470A

CASWELL FILE
9-14-93

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

SEP 14 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: **IPRODIONE** - Carcinogenicity Study in Mice [6(a)(2) Submission]

TO: Linda Deluise
PM Team Reviewer (52)
Reregistration Branch, SRRD (H7508W)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/9/93
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 9/9/93
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 9/10/93
Chief, Toxicology Branch II/HED (H7509C)

Registrant: Rhone-Poulenc
Chemical: [2-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboximide]
Synonym: Iprodione; 26019 RP
Case No.: 819434/2335
Caswell No.: 470A
Submission No.: S445133
Identifying No.: 109801
DP Barcode: D193492
MRID No.: 428250-00, -01, and -02
Action Requested: Please review MRIDs 42825001 and 42825002 for 83-2(b). Does this change the risk assessment?

Comment: The Registrant has submitted a mouse carcinogenicity study to fulfill a data gap identified in the Phase III Response for Iprodione. This study has been reviewed, and the DER is appended.

Treatment of mice with Iprodione at dose levels in the diet of 160, 800, and 4000 ppm [23, 115, & 604 mg/kg (♂♂)/27, 138, 793 mg/kg (♀♀), respectively] for 99 weeks did not have an adverse effect on survival or food consumption, but body-weight gain at the high-dose level was decreased overall in both sexes. There was an increase in the incidence of liver tumors in both sexes at the high-dose level,

which was accompanied by increases in several liver lesions [centrilobular hepatocyte enlargement/vacuolation, area(s) of enlarged eosinophilic hepatocytes, pigmented macrophages, centrilobular necrosis, and amyloid deposits]. Additionally, statistically significant increases were observed in GPT and GOT levels relative to control values at the high-dose level (♂♂ & ♀♀) at week 52 (only interval examined), and liver weight was increased in the high-dose mice (both sexes) at both sacrifices. There was also an increase in the incidence of benign ovarian tumors (luteoma) at the high dose compared to the control incidence, which was accompanied by an increase in luteinization of the interstitial cells, corpora lutea absent, and prominent granulosa cells. Also increased was the incidence of generalized vacuolation/hypertrophy of the interstitial cells of the testes in the mid- and high-dose mice. The NOEL can be set at 160 ppm [23 (♂♂)/27 (♀♀) mg/kg], the LEL at 800 ppm [115 (♂♂)/138 (♀♀) mg/kg], based on the increased incidence of centrilobular hepatocyte enlargement in females and the increased incidence of generalized vacuolation/hypertrophy of the interstitial cells in the testes.

This study is classified Core Minimum, and it satisfies the guideline requirement (83-2) for a carcinogenicity study in mice.

With regard to the question of risk assessment, both the rat chronic toxicity/carcinogenicity study and this mouse carcinogenicity study indicate that Iprodione has carcinogenic potential. In the rat study, Iprodione induced interstitial cell tumors in the testes of males, but there was no increase in ovarian tumors, although there was a slight increase in tubular hyperplasia in the ovary. In the mouse study, Iprodione induced luteomas in the ovaries of females, but there was no increase in testicular tumors, although there was an increase in the incidence of generalized vacuolation/hypertrophy of the interstitial cells of the testes. Additionally, in the mouse study, the incidence of liver tumors was increased in both sexes. The Registrant has proposed possible mechanisms that may be involved in the formation of the observed tumors; i.e., a perturbation in sex hormone regulation, and intends to conduct mechanistic studies to demonstrate the effect of Iprodione on sex hormone regulation and to establish a link between this hormonal perturbation and the increased incidence in tumor formation. The Registrant argues that quantitative carcinogen risk assessment based on the "linearized" multistage model is inappropriate in the case of Iprodione, and safety factors are considered to be an appropriate and adequate method for risk assessment of threshold carcinogens, a group to which the Registrant believes Iprodione belongs. These issues, along with the rat and mouse carcinogenicity studies and all available data on Iprodione, will be presented to the HED Carcinogenicity Peer Review Committee in the near future.

Reviewed by: Linda L. Taylor, Ph.D. 9/7/93
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel 9/9/93
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity-mouse TOX. CHEM NO: 470A

MRID NO.: 428250-02 Shaughnessy #: 109801

TEST MATERIAL: Iprodione

SYNONYMS: 26019 RP

STUDY NUMBER: RNP 359/921240

SPONSOR: Rhone-Poulenc Agrochimie

TESTING FACILITY: Huntingdon Research Centre Ltd.

TITLE OF REPORT: IPRODIONE Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice

AUTHOR(S): PR Chambers, D Crook, WA Gibson, RM Read, C Gopinath

REPORT ISSUED: May 10, 1993

Quality Assurance: A quality assurance statement and a Good Laboratory Compliance statement were provided.

