1/3/97

DATA EVALUATION RECORD

AZOXYSTROBIN

Study Type: 83-4; ICIA5504:
Multigeneration Study in the Rat

Work Assignment No. 2-22A (MRID 43678144)

Prepared for

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Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - rat

OPPTS Number: 870.3800 OPP Guideline Number: §83-4

DP BARCODE: D208319 SUBMISSION CODE: S48692
P.C. CODE: 128810 TOX. CHEM. NO.:

TEST MATERIAL (PURITY): ICIA5504 (Azoxystrobin, 96.2% a.i.)

SYNONYMS: None

CITATION: Moxon, M.E., (1994) ICIA5504: Multigeneraton Study in the Rat. Zeneca Central Toxicology Laboratory, Cheshire, UK. Laboratory Report/Study No. CTL/P/4213/RR0604, November 15, 1994. MRID 43678144. Unpublished

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EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 43678144), ICIA5504 (96.2% a.i.) was administered to 26 Alpk:APfSD (Wistar-derived) rats/sex/dose at dose levels of 0, 60, 300, and 1,500 ppm in the diet. The average (F_0 and F_1) achieved test substance intake during the premating interval was as follows: 0 6.4, 32.3, or 165.4 (males) and 0, 6.8, 33.8, or 175.0 mg/kg/day (females). Exposure to the F_0 parental animals began at 4 weeks of age and lasted for 10 weeks before they were mated to produce the F_{1a} litters. The F_1 parental animals were selected from the F_{1a} litters at 29 days of age and were mated 10 weeks after selection to produce the F_{2a} litters. All animals were mated on a 1:1 ratio. Exposures to the test material for all animals were continuous in the diet throughout the study.

At 1,500 ppm, systemic toxicity in the F_0 and F_1 adults (both sexes) was demonstrated as reduced adjusted body weights (↓3-12%, $p \leq 0.01$ or 0.05) and food consumption (↓5-14%, $p \leq 0.05$ or 0.01) during the pre-mating intervals. In addition, treatment-related increases in liver weights adjusted for final body weights were noted in the F_0 and F_1 males and females (↑15-38%, $p \leq 0.01$ or 0.05) at the 1,500 ppm dose level. Treatment-related distention of the common bile duct was also noted grossly in 12 and 42% of the F_0 and F_1 males dosed at 1,500 ppm, respectively. Treatment-

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related histopathologic lesions of the common bile duct in the adult high-dose males were characterized as epithelial hyperplasia of the intraduodenal portion, cholangitis, ulceration of the dilated region, and small basophilic deposits in the lumen. Treatment-related increases in severity of proliferative cholangitis were also observed in the livers of the F_0 and F_1 males dosed at 1,500 ppm. Both sexes in the F_{1a} and F_{2a} 1,500 ppm dose groups had treatment-related increases in the adjusted (for final body weight) liver weights (↑10-13%, p≤0.01). **The LOEL for systemic toxicity in males is 1500 ppm (163.2 mg/kg/day), based on reduced adjusted body weight means. A systemic NOEL in males was 300 ppm (33 mg/kg/day). The LOEL for systemic toxicity in females is 1,500 ppm (170.6 mg/kg/day), based on reduced adjusted body weight and feed consumption means and increased adjusted liver weights. The systemic NOEL in females is 300 ppm (33.2 mg/kg/day).**

Reproductive toxicity was demonstrated as treatment-related reductions in adjusted (for initial weight) pup body weights as observed in the F_{1a} and F_{2a} pups dosed at 1500 ppm (↓8 - 21%, p≤0.05 or p≤0.01). **The LOEL for reproductive toxicity is 1500 ppm (31.7 mg/kg/day), based on reduced adjusted pup body weights. The reproductive NOEL is 300 ppm (33 mg/kg/day).**

The reproductive study in the rat is classified as **acceptable** satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4).

