vs 1526 C] and high [916 vs 1526 C] doses and a decreased pH at the mid [6.3 vs. 6.9 C] and high [5.9 vs. 6.9 C] doses. The high dose male group on test day 93 was statistically significant [$p<0.05$] showing an increase in urine volume [21.8 vs 7.2 C], a decrease in osmolality [656 vs. 1452 C], and a decrease in pH [5.9 vs 7.3 C] (Table 5).

In female rats at the high dose, the pH was lowered on day 47 [5.2 vs. 6.6 C ($p<0.05$)] and 94 [6.1 vs. 6.9 C ($p<0.05$)]. A spurious increase in urine volume occurred at the mid dose [5.1 vs. 9.7 C ($p<0.05$)] at mid study. Increases were not seen at other dose levels and times (Table 5).

TABLE 5. Urinalysis in rats gavaged with glycolic acid for 90 days^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
Males				
Volume (ml/day)				
day 46	5.8	4.3	7.9	13.2#
day 93	7.2	6.8	8.5	21.8#
Osmol (mOsm)				
day 46	1526	1444	1017*	916*
day 93	1452	1506	1286	656*
pH				
day 46	6.9	6.7	6.3*	5.9*
day 93	7.3	6.7	7.1	5.9*
Females				
Volume (ml/day)				
day 47	5.1	2.8	9.7#	4.6
day 94	4.5	4.7	6.4	9.1
Osmol (mOsm)				
day 47	1318	1734	830	1260
day 94	1127	1274	1265	979
pH				
day 47	6.6	6.3	6.9	5.2*
day 94	6.9	6.6	6.3	6.1*

^Data were taken from Tables 20 and 21, pp. 121-126, MRID 44975304.
*Statistically significant, Dunnett's test, $p<0.05$.
#Statistically significant, Dunn's multiple comparisons, $p<0.05$

9. Sacrifice and pathology

a. Organ weight

The mean absolute and relative (to body weight) kidney weights in males in the high-dose group and the relative kidney weight of males in the mid-dose group were statistically significantly elevated compared with the control group (Table 5). The relative kidney weights in the mid- and high-dose groups were also elevated in female rats, but the mean absolute kidney weight was elevated only in the 300 mg/kg/day dose group and there was no clear dose-response relationship. Liver weights relative to body weight were also elevated in treated females although the value in the 300 mg/kg/day dose group did not attain statistical significance (Table 6).

In the male at 600 mg/kg/day, liver weights relative to brain weights were decreased [11%, $p<0.05$]. Albeit, since no other changes were noted in either the organ weights or ratios and microscopic pathology was not remarkable at other dose levels, no toxicological importance was suggested. There were no other treatment-related changes in organ weights.

b. Gross pathology

Gross examination revealed dilatation of the renal pelvis of the kidneys in male rats of the 300 (1/10) and 600 (3/10) mg/kg/day dose groups and correlated, in part, with the microscopic diagnosis of oxalate crystal nephropathy. This lesion was not present in the control and 150 mg/kg/day dose groups. In the female dilatation of the renal pelvis occurred in one animal at 300 mg/kg/day, but oxalate crystal nephropathy was not seen when the organ was microscopically examined.

TABLE 6. Terminal body weights and organ weights of rats gavaged with glycolic acid for 90 days[a] ^

Terminal body and organ weights	Dose (mg/kg/day)			
	0	150	300	600
Males				
Final body weight (g)	521.3	526.6	478.4	477.8
Kidney (absolute) (g)	3.68	3.63	3.90	4.20*
Kidney (relative) (%)	0.709	0.689	0.815*	0.885*
Liver (relative) (%)	2.84	2.76	2.74	2.82
Females				
Final body weight (g)	302.6	288.8	292.8	270.7
Kidney (absolute) (g)	2.02	2.05	2.33*	2.01
Kidney (relative) (%)	0.669	0.710	0.801*	0.744*
Liver (relative) (%)	2.71	2.92*	2.90	2.91*

^Data taken from Tables 22 and 23, pages 127-128, MRID 44975304.
[a] 9-10 animals per group.
*Significantly different from control, Dunnett's test ($p<0.05$).

c. Microscopic pathology

Non-neoplastic - Treatment related microscopic lesions were observed in the lungs and kidneys (Table 7). The lungs of mid- and high-dose animals were irritated from aspiration of the highly acidic gavage solution. Changes included bronchiolar hypertrophy/hyperplasia, centriacinar inflammation, and alveolar histiocytosis. The tracheas were also inflamed and exhibited squamous metaplasia. These changes were slightly more severe in males than in females.

