Appendix II

Histopathology Report

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE CD® (SPRAGUE-DAWLEY) RATS AFTER EXPOSURE TO SELECTED CHEMICALS ADMINISTERED BY GAVAGE ON POSTNATAL DAYS 22 THROUGH 42/43

65U-08055.001.015.0021(F)

EPL PROJECT NO. 237-004

PATHOLOGY REPORT

Submitted to

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Submitted by

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October 28, 2003

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE CD® (SPRAGUE-DAWLEY) RATS AFTER EXPOSURE TO SELECTED CHEMICALS ADMINISTERED BY GAVAGE ON POSTNATAL DAYS 22 THROUGH 42/43

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NARRATIVE SUMMARY

INTRODUCTION

The objective of this study was to examine the sensitivity of pubertal assays to the effects of a wide variety of chemicals that are known to affect the endocrine system through different pathways and/or mechanisms of action. EPA has decided to test several chemicals in multiple-dose studies which may provide greater confidence in the reliability and relevance of the assays.

For this study, the following chemicals were tested: Atrazine, Fenarimol, Methoxychlor, Bisphenol A, Ketoconazole and Propylthiouracil. The study was conducted in two components. Each component consisted of two dose groups per test material and one vehicle control group, each group comprised of 15 weight-matched F1 female weanlings, for each of the two components.

The ovaries, uterus and thyroids were examined microscopically.

SUMMARY

Administration of the test chemicals by gavage to female, CD® (Sprague-Dawley) rats, under the conditions of this study, was associated with the following histopathologic changes:

1. The presence and dose-related increased severity of cytoplasmic vacuolization of the ovarian corpora luteal cells in both the 50 and 100 mg/kg dosed Ketoconazole animals. In addition, the presence of ovarian hypoplasia in the 100 mg/kg dosed animals was observed.

2. The presence and dose-related increased severity of thyroid, follicular cell hypertrophy/hyperplasia in both the 2 and 25 mg/kg dosed Propylthiouracil animals.
3. The presence of ovarian hypoplasia in several 600 mg/kg dosed Bisphenol A animals.
4. The presence of minimal follicular cell hypertrophy in the thyroid glands of several of the 250 mg/kg dosed Fenarimol animals.
5. The presence of minimal epithelial hyperplasia within the uterus in several 50 mg/kg/day Methoxychlor animals.

DESIGN OF THE STUDY

Six test chemicals were administered via gavage once daily for 21-22 consecutive days (pnd 22 to pnd 42 or 43) to female CD® (Sprague-Dawley) rats under the study conditions outlined in the study protocol (RTI Master Protocol No.: RTI-830).

The study began with 15 weight-matched F1 females/group. The study design, test chemicals and target dose levels are presented in Table 1.

Table 1 - Study Design

Component #1

Group No.	No. F1 Females	Chemical	Dose mg/kg/day	Concentration mg/ml	Dose Volume ml/kg
1	15	Corn Oil, Vehicle Control	0	0	5
2	15	Atrazine	75	15	5
3	15	-	150	30	5
4	15	Fenarimol	50	10	5
5	15	-	250	50	5
6	15	Methoxychlor	25	5	5
7	15	-	50	10	5

Component #2

Group No.	No. F1 Females	Chemical	Dose mg/kg/day	Concentration mg/ml	Dose Volume ml/kg
1	15	Corn Oil, Vehicle Control	0	0	5
2	15	Bisphenol A	400	80	5
3	15	-	600	120	5
4	15	Ketoconazole	50	10	5
5	15	-	100	20	5
6	15	Propylthiouracil	2	0.4	5
7	15	-	25	5	5

Individual treatment groups within each component of the study were given unique five digit codes that are presented in Table 2.

Table 2 - Treatment Group Designations

Group	Component 1	Component 2
1	87668	63561
2	67607	05498
3	02227	25517
4	10935	97694
5	93970	48744
6	37738	86070
7	41133	13694

According to the study protocol, all F1 females were subjected to a complete necropsy with selected organs dissected and weighed. Protocol tissues were fixed in Bouin's fixative for 24 hours, after which they were rinsed and stored in 70% alcohol. Component #1 thyroids were fixed in Bouin's but this fixation made the later dissection of thyroids from the trachea and subsequent weighing procedures difficult because of the uniform yellow discoloration and tissue hardness. The Component #2 thyroids were fixed in 10% neutral-buffered formalin which facilitated the recognition and dissection of the thyroids from the trachea. All tissues were trimmed, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (H&E).