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: ICIA5504 (96.2% a.i.)
   Description: light brown, solid, technical
   Lot/Batch #: P49/D73534/46
   Purity: 96.2 % a.i.
   CAS #: 131860-33-8

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2. Vehicle:
diet

3. Test animals:
Species:
rat
Strain:
Alpk:APf
SD (Wistar-derived)
Age at start of dosing: (F_0) approximately 29 days
(F_1) age at weaning (22 days)
Weight at start of dosing:
(F_0) Males: 56.8-92.7 g, Females: 58.3-90.3 g
(F_1) Males: 54.1-97.3 g, Females: 51.0-98.5 g
Source: Specific Pathogen Free colony at Barriered Animal Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK
Housing: Two same sexed rats/cage in the pre-mating period, one male with one female during mating, and females individually during gestation and lactation in cages with solid stainless steel sides and floor and stainless steel mesh in the back and front
Diet: CTI diet from Special Diet Services Ltd, Stepfield, Witham, Essex, UK, ad libitum
Water: filtered tap water, ad libitum
Environmental conditions:
Temperature: 66-77°F
Humidity: 33-83%
Air changes: 15/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period (F_0): approximately one week

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group until sperm cells were observed in vaginal smears taken daily during the 3 week mating period. If sperm were not found after 21 days or if a presumed pregnant dam was not showing the expected body weight gain, the first male was removed, and after a rest period of at least 3 days, was replaced by another male with proven fertility in the same test group. Sibling matings within the F_1 generation were avoided.

After successful mating, each pregnant female was individually placed in a cage with a solid bottom and paper bedding where she was kept through gestation and lactation.

2. Study schedule: The F_0 parental animals were given test diets for 10 weeks before they were mated to produce the F_{1a} litters. The F_1 parental animals were selected from the F_{1a} litters at 29 days of age and were mated 10 weeks after selection to produce the F_{2a} litters. The F_0 and F_1 adults were approximately 14 weeks of age at mating.

3. Animal assignment: The F_0 parental animals were randomly assigned to test groups (Table 1). F_{1a} pups selected to become parents of the next generation were selected from litters containing six to eighteen pups and were note selected from litters derived from remating.

TABLE 1. Animal Assignment

Test Group	Dose in Diet[a] ppm	Animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	26	26	26	26
Low (LDT)	60	26	26	26	26
Mid (MDT)	300	26	26	26	26
High (HDT)	1,500	26	26	26	26

[a]Test diets were administered from the beginning of the study until sacrifice.

4. Dose selection rationale: A dose selection rationale was not provided.

5. Dosage preparation and analysis: Formulations were prepared by mixing appropriate amounts of test substance with the CT1 diet and were stored at room temperature. The frequencies of the test diet preparations and their storage intervals were not specified. The stability and homogeneity (top, middle, and bottom) of the test substance in the diet (60 and 1,500 ppm) were evaluated for a two year feeding study in rats (data from CTL study number PR0892). Stability was assessed for a period of 66 days at unspecified temperatures. During the study, samples of treated feed were analyzed at approximately two monthly intervals for concentration.

Results - Homogeneity Analysis: 60 ppm diet - mean concentration was 104% of nominal with % deviations of -0.8 to +0.5%; 1,500 ppm diet - mean concentration was 103% of nominal with % deviations of -1.2 to +1.2%

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Stability Analysis: 60 ppm diet - 95.0% of nominal after 66 days; 1,500 ppm diet - 95.1% of nominal after 66 days

Concentration Analysis: 90.8-104.9% of nominal

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.

C. OBSERVATIONS

1. Parental animals: Animals were observed daily for mortality and clinical signs of toxicity. Physical examinations were performed weekly at the same time body weights were recorded. Body weights were recorded weekly throughout the premating intervals starting on the first day of dosing for the F_0 animals and on the day of selection for the F_1 animals. Mated female body weights were recorded on days 1, 8, 15, and 22 of gestation and days 1, 5, 11, 16, 22, and 29 of lactation. Feed consumption was recorded weekly throughout the study.

   The reproductive performance of the parents was assessed by evaluating the success of each mating, i.e. one in which a litter with at least one viable pup was produced. In addition, the length of gestation (interval from the date of a positive vaginal smear to parturition) and precoital intervals (interval between the date of pairing to the date of a positive vaginal smear) were recorded.

2. Litter observations: The following litter observations (X) were made (Table 2).

TABLE 2. F_1/F_2 Litter Observations.[a]

Observation	Time of observation (lactation day)					
	Day 1[b]	Day 5	Day 11	Day 16	Day 22	Day 29
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

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[a]Data extracted from the study report pages 21 and 22.
[b]Day 1 is defined in the report as within 24 hours of parturition.