CONCLUSION: Treatment of mice with Iprodione at dose levels in the diet of 160, 800, and 4000 ppm [23, 115, & 604 mg/kg (♂♂)/27, 138, 793 mg/kg (♀♀), respectively] for 99 weeks did not have an adverse effect on survival or food consumption, but body-weight gain at the high-dose level was decreased overall in both sexes. There was an increase in the incidence of liver tumors in both sexes at the high-dose level, which was accompanied by increases in several liver lesions [centrilobular hepatocyte enlargement/vacuolation, area(s) of enlarged eosinophilic hepatocytes, pigmented macrophages, centrilobular necrosis, and amyloid deposits]. Additionally, statistically significant increases were observed in GPT and GOT levels relative to control values at the high-dose level (♂♂ & ♀♀) at week 52 (only interval examined), and liver weight was increased in the high-dose mice (both sexes) at both sacrifices. There was also an increase in the incidence of benign ovarian tumors (luteoma) at the high dose compared to the control incidence, which was accompanied by an increase in luteinization of the interstitial cells, corpora lutea absent, and prominent granulosa cells. Also increased was the incidence of generalized vacuolation/hypertrophy of the interstitial cells of the testes in the mid- and high-dose mice. The NOEL can be set at 160 ppm [23 mg/kg (♂♂)/27 mg/kg (♀♀)], the LEL at 800 ppm [115 mg/kg (♂♂)/138 mg/kg (♀♀)], based on the increased incidence of centrilobular hepatocyte enlargement in females and the increased incidence of generalized vacuolation/hypertrophy of the interstitial cells in the testes.

Classification: Core-Minimum. This study satisfies the guideline requirement (83-2) for a carcinogenicity study in mice.

A. MATERIALS

1. Test compound: Iprodione, CAS #: 36734-19-7; Description: cream-colored powder; Batch #: DA 604; Purity: 95.7%; Source: Sponsor. Addendum 6 provides Certificates of Analyses, which list the purity as ranging from 945 g/kg to 949 g/kg, and the appearance as a white/white-beige powder.

2. Test animals: Species: mouse; Strain: Crl: CD-1 (ICR) BR; Age: 28 days on receipt; Weight: males ≈ 5 g, females ≈ 4 g on receipt; Source: Charles River Breeding Laboratories, Portage, Michigan, USA.

B. STUDY DESIGN

1. Animal assignment

On receipt, 5 mice/sex were selected for health check purposes and were sacrificed within 24 hours of receipt at the testing facility. These were examined macroscopically, with abnormalities being immediately assessed microscopically. The lungs, liver, kidneys, spleen, and heart were preserved. The remaining mice were randomly placed into cages (individual housing). Following an acclimation period of 4 ♂♂/5 ♀♀ days, the mice were weighed and the appropriate number were selected by discarding those mice furthest from the mean body weight. Those chosen were randomly assigned to groups, stratified by body weight, with care taken to insure an initial group mean that was comparable among the groups. An additional 7-day acclimation period occurred between allocation to groups and the initiation of treatment.

There were 50 mice/sex/group in the Main phase and 15 mice/sex/group in the Satellite phase (mice utilized for blood analyses and interim sacrifice at week 52). The four treatment groups in both the Main and Satellite phases were fed diets containing 0, 160, 800, or 4000 ppm test material for at least 99 weeks, or until the 52-week interim sacrifice. The mice were housed individually and were allowed free access to tap water and powdered SDS Rat and Mouse No. 1 modified maintenance diet.

2. Diet preparation

Test material was ground directly into the powdered diet and mixed; this premix was then used to prepare the required concentrations by direct dilution with additional quantities of untreated diet and additional mixing. Treated diets were prepared weekly and stored at room temperature. Prior to study initiation, the proposed mixing procedures were checked to confirm that the methods were adequate for producing homogeneous diets and stability tests were performed (work was performed as a part of the 13-week subchronic study - HRC Report # RNP323/90667). The test diets were analyzed for test

material concentration [gas chromatography] for weeks 1, 2, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 89, and 97.

RESULTS

Analyses for stability and homogeneity performed prior to this chronic feeding study at dietary concentrations of 100 and 12000 ppm demonstrated that Iprodione was stable in the diets for at least 14 days at room temperature, and it was uniformly distributed throughout the diet. Initial (cold) extraction procedures were found to result in variable and often unacceptable analytical results during the initial weeks [1-32] of the study, and a new (hot extraction) procedure was developed. Concentrations attained ranged from 85 to 105% of the nominal concentrations over the 97-week period. The mean % of nominal concentrations attained is listed in Table 1.

Table 1. Mean Percent of Target Value Attained

Dose Level (ppm)	Mean % of Target Value
160	97.0
800	92.1
4000	93.4

3. Statistics - The following sequence of procedures was utilized: body weight, food consumption, clinical pathology and organ weight data - (1) Bartlett's test for homogeneity of variance between groups; where significant (1% level) heterogeneity found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. (2) If no significant heterogeneity was detected or if a satisfactory transformation was found, a one-way analysis of variance was performed. If significant heterogeneity of variance was present and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used. (3) Analyses of variance were followed by Student's t-test and Williams' test for a dose-related response. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the "t" test and Williams' test (Shirley's test). All statistical tests were 2-tailed, with $p \leq 0.05$ and $p \leq 0.01$ used as levels of significance.

