DATA EVALUATION RECORD

012476

PHORATE

Study Type: 83-4a; A Two-Generation (Two Litters) Reproduction Study with AC 35024 to Rats

Work Assignment No. 3-41A (MRID 44422302)

Prepared for

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Phorate (AC 35024) **Reproduction Study OPPTS 870.3800 (§83-4)**

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DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat
OPPTS Number: 870.3800 OPP Guideline Number: §83-4

DP BARCODE: D240870 SUBMISSION CODE: S533465
P.C. CODE: 057201 TOX. CHEM. NO.: 660

TEST MATERIAL (PURITY): AC 35024 (phorate 92.1% a.i.)

SYNONYMS: Phosphorodithioic acid, 0,0-diethyl S-((ethylthio)methyl) ester

CITATION: Schroeder, R.E. (1991) A Two-Generation (Two Litters) Reproduction Study with AC 35024 to Rats. Bio/dynamics Inc., East Millstone, NJ. Laboratory Project Identification 88-3350, September 23, 1991. MRID 44422302. Unpublished.

SPONSOR: American Cyanamid Company, Princeton, NJ

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID 44422302) AC 35024 (phorate, 92.1% a.i.) was administered to Sprague Dawley rats at dose levels of 0, 1, 2, 4, or 6 ppm in the diet (achieved doses at pre-mating period : 0, 0.087, 0.176, 0.359, or 0.603 mg/kg/day for males and 0, 0.103, 0.201, 0.420, or 0.727 mg/kg/day for females; gestation period: 0, 0.081, 0.164, 0.331, or 0.550 mg/kg/day, respectively). Exposure to P animals (25/sex) began at 7 weeks of age and lasted for 9 weeks before they were mated the first time to produce the F_{1a} litters. P animals were mated a second time to produce F_{1b} litters. At 28 days post partum, F_{1b} pups (1-2 pups/sex/litter) were selected to become the F_1 parents of the F_2 litters and were given the same concentration test diets as their dam. F_1 animals (25 or 30/sex/dose) were given test diets for 10 weeks prior to mating the first time to produce the F_{2a} litters. F_1 animals were mated a second time with the F_{2b} litters. Exposure of the test material to all animals was continuous in the diet throughout the study. All animals were mated on a 1:1 ratio.

Parental toxicity was noted overtly at 4 and 6 ppm and marginally at 2 ppm. At 2 ppm, plasma (↓19%) and brain (↓17%) cholinesterase levels

were reduced in the F_1 females only. The differences from controls were not statistically significant and decreases did not occur in males. There were no other signs of systemic toxicity noted at 2 ppm.

At 4 ppm, clinical signs of toxicity were noted in P and F_1 females (tremors and/or exophthalmos). Treatment-related reductions in lactational body weights (F_1 dams only, ↓8-10%, $p \leq 0.01$ or 0.05) and lactational body weight gain (P and F_1 dams, $p \leq 0.01$) were seen at 4 ppm. Reductions in plasma (↓74%, $p \leq 0.01$) and brain (↓59%, $p \leq 0.01$) cholinesterase levels were also observed in the 4 ppm F_1 females. Dose-dependent reductions in plasma (↓25%) and brain (↓14%) cholinesterase levels were noted in the 4 ppm F_1 males; differences from controls were not statistically significant.

At 6 ppm, a treatment-related increase in mortality was observed in the F_1 generation (2 males and 5 females died vs. 1 control female). Clinical signs of toxicity were noted at the high dose in females only of the P generation (tremors and exophthalmos). For the F_1 generation at 6 ppm, clinical signs were noted in both sexes and included tremors, convulsive movements, aggressive behavior, increased ano-genital staining, protruding eyes, protrusion of tissue off the cornea, and opacity of the eyes. In addition, for the F_1 animals, increased eye infections (66% males and 78% females) were observed. Related findings observed at gross necropsy and microscopic examination included opacities, anterior synechia, various types of corneal lesions, retinal atrophy and/or folds or rosettes, and secondary cataract formation. The increased incidence of eye infections at 6 ppm cannot unequivocally be considered a direct result of treatment with AC 35024, but may be due to compromised immunity resulting either from the poor health or a direct affect of AC 35024 on the immune system of the high-dose F_1 animals. Reductions in cholinesterase levels were noted in plasma (↓40-96%), brain (↓40-83%), and RBCs (↓10-11%) in the high-dose F_1 animals.

Treatment-related decreases in body weights (↓6-15%) and overall body weight gains (↓30%) were observed unequivocally in the P females only during the premating interval. For the F_1 males and females at 6 ppm, body weights during the premating interval were decreased. However, a contributing factor to these decreases was lower lactational pup body weights (↓26-45%) in the F_{1b} litters. For both sexes, greater differences from the controls in body weights were observed at the beginning than at the end of the premating interval (week 0: ↓49-51%; week 10: ↓11-16%). In addition, overall body weight gains (↑6-15%) and food consumption (↑12-61%) were increased throughout the premating interval for the F_1 rats at the high dose. Because body weight gains and food consumption were increased during the premating period and the decreases in body weights lessened over the duration of treatment, the reductions in body weights cannot unequivocally be considered a treatment-related systemic effect on the high-dose F_1 animals. During

gestation and lactation, reductions in body weights and body weight gains were observed in the P and F_1 dams with food consumption typically increased during gestation.

The LOEL for parental systemic toxicity is 4 ppm (0.4 mg/kg/day for males and females) based on clinical signs (i.e., tremors), reduced plasma and brain cholinesterase levels (F_1 females only). The NOEL is 2 ppm (0.2 mg/kg/day for males and females).

Reproductive toxicity was demonstrated at 4 ppm as treatment-related reductions in the pup survival index for days 0-4 (F_{2a} only: 79.0% vs 97.7% for controls, $p \leq 0.01$) and in pup body weights (F_{1a}, F_{1b}, and F_{2a} litters: ↓9-21%, $p \leq 0.01$, days 14-21 of lactation). At 6 ppm, treatment-related reductions in mean litter sizes were noted on day 4 pre-cull in all litters (8.0-10.2 vs. 12.3-13.6 for controls, $p \leq 0.01$) and on day 0 for the F_{2b} litters (10.8 vs. 13.6 for controls, $p \leq 0.01$). The pup survival indices for days 0-4 and 4-21 were reduced for both litters of the F_1 and F_2 generations at the high dose (52.4-85.3% vs. 97.7-100% for controls). The litter survival indices were also reduced at 6 ppm for the F_{1b} and F_{2a} litters (72.7% and 43.5% respectively vs. 100% for controls, $p \leq 0.05$ or 0.01). Treatment-related reductions in offspring body weights were noted at 6 ppm in all litters from days 4-21 of lactation (↓19-49%, $p \leq 0.01$ or 0.05).

The LOEL for reproductive toxicity is 4 ppm (0.4 mg/kg/day) based on decreased pup survival and pup body weights. The reproductive NOEL is 2 ppm (0.2 mg/kg/day).