Litters were not standardized to a maximum size. Dead pups and pups killed moribund were examined grossly.

3. Postmortem observations:

1) Parental animals: All surviving parental males were sacrificed at the same time after the mating period for each generation. Maternal animals were sacrificed after the last litter of each generation was weaned. Females exhibiting prolonged gestation or parturition difficulties were sacrificed and subjected to a full gross postmortem examinations as follows.

The following tissues (X) were prepared for microscopic examination and weighed (XX):

X	Ovaries	XX	Epididymides
X	Uterus	X	Prostate
X	Vagina/cervix	X	Seminal vesicles including coagulating gland
X	Mammary glands (females only)	XX	Testes
X	Pituitary gland	XX	Liver
X	Common bile duct	X	Duodenum
X	Lesions		

One high-dose F_0 male sacrificed moribund exhibited distention of the common bile duct. Because of this finding, the common bile duct and the duodenum were collected from all F_0 adults at terminal sacrifice and from all F_1 adults (intercurrent and terminal deaths). Histopathologic examinations were performed on the common bile duct and duodenum of adults exhibiting biliary distentions. The livers from all F_0 and F_1 adults were examined histologically. For groups that were suspected to be infertile, the following tissues were examined histologically: uterus, cervix, vagina, ovary, mammary glands (females), testis, epididymis, prostate, seminal vesicle (males), and pituitary glands (both sexes).

2) Offspring: The F_{1a} offspring not selected as parental animals and all F_{2a} offspring were sacrificed at 29 days

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of age. Five pups/sex/dose level from F_{1a} generation and 10 pups/sex/dose level from the F_{2a} generation were selected randomly and subjected to gross post mortem examinations. Any remaining pups showing abnormal clinical signs of toxicity, and, if available, two normal pups/sex/litter were also selected for necropsy. In addition, pups over 18 days of age found dead or sacrificed moribund were subjected to a gross examination. For pups under 18 days of age found dead or sacrificed moribund, abnormalities were recorded and the carcasses were discarded.

At necropsy, the following tissues (X) were prepared for microscopic examination and weighed (XX, F_{1a} generation only):

X	Ovaries	XX	Epididymides
X	Uterus	X	Prostate
X	Vagina/cervix	X	Seminal vesicles including coagulating gland
X	Mammary glands (females only)	XX	Testes
X	Pituitary gland	XX	Liver
X	Common bile duct	X	Duodenum
X	Lesions		

Histopathologic examinations were performed on livers from all F_{1a} and F_{2a} pups that were subjected to gross necropsies. Any abnormal tissues collected at necropsy were also examined histologically.

D. DATA ANALYSIS

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

2. Indices:

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

male fertility index:
fertile 100%/(#fertile + # infertile)

female mating index:
fertile 100%/(#fertile + # infertile)

Fertility was evaluated by the success of each mating,

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i.e. the production of a litter in which at least one pup was found alive at day 1.

The proportion of litters with gestational lengths of less than 22, 22, and greater than 22 days, and the proportion of litters with pre-coital intervals of 1, 2, 3, 4 and greater than 4 days were calculated.

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

proportion of pups live born:
[# pups live born/(# pups live born + # pups born dead)] x 100%

proportion of pups surviving to day 22:
(# pups live pups on day 22/# pups live pups on day 1) x 100%

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

II. **RESULTS**

A. PARENTAL ANIMALS

1. Mortality and clinical signs: There were no treatment-related clinical signs of toxicity or increases in mortality noted at any dose level. However, one F_0 and one F_1 male from the 1500 ppm group, were sacrificed moribund and exhibited treatment-related distention of the common bile duct. Therefore, these intercurrent deaths are attributed to treatment with ICIA5504 at 1,500 ppm.