Analysis of covariance was used in place of analysis of variance in the above sequence for organ-weight data. The final body weight was used as a covariate. Mortality was analyzed using log rank methods (test for heterogeneity between groups; one-tailed test for trend against dose, test for non-linearity in any dose-related trend, and one-tailed pairwise comparisons of each treated group with control [sexes separately]. The numbers of mice with tumors were analyzed by the time-to-tumor methods (IARC, 1980), and the incidence of benign alone, malignant and benign and/or malignant liver cell tumors were analyzed for each sex separately. The incidence of

ovarian luteoma was analyzed for females. Following the calculation of observed and expected numbers for each group, the following were carried out: a test for heterogeneity between the 4 treatment groups; a one-tailed test for positive trend against dose level; a test for non-linearity in any dose-related trend; and one-tailed pairwise comparisons of each treated group with the control group.

C. METHODS AND RESULTS

1. Observations

The mice were observed daily (at least once) for behavioral changes, reaction to treatment, or ill health, and additional checks (a.m. and p.m.) for moribundity and mortality were performed. Once a week a detailed palpation of each mouse was performed and the time of appearance, location, and dimension of all palpable masses were recorded. Each mouse was weighed at the time of allocation to groups, on the first day of treatment, then once a week thereafter. Individual food consumption was recorded on a weekly basis (g/mouse/week), and food conversion ratios were calculated during the first 12 weeks of treatment from body weight and food consumption data (weight of food consumed per unit gain of body weight). Group mean achieved intake of test material was calculated.

RESULTS

1. Toxicity/Mortality (survival): There were no clinical signs observed indicative of toxicity or reaction to treatment, and there was no apparent effect of treatment on survival, although the high-dose group displayed the highest mortality rate for both sexes (Table 2). The most frequently occurring probable cause of death was amyloidosis, with the high-dose males and all treated female groups (dose-related) displaying a greater incidence than their respective controls (Table 2).

Table 2. Survival/# dying on test

# Survivors/ Dose level (ppm)/Interval	MALES				FEMALES			
	0	160	800	4000	0	160	800	4000
# at Termination	29	25	32	22	25	21	32	20
1-52 weeks	1	3	3	0	3	1	1	2
53-78 weeks	12	10	6	19	7	17	10	18
79-99 weeks	8	12	9	9	15	11	17	10
% Survival	58	50	64	44	50	42	44	40
# dying due to amyloidosis (%)	11 (52)	13 (50)	8 (44)	18 (64)	7(28)	13(45)	16(55)	17 (57)

2. Bodyweight: During the first 18-week period, body-weight gains were comparable among the groups for both sexes. For the period of week 18 to 45, the high-dose mice of both sexes

displayed a statistically significant decrease in body-weight gain compared to their respective control group [56 (♂) and 53 (♀) % of control value]. For weeks 45 to 99, the males at the mid- and high-dose levels displayed negative gains and the low-dose males displayed no gain. The low- and mid-dose females showed a decreased gain (61% of control value), while the high-dose females were comparable to the controls. Overall, mice of both sexes at the high-dose level displayed a lower body-weight gain compared to their respective control group.

Table 3. Body-Weight Gains

Interval/Group/Dose	0 ppm	160 ppm	800 ppm	4000 ppm
	MALES			
0-18	11.1	11.8 (106)	12.0 (108)	11.6 (105)
18-45	7.2	7.2	7.8 (108)	4.0**(56)
45-99	0.9	0.0	-1.2	-0.6
0-99	18.9	18.2	18.4	16.2 (86)
	FEMALES			
0-18	9.6	9.4	9.9 (103)	9.5
18-45	5.5	5.7	5.4	2.9**(53)
45-99	1.8	1.1 (61)	1.1 (61)	2.1 (117)
0-99	15.6	16.4 (105)	17.2 (110)	13.9 (89)

** p<0.01

3. Food consumption and compound intake

With the exception of the first week in which the high-dose level mice displayed decreased consumption compared to their respective controls, food consumption was comparable among the groups. Food efficiency was greater for both sexes at the high-dose level compared to the control values during week 1. Subsequently, up to week 12, the efficiency of food utilization was comparable among the groups for both sexes.

Table 4. Food Consumption (g/mouse) and Conversion Ratios

Treatment Period (weeks)/Dose	MALES				FEMALES			
	0	160	800	4000	0	160	800	4000
Consumption								
1	46	49	48	43*(93)♦	47	47	45	40**(85)
2-18	660	682	697	672	695	675	695	739
19-99	3124	3210	3236	3317	3151	3105	3200	3462
1-99	3817	3904	3956	4027	3861	3803	3922	4202
Conversion Ratio								
1-12	50.5	51.4	49.0	50.0	64.5	63.9	60.9	62.9

♦ (% of control value); * P<0.05; ** p<0.01

The average test material consumption values for the 99-week study are listed below.