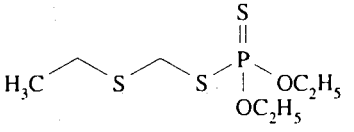
The reproductive study in the rat is classified **acceptable (§83-4)** and satisfies the requirement for a 2-generation reproductive study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 35024
   Description: Clear, colorless liquid
   Lot/Batch #: AC 6479-145
   Purity: 92.1% a.i.
   CAS #: 298-02-2
   Structure:



2. Vehicle: None

3. Test animals: Species: rat
   Strain: COBS® CD® Sprague Dawley
   Age at start of dosing: (P) 42 days; (F_1) approximately 3 wks (at weaning)
   Weight at start of dosing:
   (P) Males: 144-184 g; Females: 108-154 g
   (F_1) Males: 52-196 g; Females: 39-158 g
   Source: Charles River Laboratories, Inc., Portage, MI
   Housing: Stainless steel, wire mesh bottom cages; stainless steel floor pans and hardwood shavings were placed in each cage on day 20 of gestation; 1/cage, individually with their litters during lactation
   Diet: Purina Certified Rodent® Chow No. 5002, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 50-80°F
   Humidity: 16-79%
   Air changes: Not reported
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): 2 weeks

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group until a copulation plug or sperm in the vaginal smear were observed. If sperm were not found after 10 days of observation, the first male was removed and replaced by another male from the same dosage group for 10 days. If two attempts at mating were unsuccessful, no further matings were tried. Sibling matings within the F_1 generation were avoided.

   Solid bottoms were placed in the cages of each pregnant female on day 20 of gestation.

2. Study schedule: Starting at 7 weeks of age, P animals were given test diets for 9 weeks before they were mated the first time to produce the F_{1a} litters. P were mated a second time to produce F_{1b} litters. At 28 days of age, F_{1b} pups (1-2 pups/sex/litter) were selected to become the F_1 parents of the F_2 generations and were given the same concentration test diets as their dam. F_1 animals were given test diets for 10 weeks prior to mating the first time to produced the F_{2a} litters. F_1 animals were mated a second time to produce the F_{2b} litters. Exposure of all animals to the test material in the diet was continuous throughout the study.

3. Animal assignment: P and F_1 animals were randomly assigned (stratified by body weight for P rats) to test groups as seen in Table 1.

Table 1. Animal assignment

Test Group	Dose in Diet[a] (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	25	25	25	25
Low	1	25	25	25	25
Mid-1	2	25	25	25	25
Mid-2	4	25	25	25	25
High	6	25	25	30	30

a Diets were administered from the beginning of the study until sacrifice.

4. Dose selection rationale: A range-finding study was not included in the current submission. However, as the dose levels selected achieved systemic and reproductive LOELs and NOELs, this is not a major deficiency.

5. Dosage preparation and analysis

Formulations were prepared weekly by dissolving appropriate amounts of test substance in acetone, then mixing with Purina Certified Rodent Chow No. 5002. Feed jars were changed weekly. Prior to the start of the study, the stability of the test substance in the diet (1 and 6 ppm diets) was evaluated for a period of up to 14 days at ambient and freezer temperatures. Homogeneity (left: top,

middle, bottom; right: top, middle, bottom) was also evaluated prior to the start of the study for the 1 and 6 ppm diets. During the study, samples of treated feed at each dose level were analyzed for concentration every week for the first month, on study week 8, and weekly thereafter.

Results - Homogeneity Analysis - 1 ppm diet: 88.9-100% of nominal, coefficient of variation (c.v.) = 3.3%; 6 ppm diet: 90.5-98.2% of nominal, c.v. = 3.8% of nominal

Stability Analysis: Stable in the diet for up to 14 days at freezer temperatures and for 7 days at ambient temperatures (see Table 2).

Table 2. Stability analysis of AC 35024 in the 1 and 6 ppm test diets. [a]

Storage interval (days)	% Nominal[b]	
	Ambient temperatures	Frozen
1	111.8, 93.3	105.5, 91.3
3	101.7, 90.8	103.5, 94.2
5	96.0, 83.5	99.0, 104.8
7	97.0, 85.7	94.0, 103.2
10	79.8, 69.3	98.9, 97.5
14	72.2, 64.0	124.0, 97.7

a Data extracted from the study report page 2242.
b Values listed are the % nominal of the 1 and 6 ppm diets, respectively.

Concentration Analysis: average concentrations for the 1, 2, 4, and 6 ppm diets were 96.3-97.9% of nominal with c.v.'s of 7.8-13.3%.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the test substance was demonstrated to be stable in the test diets as they were prepared and stored in the study, i.e. no diet at ambient temperature longer than 1 week.

C. OBSERVATIONS

1. Parental animals: Animals were observed for mortality and clinical signs twice daily. Physical examinations were performed weekly. Males were weighed weekly throughout the study. Females were weighed weekly throughout the pre-mating interval, on gestational days 0, 7, 14, and 20, and on lactational days 0, 4, 7, 14, and 21. Food consumption was recorded weekly throughout the study except during mating and lactation. During gestation, maternal food consumption was recorded for days 0-7, 7-14, and 14-20. During lactation, food consumption was recorded for days 0-4, 4-7, 7-14 and 14-21.

   Ophthalmoscopic examinations were performed on the P females just prior to sacrifice and on the F_1 males and females at the beginning of the pre-mating interval and again approximately 2 weeks prior to sacrifice.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 3):

Table 3. F_1/F_2 Litter observations.[a]

Observation	Time of observation (lactation day)					
	Day 0	Day 4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	x	x	x	x	x	x
Pup weight	x	x	x	x	x	x
External alterations	x	x	x	x	x	x
Number of dead pups	x	x	x	x	x	x
Sex of each pup (M/F)	x	x	x	x	x	x

a Data extracted from the study report page 27.
b Before standardization (culling).
c After standardization (culling).

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter with 4/sex/litter, as nearly as possible.

3. Postmortem observations:

1) Parental animals: P males were sacrificed 3 weeks after mating to produce the F_{1b} litters. F_1 males and females were sacrificed following a 4-week post-weaning period of the F_{2b} litters. P dams were sacrificed after the last F_1 litters were weaned. These animals were subjected to postmortem examinations as follows:

Gross necropsy consisted of external and internal examinations. The following tissues (X) from all males and/or females of the P and F_1 generations were prepared for microscopic examination. Histological examinations were performed on tissues collected from the control and high-dose groups. Gross lesions were examined histologically regardless of treatment group.

X	Ovaries	X	Epididymides
X	Uterus	X	Prostate
X	Vagina	X	Seminal vesicles
X	Pituitary	X	Testes
X	Lesions		

The following tissues were prepared from P females; histological examinations were performed on the control and high-dose groups.

X	Eyes	X	Lacrimal glands
X	Harderian glands		

The following tissues were prepared from F_1 males and females; histological examinations were performed on the control and all treatment groups.

X	Eyes	X	Retina
X	Intra-ocular muscles	X	Optic nerve
X	Extra-ocular muscles	X	Lacrimal glands
X	Harderian glands		

In addition for the F_1 parental generation, cholinesterase levels in the plasma, erythrocytes, and brain were determined for 10 rats/sex/group at sacrifice.