Treatment-related changes were present in the kidneys of mid- and high-dose male rats. These changes included unilateral hydronephrosis, transitional cell hyperplasia, and oxalate crystal nephrosis. The high-dose male rat that died on test day 46 had oxalate crystal nephropathy (urogenital inflammation/ obstruction/ calculi). Oxalate crystal formation was not present in female rats and hydronephrosis and transitional cell hyperplasia did not occur in females in a dose-dependent manner (Table 7).

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TABLE 7. Subchronic toxicity: Microscopic changes in rats gavaged with glycolic acid for 90 days[a]^

Organ: lesion	Dose (mg/kg/day)			
	0	150	300	600
Males				
Lungs				
bronchiolar hyperplasia/hypertrophy	0/10	1/10	5/10	6/10
centriacinar inflammation	0/10	0/10	4/10	2/10
peribronchiolar eosinophilic inflammation	1/10	0/10	3/10	2/10
alveolar histiocytosis	3/10	2/10	5/10	5/10
bronchiolar inflammation/necrosis	0/10	0/10	1/10	2/10
Kidneys				
unilateral hydronephrosis	0/10	0/10	4/10	7/10
transitional cell hyperplasia	0/10	0/10	4/10	6/10
oxalate crystal nephrosis	0/10	0/10	6/10	10/10
Females				
Lungs				
bronchiolar hyperplasia/hypertrophy	0/10	3/10	5/10	4/10
centriacinar inflammation	0/10	1/10	3/10	0/10
alveolar histiocytosis	1/10	3/10	6/10	5/10
peribronchiolar eosinophilic inflammation	0/10	2/10	2/10	2/10
Kidneys				
unilateral/bilateral hydronephrosis	0/10	0/10	2/10	0/10
transitional cell hyperplasia	0/10	0/10	2/10	0/10
oxalate crystal nephrosis	0/10	0/10	0/10	0/10

^Data were taken from Tables 26 and 27, pp. 131-140, MRID 44975304.
[a] Number of animals affected/total number of animals

Neoplastic - There were no neoplastic findings.

B. IMMUNOTOXICITY

No deaths were recorded in this study.

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1. Organ Weights

The mean absolute spleen weight was significantly lower in males administered 600 mg/kg/day. The other three groups were unaffected. Spleen weights in the 0, 150, 300, and 600 mg/kg/day dose groups were 0.874, 0.883, 0.856, and 0.724 g (-17%, $p<0.05$), respectively. There were no statistical differences in mean spleen weights relative to body weights. For males, absolute and relative thymus weights were lower in the 300 and 600 mg/kg/day groups than respective control weights, but the differences were not statistically significant. There was no affect on the absolute or relative weight of the spleen or thymus in females, although absolute and relative thymus weights were lower than respective control values for females in the 150 and 600 mg/kg/day dose groups.

2. Immune Response to SRBC

There were no statistically significant differences in the primary humoral immune response to SRBC for male or female rats as measured by the SRBC-specific serum IgM titer. Values, expressed as mean $\log_2$ of the SRBC-specific serum IgM titer, were 90-96% and 106-110% of the respective control values for males and females, respectively. A positive response was elicited for male and female rats dosed for 6 days with 20 mg/kg/day of cyclophosphamide, the positive control material. The decreases compared to controls were 56% and 66% for males and females, respectively. The responses for the positive control group showed that the ELISA was capable of determining a positive response and therefore was valid for glycolic acid.

C. NEUROTOXICITY

Two male rats, one in the control group and one in the 300 mg/kg/day group died as a result of gavage trauma (test days 75 and 90, respectively). Four female rats, one in the 300 mg/kg/day group and three in the 600 mg/kg/day group died or were sacrificed *in extremis* as the result of gavage trauma (test days 26, 13, 35, and 39, respectively).