Table 5. Average Test Material Consumption (mg/kg/day)

Interval (weeks)/Dose (ppm)	MALES			FEMALES		
	160	800	4000	160	800	4000
1-26	27	137	664	32	167	881
27-52	22	108	574	26	130	772
53-78	22	108	596	25	130	769
79-99	21	107	577	24	123	737
1-52	25	123	619	29	148	827
1-78	24	118	611	28	142	807
1-99	23	115	604	27	138	793

4. Hematology

Blood was obtained from the orbital sinus of 10 mice/sex/group from the Satellite group during week 52. The biochemical parameters determined were: alkaline phosphatase, Glutamic-pyruvic transaminase (alanine aminotransferase), Glutamic-oxaloacetic transaminase (aspartate aminotransferase) and γGlutamyl transferase. Additionally, venous blood smears were prepared from all Main group mice killed during the study and from 10 mice/sex/group surviving at weeks 52, 80, and 100, and a differential WBC count [neutrophils, lymphocytes, eosinophils, basophils, and monocytes] and cell morphology were examined.

RESULTS

There were no apparent differences among the groups of either sex with respect to the blood smears examined at week 52; however, since total leukocyte counts were not provided and absolute counts could not be calculated, no definitive assessment of these data is possible (Table 6). Statistically significant increases in GPT and GOT levels were observed in the high-dose mice of both sexes at week 52 compared to the control values (Table 7). No other differences were reported.

Table 6. Differential White Cell Count (%)

Interval/ Group/ Dose (ppm)	N	L	E	B	M
Control ♂♂					
52	36	60	4	0	1
80	41	55	3	0	0
100	40	56	3	0	1
High-dose ♂♂					
52	37	61	1	0	1
80	31	69	0	0	1
100	30	69	1	0	1

Interval/ Group/ Dose (ppm)	Table 6. Differential White Cell Count (%)				
	N	L	E	B	M
Control ♀♀					
52	33	65	3	0	0
80	44	55	1	0	0
100	46	53	1	0	0
High-dose ♀♀					
52	32	67	0	0	0
80	38	61	1	0	0
100	38	62	1	0	0

Table 7. Biochemistry Values

Parameter/ Group/ Dose (ppm)	MALES				FEMALES			
	0	160	800	4000	0	160	800	4000
AP (mU/mL)	93	117	83	83(89)♦	82	105	89	71(87)
GPT (mU/mL)	27	38	31	83**(307)	23	23	23	59**(257)
GOT (mU/mL)	37	45	40	53*(143)	38	42	42	56**(147)

♦ (% of control value); * $p<0.05$; ** $p<0.01$

5. Sacrifice and Pathology

All animals that died or were sacrificed on schedule were subjected to a complete gross pathological examination, which included a visual examination of all superficial tissues (urogenital orifices and tail, each pinna, eye, and external auditory meatus, as well as the mammary tracts and the subcutaneous structures) and palpation for distortion, swelling, or other evidence of tumor formation. The external nares, buccal cavity, and tongue were then examined, and the cranial roof removed to allow observation of the brain, pituitary gland, and cranial nerves. After ventral midline incision and skin reflection, all subcutaneous tissues were examined, including nodes, mammary and thyroid/parathyroid glands. The condition of the thoracic viscera, thymus, lymph nodes, and heart were noted also. The abdominal viscera were examined before and after removal; the urinary bladder was distended briefly with fixative, opened, and examined under low-power magnification. The stomach, cecum, and kidneys, and portions of the duodenum, jejunum, ileum, colon, and esophagus were incised and examined. The lungs were removed and all pleural surfaces were examined under illumination. The liver was sectioned at intervals of a few millimeter. Any abnormalities in the appearance and size of the gonads, adrenals, uterus, intra-abdominal lymph nodes, and accessory reproductive organs were recorded, and the location, size, and multiplicity of any lesion suggestive of neoplasia (and any evidence of adhesion or possible invasion to adjacent structures) were noted. The adrenals, brain, kidneys, liver, heart, spleen, pituitary, ovaries [♀♀], uterus [♀♀], thyroid,

and testes (with epididymides; ♂♂) of all mice sacrificed at 12 months and at study termination were weighed, and the weights of the major organs of those dying on test/sacrificed moribund were recorded at pathologist's discretion. The CHECKED (X) tissues were collected from all animals. All tissues required by the EPA FIFRA guidelines of the control and high-dose groups in both the Main and Satellite groups dying on test, sacrificed at 52-week interim or terminal sacrifice, and all low- and mid-dose mice dying on test were examined microscopically. Additionally, the liver, lungs, kidneys, and gross lesions from Main and Satellite group low- and mid-dose mice sacrificed at 52 weeks or at termination were examined microscopically. Any tissue displaying treatment-related changes at the high-dose level was examined from all mice at the low- and mid-dose levels at both sacrifices [testes (♂♂), and ovaries, uterus, cervix, vagina, and adrenals (♀♀) - both sacrifices; epididymides and stomach (♂♂) and spleen (♀♀) -terminal sacrifice].