Histopathological examination of selected organs was conducted on the protocol-required tissues. The protocol-required tissues were: ovaries, uterine horns, and thyroid glands.

The gross and histopathologic data were entered in the Experimental Pathology Laboratories, Inc. Computerized Pathology Reporting System. Each lesion was graded according to a four-grade severity scale (1-4). "Decidual Alteration" of the uterus was designated only as "Present".

RESULTS

The individual animal data are presented by group in the Histopathology Incidence Tables (HIT) and the group summary data in the Summary Incidence Tables (SIT). Gross necropsy findings were correlated to the microscopic

findings, whenever possible. These findings are presented in the section "Correlation of Gross and Microscopic Findings Tables".

A limited number of histopathologic changes were observed in both control and treated animals. For the most part, these changes were typical of the spontaneous type of microscopic pathology that can be observed at this age and in this strain of rat. During the microscopic examination of ovaries, attempts to quantify the number of corpora lutea were not performed because of the variation that one may observe in any one cross-section of ovary. However, each ovary was examined for the presence of primary, growing and pre-ovulatory follicles.

The following chemicals were not associated with any treatment-related histopathologic changes: Component 1 = Atrazine (75 and 150 mg/kg), Fenarimol (50 mg/kg), and Methoxychlor (25 and 50 mg/kg); Component 2 = Bisphenol A (400 mg/kg).

TREATMENT-RELATED FINDINGS BY CHEMICAL

Fenarimol:

Exposure to Fenarimol (250 mg/kg) was associated with follicular cell hypertrophy of the thyroid gland. This lesion was characterized by a subtle increase in cell size, particularly in the height of the follicular lining cells. In addition, the lumen of affected thyroid follicles was slightly reduced in size and the amount and staining intensity of the thyroid colloid was less. The incidence and severity of follicular cell hypertrophy are presented in Table 3.

Table 3 – Incidence and Severity of Follicular Cell Hypertrophy – Fenarimol

Dose (mg/kg)	0	250
THYROID (No. Examined)	(15)	(15)
Hypertrophy, Follicular Cell	0	5
Minimal	0	5

As can be seen in the above Table, 5 out of 15 animals had minimal follicular cell hypertrophy. The toxicological or biological significance of this lesion is not clear since only 1/3 of the animals had hypertrophy and there was no apparent increased thyroid weight changes noted. However in this dose group, the level of thyroxine hormone (4) was decreased and thyroid stimulating hormone (TSH) was increased.

Bisphenol A:

Exposure to Bisphenol A (600 mg/kg) was associated with the presence of ovarian hypoplasia in 3 out of 14 animals examined. Ovarian hypoplasia was characterized by the complete absence of corpora lutea (CL's) and an apparent reduction or absence of the large pre-ovulatory follicles (Graffian Follicles). Mild hypoplasia seemingly had fewer large follicles and with moderate hypoplasia no large pre-ovulatory follicles were observed. This appearance suggested that some inhibition or delay of follicle development and/or ovulation had occurred. Hypoplasia was used in this context since evidence of complete ovarian maturity and subsequent atrophy was not observed (Davis et al, 1999). According to the organ weight data, animals exposed to 400 and 600 mg/kg of Bisphenol A had lower ovary weights but also had significantly lower body weights.

It has been suggested that significantly reduced body weights, associated with chemical administration, may result in "stress" to the animal decreasing the frequency and amplitude of luteinizing hormone (LH) thus altering the reproductive cycle (Yuan et al, 2002). Although the small incidence of ovarian hypoplasia might have been associated with decreased body weights, it is not clear if other mechanisms might be involved as well.

Ketoconazole:

Exposure to 50 and 100 mg/kg of Ketoconazole was associated with cytoplasmic vacuolization of ovarian corpora luteal cells and ovarian hypoplasia in the 100 mg/kg dosed animals. Cytoplasmic vacuolization was characterized by the presence of clear, variably sized vacuoles within the cytoplasm of corpora luteal cells. Occasionally, small cytoplasmic vacuoles may be noted in some degenerating cells within normal corpora lutea, but the vacuoles in Ketoconazole-exposed animals tended to be larger and more dispersed within the corpora lutea. The severity of cytoplasmic vacuolization was graded according to a subjective evaluation based on the average number and size of the vacuolated cells in a corpora lutea (minimal = 6-25%; mild = 26-50%; moderate = 51-75%; and marked 76-100%). The pathogenesis of the vacuolization could not be determined but was thought to involve the alteration of steroid metabolism at the cellular level.