2) Offspring: For pups not culled on day 4 of lactation, F_{1b} offspring not selected as parental animals and F_{1a}, F_{2a}, and F_{2b} offspring were sacrificed on day 21-28 of lactation. These pups were examined externally and those with findings were given a gross internal exam; lesions were preserved. In addition, 1 pup/sex/litter of the F_{1b} and F_{2b} generations were given detailed internal and external examination. Pups culled on day 4, pups found dead, and stillborn pups were given a gross internal and external examination. Lesions were preserved in 10% neutral buffered formalin.

D. DATA ANALYSIS

1. Statistical analyses: All collected data were subjected to routine appropriate statistical procedures.

2. Indices:

Reproductive indices: The following reproductive indices as presented in the study report (page 213 and 214) were calculated for the P and F_1 adults:

female mating index = # of females showing evidence of mating/total # of females x 100%
male mating index = # of males for which mating was confirmed in at least one female/total # of males x 100%
female pregnancy index = # of females showing evidence of parturition/# of females mated x 100%
male fertility index = # of males mated with at least one female showing evidence of parturition/# of males mated x 100%

Offspring viability indices: The following viability indices as presented in the study report (page 231 and 232) were calculated for the F_1 and F_2 litters:

pup viability index = total # live pups at day 0/total # of pups born
pup survival index (days 0-4) = total # live pups at day 4 (pre-cull)/total # live pups at day 0
pup survival index (days 4-21) = total # live pups at day 21/total # live pups at day 4 (post-cull)
litter survival index = total # of litters at weaning (day 21)/total # of litters with live pups at day 0

3. Historical control data: Historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: There were no treatment-related deaths in the P generation males or females. One high-dose P female died on day 16 of lactation for the F_{1a} litters; the cause of death was not determined. One high-dose female with ocular lesions was sacrificed moribund after weaning its F_{1b} litter. No other P generation animals died at any dose level. Because only one death occurred in the P generation, it was not considered treatment-related.

   Treatment-related clinical signs of toxicity were noted in the females of the P generation at 4 and 6 ppm. Three females at 4 ppm and 23 females at 6 ppm were noted with tremors at one or more weekly intervals. Exophthalmos was also observed at 4 ppm (one female) and at 6 ppm (20 females). There were no treatment-related ocular lesions noted at ophthalmoscopic evaluation of the P generation animals (see below). There were no treatment-related clinical signs of toxicity in the P males at any dose level.

   For the F_1 generation, a treatment-related increase in mortality was observed at 6 ppm. Two males and five females died at 6 ppm (6.9 and 20.7% mortality, respectively) while only one control female died. Five of these seven deaths occurred when the animals were young (prior to or within the first two weeks of the premating-interval). No treatment-related deaths occurred at dose levels of ≤4 ppm in the F_1 generation.

   Treatment-related clinical signs of toxicity were noted at 4 ppm in the F_1 females during the lactation interval of the F_{2a} litters (tremors in three females). There were no other treatment-related clinical signs of toxicity noted in the F_1 animals at dose levels of ≤4ppm.

   At 6 ppm, treatment-related clinical signs of toxicity were noted in the F_1 males and females. Clinical signs included tremors (11 males, 27 females), convulsive movements (2 males), and aggressive behavior (10 males, 12 females). In

addition, ocular observations in the high-dose males and females included protruding eyes, protrusion of tissue off the cornea, and opacities. Some of these ocular findings were consistent with the observations made at ophthalmoscopic evaluation of the F_1 generation animals (see below). Increased ano-genital staining was also observed in the high-dose F_1 females.

2. Ophthalmoscopic evaluations: There were no treatment-related changes noted at any dose level in the eyes of the P generation females at the ophthalmoscopic examinations performed just prior to sacrifice.

   For the F_1 generation, active and/or resolved infectious disease with related lesions were noted in the eyes of animals from all groups. An increase in the incidence of the infectious disease with lesions was noted in the high-dose group (66% of the males and 78% of the females) at the first and second ophthalmoscopic evaluations (beginning of the pre-mating interval and again approximately 2 weeks prior to sacrifice). The severity of the disease and lesions were also increased at the high dose. The increased incidence of infectious disease in the eyes at 6 ppm cannot unequivocally be considered a direct result of treatment with AC 35024. The increased incidence of infections may be due to compromised immunity resulting either from the poor health of the 6 ppm animals or a direct affect of AC 35024 on the immune system of these animals. There were no treatment-related effects noted at dose levels of ≤4 ppm at ophthalmoscopic exam in the F_1 generation.

3. Body weight and food consumption: Body weight and food consumption data for the premating intervals of the P and F_1 generations are presented in Tables 4a and 4b, respectively. Treatment-related decreases in body weights and body weight gains were observed at 6 ppm in the P generation (females only). Changes in food consumption observed in the P generation were not attributed to treatment at any dose level. Body weights, body weight gains, and food consumption were unaffected by treatment at dose levels of ≤4 ppm for the P generation.

   For the F_1 generation, body weights during the premating interval were decreased at 6 ppm (males and females).

However, a contributing factor to these decreases was lower pup body weights at weaning that were possibly caused by poor maternal health in the 6 ppm dams (P generation). In addition, compensatory increases in food consumption and body weight gains were noted for the high-dose F_1 males and females during the premating interval. At 4 ppm, food consumption and body weight gains were increased and body weights were comparable to the controls. These changes at 4 ppm were also attributed to lower pup body weights at weaning that may have been caused by poor maternal health in the 4 ppm P dams during lactation.

The following changes were noted in the P generation animals in the 9-week premating interval:

For the P females dosed at 6 ppm, treatment-related decreases in body weights (↓6-15%, $p \leq 0.01$ or 0.05, weeks 1, 3, 5, 7-9) and in overall body weight gains (↓30%, $p \leq 0.01$) were noted during the premating interval. There were no changes in body weights or overall body weight gains noted in the P females dose at ≤4 ppm. There were no treatment-related changes in body weights or body weight gains observed in the P generation males at any dose level.

There were no treatment-related effects noted in food consumption (g/kg/day) for the P males or females at any dose level. Several slight, but statistically significant ($p \leq 0.05$ or 0.01) changes in food consumption were noted for the P males (2 ppm: ↓3%, week 1; 4 ppm: ↑4%, week 8; 6 ppm: ↑4-15%, weeks 5 and 8). As these changes were not consistent and/or dose-dependent, they are not considered treatment-related. Several statistically significant ($p \leq 0.01$) changes in food consumption were observed for the P females at 6 ppm (↑11-14% weeks 3, 4, 6, 8, and 9; ↓23%, week 7). As food consumption was not consistently increased or decreased in the females, the changes also are not considered treatment-related.

The following changes were noted in the F_1 generation animals during the 10-week premating treatment interval.

For males dosed at 6 ppm, decreases in body weights were noted throughout the premating interval ($p \leq 0.01$, weeks 0-10). The differences in body weights lessened over the pre-mating interval with a 49% decrease observed at week 0 and an 11% decreased observed at week 10. Overall body weight

gains (↑6%, not statistically significant) and food consumption (↑12-48%, p≤0.01) were increased throughout the premating interval (weeks 0-10). Analogous results were noted in the high-dose F_1 females. Female body weights were decreased (p≤0.01, weeks 0-10) with a greater difference observed at week 0 (↓51%) than at week 10 (↓16%) and increases were seen in overall body weight gains (↑15%, p≤0.01) and food consumption (↑21-61%, p≤0.01, weeks 0-10). Lactational pup body weights (average of both sexes) for the F_{1b} high-dose group were lower than the controls (↓26-45%, p≤0.01, days 4-21 of lactation).