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta	X	Brain (3 levels)
X	Salivary glands	X	Heart	X	Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes*	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone, femur w/joint
Respiratory		X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Corpus/cervix	X	All gross lesions and masses
X	Nasal passages	X	Vagina		
X	Pharynx	X	Preputial glands	X	Harderian glands
X	Larynx			X	Head/middle ear/oral cavity
X	Zymbal's gland				

* cervical and mesenteric

RESULTS

a. Organ weight - **12-Month Interim Sacrifice:** Liver weights were increased in both sexes at the high-dose level, and when adjusted for body weight, statistical significance was attained. Relative (to body weight) liver weights were also increased, but statistical significance was not attained. The high-dose males displayed a statistically significant increase

in absolute adrenal weight, but the increase in the relative adrenal weight in this group was not statistically significant. The mid- and low-dose males also displayed increases in adrenal weight, but there was no dose response and a $p<0.05$ was not attained. The weight of the uterus was decreased in the high-dose females (absolute, adjusted, and relative) but statistical significance was not attained. Absolute and relative ovarian weights were decreased at all dose levels compared to the control value, but there was no dose response. Absolute thyroid weight was significantly decreased at all dose levels in females, and the relative thyroid weights were decreased in females at the mid- and high-dose levels ($p<0.05$ was not attained). The authors attributed this to the high value in the control group rather than to an effect of treatment and stated that 2 of the 13 control females displayed enlarged thyroids at necropsy and 1 of 13 high-dose females displayed enlarged thyroids. TB II notes that more of the control values are high [for example, 5 control and 1 in each of the treated groups displayed values ≥ 6] compared to the treated groups (Table 8), although the control does have 2 "outliers". No historical control data regarding thyroid weight in females were presented to support the contention that the control thyroid weight is unusual, but TB II notes that the control male thyroid weight is also less than the control female thyroid weight. Table 8 illustrates how more low values occurred in the treated females than in the control, as well as the occurrence of low values in the control males, which supports the contention that the decrease in females is not related to treatment.

Table 8. INTERIM SACRIFICE

Parameter / Group / Dose	# Mice With Thyroid Weight as Indicated (g)			
	0 ppm	160 ppm	800 ppm	4000 ppm
MALES N=	13	15	14	12
≤ 4	4	4	3	6
≥ 5	2	7	10	3
≥ 6	0	2	4	1
> 10	0	1	0	0
2 highest values (g)	5.9/5.0	12.9/6.2	9.4/7.6	6.1/5.6
lowest value (g)	3.4	2.7	2.9	2.8
FEMALES N=	13	15	15	13
≤ 4	6	6	8	10
≥ 5	5	4	5	3
≥ 6	5	1	1	1
> 10	1	0	0	1
2 highest values (g)	12.3/9.5	7.8/5.9	6.1/5.6	10.1/5.7
lowest value (g)	2.9	1.9	2.1	1.8

Table 9. Interim Organ Weight Data - Females

Parameter/dose (ppm)	FEMALES [12-month sacrifice]			
	0	160	800	4000
Liver				
absolute (g)	1.94	1.90	1.93	2.83 (146)♦
adjusted (g)	1.80	1.94	1.89	2.98**(166)
relative-body♦	584	511	598	1742 (298)
Uterus				
absolute (g)	0.501	0.526	0.605 (121)	0.278 (55)
adjusted (g)	0.457	0.536 (117)	0.595 (130)	0.321 (70)
relative-body♦	304	317	373 (123)	135 (44)
Ovaries				
absolute (g)	37.8	26.3 (70)	31.2 (83)	22.2 (59)
adjusted (g)	-	-	-	-
relative-body♦	71.7	55.7 (78)	76.0 (106)	33.8 (47)
Adrenals				
absolute (g)	8.8	7.8 (89)	8.7	10.3 (117)
adjusted (g)	-	-	-	-
relative-body♦	2.9	2.7 (93)	2.7 (93)	3.3 (114)
Thyroids				
absolute (g)	5.8	4.1*(71)	4.1*(71)	3.9*(67)
adjusted (g)	-	-	-	-
relative-body♦	1.5	1.2 (80)	1.1 (73)	1.2 (80)

Table 10. Interim Organ Weight Data - Males

Parameter/dose (ppm)	MALES [12-month sacrifice]			
	0	160	800	4000
Liver				
absolute (g)	2.15	2.29 (103)♦	2.51 (117)	3.08 (143)
adjusted (g)	2.19	2.20	2.35 (107)	3.33**(152)
relative-body♦	509	516 (101)	548 (108)	789 (155)
Testes/Epi.				
absolute (g)	0.368	0.380 (103)	0.371 (101)	0.408 (111)
adjusted (g)	-	-	-	-
relative-body♦	87.7	86.9 (99)	81.8 (93)	105.5 (120)
Adrenals				
absolute (g)	3.6	4.5 (125)	4.0 (111)	6.0 **(167)
adjusted (g)	-	-	-	-
relative-body♦	0.9	1.1 (122)	0.9	1.6 (178)
Thyroids				
absolute (g)	4.5	5.2 (107)	5.5 (122)	4.0 (89)
adjusted (g)	-	-	-	-
relative-body♦	1.1	1.2	1.2	1.0 (91)
Spleen				
absolute (g)	0.083	0.117 (141)	0.095 (114)	0.101 (122)
adjusted (g)	0.084	0.116 (138)	0.091 (108)	0.106 (126)
relative-body♦	20	27 (135)	21 (105)	26 (130)