During the open field part of the FOB on day 90, labored respiration was observed in one male rat in both the 150 and 300 mg/kg/day dose groups and three male rats in the 600 mg/kg/day dose group. No gross or microscopic lesions were observed in tissues selected for neurotoxicity evaluation on days 94 and 95 (6 rats/sex/group).

1. Acute Neurotoxicity

Observations were not remarkable.

2. Functional observational battery

Forelimb and hindlimb grip strength and foot splay were unaffected by treatment. A statistically significant decrease in foot splay in the 300 mg/kg/day group of males on day 90 was not dose related. An increased number of defecations for treated male rats in the open field on day 90 was attributable to the unusually low number for the control group. No treatment-related affects were observed in males or females for any of the other 37 parameters in the FOB.

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3. Motor activity

No treatment-related differences were observed in either mean duration of movements or mean number of movements in either sex at any test time interval (Table 8). A statistically significant increase in the mean duration of movements in male rats in the 150 mg/kg/day dose group at the 45-day evaluation compared to the control value was

not dose-related. A statistically significant decrease in mean number of movements of females during the fourth time interval of week 2 in the 600 mg/kg/day

TABLE 8. Motor activity of rats gavaged with glycolic acid for 90 days^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
Males				
Mean duration of movements (sec)				
baseline	955 (287)[a]	1091 (275)	1133 (308)	1278 (432)
week 2	1335 (312)	1385 (430)	1192 (264)	1289 (411)
day 45	1243 (275)	1680 (444)*	1219 (347)	1088 (496)
day 90	1529 (276)	1778 (407)	1244 (382)	1503 (493)
Mean number of movements				
baseline	426 (139)	397 (100)	501 (97)	497 (136)
week 2	575 (152)	532 (186)	555 (117)	514 (154)
day 45	547 (133)	655 (148)	550 (117)	453 (182)
day 90	689 (148)	722 (132)	561 (118)	609 (160)
Females				
Mean duration of movements (sec)				
baseline	1112 (340)	1094 (293)	1147 (167)	1041 (278)
week 2	1324 (135)	1181 (335)	1181 (248)	1164 (360)
day 45	1306 (264)	1212 (333)	1263 (405)	1436 (307)
day 90	1590 (310)	1207 (251)	1276 (224)	1492 (452)
Mean number of movements				
baseline	505 (94)	511 (151)	543 (103)	486 (100)
week 2	651 (122)	578 (113)	564 (110)	543 (159)
day 45	627 (98)	606 (128)	619 (161)	694 (74)
day 90	737 (131)	618 (156)	652 (109)	688 (108)

^Data were taken from Tables 45 and 46, pp. 190-199, MRID 44975304.
[a]Standard deviation in parentheses.
*Statistically significant, $p<0.05$.

group can be considered spurious as the total number of movements for the other time intervals were similar to that of the control values and values for total number of movements were similar to control numbers at the later test times.

D. ONE GENERATION REPRODUCTIVE AND FERTILITY EFFECTS

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1. Parental animals

Mortality and clinical signs. One male rat [#616656] in the 600 mg/kg/day group was found dead on test day 73; death was preceded by gasping and lung noise. Extensive autolysis prevented microscopic examination. Another male rat [#616645] in this group was sacrificed *in extremis* on test day 99; death was preceded by lung noise and irregular respiration. Kidney pathology was seen. No statistical differences were

observed in any other clinical signs among treatment groups in female rats during the gestation or lactation phases of the study.

Body weights and food consumption. During several intervals of the gestation period, the mean body weights of females in the 300 and 600 mg/kg/day groups were significantly lower than control values, but the differences were not significant for either group by day 21 of gestation and mean total weight gains were similar among control and treated groups (92-104% of the control value) (Table 8). It should be noted that at the start of the gestation period (gestation day 0), following 90 days of treatment with the test material, the mean body weights of the groups receiving 300 and 600 mg/kg/day were lower than that of the control group although the differences did not reach statistical significance (the differences had attained statistical significance for the 300 and 600 mg/kg/day groups in the 92-day subchronic study [see Table 2]). Mean daily food consumption and food efficiencies were similar among treated and control groups during the gestation period (Table 9). Although the mean body weight of females in the 600 mg/kg/day group was significantly lower than that of the control group on lactation day 0, the mean body weight gain over the lactation period was higher than that of the control group (which had a negative weight gain) and the difference was no longer significant by lactation day 21.