As previously mentioned, 5 out of 15 high-dosed animals also had mild ovarian hypoplasia which meant there were no corpora lutea present and subsequently, no cytoplasmic vacuolization to evaluate and grade.

The incidence and severity of corpora luteal cytoplasmic vacuolization and ovarian hypoplasia is presented in Table 4.

Table 4 – Incidence and Severity of Corpora Luteal Cytoplasmic Vacuolization and Ovarian Hypoplasia – Ketoconazole

Dose (mg/kg)	0	50	100
Animals (No. Examined)	(14)	(15)	(15)
Vacuolization Cytoplasmic, Corpora Luteum	0	12	9
Minimal	0	6	1
Mild	0	4	4
Moderate	0	2	3
Marked	0	0	1
Hypoplasia	0	0	5
Mild	0	0	5

According to the organ weight data, some evidence of decreased ovarian weight was apparent in the 100 mg/kg dosed animals. This may be related to the ovarian hypoplasia seen at this dose level. However, the apparent cytoplasmic vacuolization apparently did not result in any ovarian weight change.

Propylthiouracil:

Administration of both 2 and 25 mg/kg Propylthiouracil was associated with the presence and dose-related increased severity of thyroid follicular cell hypertrophy/hyperplasia which was clearly related to increased thyroid weights and levels of TSH and decreased levels of T4.

Follicular cell hypertrophy/hyperplasia was characterized by a spectrum of histologic changes including the increased size and apparent number of follicular cells, the reduction in follicular lumen size, the presence of pale staining colloid and/or the reduction or absence of colloid within some thyroid follicles. The severity of hypertrophy/hyperplasia was subjectively based on a number of criteria: minimal = multifocal follicles affected, size and number of follicular cells slightly enlarged and increased; mild = diffuse change with further increased cell

size and hyperplasia; moderate = enhanced severity with the presence of notable numbers of follicular cell mitoses; marked = increased mitotic rate, some degenerative cells (deeply eosinophilic cytoplasm and pyknotic nuclei) within the follicular epithelium, and obvious thyroid gland size and shape enlargement. No alteration of the thyroid vasculature was noted.

The incidence and severity of thyroid hypertrophy/hyperplasia is presented in Table 5.

Table 5 – Incidence and Severity of Thyroid Hypertrophy/Hyperplasia – Propylthiouracil

Dose (mg/kg)	0	2	25
Animals (No. Examined)	(14)	(15)	(15)
Hypertrophy/Hyperplasia	0	15	15
Minimal	0	1	0
Mild	0	13	2
Moderate	0	1	9
Marked	0	0	4

Results of the microscopic examination of the thyroid gland are compatible with previous reports on the direct action of Propylthiouracil on the thyroid gland (Thomas and Williams, 1999).

Methoxychlor:

Administration of Methoxychlor was associated with the presence of epithelial hyperplasia of the uterine epithelium.

In control animals, the uterine surface epithelial lining was characterized by having a single layer of columnar cells with a cytoplasmic to nuclear ratio of around 1.5. The height and presence of vacuolar to necrotic changes and the presence of inflammatory cells depended upon the stage of the estrous (reproductive) cycle. In addition, mitotic figures were common in the estrous portion of the cycle (Yuan and Foley, 2002).

In the 50 mg/kg/day dosed group, some animals had changes of the uterine surface epithelium that were diagnosed as epithelial hyperplasia. In these cases, the lining epithelium was generally diffusely affected. This change was characterized by taller columnar cell height and nuclear crowding indicative

of more cells. In addition, these cells tended to have more cytoplasm (hypertrophy) which appeared lightly basophilic. Mitotic figures were occasionally noted, however, vacuolar to necrotic changes normally associated with the estrous cycle were not observed.

All cases of epithelial hyperplasia were diagnosed as minimal which was a change barely detectable.

The appearance of the lesion suggested a hormonal imbalance as a possible mechanism.

The incidence and severity of uterine epithelial hyperplasia is presented in Table 6.