2. Body weight and feed consumption: Selected body weight and feed consumption data are summarized in Tables 3a and

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TABLE 3a. Body Weight and Feed Consumption - F_0 Generation Pre-mating.[a]

Observations/study week	Dose Group			
	0	60	300	1,500
F_0 Generation Males - Pre-mating				
Mean body weight (g) Week 1	74.3	74.6	74.4	76.8
Adjusted mean body weight (g) Week 2	125.1	125.4	124.4	117.4**
Adjusted mean body weight (g) Week 5	289.1	288.0	287.1	264.4**
Adjusted mean body weight (g) Week 11	449.9	448.3	444.3	404.5**
Mean feed consumption (g/animal/day) Week 1	18.3	18.7	18.7	17.4*
Mean feed consumption (g/animal/day) Week 2	24.1	24.5	24.7	23.7
Mean feed consumption (g/animal/day) Week 5	30.8	30.7	31.0	29.0**
Mean feed consumption (g/animal/day) Week 10	29.7	29.9	30.2	27.6**
F_0 Generation Females - Pre-mating				
Mean body weight (g) Week 1	73.1	72.5	72.0	71.5
Adjusted Mean body weight (g) Week 2	113.1	114.3	112.9	107.2**
Adjusted Mean body weight (g) Week 5	197.3	199.1	194.9	180.4**
Adjusted Mean body weight (g) Week 11	266.8	262.9	262.3	236.6**
Mean feed consumption (g/animal/day) Week 1	17.1	17.3	17.0	15.6**
Mean feed consumption (g/animal/day) Week 2	20.9	20.2	20.1	19.9
Mean feed consumption (g/animal/day) Week 5	22.4	22.3	21.6*	19.5**
Mean feed consumption (g/animal/day) Week 10	21.0	20.7	21.1	19.6**

[a]Data extracted from the study report pages 97-100, 105, and 106; mean body weights were adjusted for initial weights.
*Statistically different from control, p≤0.05.
**Statistically different from control, p≤0.01.

3b. Body weight data were adjusted for initial body weights.

At 1,500 ppm, mean adjusted body weights were reduced by 3-12%, ($p \leq 0.01$ or 0.05) for both sexes of the F_0 and F_1 generations at weeks 2 through 11 (pre-mating treatment interval). The initial mean body weights of the F_1 generation high-dose animals were reduced relative to the controls by 17 and 18% ($p \leq 0.01$). Treatment-related reductions in mean feed consumption were also noted in the F_0 and F_1 males and females during the premating period at the 1,500 ppm dose level (↓5-14%, $p \leq 0.05$ or 0.01).

At 300 ppm, adjusted mean body weights were comparable to controls for the F_0 males and were sporadically, slightly reduced for the F_0 females (↓3%, $p \leq 0.05$ at weeks 7 and 8). For the F_1 generation, the adjusted mean body weights were reduced in males at weeks 3 and weeks 5 through 11 (↓3-6%, $p \leq 0.05$ or 0.01) and were comparable to the controls for the females. Feed consumption was comparable to the controls for the F_0 males and the F_1 females dosed at 300 ppm. For the F_0 females and the F_1 males dosed at 300 ppm, feed consumption was reduced and differences from the controls were occasionally statistically significant (↓4-5%, $p \leq 0.01$ or 0.05).

At 60 ppm, body weights were comparable to the controls for the F_0 generation males and females. For the F_1 generation, reductions in the adjusted mean body weights were noted in males at weeks 5 through 11 (↓3-6%, $p \leq 0.05$ or 0.01) and slightly reduced in females at week 4 (↓3%, $p \leq 0.05$). No treatment-related changes were noted in the feed consumption for either generation or sex dosed at 60 ppm.

The reductions in the adjusted mean body weight noted at 1,500 ppm were statistically significant, greater than 10%, noted in both generations and sexes, and were therefore considered to have been be treatment-related. The reductions noted in adjusted mean body weights at 60 or 300 ppm for the F_0 or F_1 females were sporadic and slight, and therefore cobsidered to have been unrelated to treatment. The study author's stated that the reductions noted the adjusted mean body weights of the F_1 males at 60 and 300 ppm were unrelated to treatment because they were comparable to the historical controls. The historical control data were only provided in graphic format and numerical raw data were not provided. Thus,

TABLE 3b. Body Weight and Feed Consumption - F_1 Generation Pre-mating.[a]