TABLE 9. Mean body weights (g), mean body weight gain (g), mean food consumption (g), and mean food efficiency of females during gestation and lactation^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
	Number of females			
	6	8	9	6
Mean body weight - gestation				
day 0	313.2	301.7	284.9	277.1
day 7	347.8	326.4	310.2*	306.7*
day 14	372.9	355.1	339.4	327.6*
day 18	412.8	399.0	382.2	363.0*
day 21	447.9	441.5 (99)	416.1 (93)	401.1 (90)
Mean body weight gain, gestation days 0-21	134.6	139.9 (104)	131.2 (97)	124.0 (92)
Mean body weight - lactation				
day 0	345.4	328.7	309.0	276.9*
day 7	339.9	323.5	330.5	321.9
day 14	360.9	349.9	339.6	332.8
day 21	343.4	327.3 (95)	325.5 (95)	315.8 (92)
Mean body weight gain, lactation days 0-21	-2.1	-1.5	16.5	38.9
Mean food consumption during gestation	26.1	24.3	25.3	24.4
Mean food efficiency during gestation	0.251	0.282	0.259	0.243

^Data taken from Tables 51-53, pages 208-210, MRID 44975304.
*Statistically significant, $p<0.05$.
() percent of control.

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TABLE 10. Reproductive indices, implantation site numbers, and implantation efficiency of female rats following 90 days of gavage treatment with glycolic acid^

Parameter	Dose (mg/kg/day)			
	0	150	300	600
Mating index (%)	80 (8/10)[a]	90 (9/10)	100 (10/10)	90 (9/10)
Fecundity index (%)	75 (6/8)	89 (8/9)	90 (9/10)	67 (6/9)
Gestation length (days)	22.3	22.6	22.4	22.7
Number of implantation sites	13.8	13.6	13.8	13.3
Implantation efficiency (%)	84.4	97.8	88.0	87.1

^Data taken from Tables 56 and 57, pages 213 and 214, MRID 44975304.
[a]Incidences in parentheses.

Reproductive indices. There were no biologically, statistically significant, or dose-related differences in mating, fecundity, number of implantation sites, implantation efficiency or gestation length in rats administered glycolic acid for 90 days prior to mating (Table 10).

Sacrifice and pathology. Death of one male rats in the 600 mg/kg/day group [day 73] was attributed to gavage trauma as the lungs were dark red upon examination. The male rat that was sacrificed *in extremis* on day 99* had pale discolored kidneys; microscopic examination revealed minimal transitional cell hyperplasia and severe oxalate crystal nephrosis. Three additional male rats [scheduled sacrificed at day 131] in the 600 mg/kg/day group had compound-related gross lesions of the kidneys including dilatation of the pelvis, calculus, and chronic progressive nephropathy. Microscopically, these lesions correlated with oxalate crystal nephropathy. No compound-related lesions were found in males in the lower dose groups or in females of any dose group.

Male rats were sacrificed following production of litters in the female (study day 131), and females were sacrificed on lactation day 21. Body weights were statistically significantly lower for males in all treatment groups (Table 11) and for females in the high dose group (318.9 g compared with the mean control value of 363.3 g ($p<0.05$); data not shown). For males, mean testes weights relative to body weight were increased in all dose groups relative to the control value ($p<0.05$) in this subset (Table 11).

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* in the original toxicology report, this animal was listed as being dead.

TABLE 11. Terminal body weights and testes weights of male rats in the reproduction study[a]^				
Terminal body and organ weights	Dose (mg/kg/day)			
	0	150	300	600
Final body weight (g)	633.7	558.5*	549.4*	500.7*
Testes (absolute) (g)	3.548	3.596	3.664	3.702
Testes (relative) (%)	0.563	0.646*	0.670*	0.741*

^Data taken from Tables 61, page 218, MRID 44975304.
[a] 10, 10, 10, and 8 animals per group, respectively.
*Significantly different from control, Dunnett's test ($p<0.05$).

2. Offspring

Viability. Jonckheere's trend test showed a statistically significant difference in the number of pups born and pups born alive in the 300 and 600 mg/kg/day groups ($p<0.05$) (Table 12); however, the disparity was small and numbers were within the range of historical control data. Using pair-wise comparison test, no statistical significance was seen for pups born and pups born alive. Furthermore, when the relatively high number for the 150 mg/kg/day group was removed, the trend test was no longer significant. At all other time points, the size and survival of the pups were comparable to the control group.