Table 6 – Incidence and Severity of Uterine Epithelial Hyperplasia – Methoxychlor

Dose (mg/kg)	0	25	50
Animals (No. Examined)	(15)	(15)	(15)
Hyperplasia, Epithelial	0	0	5
Minimal	0	0	5

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October 28, 2003
Date

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Study Design

Component #1

Group No.	No. F1 Females	Chemical	Dose mg/kg/day	Concentration mg/ml	Dose Volume ml/kg
1	15	Corn oil, Vehicle Control	0	0	5
2	15	Atrazine	75	15	5
3	15	-	150	30	5
4	15	Fenarimol	50	10	5
5	15	-	250	50	5
6	15	Methoxychlor	25	5	5
7	15	-	50	10	5

SUMMARY INCIDENCE TABLES

COMPONENT #1

SUMMARY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
OVARY (NO. EXAMINED)	(15)	(15)	(15)	(15)	(15)	(15)
Cyst, Follicle	1	1		1		
Cyst, Luteal	1	1			2	
Hypoplasia					1	
THYROID (NO. EXAMINED)	(15)	(15)	(15)	(15)	(15)	(15)
Hypertrophy, Follicular Cell					5	
Inflammation, Chronic						
UTERUS (NO. EXAMINED)	(15)	(15)	(15)	(15)	(15)	(15)
Decidual Alteration			1	1		
Hemorrhage, Endometrium						
Hyperplasia, Epithelium						

SUMMARY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 7					
OVARY (NO. EXAMINED)	(15)					
Cyst, Follicle						
Cyst, Luteal						
Hypoplasia						
THYROID (NO. EXAMINED)	(15)					
Hypertrophy, Follicular Cell						
Inflammation, Chronic	1					
UTERUS (NO. EXAMINED)	(15)					
Decidual Alteration						
Hemorrhage, Endometrium	1					
Hyperplasia, Epithelium	5					

HISTOPATHOLOGY INCIDENCE TABLES

COMPONENT #1

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 1

ANIMAL	1	14	15	28	29	35	48	49	62	63	76	77	90	91	104					
OVARY	X	Xm	X	X		X	X		X	X	X	X	X	X	X					
Cyst, Follicle								2												
Cyst, Luteal					1															
Hypoplasia																				
THYROID	X	Xm	X	X	Xm	X	X	X	X	X	X	X	X	X	X					
Hypertrophy, Follicular Cell																				
Inflammation, Chronic																				
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Decidual Alteration																				
Hemorrhage, Endometrium																				
Hyperplasia, Epithelium																				

EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 2																		
ANIMAL	2	13	16	27	30	36	47	50	61	64	75	78	89	92	103				
OVARY	X	X	X	Xm	X	X	m	X		X	X	X	X	X	X				
Cyst, Follicle									1										
Cyst, Luteal							1												
Hypoplasia																			
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Hypertrophy, Follicular Cell																			
Inflammation, Chronic																			
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Decidual Alteration																			
Hemorrhage, Endometrium																			
Hyperplasia, Epithelium																			

EPL
Experimental Pathology Laboratories, Inc.

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1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 3																		
ANIMAL	3	12	17	26	31	37	46	51	60	65	74	79	88	93	102				
OVARY	X	X	X	X	X	X	X	X	X	Xm	X	X	X	X	X				
Cyst, Follicle																			
Cyst, Luteal																			
Hypoplasia																			
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Hypertrophy, Follicular Cell																			
Inflammation, Chronic																			
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X		X				
Decidual Alteration														P					
Hemorrhage, Endometrium																			
Hyperplasia, Epithelium																			

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 4

ANIMAL	4	11	18	25	32	38	45	52	59	66	73	80	87	94	101					
OVARY		X	X	X	X	X	X	Xm	X	Xm	X	X	X	X	X					
Cyst, Follicle	1																			
Cyst, Luteal																				
Hypoplasia																				
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Hypertrophy, Follicular Cell																				
Inflammation, Chronic																				
UTERUS		X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Decidual Alteration	P																			
Hemorrhage, Endometrium																				
Hyperplasia, Epithelium																				

EPL
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1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 5

ANIMAL	5	10	19	24	33	39	44	53	58	67	72	81	86	95	100					
OVARY	X		X		X	X	m	X	Xm	X	X	X	X	X	X					
Cyst, Follicle																				
Cyst, Luteal				1			1													
Hypoplasia		2																		
THYROID	X	X	X	X	X	X	X			X			X		X					
Hypertrophy, Follicular Cell								1	1		1	1		1						
Inflammation, Chronic																				
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Decidual Alteration																				
Hemorrhage, Endometrium																				
Hyperplasia, Epithelium																				

EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 6																		
ANIMAL	6	9	20	2u3	34	40	43	54	57	68	71	82	85	96	99				
OVARY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Cyst, Follicle																			
Cyst, Luteal																			
Hypoplasia																			
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Hypertrophy, Follicular Cell																			
Inflammation, Chronic																			
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Decidual Alteration																			
Hemorrhage, Endometrium																			
Hyperplasia, Epithelium																			

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1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 7																		
ANIMAL	7	8	21	22	41	42	55	56	69	70	83	84	97	98	105				
OVARY	X	X	X	X	Xm	X	X	X	X	X	X	X	X	X	X				
Cyst, Follicle																			
Cyst, Luteal																			
Hypoplasia																			
THYROID	X		X	X	X	X	X	X	X	X	X	X	X	X	X				
Hypertrophy, Follicular Cell																			
Inflammation, Chronic		1																	
UTERUS	X			X			X	X	X	X	X	X	X						
Decidual Alteration																			
Hemorrhage, Endometrium														1					
Hyperplasia, Epithelium		1	1		1	1									1				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

COMPONENT #1

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 1

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
63	UTERUS	Fluid filled	No Correlating Lesion
90	KIDNEY	Right side, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 2

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
103	UTERUS	Fluid filled - clear	No Correlating Lesion

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 3

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
51	UTERUS	Pulled apart above cervix (2 sections)	No Comment Required
93	UTERUS	Right horn, cyst 5mm in diameter	Decidual Alteration
102	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 4

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
4	UTERUS	Uterine horn, right, cysts, two (solid), 3mm in diameter	Cyst, Follicle (OVARY)
25	UTERUS	Fluid filled	No Correlating Lesion
45	UTERUS	Fluid filled - clear	No Correlating Lesion
52	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 5

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
33	UTERUS	Fluid filled	No Correlating Lesion
67	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled
72	UTERUS	Right horn, 1/2 of length, reduced in size	No Correlating Lesion
	UTERUS	Left side, fluid filled	No Correlating Lesion
86	UTERUS	Fluid filled	No Correlating Lesion
95	UTERUS	Fluid filled	No Correlating Lesion

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 6

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
6	UTERUS	Uterine horns, fluid filled	No Correlating Lesion
23	LUNG	Congested oil present - probable aspiration of dosing suspension	Intentionally Not Sampled
43	UTERUS	Fluid filled	No Correlating Lesion
68	KIDNEY	Left, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 7

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
8	UTERUS	Fluid filled	No Correlating Lesion
21	UTERUS	Fluid filled	No Correlating Lesion
22	UTERUS	Fluid filled	No Correlating Lesion
41	UTERUS	Fluid filled - clear	No Correlating Lesion
69	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled
70	UTERUS	Fluid filled	No Correlating Lesion
105	UTERUS	Fluid filled - clear	No Correlating Lesion

Study Design

Component #2

Group No.	No. F1 Females	Chemical	Dose mg/kg/day	Concentration mg/ml	Dose Volume ml/kg
1	15	Corn oil, Vehicle Control	0	0	5
2	15	Bisphenol A	400	80	5
3	15	-	600	120	5
4	15	Ketoconazole	50	10	5
5	15	-	100	20	5
6	15	Propylthiouracil	2	0.4	5
7	15	-	25	5	5

SUMMARY INCIDENCE TABLES

COMPONENT #2

SUMMARY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
OVARY (NO. EXAMINED)	(14)	(11)	(14)	(15)	(15)	(15)
Cyst, Luteal				1		
Hypoplasia			3		5	
Vacuolization Cytoplasmic, Corpus Luteum				12	9	
THYROID (NO. EXAMINED)	(13)	(11)	(14)	(15)	(15)	(15)
Hypertrophy/Hyperplasia						15
Inflammation, Chronic	1	1				
UTERUS (NO. EXAMINED)	(14)	(11)	(14)	(15)	(15)	(15)
Dilatation, Lumen, Unilateral	1					
Hemorrhage, Endometrium						1
Hyperplasia, Epithelium				1		
Hypoplasia			1			