♦ % x 100; ♦ % of control value; * $p<0.05$; ** $p<0.01$

Terminal Sacrifice: Liver weight was increased in both sexes [absolute for ♀♀/when adjusted for body weight for ♂♂] at the high-dose level; relative liver weight was increased also, although statistical significance was not attained. There was a dose-related increase in thyroid weights [adjusted for body weight] in both sexes, but only the high-dose males attained statistical significance. High-dose females displayed a significant decrease [41 % of control value] in uterine weight

[adjusted for body weight] and a decrease [47% of control value] in ovarian [absolute and relative] weight ($p<0.05$ was not attained). Adjusted kidney weight was slightly increased in the high-dose females compared to the control value. There was a slight increase in testes weight at the mid- and high-dose levels, but a $p<0.05$ was not attained. Other differences are listed in Tables 11 & 12. Table 11 is similar to Table 8, illustrating the occurrence of higher thyroid weight values in treated mice compared to the controls.

Table 11. Terminal Thyroid Weight Values

Parameter Group Dose	# Mice With Thyroid Weight as Indicated (g)			
	0 ppm	160 ppm	800 ppm	4000 ppm
MALES N=	29	24	32	22
≤ 4	5	4	2	2
≥ 5	15	17	19	16
≥ 6	7	9	13	10
> 10	0	0	9	0
2 highest values (g)	8.2/7.3	7.4/7.1	8.2/8.9	9.4/9.3
lowest value (g)	1.9	3.7	3.1	2.8
FEMALES N=	25	21	21	20
≤ 4	5	9	4	2
≥ 5	12	11	12	13
≥ 6	8	8	10	4
> 10	0	0	1	2
2 highest values (g)	7.8/7.2	9.3/8.4	10.5/9.2	13.7/11.7
lowest value (g)	2.3	3.0	3.1	3.0

Table 12. Terminal Organ Weight Data - Females

Parameter/dose (ppm)	FEMALES [terminal sacrifice]			
	0	160	800	4000
Liver				
absolute (g)	2.07	1.80 (87)	2.13 (103)	5.82**(281)
adjusted (g)	-	-	-	-
relative-body♦	584	511 (88)	598 (103)	1742 (298)
Uterus				
absolute (g)	1.049	1.236 (118)	1.309 (125)	0.454 (43)
adjusted (g)	0.824	0.683 (83)	0.810 (98)	0.337**(41)
relative-body♦	304	317 (104)	373 (123)	135 (47)
Ovaries				
absolute (g)	241.9	196.7 (81)	250.7 (104)	113.1 (47)
adjusted (g)	-	-	-	-
relative-body♦	71.7	55.7 (78)	76.0 (106)	33.6 (47)
Adrenals				
absolute (g)	9.9	9.3 (94)	9.6 (97)	10.5 (106)
adjusted (g)	-	-	-	-
relative-body♦	2.9	2.7 (93)	2.7 (93)	3.3 (114)
Thyroids				
absolute (g)	5.1	5.3 (104)	6.0 (118)	5.9 (116)
adjusted (g)	5.1	5.3	5.9 (116)	6.1 (120)
relative-body♦	1.5	1.5	1.7 (113)	1.8 (120)
Kidneys				
absolute (g)	0.496	0.491	0.499 (101)	0.529 (107)
adjusted (g)	0.496	0.486 (98)	0.494	0.541*(109)
relative-body♦	144	140 (97)	141 (98)	162 (113)

Table 13. Terminal Organ Weight Data - Males

Parameter/dose (ppm)	MALES [terminal sacrifice]			
	0	160	800	4000
Liver				
absolute (g)	2.39	2.18 (91)	2.72 (114)	6.30 (264)
adjusted (g)	2.16	2.05 (95)	2.48 (115)	6.23**(288)
relative-body♦	568	523 (92)	660 (116)	1603 (282)
Testes/Epi.				
absolute (g)	0.336	0.336	0.359 (107)	0.382 (114)
adjusted (g)	-	-	-	-
relative-body♦	81.7	81.9	88.2 (108)	95.7 (117)
Adrenals				
absolute (g)	5.8	5.3 (91)	5.4 (93)	6.6 (114)
adjusted (g)	5.8	5.3 (91)	5.4 (93)	6.5 (112)
relative-body♦	1.4	1.3 (93)	1.3 (93)	1.7 (121)
Thyroids				
absolute (g)	5.1	5.5 (108)	5.6 (110)	5.9 (116)
adjusted (g)	5.1	5.5 (108)	5.6 (110)	6.0*(118)
relative-body♦	1.2	1.4 (117)	1.4 (117)	1.5 (125)
Spleen				
absolute (g)	0.094	0.083 (88)	0.135 (144)	0.129*(137)
adjusted (g)	-	-	-	-
relative-body♦	22	20 (91)	33 (150)	32 (145)
Brain				
absolute (g)	0.518	0.524 (101)	0.514 (99)	0.492 (95)
adjusted (g)	0.517	0.524 (101)	0.514 (99)	0.494**(96)
relative-body♦	126	128 (102)	126	127 (101)