TABLE 12. Litter size and pup survival in F_1 rats^				
Parameter	Dose (mg/kg/day)			
	0	150	300	600
Mean number pups born/litter	13.3	14.1	12.3*	11.7*
Mean number pups born alive/litter	13.3	14.1	12.3*	11.5*
Mean percent born alive	100.0	100.0	100.0	98.6
Gestation index	100.0	100.0	100.0	100.0
Viability index	97.5	98.3	100.0	100.0
Sex ratio (males)	0.58	0.52	0.41	0.48
Lactation index	100.0	100.0	98.6	100.0
Litter survival (%)	100.0	100.0	100.0	100.0

^Data taken from Table 58, page 215, MRID 44975304.
*Statistically significant trend, Jonckheere's test ($p<0.05$).

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Body weight. Beginning with day 0, pups were weighed during the lactation period (Table 13). Although pups from litters of dams treated with 600 mg/kg/day prior to

TABLE 13. Mean pup weights of F_1 generation^				
Day	Dose (mg/kg/day)			
	0	150	300	600
Day 0	6.6	6.9	6.9	6.7
Day 4 preculling	10.9	10.8	11.4	10.7
Day 4 postculling	10.8	10.9	11.5	10.7
Day 7	16.8	17.6	18.0	16.3
Day 14	36.4	36.2	35.9	32.6
Day 21	58.4	58.1	59.0	54.7

^Data taken from Table 59, page 216, MRID 44975304.

mating gained less weight than their litter mates, the difference was not statistically different.

Sacrifice and pathology. No compound-related deaths occurred in the F_1 weanlings. Dilatation of the renal pelvis was observed grossly in both male and female weanling rats. These lesions occurred primarily in the weanlings whose parents were administered 150 or 300 mg/kg/day. Therefore, there was no dose-response relationship. Furthermore, the kidneys of the F_1 weanlings were examined microscopically and no compound-related microscopic changes were observed.

III. DISCUSSION

A. DISCUSSION

1. Subchronic toxicity

Eight animals, one in the subchronic study, six in the neurotoxicity study, and one in the reproduction study, died as a result of gavage accidents. At sacrifice, these animals had obvious gavage related injuries including perforation of the esophagus and/or dark red discoloration of the lungs. Additional animals, primarily in the mid- and high-dose groups, exhibited clinical signs related to gavage treatment with a solution of low pH. These included lung noises and irregular respiration. Examination of these animals at sacrifice revealed bronchiolar hypertrophy/ hyperplasia, centriacinar inflammation, and alveolar histiocytosis. These signs and lesions are attributable to the aspiration of the low pH dosing solution with resulting irritation of the respiratory tract; these intermittent signs are related to the route of administration rather than being primary systemic effects. There were no clinical signs related to systemic toxicity. One female in the subchronic study died of renal bilateral hydronephrosis unrelated to test substance administration.

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Statistically significant, treatment-related decreases in mean body weight were observed in the mid- and high-dose groups during later weekly intervals which resulted in lower body weights in males of 8 and 13%, respectively and in females of 6 and 11%, respectively, at study day 92. The lower body weights correlated with lower mean food consumption and food efficiencies in these two dose groups. These parameters were not affected in the 150 mg/kg/day dose group of either sex.

Treatment-related changes were observed in the kidneys of primarily mid- and high-dose male rats. The absolute kidney weight of males in the 600 mg/kg/day group and the relative kidney weights of the 300 and 600 mg/kg/day males and females were statistically significantly higher than control values. The weight change was accompanied by grossly observed renal pelvis dilatation and correlated with hydronephrosis, transitional cell hyperplasia, and oxalate crystal nephropathy in males of the mid- and high-dose groups. Crystal deposition occurred in all zones of the kidneys but was most abundant at the tip of the renal papilla. Hyperplasia of the transitional epithelium of the pelvis was probably secondary to mucosal irritation created by passage of the crystals from the kidney. The death of one male rat in the 600 mg/kg/day dose group [#616680] was attributed to oxalate crystal nephrosis. These lesions were not present in female rats. The destructive effects on the kidney resulted in renal insufficiency as evidenced by increased urine volume with decreased osmolality. Microscopic changes related to crystal formation were not observed in male rats in the 150 mg/kg/day dose group. Although relative kidney weights were increased in females in the 300 and 600 mg/kg/day groups, there were no gross or microscopic findings indicative of an effect on the kidneys in any female group. The slight increase in liver weights in females in all dose groups are not considered toxicologically significant.