Because body weight gains and food consumption were increased during the premating period and the decreases in body weights lessened over the duration of treatment, the reductions in body weights cannot unequivocally be considered a treatment-related systemic effect on the high-dose F_1 animals. In addition, a contributing factor to the lower body weights during premating was the decreased lactational body weights which caused reduced weights at the start of the premating interval. The lower body weights during premating at 6 ppm, therefore may have been caused by poor maternal health in the high-dose P dams during lactation.

Similar trends in body weights, body weight gains and food consumption were noted at 4 ppm in the F_1 generation. Pup body weights were decreased at 4 ppm (↓9-12%, p≤0.01, days 14 and 21 of lactation). Body weight gains for the F_1 females at 4 ppm were increased during the premating period (↑18%, p≤0.01). Food consumption was also increased (males: ↑6-11%, p≤0.05, weeks 2 and 6-8; females: ↑12-25%, p≤0.01 or 0.05, weeks 1-4, 6, and 8). Body weights were comparable to the controls throughout the premating interval for the males and females at 4 ppm. The increases in food consumption and body weight gains at 4 ppm are attributed to lower pup body weights at weaning that may have been caused by poor maternal health in the 4 ppm P generation dams during lactation.

Body weights and body weight gains were comparable to the controls throughout the premating interval at 1 and 2 ppm for both sexes of the F_1 generation. Food consumption was increased at 2 ppm (males: ↑8 and 4%, weeks 2 and 6, p≤0.05) and at 1 ppm (females: ↑15%, week 1, p≤0.05). However, these are not considered treatment-related effects because

the increases were marginal and sporadic.

Table 4a. Mean body weights (g), gains (g), and food consumption (g/kg/day) - P generation pre-mating.[a]

Observation/Study Weeks	Dose Group (ppm)				
	0	1	2	4	6
P Generation Males - Pre-mating					
Mean body weight/Week 0	165.8	165.2	164.6	165.3	166.3
Mean body weight/Week 5	391.8	334.3	379.7	392.1	392.3
Mean body weight/Week 9	468.1	463.2	454.6	466.2	460.5
Mean weight gain/Weeks 0-3 b	159.6	154.8	148.1	159.1	157.1
Mean weight gain/Weeks 3-6 b	93.7	92.4	90.6	93.3	95.2
Mean weight gain/Weeks 6-9 b	49.0	50.8	51.3	48.5	41.9
Mean weight gain/Weeks 0-9	302.3	298.0	290.0	300.9	294.2
Mean food consumption/Week 0	156.9	156.5	156.4	156.7	155.5
Mean food consumption/Week 5	76.2	77.5	76.8	77.9	79.2*
Mean food consumption/Week 9	65.8	66.4	66.1	66.0	67.2
P Generation Females - Pre-mating					
Mean body weight/Week 0	134.2	132.8	135.9	132.4	132.4
Mean body weight/Week 5	230.3	227.0	241.4	233.7	212.6**
Mean body weight/Week 9	262.9	255.4	273.1	268.5	222.8**
Mean weight gain/Weeks 0-3 b	66.9	65.7	73.7	71.2	56.7
Mean weight gain/Weeks 3-6 b	39.2	41.0	41.0	42.2	45.9
Mean weight gain/Weeks 6-9 b	22.4	15.9	22.5	22.7	-12.2
Mean weight gain/Weeks 0-9	128.6	122.6	137.2	136.1	90.4**
Mean food consumption/Week 0	163.0	160.6	154.8	155.7	157.0
Mean food consumption/Week 5	89.4	93.4	88.6	89.2	91.7
Mean food consumption/Week 9	80.5	81.4	79.5	80.4	89.3**

a Data extracted from the study report pages 75-80, and 108-111.
b Calculated by the reviewer from the mean data presented in the study report; statistical analyses not performed.
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

Table 4b. Mean body weights (g), gains (g), and food consumption (g/kg/day) - F_1 generation pre-mating.[a]

Observation/Study Weeks	Dose Group (ppm)				
	0	1	2	4	6
F_1 Generation Males - Pre-mating					
Mean body weight/Week 0	163.5	159.2	149.2	150.9	83.2**
Mean body weight/Week 5	386.9	376.2	374.0	376.5	306.9**
Mean body weight/Week 10	493.3	476.4	487.3	483.1	436.7**
Mean weight gain/Weeks 0-3 b	157.9	152.3	157.7	158.8	145.8
Mean weight gain/Weeks 3-6 b	94.0	92.1	98.5	95.4	112.4
Mean weight gain/Weeks 6-10 b	77.9	72.8	81.9	78.0	95.3
Mean weight gain/Weeks 0-10	329.8	317.2	338.0	332.2	350.7
Mean food consumption/Week 1	133.1	132.1	142.0	147.7	191.8**
Mean food consumption/Week 5	86.5	87.6	90.3	92.7	112.3**
Mean food consumption/Week 6	73.6	77.1	76.8*	77.9*	93.7**
Mean food consumption/Week 10	62.4	62.6	62.5	63.1	70.7**
F_1 Generation Females - Pre-mating					
Mean body weight/Week 0	131.3	125.5	125.7	124.3	64.0**
Mean body weight/Week 5	229.6	225.2	221.9	234.9	169.0**
Mean body weight/Week 10	270.5	267.5	262.8	288.8	227.2**
Mean weight gain/Weeks 0-3 b	72.3	72.0	70.3	79.5	72.2
Mean weight gain/Weeks 3-6 b	38.3	40.9	37.6	47.2	49.9
Mean weight gain/Weeks 6-10 b	28.6	29.1	29.2	37.8	41.1
Mean weight gain/Weeks 0-10	139.2	142.0	137.2	164.5**	160.5**
Mean food consumption/Week 1	130.4	150.0*	149.7	163.3**	185.0**
Mean food consumption/Week 5	102.4	105.9	101.9	110.9	144.4**
Mean food consumption/Week 6	88.5	95.1	92.6	100.1**	126.3**
Mean food consumption/Week 10	74.2	78.4	77.0	77.7	94.4**

a Data extracted from the study report pages 82-87, and 113-116.
b Calculated by the reviewer from the mean data presented in the study report; statistical analyses not performed.
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

At the high-dose, treatment-related reductions in body weights were noted during the gestation and lactation of both litters for the P and F_1 dams (↓10-28%, $p \leq 0.01$ or 0.05). Maternal body weight gains were also reduced during gestation for the P and F_1 dams (↓15-31%, $p \leq 0.01$, F_{1a}, F_{2a},

and F_{2b} litters). Body weight gains during lactation were also reduced for the P and F_1 dams (dams lost weight during lactation of F_{1a}, F_{1b}, F_{2b} litters, $p \leq 0.01$). Food consumption was typically increased at the high dose for the P and F_1 dams during gestation of both litters (↑7-31%, $p \leq 0.01$ or 0.05, days 0-20, 0-14, and/or 7-14).