♦ % x 100; ♦ % of control value; * $p<0.05$; ** $p<0.01$

b. Gross pathology - **Interim Sacrifice:** Enlarged livers were observed in 1 of 14 mid-dose males, 3 of 12 high-dose males, and 7 of 13 high-dose females compared to 0 of 13/sex in the control mice. Accentuated lobular markings were noted only in treated mice of both sexes. There was a decreased incidence of thickened uteri in high-dose females compared to the control. Other findings in common with those found at termination are listed in Table 14.

Group Dose (ppm) Lesion	Table 14. Interim Macroscopic Findings♦			
	0	160	800	4000
MALES				
Liver				
enlarged	0/13	0/15	1/14	3/12
accentuated lobular markings	0/13	1/15	3/14	2/12
Adipose Tissue				
minimal	1/13	0	0	2/12

Group Dose (ppm) Lesion	Table 14. Interim Macroscopic Findings♦			
	0	160	800	4000
FEMALES				
Liver				
enlarged	0/13	0/15	0/15	7/13
accentuated lobular markings	0/13	0/15	1/15	2/13
Kidneys				
irregular cortical scarring	0/13	0/15	0/15	1/13
Uterus				
thickened	7/13	9/15	6/15	3/13
Forestomach				
thickened	0/13	0/15	0/15	2/13
Adipose Tissue				
minimal	0	2/15	1/15	5/13

♦ # with lesion/# examined

Terminal Sacrifice: Enlarged livers were found in 6/50 male and 7/50 female high-dose mice compared with 2/50/sex in the controls, and liver masses occurred more frequently in the mid-dose males and high-dose mice of both sexes. Fewer high-dose females displayed uteri that were thickened compared to the control. There was an increased incidence of thickened and/or white forestomachs at the high-dose level in both sexes compared to the controls. Irregular cortical scarring of the kidneys was increased at the high-dose in females but not males, and misshapen kidneys were observed more frequently in high-dose females than control females. A greater number of masses were noted in the testes of the high-dose males compared to the control males, and smaller and/or flaccid testes were found more frequently at the high dose than in controls. The incidence of other macroscopic lesions observed at termination appears comparable among the groups, with no dose response evident in the differences noted. The Registrant stated that the findings were within the expected background range, but no historical control data were provided.

Group Dose (ppm) Lesion	Table 15. Incidence of Lesions 0	160	800	4000
MALES	**50**	**50**	**50**	**50**
Liver				
masses	8	7	14	26
enlarged	2	1	2	6
Thyroids				
enlarged	1	0	2	3
Forestomach				
thickened	4	2	4	10
white	3	1	3	12
Adrenals				
enlarged	1	0	0	4
Kidneys				
masses	0	0	0	2
irreg.cort. scarring	9	9	9	4
Testes				
masses	1	1	3	5
flaccid	4	6	5	14
small	2	4	2	6
Adipose Tissue				
minimal	3	4	1	6
FEMALES	**50**	**50**	**50**	**50**
Liver				
masses	4	4	2	22
enlarged	2	6	6	7
Uterus				
thickened	39	34	36	16
Forestomach				
thickened	3	2	5	9
white	3	1	5	11
Adrenals				
enlarged	0	1	0	5
Kidneys				
enlarged	1	1	2	2
irreg. cort. scarring	6	2	3	12
misshapen	1	4	3	9
Ovaries				
enlarged	0	1	1	2
Heart				
pale	0	2	2	4
Adipose Tissue				
minimal	6	3	7	10

c. Microscopic pathology: **Interim Sacrifice** - 1) Non-neoplastic: In the liver, mice of both sexes displayed an increase in the incidence and degree of centrilobular hepatocyte enlargement compared to the controls, and centrilobular hepatocyte vacuolation was observed in the majority of high-dose females compared to the control incidence. These findings are consistent with the increases in liver weight and plasma GPT and GOT observed in the groups. Although the incidence and degree of fat in the hepatocytes was similar among the groups, a difference in distribution was noted; i.e., control, low- and mid-dose mice displayed fat in all zones while in the high-dose mice, fat was confined to the periportal hepatocytes.

The majority of high-dose females displayed hypertrophy of the cells of the zona fasciculata of the adrenal gland. No other group displayed this lesion. The lesion correlates with the increased adrenal weight observed in these females, but no morphological correlation was observed to account for the

increased adrenal weight observed in high-dose males.

Only high-dose males displayed generalized vacuolation and hypertrophy of the interstitial cells of the testes. In females, only high-dose mice displayed luteinization of the interstitial cells of the ovary. In the cervix and vagina, epithelial thickening, usually with keratinization, was observed more frequently in the treated females compared to the controls, but no dose-response was evident.