Observations/study week	Dose Group			
	0	60	300	1,500
F_1 Generation Males - Pre-mating				
Mean body weight (g) Week 1	78.6	85.2**	79.4	64.6**
Adjusted mean body weight (g) Week 2	126.2	127.0	124.3	120.6**
Adjusted mean body weight (g) Week 5	292.6	284.0*	280.0**	267.7**
Adjusted mean body weight (g) Week 11	466.9	439.7**	439.3**	411.0**
Mean feed consumption (g/animal/day) Week 1	17.6	18.9**	18.3	15.4**
Mean feed consumption (g/animal/day) Week 2	24.3	25.5*	24.4	21.6**
Mean feed consumption (g/animal/day) Week 5	30.8	30.2	29.6*	28.0**
Mean feed consumption (g/animal/day) Week 10	30.3	30.2	29.3	26.1**
F_1 Generation Females - Pre-mating				
Mean body weight (g) Week 1	73.9	80.3**	74.3	61.0**
Adjusted mean body weight (g) Week 2	111.4	111.2	111.2	108.1**
Adjusted mean body weight (g) Week 5	193.5	187.8	191.9	177.8**
Adjusted mean body weight (g) Week 11	254.3	254.8	256.5	233.1**
Mean feed consumption (g/animal/day) Week 1	15.6	16.6*	16.1	13.9**
Mean feed consumption (g/animal/day) Week 2	20.4	21.3	20.5	18.8**
Mean feed consumption (g/animal/day) Week 5	20.4	20.7	20.3	18.5**
Mean feed consumption (g/animal/day) Week 10	19.4	20.5*	19.9	18.2**

[a]Data extracted from the study report pages 101-104 and 107-108.
*Statistically different from control, p≤0.05.
**Statistically different from control, p≤0.01.

it was not possible to determine from the graphs if the data were for adjusted mean body weights; the age of the rats also was not indicated. The reductions in the adjusted mean body weights of the F_1 males were persistent, dose-related, and were considered treatment-related.

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During gestation of the high-dose F_0 and F_1 animals, adjusted mean body weights were slightly reduced (↓≤5%, p≤0.01 or 0.05) and feed consumption was reduced (↓9-15%, p≤0.01). Lactational adjusted mean body weights were reduced for the F_0 females (↓≤9%, p≤0.01 or 0.05) and were unaffected by treatment for the F_1 generation. Feed consumption during lactation was statistically comparable to the controls.

For animals dosed at 300 and 60 ppm, body weights and feed consumption during gestation and lactation were comparable to the controls.

3. Test Substance Intake: The doses expressed as mean daily mg test substance/kg body weight during the 10 week pre-mating period are based on feed consumption and body weight data and the nominal dietary concentrations (Table 4). The values for the F_0 and F_1 generation are considered to be representative of the test substance intake for the entire study.

TABLE 4. Test Substance Intake (mean mg/kg body weight/day).[a]

Male			Female		
60 ppm	300 ppm	1,500 ppm	60 ppm	300 ppm	1,500 ppm
F_0 Generation					
6.5	33.0	162.3	6.9	34.4	170.6
F_1 Generation					
6.3	31.7	168.4	6.7	33.2	179.4
Aveage of Both Generations					
6.4	32.3	165.4	6.8	33.8	175.0

[a]Data extracted from the study report pages 203-206.

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 female abnormalities. Pre-coital intervals were unaffected by treatment for

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both generations and at all dose levels.

b. Sperm measures: No sperm parameter observations were made in this study. However, there were no indications of treatment-related male fertility abnormalities.

c. Sexual maturation (F_{1a}): No observations were made pertaining to the sexual maturation rates of the F_{1a} or F_{2a} litters.

5. Reproductive performance: Reproductive performance results are presented in Table 5a and 5b. There were no treatment-related effects noted in the reproductive performance of the F_0 or F_1 adults.

TABLE 5a. Reproductive Performance.[a]

Observation	Dose Group (ppm)			
	0	60	300	1,500
F_0 Generation - Litter F_{1a}				
Mean precoital interval (days)	2.33	3.38	2.90	2.31
Proportion 1 day (%)	22.2	27.6	40.0	37.9
Proportion 2 days (%)	44.4	20.7	16.7	31.0
Proportion 3 days (%)	18.5	24.1	16.7	6.9
Proportion 4 days (%)	11.1	17.2	13.3	17.2
Proportion >4 days (%)	3.7	10.3	13.3	6.9
Median gestation interval (days)	22.3	22.2	22.3	22.3
Proportion 22 days (%)	72.0	79.2	65.2	66.7
Proportion >22 days (%)	28.0	20.8	34.8	33.3
Number of litters	25	24	23	24
MALES				
Fertility index (%)	92.3	87.5	86.4	84.0
Intercurrent deaths	1	0	1	2
FEMALES				
Fertility index (%)	100	96	100	96.2
Intercurrent deaths	0	0	2	0

[a]Data extracted from the study report pages 83, 87, 119, 120, and 121.
*Statistically different from control, $p<0.05$.
**Statistically different from control, $p<0.01$.