At 4 ppm, gestational body weights, gains, and food consumption were unaffected by treatment for the P and F_1 dams (both litters). During lactation of the F_{1a}, F_{1b}, and F_{2b} litters, P and F_1 dams either lost more weight than the controls or experienced a weight loss while the controls gained weight ($p \leq 0.01$). Body weights of the F_1 dams were also reduced at day 21 of lactation for the F_{2a} and F_{2b} litters (↓8-10%, $p \leq 0.01$ or 0.05).

There were no treatment-related effects on body weights, body weight gains, or food consumption at dose levels of ≤2 ppm for the P or F_1 dams.

3. Test Substance Intake: Based on food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the pre-mating period (9 weeks for P and 10 weeks for F_1) and gestation period are presented in Table 5. The values are considered to be representative of the test substance intake for the entire study.

Table 5. Test substance intake (mg/kg/day)

Dose (ppm)	Pre-mating				Gestation	
	F0 G		F1 G		F0	F1
	M	F	M	F		
1	0.086	0.101	0.088	0.105	0.082	0.080
2	0.171	0.197	0.181	0.205	0.165	0.162
4	0.347	0.401	0.370	0.439	0.330	0.332
6	0.523	0.622	0.683	0.831	0.558	0.542
Average	M		F			
1	0.087		0.103		0.081	
2	0.176		0.201		0.164	
4	0.359		0.420		0.331	
6	0.603		0.727		0.550	

Data extracted from the study report pages 39 & 49.

4. Reproductive function:

   a. Estrous cycle length and periodicity: No observations were made pertaining to the estrous cycle length and periodicity in this study. However, there were no indications of treatment-related abnormalities in female reproductive function during the study.
   b. Sperm measures: No sperm parameter observations were made in this study. However, there were no indications of treatment-related male fertility abnormalities during The study.
   c. Sexual maturation (F_1): No observations were made pertaining to the sexual maturation rates of the F_1 or F_2 litters.

5. Reproductive performance: Reproductive performance results are presented in Tables 6a and 6b. There were no treatment-related effects noted in the reproductive performance of the P or F_1 adults at any dose level.

Table 6a. Reproductive performance.[a]

Observation	Dose Group (ppm)				
	0	1	2	4	6
P Generation - Litter F_{1a}					
Female Mating Index (%)	100	100	100	100	100
Male Mating Index (%)	92.0	92.0	100	100	96.0
Pregnancy Index (%)	88.0	100	88.0	100	92.0
Male Fertility Index (%)	87.0	100	88.0	100	95.8
Median Gestation Interval (Days)	21.9	21.8	21.8	22.0	21.9
Number of Litters	22	25	22	25	23
P Generation - Litter F_{1b}					
Female Mating Index (%)	100	100	96.0	100	100
Male Mating Index (%)	96.0	88.0	92.0	96.0	100
Pregnancy Index (%)	96.0	88.0	91.7	92.0	95.7
Male Fertility Index (%)	95.8	95.5	95.7	91.7	95.7
Median Gestation Interval (Days)	22.0	22.0	21.9	22.0	22.1
Number of Litters	24	22	22	23	22

a Data extracted from the study report pages 213, 216, and 217.

Table 6b. Reproductive performance.[a]

Observation	Dose Group (ppm)				
	0	1	2	4	6
F_1 Generation - Litter F_{2a}					
Female Mating Index (%)	96.0	96.0	100	100	100
Male Mating Index (%)	92.0	92.0	92.0	96.0	96.2
Pregnancy Index (%)	87.5	100	100	80.0	88.5
Male Fertility Index (%)	87.0	100	100	79.2	88.0
Median Gestation Interval (Days)	22.2	22.0	21.9	22.1	22.0
Number of Litters	21	24	25	20	23
F_1 Generation - Litter F_{2b}					
Female Mating Index (%)	96.0	96.0	100	84.0	92.0
Male Mating Index (%)	88.0	96.0	84.0	80.0	88.0
Pregnancy Index (%)	83.3	91.7	84.0	76.2	82.6
Male Fertility Index (%)	86.4	91.7	81.0	80.0	81.8
Median Gestation Interval (Days)	22.2	22.0	22.0	22.1	22.1
Number of Litters	20	22	21	16	19

a Data extracted from the study report pages 214, 218, and 219.

5. Parental postmortem results

a) Cholinesterase determinations: Cholinesterase levels in plasma, red blood cells, and brain were determined at sacrifice for the F_1 generation only (Table 7).

At 2 ppm, reductions in plasma (↓19%) and brain (↓17%) cholinesterase levels were noted in females only. The differences from controls were not statistically significant, not observed in males, and not considered to be biological significant.

At 4 ppm, treatment-related reductions in plasma (↓74%, $p \leq 0.01$) and brain (↓59%, $p \leq 0.01$) cholinesterase levels

were noted in females. Dose-dependent and treatment-related reductions in plasma (↓25%) and brain (↓14%) cholinesterase levels were also noted in males at 4 ppm; differences from controls were not statistically significant.

In animals dosed at 6 ppm, treatment-related reductions in cholinesterase levels were noted in plasma (males: ↓40%, $p \leq 0.01$; females: ↓96%, $p \leq 0.01$), brain (males: ↓40%, $p \leq 0.01$; females: ↓83%, $p \leq 0.01$), and RBCs (males: ↓10%, $p \leq 0.05$; females: ↓11%, not statistically significant).

Table 7. Cholinesterase levels in plasma, red blood cells and brain of F_1 animals at sacrifice.[a]

Mean levels	Dose Group (ppm)				
	0	1	2	4	6
Males					
Plasma (IU/ml)	0.496	0.599	0.485	0.371	0.296**
RBC (IU/ml)	7.9	8.0	7.9	7.4	7.1*
Brain (IU/g)	8.3	8.6	8.5	7.1	5.0**
Females					
Plasma (IU/ml)	2.473	2.555	1.999	0.633**	0.104**
RBC (IU/ml)	7.3	7.1	7.2	7.0	6.5
Brain (IU/g)	8.6	8.5	7.1	3.5**	1.5**

a Data extracted from the study report pages 235 and 236.
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

b) Organ weights: No organ weight data were collected in this study.

c) Pathology

1) Macroscopic examination: There were no treatment-related macroscopic findings for any treatment group in the P generation. At 6 ppm, opacity of the eyes were

observed in 14 F_1 males and 13 F_1 females. This finding is consistent with the observations made during the in-life ophthalmoscopic evaluations where an increased incidence of infectious disease of the eye was observed at the high dose in the F_1 generation. The increased incidence of eye infections at 6 ppm cannot unequivocally be considered a direct result of treatment with AC 35024, but may be due to the poor health of the 6 ppm animals. There were no treatment-related histopathologic findings noted in the F_1 generation at dose levels of ≤4 ppm.