Changes in hematology and clinical chemistry parameters correlated with the observed gross and microscopic changes in the lungs and kidneys. Increased neutrophils, indicative of inflammation, were moderately elevated in males in the 300 (transient) and 600 mg/kg/day dose groups and non-significantly in females in the 600 mg/kg/day group (study termination only). The study authors considered these increases a response to the inflammation of the lungs (all treatment groups) and kidney (primarily mid- and high-dose males). The decrements in hemoglobin were not toxicologically significant. The increased BUN in the high dose males at termination when correlated with the kidney histopathology was an indication of decreased glomerular filtration.

The decreases in total protein, reflected by decreases in albumin and globulin, in both sexes were considered mild and of no toxicological significance.

The LOAEL for systemic toxicity is 300 mg/kg/day and is based on decreased body weight, body weight gain as a result of decreased food consumption and food efficiency in both males and females. Renal insufficiency [high urine volume/low urine osmolality] occurred in the mid [day 46] and high dose males [days 47 and 94] which was correlated with hyperplasia of the renal pelvis transitional epithelium, unilateral hydronephrosis, and oxalate crystal nephrosis in male rats. The NOAEL for systemic toxicity is 150 mg/kg/day in male and female rats.

This study is considered to be **Acceptable/Guideline** and fulfills guideline [OPPTS 870.3100 (§82-1)] for a subchronic oral toxicity study in the rat.

2. Immunotoxicity

The only potentially adverse affect observed in the immunotoxicity study was the reduced mean absolute spleen weight [-17%, $p \leq 0.05$] in male rats administered 600 mg/kg/day of glycolic acid. As spleen weight relative to body weight was not statistically significantly affected (0.201, 0.203, 0.206, and 0.184 in the 0, 150, 300, and 600 mg/kg/day dose groups), the reduced absolute mean weight could be attributed to the reduced body weight in this group. Spleens and thymuses were examined microscopically in the subchronic study but not in the immunotoxicity study. There were no microscopic correlates observed in the subchronic study for the reduced spleen and thymus (non-significant reductions in mean absolute and relative weights in both sexes) weights observed in the immunotoxicity study. The SRBC assay confirmed that there was no affect of glycolic acid on the humoral immune system. The ability to detect an affect was confirmed by the positive results with cyclophosphamide.

A LOAEL for immunotoxicity was not attained. The NOAEL for immunotoxicity is ≥600 mg/kg/day [HDT] based on no effects of glycolic acid on the humoral immune response to SRBC at the highest dose tested.

This study is considered **Acceptable/Guideline** and fulfills the guidelines [OPPTS 870.7800 (§85-7)] for a immunotoxicity study the rat.

3. Neurotoxicity

The neurotoxicity observations and tests showed no treatment-related effects on male or female rats. The few statistically significant observations at various time points were not dose-related and so can be attributed to chance variation. Furthermore, there were no neuropathological changes in the tissues selected for neuropathological evaluation. Based on the absence of affects on behavior, activity, and tissues/organs,

the NOAEL for neurotoxicity for both males and females in this study is ≥600 mg/kg/day {HDT).

There was a lack of recent positive control data for the motor activity portion of the test as specified by the Guidelines. The reviewer is of the opinion that this omission would not affect the integrity of the study and the study is considered Acceptable/ Guideline.

A LOAEL for neurotoxicity was not attained. Based on the absence of clinical signs related to neurotoxicity, effects on behavior and activity, and lesions in neurological tissue, the NOAEL for neurotoxicity is ≥600 mg/kg/day [HDT].

This study is considered **Acceptable/Guideline** and fulfills [OPPTS 870.6200 (§81-1, §82-7, §83-8)] guidelines for a neurotoxicity study in the rat.

