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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

BATTELLE PACIFIC-NORTHWEST NATIONAL
LABORATORIES
STUDY NUMBER WA 2-17
EPL PROJECT NUMBER 237-010

AVIAN DOSING STUDY

DRAFT PATHOLOGY REPORT

Submitted by:

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DRAFT PATHOLOGY SUMMARY

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The purpose of this study was to establish appropriate exposure scenario(s) during a two-generation test to be used by the EPA and OECD in the development of a test guideline to assess the impact of chemicals on the reproduction and development of birds over two generations.

The study design was as follows:

Onset of Exposure	Pens per P1 or F1 Group (1 cock/1 hen per pen)	EPL Group ID	17β-Estradiol Exposure Concentration (ppm)			
			Adults	F1a	F1b	F2
P1A (pre-breeding; 2-3 wks old)	8	1	0	0	-- ²	0
	8	2	5.0	5.0	0	0
	8	3	1.25	1.25	0	0
	8	4	0.31	0.31	0	0
	8	5	0.078	0.078	0	0
P1B (adult; proven breeders)	8	1	0	0	-- ²	0
	8	2	5.0	5.0	0	0
	8	3	1.25	1.25	0	0
	8	4	0.31	0.31	0	0
	8	5	0.078	0.078	0	0

¹ Ten pairs were established in each group initially to provide for at least eight pairs of breeding pairs during treatment. (Both birds of a pair were removed if one of a pair died or was injured).

² No additional control group was used. The F1^a control groups served as controls for both F1^a and F1^b populations.

MATERIALS AND METHODS

A necropsy was performed by the sponsor, Battelle Pacific-Northwest National Laboratories. Protocol-required tissues were collected and preserved in fixative. The fixed tissues from all quail were shipped to Experimental Pathology Laboratories, Inc. (EPL[®]) and were trimmed, processed, embedded in paraffin, microtomed, placed on glass microscope slides, and stained with hematoxylin and eosin. The following protocol-required tissues were evaluated, as available, from all animals, both males and females, in the control and 5 ppm level adult P1 and F1 generations: adrenal, brain, liver, pineal gland, pituitary, and thyroid. The following tissues were processed and evaluated, as available, from all male animals only in the P1, F1 and F2 generations: cloacal gland, epididymis, and testes. The following tissues were processed and evaluated, as available, from all female animals only in the P1, F1 and F2 generations: ovary and oviduct. The following protocol-required tissues were processed and evaluated, as available, from all animals, both males and females, in the control and 5 ppm level F2 generations: thyroid. Gross lesions from all animals were also processed and examined histologically. The microscopic examination was conducted by Peter C. Mann, DVM, Diplomate, ACVP.

Microscopic findings for each animal are listed in the Histopathology Incidence Tables and are graded one to five depending on severity. Findings that could not be graded, such as immature reproductive organs, are listed as Present (P). All findings for all animals are summarized by sex, generation, and treatment group in the Summary Incidence Tables, together with the total number of animals in each group. A correlation of gross and microscopic findings is provided in the Correlation of Gross and Microscopic Findings tables.

RESULTS**PI GENERATION****Males**

Testes: There was diffuse degeneration of the seminiferous tubules in the testes of treated males in the P1 generation. This degeneration consisted of a reduction in the number of primary and secondary spermatocytes, as well as an increased granularity to the cytoplasm of the affected cells. In addition, there was increased vacuolation (possibly of Sertoli cell cytoplasm) in the testes of P1A males treated with 5.0 ppm. The degeneration was present in all treated dose groups in the P1A males, but only at 5.0 ppm in the P1B males.

Epididymis: Hypospermia (a reduction in the number of mature spermatids in the epididymal lumens) was noted in many of the male quail. In some animals, several sections of epididymis were present - one set adjacent to the testes; another associated with the adrenals; and in some cases, a third set adjacent to the cloaca. It was not unusual for one set of epididymides to be devoid of sperm, while a second set from the same animal to be filled with normal, mature sperm. In the P1A males, there was an apparent effect of treatment - most of the treated animals showed hypospermia. This effect was not present in the P1B males, where the incidence of hypospermia was similar in the control and the 5.0 ppm groups.

Cloacal Glands: In the P1A males, there was some degree of atrophy of the submucosal glands in the cloaca. The incidence and severity were slightly higher in the 5.0 ppm group. This effect was not present in the P1B groups.

Adrenal Glands: Diffuse hypertrophy, affecting both the cortical and medullary cells similarly, was present in the P1A males treated with 5.0 ppm.

This change was not present in any of the other P1A treated males, or in any of the P1B males.

Females

Ovary: Two changes were noted with an increase with treatment: increased follicles and degenerating follicles. Both of these changes are likely associated with normal reproductive activity in quail, but the increases appeared to be the result of treatment. Increased follicles were diagnosed when the number of small follicles in the ovary was increased over baseline, and the mature follicles adjacent to the ovary were also enlarged. Degenerating follicles were diagnosed when there were increased numbers of follicles undergoing degeneration. In the P1A group, the incidence of increased follicles was higher than controls in all treated groups. This effect was not noted in the P1B females. The incidence of degenerating follicles was increased in the treated P1A females, but not in the P1B females.