2) Microscopic examination: There were no treatment-related microscopic findings for any treatment group in the P generation. Histopathology noted in the eyes in the F_1 high-dose animals included anterior synechia, various types of corneal lesions, retinal atrophy and/or folds or rosettes, and secondary cataract formation. There were no histopathologic findings in the intra- or extra-ocular muscles or of the optic nerves. The histopathology of the eyes in the high-dose F_1 animals was consistent with the findings at necropsy and the ophthalmoscopic evaluations. The findings were considered "end stage lesions" which remained after active infection had taken place and cannot unequivocally be considered a direct result of treatment with AC 35024. The increased incidence of eye infections at the high dose may be due to compromised immunity resulting either from the poor health of the 6 ppm animals or a direct affect of AC 35024 on the immune system of these animals. There were no treatment-related histopathologic findings noted in the F_1 generation at dose levels of ≤4 ppm.

B. OFFSPRING

1. Viability and clinical signs: There were no treatment-related clinical signs reported in the pups at any dose level for either the F_1 or F_2 generations. Mean litter size and viability results from F_1 and F_2 pups during lactation are summarized in Tables 8a and 8b.

At 6 ppm, treatment-related reductions in mean litter size and pup viability throughout lactation were noted in both litters of both generations. For the F_{1a}, F_{1b}, and F_{2a} litters, mean litter sizes at day 0 were comparable to the controls at all dose levels. For the high-dose F_{2b} litters, the mean litter size on day 0 was less than controls (10.8 vs. 13.6, $p \leq 0.01$). By day 4 pre-cull, mean litter sizes at the high-dose were reduced for the F_{1a}, F_{1b}, F_{2a}, and F_{2b} litters (8.0-10.2 vs. 12.3-13.6 for controls, $p \leq 0.01$). The pup survival indices for days 0-4 and 4-21 were also reduced for both litters of the F_1 and F_2 generations at the high dose (52.4-85.3% vs. 97.7-100% for controls). In addition at the high dose, the litter survival indices (total # of litters at weaning/total # of litters with live pups at day 0) were reduced for the F_{1b} and F_{2a} generations (72.7% and 43.5%, respectively, vs. 100% for controls, $p \leq 0.05$ or 0.01).

At 4 ppm, a treatment-related reduction in the pup survival index for days 0-4 was noted for the F_{2a} litters only (79.0% vs 97.7% for controls, $p \leq 0.01$). The pup viability index (total # live pups at day 0/total # of pups born) and pup survival index for days 4-21 were also statistically significantly ($p \leq 0.01$ or 0.05) lower than the controls for the F_{2a} litters, although the differences were not great (89.3-95.9% vs. 100-99.6% for controls). There were no other treatment-related effects on pup viability or litter sizes for either generation of litters at any dose level.

Marginal, statistically significant ($p \leq 0.01$ or 0.05) reductions in pup survival indices for days 0-4 and/or days 4-21 were noted at 2 and 4 ppm for the F_{1a} and F_{1b} litters and at 2 ppm for the F_{2a} litters. The pup survival indices for these groups (93.0-95.9%) were within the historical control ranges (80.3-100%, 10 studies conducted in 1985-1989). The statistically significant differences are therefore attributed to high survival indices in the concurrent controls (97.7-100%) and not to treatment with AC 35024.

Table 8a. Mean litter size and viability.[a]

Observation	Dose Group (ppm)				
	0	1	2	4	6
F_{1a} Litters					
Mean litter size					
Day 0	13.9	13.2	13.6	12.7	12.6
Day 4[b]	13.6	12.8	13.1	11.8	10.2**
Day 4[c]	8.0	7.9	7.9	7.8	7.6
Day 7	8.0	7.9	7.9	7.6	7.2
Day 14	8.0	7.9	8.0	7.5	7.1
Day 21	8.0	7.9	7.9	7.5	6.7
Number live pups					
Day 0	305	329	300	318	277
Day 4[b]	299	319	288	296	224
Day 4[c]	176	198	173	195	167
Day 21	176	198	165	187	128
Number deaths[d]					
Days 0-4	6	10	12	22	53
Days 4-21	0	0	8	8	39
Indices					
pup viability	98.7	98.2	99.0	97.5	97.6
pup survival days 0-4	98.0	97.0	96.0	93.1**	77.2**
pup survival days 4-21	100.0	100.0	95.4*	95.9*	80.5**
litter survival	100.0	100.0	95.5	100.0	86.4
F_{1b} Litters					
Mean litter size					
Day 0	13.1	13.1	13.5	12.6	12.4
Day 4[b]	13.0	13.0	13.2	12.0	9.9**
Day 4[c]	8.0	7.7	8.0	7.8	7.2
Day 7	7.9	7.7	8.0	7.8	7.0
Day 14	7.9	7.7	8.0	7.6	7.1
Day 21	7.9	7.7	7.9	7.6	7.0
Number live pups					
Day 0	314	289	298	289	244
Day 4[b]	312	285	277	276	205
Day 4[c]	191	170	168	179	158
Day 21	189	169	166	175	112
Number deaths[d]					
Days 0-4	2	4	21	13	39
Days 7-21	2	1	2	4	46
Indices					
pup viability	98.4	99.3	98.3	99.0	97.5
pup survival days 0-4	99.4	98.6	93.0**	95.5**	79.8**
pup survival days 4-21	99.0	99.4	98.8	97.8	70.9**

a Data extracted from the study report pages 216, 217, 228, and 231.
b Before standardization (culling)
c After standardization (culling)
d Calculated by the reviewer; statistical analyses not performed.
* $p \leq 0.05$; ** $p \leq 0.01$

Table 8b. Mean Litter Size and Viability.[a]

Observation	Dose Group (ppm)				
	0	1	2	4	6
F_{2a} Litters					
Mean litter size					
Day 0	12.6	12.6	13.2	11.7	10.0
Day 4[b]	12.3	12.0	13.0	10.8	8.0**
Day 4[c]	7.4	7.5	7.8	7.7	6.6
Day 7	7.4	7.5	7.5	7.6	6.2
Day 14	7.4	7.5	7.4	7.4	6.1
Day 21	7.4	7.6	7.4	6.9	6.0
Number live pups					
Day 0	264	303	331	233	222
Day 4[b]	258	288	324	184	120
Day 4[c]	156	181	194	131	99
Day 21	156	175	184	117	60
Number deaths[d]					
Days 0-4	6	15	7	49	102
Days 4-21	0	6	10	14	39
Indices					
pup viability	99.6	99.0	98.5	95.9*	98.7
pup survival days 0-4	97.7	95.0	97.9	79.0**	52.4**
pup survival days 4-21	100	96.7	94.8**	89.3**	60.6**
litter survival	100	95.8	100	85.0	43.5**
F_{2b} Litters					
Mean litter size					
Day 0	13.6	12.7	13.6	13.1	10.8**
Day 4[b]	13.5	12.4	13.2	12.6	9.8**
Day 4[c]	7.6	7.3	8.0	7.8	7.6
Day 7	7.6	7.3	8.0	7.7	7.1
Day 14	7.5	7.3	8.0	7.6	6.9
Day 21	7.5	7.3	8.0	7.6	6.9
Number live pups					
Day 0	259	280	285	210	206
Day 4[b]	256	272	278	201	167
Day 4[c]	144	161	168	125	129
Day 21	142	161	167	122	110