SUMMARY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 7					
OVARY (NO. EXAMINED)	(15)					
Cyst, Luteal						
Hypoplasia						
Vacuolization Cytoplasmic, Corpus Luteum						
THYROID (NO. EXAMINED)	(15)					
Hypertrophy/Hyperplasia	15					
Inflammation, Chronic						
UTERUS (NO. EXAMINED)	(15)					
Dilatation, Lumen, Unilateral	1					
Hemorrhage, Endometrium						
Hyperplasia, Epithelium	1					
Hypoplasia						

HISTOPATHOLOGY INCIDENCE TABLES

COMPONENT #2

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 1																		
ANIMAL	14	15	28	30	43	44	57	58	71	72	85	86	96	97					
OVARY	X	X	X	X	Xm	X	X	X	X	X	X	X	X	X					
Cyst, Luteal																			
Hypoplasia																			
Vacuolization Cytoplasmic, Corpus Luteum																			
THYROID	X	X	X	X	X	X	X		X	X	X	X	X	N					
Hypertrophy/Hyperplasia																			
Inflammation, Chronic								1											
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X						
Dilatation, Lumen, Unilateral														2					
Hemorrhage, Endometrium																			
Hyperplasia, Epithelium																			
Hypoplasia																			

EPL
Experimental Pathology Laboratories, Inc.

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 2

ANIMAL	2	13	31	42	45	56	70	73	84	95	98
OVARY	X	X	X	X	X	X	X	X	X	X	X
Cyst, Luteal											
Hypoplasia											
Vacuolization Cytoplasmic, Corpus Luteum											
THYROID	X	X	X		X	X	X	X	X	X	X
Hypertrophy/Hyperplasia											
Inflammation, Chronic				1							
UTERUS	X	X	X	X	X	X	X	X	X	X	X
Dilatation, Lumen, Unilateral											
Hemorrhage, Endometrium											
Hyperplasia, Epithelium											
Hypoplasia											

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
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m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 3

ANIMAL	3	12	17	26	32	41	55	60	69	74	83	88	94	99
OVARY	X		X		X	X		X	X	X	X	X	X	X
Cyst, Luteal														
Hypoplasia		2		3			2							
Vacuolization Cytoplasmic, Corpus Luteum														
THYROID	X	X	X	Xm	X	X	X	X	X	X	X	X	X	X
Hypertrophy/Hyperplasia														
Inflammation, Chronic														
UTERUS	X	X	X		X	X	X	X	X	X	X	X	X	X
Dilatation, Lumen, Unilateral														
Hemorrhage, Endometrium														
Hyperplasia, Epithelium														
Hypoplasia				2										

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HISTOPATHOLOGY INCIDENCE TABLE

N/A.
F1 Sacrifice
Female Rat

GROUP 4

ANIMAL	4	11	18	25	29	33	40	47	54	61	68	75	82	89	93					
OVARY	X				m	X						m	X							
Cyst, Luteal															1					
Hypoplasia																				
Vacuolization Cytoplasmic, Corpus Luteum		1	1	1	1		3	2	1	3	2	2		1	2					
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Hypertrophy/Hyperplasia																				
Inflammation, Chronic																				
UTERUS	X	X	X	X	X	X	X	X	X		X	X	X	X	X					
Dilatation, Lumen, Unilateral																				
Hemorrhage, Endometrium																				
Hyperplasia, Epithelium										1										
Hypoplasia																				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
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P=Present B=Benign M=Malignant
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HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 5																		
ANIMAL	5	10	19	24	34	39	48	53	62	67	76	81	90	100	105				
OVARY			X							m									
Cyst, Luteal																			
Hypoplasia								2	2	2	2				2				
Vacuolization Cytoplasmic, Corpus Luteum	3	1		2	2	2	2					4	3	3					
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Hypertrophy/Hyperplasia																			
Inflammation, Chronic																			
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Dilatation, Lumen, Unilateral																			
Hemorrhage, Endometrium																			
Hyperplasia, Epithelium																			
Hypoplasia																			

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HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

	GROUP 6																		
ANIMAL	6	9	20	23	35	38	49	52	63	66	77	80	91	101	104				
OVARY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Cyst, Luteal																			
Hypoplasia																			
Vacuolization Cytoplasmic, Corpus Luteum																			
THYROID																			
Hypertrophy/Hyperplasia	2	2	2	2	2	1	2	2	2	3	2	2	2	2	2				
Inflammation, Chronic																			
UTERUS	X	X	X	X	X	X		X	X	X	X	X	X	X	X				
Dilatation, Lumen, Unilateral																			
Hemorrhage, Endometrium							1												
Hyperplasia, Epithelium																			
Hypoplasia																			