TABLE 5b. Reproductive Performance.[a]

Observation	Dose Group (ppm)			
	0	60	300	1,500
F_1 Generation - Litter F_{2a}				
Mean precoital interval (days)	2.23	3.44	3.57	2.52
Proportion 1 day (%)	26.9	29.6	21.4	28.0
Proportion 2 days (%)	38.5	33.3	32.1	40.0
Proportion 3 days (%)	19.2	7.4	14.3	16.0
Proportion 4 days (%)	15.4	14.8	17.9	12.0
Proportion >4 days (%)	0	14.8	14.3	4.0
Median gestation interval (days)	22.1	22.1	22.2	22.1
Proportion <22 days (%)	0	3.8	0	0
Proportion 22 days (%)	87.5	84.6	84.0	87.0
Proportion >22 days (%)	12.5	11.5	16.0	13.0
Number of litters	24	26	25	23
MALES				
Fertility index (%)	100	96.0	92.0	100
Intercurrent deaths	2	1	0	1
FEMALES				
Fertility index (%)	100	100	96.2	100
Intercurrent deaths	2	0	1	3

[a]Data extracted from the study report pages 91, 94, 119,120, and 121.
*Statistically different from control, p≤0.05.
**Statistically different from control, p≤0.01.

6. Parental postmortem results

a) Organ weights: Treatment-related increases in liver weight adjusted for final body weight were noted in the F_0 and F_1 males and females dosed at 1,500 ppm (↑15-38%, p≤0.01). Absolute liver weightwere also increased (↑8%, p≤0.05) for the F_0 males dosed at 1,500 ppm. Corroborative histopathologic changes were noted in the livers of the F_0 and F_1 males dosed at 1,500 ppm.

A statistically significant decrease (↓8%, p≤0.01) was noted in the absolute epididymis weight for the F_1 males dosed at 1,500 ppm. As no corroborative gross or

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histopathology was noted in the epididymis at any dose level, this finding was considered incidental and not attributed to treatment. Testicular weights were comparable to controls for both generations at 1,500 ppm.

No treatment-related effects on organ weights (liver, testes, or epididymes) were noted at the 300 and 60 ppm dose levels. (Data were extracted from the study report pages 137-142).

b) Pathology

1) Macroscopic examination: Treatment-related gross pathologic changes were noted in the 1,500 ppm F_0 and F_1 males in the common bile duct. Three F_0 males (12%) and 11 F_1 males (42%) exhibited gross distention of the common bile duct. The common bile duct was unremarkable in all control rats.

2) Microscopic examination: Treatment-related histopathologic changes were noted in the livers and common bile ducts of the F_0 and F_1 males dosed at 1,500 ppm (Table 6). Histopathologic lesions of the common bile duct of adults exhibiting biliary distention were characterized as epithelial hyperplasia of the intraduodenal portion, cholangitis, and ulceration of the dilated region. The lumen of some affected bile ducts also contained small basophilic deposits. Treatment-related histopathologic lesions of the liver were characterized as an increases in severity of proliferative cholangitis.

B. OFFSPRING

1. Viability and clinical signs: Mean litter size and viability data from pups during lactation are summarized in Tables 7a and 7b. There were no treatment-related changes noted in mean litter sizes, viability parameters, or clinical signs of toxicity at any dose level for either generation.

TABLE 6. Incidence of Selected Histopathologic Findings in Rats Fed ICIA55504.[a]

Site & Lesion	No. Observed/No. Examined		
Dose Level (ppm)	0	300	1,500
F_0 - Males			
Common bile duct: (# examined)	(3)	(0)	(3)
Dilation	0	0	3
Epithelial hyperplasia	0	0	3
Cholangitis	0	0	2
Basophilic deposits in lumen	0	0	2
Ulceration	0	0	1
Liver: (# examined)	(26)	(26)	(26)
Proliferative cholangitis - total	12	12	15
minimal	11	11	6
slight	1	1	4
moderate	0	0	2
marked	0	0	3
F_1 - Males			
Common bile duct: (# examined)	(5)	(0)	(11)
Dilation	0	0	11
Epithelial hyperplasia	0	0	10
Cholangitis	0	0	11
Basophilic deposits in lumen	0	0	3
Ulceration	0	0	10
Liver: (# examined)	(26)	(26)	(26)
Proliferative cholangitis	2	1	17
minimal	2	1	5
slight	0	0	2
moderate	0	0	8
marked	0	0	2

[a]These data were extracted the study report pages 165-171.