Number deaths[d]					
Days 0-4	3	8	7	9	39
Days 7-21	2	0	1	3	19
Indices					
pup viability	98.1	98.6	98.6	97.7	99.5
pup survival days 0-4	98.8	97.1	97.5	95.7	81.1**
pup survival days 4-21	98.6	100	99.4	97.6	85.3**
litter survival	100	100	100	100	84.2

a Data extracted from the study report pages 218, 219, 229, and 232.
b Before standardization (culling).
c After standardization (culling)
d Calculated by the reviewer; statistical analyses not performed.
*p≤0.05; **p≤0.01

2. Body weight: Treatment-related reductions in offspring body weights were noted at 6 ppm in all litters from days 4-21 of lactation (↓19-49%, p≤0.01 or 0.05). Pup body weights at day 0 were comparable to the controls at all dose levels in all litters. At 4 ppm, treatment-related reductions in pup body weights were noted in the F_{1a}, F_{1b}, and F_{2a} generations near the end of lactation (↓9-21%, p≤0.01, days 14-21). Pup body weights were comparable to the controls throughout lactation in all litters at dose levels of ≤2 ppm. Selected mean pup body weight data are presented in Tables 9a and 9b.

Table 9a. Mean pup body weights (g).[a]

Day of lactation	Dose Group (ppm)				
	0	1	2	4	6
	F_{1a} Generation				
Day 0	5.9	6.0	5.9	6.0	5.5
Day 4[b]	9.0	9.3	8.8	8.9	6.9**
Day 4[c]	9.0	9.3	8.8	8.9	6.9**
Day 7	14.5	14.9	14.7	13.8	9.5**
Day 14	29.7	30.8	30.1	26.6**	16.8**
Day 21	46.8	47.5	46.9	39.8**	23.9**
	F_{1b} Generation				
Day 0	6.0	6.0	5.9	6.1	5.8
Day 4[b]	9.3	9.3	9.3	9.0	6.9**
Day 4[c]	9.3	9.4	9.4	9.0	6.9**
Day 7	15.2	15.3	15.2	14.1	10.1**
Day 14	31.3	31.9	31.4	28.4**	18.6**
Day 21	50.0	51.2	50.3	44.0**	27.4**

a Data extracted from pages 222 and 223.
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

Table 9b. Mean litter weights (g).[a]

Day of lactation	Dose Group (ppm)				
F_{2a} Generation					
Day 0	6.2	6.0	5.9	5.8	5.8
Day 4[b]	9.0	8.8	8.7	8.4	7.3*
Day 4[c]	9.1	8.8	8.7	8.4	7.3*
Day 7	14.5	14.1	13.9	12.4	10.9**
Day 14	29.5	28.5	29.1	23.2**	17.4**
Day 21	46.5	45.8	45.3	36.6**	27.3**
F_{2b} Generation					
Day 0	6.2	6.0	6.1	6.2	5.8
Day 4[b]	9.5	9.3	9.5	9.3	7.5**
Day 4[c]	9.6	9.3	9.6	9.3	7.5**
Day 7	15.0	15.0	15.7	15.0	10.6**
Day 14	29.9	30.8	31.9	29.5	19.7**
Day 21	47.3	48.2	49.3	46.6	25.8**

a Data extracted from pages 224 and 225.
b Before standardization (culling)
c After standardization (culling)
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

3. Offspring postmortem results:

a) Organ weights: Organ weights were not recorded for the offspring of either generation in this study.

b) Pathology

1) Macroscopic examination: There were no treatment-related macroscopic findings in either the F_1 or F_2 pups at any dose level.

2) Microscopic examination: Histopathology was not performed on any of the tissues collected from the F_1 or F_2 pups for any dose level.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that parental toxicity was noted at 4 and 6 ppm. At 4 ppm, clinical signs of toxicity (tremors) were noted in females of the F_1 and P generations. For the P and F_1 dams dosed at 4 ppm, reduced body weight gains were noted during the lactation of each litter. In addition, plasma and brain cholinesterase levels were reduced for the F_1 females at 4 ppm. At 6 ppm, parental toxicity was demonstrated as increased mortality (F_1 animals), clinical signs of toxicity (tremors, P females, F_1 males and females), reduced premating body weights (P females, F_1 males and females), reduced premating body weight gains (P females), and reduced gestational and lactational body weights (P and F_1, both litters). Cholinesterase levels were also reduced in plasma, RBCs, and brain for the high-dose F_1 males and females. Ocular lesions attributed to infectious disease processes were increased at the high dose in the F_1 generation, and may have been caused by a reduced immunity due to treatment with AC 35024. The LOEL for parental toxicity was 4 ppm and the NOEL was 2 ppm.

Reproductive toxicity was demonstrated at 4 ppm as reduced pup weights during lactation (F_{1a}, F_{1b}, and F_{2a} litters) and reduced pup survival indices (F_{2a} litter only). At 6 ppm, treatment-related reductions in litter size at birth was noted for the F_{2a} and F_{2b} litters. Pup body weights, pup survival indices, and litter survival indices were reduced for all litters at the high-dose. The reproductive LOEL was 4 ppm and the NOEL was 2 ppm.

B. REVIEWER'S DISCUSSION: Over the course of the 2-generation reproduction study, AC 35024 was administered to Sprague Dawley rats at dose levels of 0, 1, 2, 4, or 6 ppm in the diet (achieved doses at pre-mating period : 0, 0.087, 0.176, 0.359, or 0.603 mg/kg/day for males and 0, 0.103, 0.201, 0.420, or 0.727 mg/kg/day for females; Gestation period: 0, 0.081, 0.164, 0.331, or 0.550 mg/kg/day, respectively). Exposure to P animals (25/sex) began at 7 weeks of age and lasted for 9 weeks before they were mated the first time to produced the F_{1a} litters. P animals were mated a second time to produce F_{1b} litters. At 28 days post partum, F_{1b} pups (1-2 pups/sex/litter) were selected to become the F_1 parents of the F_2 litters and were given the same concentration test diets as their dam. F_1 animals (25 or 30/sex/dose) were given test

diets for 10 weeks prior to mating the first time to produce the F_{2a} litters. F_1 animals were mated a second time with F_{2b} litters produced. Exposure of the test material to all animals was continuous in the diet throughout the study.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. In addition, the test substance was demonstrated to be stable in the test diets as they were prepared and stored in the study (i.e., no diet at ambient temperature longer than 1 week).

1. Parental Toxicity. Parental toxicity was noted overtly at 4 and 6 ppm and marginally at 2 ppm.

At 2 ppm, reductions in plasma (↓19%) and brain (↓17%) cholinesterase levels were noted in the F_1 females only. The study author did not consider these decreases to be treatment-related because the differences from controls were not statistically significant and the decreases were not noted in males. This reviewer agreed with the conclusion that the ChE effect at 2 ppm was not biologically significant. There were no other signs of systemic toxicity noted at 2 ppm in either generation. Cholinesterase levels were not determined for the P generation.