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

N/A
F1 Sacrifice
Female Rat

GROUP 7

ANIMAL	7	8	21	22	36	37	50	51	64	65	78	79	92	102	103					
OVARY	X	X	X	Xm	X	X	X	Xm	X	X	X	X	X	X	X					
Cyst, Luteal																				
Hypoplasia																				
Vacuolization Cytoplasmic, Corpus Luteum																				
THYROID																				
Hypertrophy/Hyperplasia	2	3	3	3	3	3	4	3	4	4	3	4	3	3	2					
Inflammation, Chronic																				
UTERUS	X		X	X	X	X	X	X	X		X	X	X	X	X					
Dilatation, Lumen, Unilateral										1										
Hemorrhage, Endometrium																				
Hyperplasia, Epithelium		1																		
Hypoplasia																				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

COMPONENT #2

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 1

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
57	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled
85	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled
86	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 3

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
12	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled
41	KIDNEY	Left, hydronephrosis	Intentionally Not Sampled
74	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 4

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
4	ADRENAL	Pale in color	Intentionally Not Sampled
11	ADRENAL	Pale in color	Intentionally Not Sampled
18	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled
25	ADRENAL	Pale in color	Intentionally Not Sampled
	KIDNEY	Both, hydronephrosis	Intentionally Not Sampled
40	ADRENAL	Bilateral, enlarged	Intentionally Not Sampled
61	ADRENAL	Enlarged	Intentionally Not Sampled
68	ADRENAL	Pale	Intentionally Not Sampled
82	ADRENAL	Enlarged and pale	Intentionally Not Sampled
93	ADRENAL	Enlarged	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 5

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
39	ADRENAL	Bilateral, increased in size	Intentionally Not Sampled
48	ADRENAL	Bilateral, increased in size	Intentionally Not Sampled
53	ADRENAL	Enlarged	Intentionally Not Sampled
62	ADRENAL	Enlarged	Intentionally Not Sampled
105	ADRENAL	Both, pale and gray in color	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 6

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
77	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled
91	KIDNEY	Right, hydronephrosis	Intentionally Not Sampled
104	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled

N/A
F1 Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 7

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
37	THYROID	Reddened and enlarged	Hypertrophy/Hyperplasia
50	THYROID	Bilateral, increased in size	Hypertrophy/Hyperplasia
65	UTERUS	Fluid filled	Dilatation, Lumen, Unilateral
79	KIDNEY	Bilateral, hydronephrosis	Intentionally Not Sampled
102	KIDNEY	Right, pale and mishaped - polycystic	Intentionally Not Sampled

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Assessment of Pubertal Development and Thyroid Function in Juvenile Female CD® (Sprague-Dawley) Rats After Exposure to Selected Chemicals Administered by Gavage on Postnatal Days 22 Through 42/43

Client Study: RTI Contract 65U-08055.001.015.002(F) EPL Project Coordinator: Dr. John Curtis Seely

EPL Project Number: 237-004 EPL Pathologist: Dr. John Curtis Seely

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	Dates Reporting
EPL Project Sheets	October 7, 2002; January 13, 2003; May 30, 2003	October 7, 2002; January 13, 2003; May 30, 2003
Project Setup	October 28, 2002; January 8, 2003	October 28, 2002; January 8, 2003
Histology Setup	October 28, 2002; January 14, 2003	October 28, 2002; January 14, 2003
Data Review	December 17, 2002; February 18, 2003	December 17, 2002; February 18, 2003
Phase/Data Review	November 25, 2003; December 11, 2002; January 14, 2003; January 20, 2003; February 18, 2003	November 25, 2003; December 11, 2002; January 14, 2003; January 20, 2003; February 18, 2003
Draft Report	June 12&13, 2003	June 13, 2003
Final Report	October 24, 2003	October 24, 2003

Date Reported to Study Director/Management: October 28, 2003

Date of last quarterly facility inspection: August 2003

Jane J. Hollingsworth
EPL Quality Assurance Unit

October 28, 2003
Date

Form No. 6-2 (09/10/99)