4. Reproductive and Fertility Effects

Parental animals. Female rats that had been administered glycolic acid at doses up to 600 mg/kg/day prior to mating showed no clinical signs during gestation or lactation. At the beginning of the gestation period, body weights of dams in the high-dose group lagged behind those of the other groups in a dose-dependent manner. This trend continued through gestation. During lactation this group had a greater weight gain than the other groups, although the mean final body weight on lactation day 21 still lagged behind the other groups (Table 8). Food consumption and food efficiency in the 600 mg/kg/day group were only slightly less than that of the control group. Therefore, the lower body weights at sacrifice in the 600 mg/kg/day group were due to the treatment previous to pregnancy and were not impacted negatively by pregnancy.

At sacrifice on study day 131, final body weights of male rats were significantly reduced in all treatment groups which correlated with the significantly reduced body weights in the two higher dose groups in the subchronic study at day 92. Weight gains between days 92 and 131 for all treated groups were smaller than that of the control group (Table 10), perhaps reflecting poorly functioning kidneys. Although oxalate crystal nephropathy was observed in the kidneys of only 4/10 males in the 600 mg/kg/day group in the reproduction study, functional changes without histological correlates may have been present at the lower doses. The increase in relative testes weights in all treated groups [$p<0.05$] was attributable to decreased body weights, for this change was not present in the subchronic study and no compound related changes were seen in the testes examined microscopically during the subchronic study. [Testes were not microscopically examined in the reproductive phase of the study]. No compound related changes were observed in the P_1 females.

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Reproductive indices. Treatment of the P_1 generation with glycolic acid prior to mating did not affect mating success, fecundity, gestation length, number of implantation sites, or implantation efficiency.

Offspring. Although Jonckheere's trend test showed statistically significant decreases in the number of pups born and number of pups born alive in the two higher dose groups, the differences were small in the mid- and high-dose groups (12.3 and 11.7 C for pups born and 12.3 and 11.5 C for pups born alive in the two groups, respectively) and the litter sizes were within the cited historical control data (11.2-15.3 for pups born and 10.2-15.2 for pups born alive). Therefore, the differences should not be considered a result of treatment. All other parameters reflecting litter size and pup survival were unaffected by treatment.

Mean body weight in the high-dose group was non-significantly lower than the mean control weight by day 21, but there were no clinical signs or grossly observed correlates. Therefore, it appears that fetal growth is affected if the test article is given during gestation; the effects may be a consequence of maternal toxicity and not a direct effect of the chemical.

All kidneys with grossly observed dilation of the pelvis were examined microscopically since the kidneys are a target organ for this compound. Microscopically all of the kidneys were typical of the immature kidney found in this age animal. No compound associated lesions (oxalate crystal nephropathy), as seen in male rats from the subchronic study, were present in the kidneys of the weanlings. Based on the absence of a dose response or correlative compound related findings, dilation of the renal pelvis in F_1 weanlings was not considered to be compound related.

Parental NOAEL/LOAEL: Maternal/Paternal - see subchronic toxicity. Reproductive Effects: A LOAEL for reproductive effects was not attained. Based on the absence of effects on reproduction indices and on pup litter size, survival and weight, the NOAEL for reproductive toxicity is ≥600 mg/kg/day [HDT].

This study is considered **unacceptable/upgradable**. The study was intended as a screening study to measure potential reproductive effects from oral administration of glycolic acid. While the data showed no significant reproductive effects, data on histopathological examination of the ovaries, testes, and epididymides was not provided per guideline requirements and is needed for a complete screening evaluation. The study can be upgraded to acceptable if detailed histopathological information is provided for the ovaries, testes, and epididymides of the high dose and control parental animals as detailed in the guideline [§870.3550].

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B. STUDY DEFICIENCIES

The deficiencies noted below were considered to be minor and did not adversely impact the integrity of the data.

A batch or lot number for the test material was not provided.

No blood clotting measurements were performed.

Positive control data for neurotoxicity studies (MRID 44660601, 44628701, 44628702, and 44628703) are referred to in the study report, however, the data were not provided to the reviewer and the cited studies were older than two years.

The one-generation reproductive toxicity study is considered unacceptable but upgradable. Data on histopathological analysis of the ovaries, testes, and epididymides from control and high dose animals are required in order to upgrade the study to meet the §870.3550 guideline.