At 4 ppm, clinical signs of toxicity were noted in P females (3 with tremors, 1 with exophthalmos) and F_1 females (3 with tremors). There were no treatment-related clinical signs of toxicity in the P males at any dose level. Body weights, body weight gains, and food consumption were unaffected by treatment at dose levels of ≤4 ppm for the P and F_1 animals during premating and gestation. Lactational body weights were reduced at 4 ppm (F_1 dams only, both litters, day 21, ↓8-10%, $p \leq 0.01$ or 0.05). In addition, lactational body weight gains were reduced for both generations; P and F_1 dams either lost more weight than the controls or experienced a weight loss while the controls gained weight (F_{1a}, F_{1b}, and F_{2b} litters, $p \leq 0.01$). Treatment-related reductions in plasma (↓74%, $p \leq 0.01$) and brain (↓59%, $p \leq 0.01$) cholinesterase levels were observed in the F_1 females at 4 ppm. Dose-dependent and treatment-related reductions in plasma (↓25%) and brain (↓14%) cholinesterase levels were also noted in F_1 males at 4 ppm; differences from controls were not statistically significant.

At 6 ppm, a treatment-related increase in mortality was observed in the F_1 generation (2 males and 5 females died vs. 1 control female that died). Five of these seven deaths occurred when the animals were young (prior to or within the first two weeks of the premating-interval). There were no treatment-related deaths in the P generation males or females at any dose level. Clinical signs of toxicity were noted at the high-dose in females only of the P generation (23 with tremors, 20 with exophthalmos). For the F_1 generation at 6 ppm, clinical signs were noted in both sexes and included tremors (11 males, 27 females), convulsive movements (2 males), aggressive behavior (10 males, 12 females), and increased ano-genital staining (females only). In addition, protruding eyes, protrusion of tissue off the cornea, and opacities were noted the high-dose F_1 males and females.

Unequivocal treatment-related decreases in body weights (↓6-15%, $p \leq 0.01$ or 0.05, weeks 1, 3, 5, 7-9) and overall body weight gains (↓30%, $p \leq 0.01$) were observed in the P females only at 6 ppm during the premating interval. There were no treatment-related effects noted in food consumption (g/kg/day) for the P males or females at any dose level during premating.

For the F_1 generation, body weights during the premating interval were decreased at 6 ppm (males and females). For both sexes, greater differences from the controls were observed at week 0 (↓49-51%) than at week 10 (↓11-16%). Overall body weight gains (males: ↑6%, not statistically significant; females: ↑15%, $p \leq 0.01$) and food consumption (males: ↑12-48%, $p \leq 0.01$; females: ↑21-61%, $p \leq 0.01$) were increased throughout the premating interval (weeks 0-10) at the high dose. Because body weight gains and food consumption were increased during the premating period and the decreases in body weights lessened over the duration of treatment, the reductions in body weights cannot unequivocally be considered a treatment-related systemic effect on the high-dose F_1 animals. A contributing factor to the decreased body weights during the premating interval was lower lactational pup body weights (↓26-45%, $p \leq 0.01$, days 4-21) that were possibly caused by poor maternal health in the 6 ppm dams (P generation).

During the gestation and lactation of both litters for the P and F_1 high-dose dams, treatment-related reductions in body weights were noted (↓10-28%, $p \leq 0.01$ or 0.05). Maternal body weight gains were also reduced during gestation for the P and

F_1 dams at 6 ppm (↓15-31%, p≤0.01, F_{1a}, F_{1b}, and F_{2b} litters). Maternal lactation body weight gains were reduced for the both generations (F_{1a}, F_{1b}, F_{2b} litters, p≤0.01). Food consumption was typically increased at the high dose for the P and F_1 dams during gestation of both litters (↑7-31%, p≤0.01 or 0.05, days 0-20, 0-14, and/or 7-14).

In addition, treatment-related reductions in cholinesterase levels were noted in plasma (males: ↓40%, p≤0.01; females: ↓96%, p≤0.01), brain (males: ↓40%, p≤0.01; females: ↓83%, p≤0.01), and RBCs (males: ↓10%, p≤0.05; females: ↓11%, not statistically significant) for the F_1 animals dosed at 6 ppm.

An increased incidence of eye infections was observed at the high dose in the F_1 generation at ophthalmoscopic evaluations (66% males and 78% females). Associated findings at necropsy included, macroscopically, opacity of the eyes and, microscopically, anterior synechia, various types of corneal lesions, retinal atrophy and/or folds or rosettes, and secondary cataract formation. The increased incidence of eye infections at 6 ppm cannot unequivocally be considered a direct result of treatment with AC 35024. The increased incidence of infections may be due to compromised immunity resulting either from the poor health or a direct affect on the immune system of the high-dose F_1 animals. There were no other treatment-related macroscopic or microscopic findings for any treatment group for the P or F_1 generations at any dose level.

There were no treatment-related effects noted in the reproductive function or performance of the P or F_1 adults at any dose level.

2. Reproductive Toxicity. Reproductive toxicity was demonstrated at 4 ppm as a treatment-related reduction in the pup survival index (days 0-4) for the F_{2a} litters only (79.0% vs 97.7% for controls, p≤0.01). The pup viability index (total # live pups at day 0/total # of pups born) and pup survival index for days 4-21 were also statistically significantly (p≤0.01 or 0.05) lower than the controls for the F_{2a} litters, although the differences were not great (89.3-95.9% vs. 100-99.6% for controls). Treatment-related reductions in pup body weights were noted at 4 ppm in the F_{1a}, F_{1b}, and F_{2a} generations near the end of lactation (↓9-21%, p≤0.01, days 14-21).

At 6 ppm, treatment-related reductions in mean litter sizes and pup viability throughout lactation were noted in both litters of both generations. For the F_{1a}, F_{1b}, and F_{2a} litters, mean litter sizes at day 0 were comparable to the controls at all dose levels. For the high-dose F_{2b} litters, the mean litter size on day 0 was less than controls (10.8 vs. 13.6, $p \leq 0.01$). By day 4 pre-cull, mean litter sizes at the high-dose were reduced for all litters (8.0-10.2 vs. 12.3-13.6 for controls, $p \leq 0.01$). The pup survival indices for days 0-4 and 4-21 were reduced for both litters of the F_1 and F_2 generations at the high dose (52.4-85.3% vs. 97.7-100% for controls). In addition at the high dose, the litter survival indices (total # of litters at weaning/total # of litters with live pups at day 0) were reduced for the F_{1b} and F_{2a} generations (72.7% and 43.5%, respectively, vs. 100% for controls, $p \leq 0.05$ or 0.01). Treatment-related reductions in offspring body weights were noted at 6 ppm in all litters from days 4-21 of lactation (↓19-49%, $p \leq 0.01$ or 0.05). Pup body weights at day 0 were comparable to the controls at all dose levels in all litters.

There were no treatment-related macroscopic findings in either the F_1 or F_2 pups at any dose level.

The LOEL for parental systemic toxicity is 4 ppm based on clinical signs (tremors), reduced plasma and brain cholinesterase levels (F_1 females only). The systemic NOEL is 2 ppm.

The LOEL for reproductive toxicity is 4 ppm based on decreased pup survival and pup body weights. The reproductive NOEL is 2 ppm.

C. STUDY DEFICIENCIES: A range-finding study was not included in the current submission. However, as the dose levels selected achieved systemic and reproductive LOELs and NOELs, this is not a major deficiency.