Oviduct: The epithelial cells lining the lumen of the oviduct underwent varying degrees of hypertrophy and hyperplasia in treated females. In the P1A females, this increase was present in all treated groups. In the P1B females a similar effect was not present.

Adrenal Glands: Diffuse hypertrophy, affecting both the cortical and medullary cells similarly, was present in a small number of the P1A females treated with 5.0 ppm and in all the P1B females treated with 5.0 ppm.

There were no treatment-related changes in any of the additional organs examined in either the male or female P1A or the P1B groups.

FI GENERATION**Males**

Testes: There was a lower incidence of diffuse degeneration of the seminiferous tubules in the testes of treated males in the F1 generation than in the P1 generation. The incidence of degeneration was slightly increased only in the treated males in the PBFB group as compared to the F1 controls; however, there was no clear dose response.

Epididymis: The incidence of hypospermia was slightly increased only in the treated males in the PBFB group as compared to the F1 controls; however, there was no clear dose response. The incidence of hypospermia was lower in the F1 generation than in the P1 generation.

Cloacal Glands: The incidence of hypertrophy of the submucosal glands in the cloaca was increased in the treated PAFB groups as compared to the control group, but there was no clear dose response. There was also an increase in dilatation of the lumens of the submucosal glands in the treated PBFA and PBFB groups.

Adrenal Glands: Diffuse hypertrophy was present in the PAFB males and the PBFB males treated with 5.0 ppm.

Females

Female Reproductive Tract: The changes in the ovary and oviduct (increased follicles and degenerating follicles in the ovary, and epithelial hyperplasia/hypertrophy in the oviduct in the F1 generation), had a higher incidence in the control group than in any of the treated groups, including consideration of the variation between the numbers of tissues available in the different treatment groups. This suggests that these changes were part of the

normal reproductive activity of quail, and not a change specifically associated with treatment with 17 β -Estradiol.

Adrenal Glands: Diffuse hypertrophy was present in 1/4 of the PAFB females whose parents received 5.0 ppm, in 5/8 PBFA females treated with 5.0 ppm, and in 4/6 PAFA females whose parents received 5.0 ppm.

Liver: There was focal mineralization in the liver in 3/4 of the PAFB females whose parents received 5.0 ppm, in 6/8 PBFA females treated with 5.0 ppm, and in 6/6 PFBF females treated with 5.0 ppm.

There were no treatment-related changes in any of the additional organs examined in either the male or female F1 generation animals, either those which received treatment, or those whose parents were treated with 17 β -Estradiol.

F2 GENERATION

The reproductive organs of the quail in the F2 generation (testes, epididymis, cloacal gland, ovary, and oviduct) were all immature. Thus, it was not possible to determine any effects on the rapidly growing cells of the testes or ovary, since they had not yet begun to proliferate. In the cloaca, the submucosal glands were also immature with much smaller glandular areas and smaller individual cells. There were no treatment-related effects in any of the organs examined in the F2 generation.

DISCUSSION AND CONCLUSION

There was a great deal of variation in the fixation quality of the testes in the P1 and F1 generations, which made diagnoses of subtle differences in the structure of the developing spermatids (spermatogonia, spermatocytes, and spermatids) extremely difficult.

In addition, several of the changes seen in the reproductive organs of both male and female quail were present in some control groups and not in others, suggesting that some of these changes were actually normal changes associated with different aspects of the reproductive cycle.

In the P1 generation, there was diffuse degeneration of seminiferous tubules in the testes at 5.0 ppm in both the males which were exposed when two to three weeks old, and those exposed at the age when the females were proven layers (P1A and P1B, respectively). Hypospermia was noted in the epididymis of P1A males in all treated groups. There was some degree of submucosal cloacal gland atrophy in the P1A males.

In P1 females, there were increased follicles as well as an increased incidence of degenerating follicles in the P1A group as compared to the controls, but not in the P1B group. It, therefore, appears that the P1A group is more sensitive to effects on the reproductive tract than those in the P1B group.

There was some degree of diffuse hypertrophy in the adrenal gland in both male and female P1 generation quail, but there was no clear dose-relationship.

In the F1 generation, the effects on the male reproductive system appeared to be more evident in the PBFB groups (parents exposed when proven layers, no exposure to the F1 generation). There were no treatment-related effects in the female reproductive tracts of treated quail in the F1 generation.

There was diffuse hypertrophy in the adrenal glands in some male and female quail in the F1 generation.

Focal mineralization in the liver was present in many of the F1 generation females (PAFB, PBFB, and PBFA groups).

The reproductive organs of the quail in the F2 generation were all immature and showed no effects of treatment.

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In conclusion, it appears that treatment of quail with 17 β -Estradiol causes histopathologic changes in the reproductive organs of both males and females in the P1 generation, adrenal changes in both the P1 and F1 generations, and changes in the liver of females in the F1 generation.

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Veterinary Pathologist

Date

PCM/skh

5/11/05 DRAFT

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Avian Dosing Study

Client Study: WA 2-17

EPL Project Coordinator: Kristi Larson

EPL Project Number: 237-010

EPL Pathologist: Dr. Peter Mann

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting

EPL Project Sheets

Project Setup

Histology Setup

Data Review

Draft Report

Final Report

Date reported to Study Director/Management _____ XXX

Date of last quarterly facility inspection _____ 4/05

EPL Quality Assurance Unit

Date