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TABLE 7a. Mean Litter Size and Viability Data of F_{1a} Generation.[a]

Observation	Dose Group (ppm)			
	0	60	300	1,500
Mean litter size				
Day 1	11.2	10.0	10.9	10.4
Day 5	9.5	8.7	9.8	9.5
Day 11	10.1	9.4	10.0	9.9
Day 16	10.1	9.4	10.0	9.9
Day 22	10.1	9.3	10.0	9.9
Day 29	10.0	9.3	10.0	9.8
Number live pups[b]				
Day 1	291	240	251	260
Day 5	247	209	216	238
Day 11	242	207	210	238
Day 16	242	207	210	238
Day 22	242	205	210	238
Day 29	240	205	210	235
Number deaths[b]				
Days 1-5	44	31	35	22
Days 5-22	5	4	6	0
Proportion of pups surviving to day 22 (%)	80.5	86.0	83.0	92.3*

[a]Data extracted from the study report pages 124 and 126.
[b]Calculated by the reviewer from the mean data.
*Statistically different from control, $p \leq 0.05$.

TABLE 7b. Mean Litter Size and Viability Data of F_{2a} Generation.[a]

Observation	Dose Group (ppm)			
	0	60	300	1500
Mean litter size				
Day 1	11.9	12.1	11.2	11.0
Day 5	11.3	11.7	10.8	10.5
Day 11	11.3	11.5	10.7	10.5
Day 16	11.2	11.5	10.7	10.5
Day 22	11.2	11.5	10.7	10.5
Day 29	11.2	11.5	10.7	10.5
Number live pups [b]				
Day 1	286	315	280	253
Day 5	271	304	270	242
Day 11	271	299	268	242
Day 16	269	299	268	242
Day 22	269	299	268	242
Day 29	269	299	268	242
Number deaths [b]				
Days 1-5	15	11	10	11
Days 5-22	2	5	2	0
Proportion of pups surviving to day 22 (%)	95.0	93.1	96.6	96.5

[a]Data extracted from the study report pages 125 and 126.
[b]Calculated by the reviewer from the mean data.

2. Body weight: Selected mean pup body weight data are presented in Table 8. For the F_{1a} males and females, mean pup body weights, adjusted for initial weights were reduced from day 16 through 29 at the 1,500 ppm dose level (↓8-17%, $p \leq 0.01$ or 0.05). F_{1a} offspring body weights were unaffected by treatment at dose levels of ≤300 ppm.

For the F_{2a} pups, the adjusted mean pup body weights were reduced from day 11 through 29 at the 1,500 ppm dose level (↓8-21%, $p \leq 0.01$ or 0.05). At 300 ppm, the adjusted mean pup body weights were reduced for females on days 5, 22 and 29 (↓6-7%, $p \leq 0.05$) and for males on days 5 and 29 (↓6%, $p \leq 0.05$). For the F_{2a} females dosed at 60 ppm, a reduction in the adjusted mean body weights were noted on day 29 (↓5%, $p \leq 0.05$).

TABLE 8. Mean Pup Body Weights.[a]

Day of lactation	Dose Group (ppm)							
	0		60		300		1,500	
	F_{1a} Generation							
	Males	Females	Males	Females	Males	Females	Males	Females
Day 1	6.0	5.5	6.1	5.7	5.9	5.6	6.1	5.7
Day 5	8.1	7.7	8.9*	8.4*	8.4	7.9	8.9*	8.4*
Day 11	16.7	16.0	18.5**	17.5*	17.7	16.5	16.7	15.9
Day 16	26.0	25.0	28.6**	27.2*	27.0	25.7	24.0*	22.9*
Day 22	41.1	39.5	45.2**	43.0*	42.1	39.3	34.3**	32.8**
Day 29	77.0	72.2	83.5**	77.0*	78.1	72.9	63.9**	60.1**
	F_{2a} Generation							
Day 1	6.2	5.9	6.3	5.9	6.3	5.9	6.4	6.1
Day 5	9.4	9.0	9.5	9.0	8.8*	8.4*	9.3	8.8
Day 11	19.1	18.5	19.0	18.1	18.4	17.9	17.6*	16.9**
Day 16	27.6	26.6	27.4	26.0	26.7	25.9	24.2**	23.2**
Day 22	42.9	41.5	41.9	39.7	40.2	38.8*	34.1**	32.6**
Day 29	81.2	75.8	78.5	72.1*	76.4*	71.3*	63.9**	60.5**

[a]Data extracted from the study report pages 127-130; Day 1 body weights are absolute means, Days 5-29 body weights are adjusted for initial body weights.
*Statistically different from control, $p \leq 0.05$
**Statistically different from control, $p \leq 0.01$

3. Offspring postmortem results:

a) Organ weights: As with the F_0 and F_1 adults, the offspring organ weights were adjusted for final body weights. Treatment-related increases in the adjusted mean liver weights were noted in the F_{1a} and F_{2a} males and females dosed at 1,500 ppm (↑10-13%, $p \leq 0.01$). A statistically significant increase (↑16%, $p \leq 0.01$) was noted in the mean adjusted testicular weight for the F_{1a} males dosed at 1,500 ppm. For the F_{2a} males dosed at 1,500 ppm, the mean adjusted testicular weight was comparable to controls and the mean absolute testicular weight was reduced (↓14%, $p \leq 0.05$). As the changes in testicular weights were not consistent for both generations, they were not considered to have been treatment-related. The absolute and adjusted epididymis weights were comparable to the controls for the F_{1a} and F_{2a} males dosed at 1,500 ppm.

No treatment-related effects on adjusted organ weights (liver, testes, or epididymis) were noted at the 300 and 60 ppm dose levels. (Data were extracted from the

study report pages 143-148).

b) Pathology:

1) Macroscopic examinations: There were no treatment-related macroscopic findings noted for either the F_{1a} or F_{2a} litters at any dose level.

2) Microscopic examinations: There were no treatment-related microscopic findings noted for either the F_{1a} or F_{2a} litters at any dose level.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that the parental LOEL was 1,500 ppm based on reduced body weight gain and feed consumption, increases in liver weight in the F_0 and F_1 adults (both sexes), gross distension of the common bile duct with associated epithelial hyperplasia and cholangitis and an increase in the severity of hepatic proliferative cholangitis. The parental NOEL was 300 ppm (approximately 32 mg/kg/day).

Reproductive toxicity was observed as a reduction in pup weights for both the F_{1a} and F_{2a} litters in the 1,500 ppm dose group. In addition, liver weights were increased in the F_{1a} and F_{2a} pups dosed at 1,500 ppm. The reproductive LOEL was 1,500 ppm and the NOEL was 300 ppm.

B. REVIEWER'S DISCUSSION: In a 2-generation reproduction study, ICIA5504 (96.2 % a.i.) was administered to 26 Alpk:APfSD (Wistar-derived) rats/sexes/dose at dose levels of 0, 60, 300, and 1,500 ppm in the diet. Exposure to the F_0 parental animals began at 4 weeks of age and lasted for 10 weeks before they were mated to produce the F_{1a} litters. The F_1 parental animals were selected from the F_{1a} litters at 29 days of age and were mated 10 weeks after selection to produce the F_{2a} litters. All animals were mated on a 1:1 ratio. Exposure to the test material for all animals was continuous in the diet throughout the study. The average achieved test substance intakes during the premating interval were as follows: F_0 generation, 0, 6.5, 33.0, or 162.3 mg/kg/day (males) and 0, 6.9, 34.4, or 170.6 mg/kg/day (females); F_1 generation, 0, 6.3, 31.7, or 168.4 mg/kg/day (males) and 0, 6.7, 33.2, 179.4 mg/kg/day (females).

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.

TB1 concurs with the Submitter's conclusions regarding parental and reproductive toxicities, and the levels at which they occurred.

C. STUDY DEFICIENCIES: No major deficiencies were noted.