Table 16. Total Incidence of Non-Neoplastic Lesions - **INTERIM**

Group/Lesion	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
LIVER - N=	13	13	15	15	14	15	12	13
centrilobular hepatocyte enlargement								
total	7	0	5	0	6	2	12	13
minimal	6	0	5	0	3	1	1	4
moderate	1	0	0	0	3	1	7	8
marked	0	0	0	0	0	0	4	1
centrilobular hepatocyte vacuolation								
total	2	1	2	4	3	5	0	10
minimal	2	1	2	3	0	3	0	8
moderate	0	0	0	1	1	2	0	2
marked	0	0	0	0	2	0	0	0
ADRENALS - N=	13	15	14	12	13	15	15	13
hypertrophy of cells of zona fasciculata	0	0	0	0	0	0	0	12
TESTES - N=	13	-	15	-	14	-	12	-
gen. vacuolation/hypertrophy-interstitial cells	0	-	0	-	0	-	9	-
OVARIES - N=	-	13	-	15	-	15	-	13
luteinization of interstitial cells	-	0	-	0	-	0	-	8
corpora lutea absent	-	0	-	0	-	0	-	2
prominent granulosa cells	-	0	-	0	-	0	-	1
UTERUS - N=	-	13	-	14	-	15	-	13
endometrial gland hyperplasia								
total	-	5	-	0	-	0	-	8
minimal	-	3	-	0	-	0	-	5
moderate	-	2	-	0	-	0	-	3
CERVIX - N=	-	13	-	15	-	15	-	13
epithelial thickening	-	3	-	8	-	3	-	8
keratinization	-	1	-	6	-	3	-	8
squamous epithelium w/ keratinization	-	0	-	2	-	2	-	2
VAGINA - N=	-	13	-	15	-	15	-	13
epithelial thickening	-	2	-	8	-	3	-	8
keratinization	-	0	-	2	-	2	-	2
squamous epithelium w/ keratinization	-	1	-	6	-	3	-	8

2) Neoplastic: Very few tumors were observed at the interim sacrifice. Pulmonary adenoma [benign] was observed in one low-dose female and one mid-dose female, and pulmonary adenocarcinoma [malignant] was observed in one control male and one high-dose male. One male in the mid- and one male in the high-dose groups displayed a benign liver cell tumor. One benign cystadenoma was observed in the low-dose male group.

Terminal - 1) Non-neoplastic: LIVER - At the high-dose level

(both sexes), there was a significantly increased incidence of single and multiple areas of enlarged eosinophilic hepatocytes and focal fat-containing hepatocytes compared to the control values. The incidence and degree of centrilobular hepatocyte enlargement were increased significantly at the high-dose level in both sexes, and the incidence of minimal centrilobular hepatocyte enlargement was increased at the mid-dose level in females compared to the control mice. Additionally, at the high-dose level, the incidence and degree of pigmented macrophages and the degree of centrilobular hepatocyte vacuolation were increased significantly in male mice compared to the control male mice. TESTES - There was an increased incidence of generalized vacuolation/hypertrophy of the interstitial cells of the testes in the mid- and high-dose mice. Other findings are listed in Table 17, below.

Table 17. Total Incidence of Non-Neoplastic Lesions -TERMINAL

Group/Lesion	MALES				FEMALES			
	0	160	800	4000	0	160	800	4000
LIVER - N=	50	50	50	50	50	50	50	50
centrilobular hepatocyte enlargement								
total	12	12	20	38**	3	3	12*	27**
minimal	10	7	14	6	2	3	10*	7
moderate	2	4	6	15**	1	0	2	14**
marked	0	1	0	17**	0	0	0	6*
centrilobular hepatocyte vacuolation								
total	11	7	12	20*	-	-	-	-
minimal	7	6	12	3	-	-	-	-
moderate	4	0	0	17**	-	-	-	-
marked	0	1	0	0	-	-	-	-
areas of enlarged eosinophilic hepatocytes	2	1	0	10*	0	0	0	8**
area of enlarged eosinophilic hepatocytes	0	3	4	7**	0	0	0	5*
pigmented macrophages	1	2	4	11**	-	-	-	-
centrilobular necrosis	0	0	0	2	0	0	0	2
amyloid deposits	9	7	3	15	9	10	10	14
ADRENALS - N=	50	26	18	50	50	49	50	50
hypertrophy of cells of zona fasciculata	5	0	6	6	9	11	15	11
amyloid deposits	11	12	9	19	9	13	16	18
TESTES - N=	50	50	50	50	-	-	-	-
gen. vacuolation/hypertrophy-interstitial cells	4	0	25**	27**	-	-	-	-
amyloid deposits	3	3	3	14	-	-	-	-
degenerate tubules	0	3	4	6	-	-	-	-
tubular atrophy	9	15	14	14	-	-	-	-
OVARIES - N=	-	-	-	-	50	49	50	50
luteinization of interstitial cells	-	-	-	-	6	10	11	23
corpora lutea absent	-	-	-	-	12	16	11	23
prominent granulosa cells	-	-	-	-	1	0	0	2
amyloid deposits	-	-	-	-	9	10	12	11
UTERUS - N=	-	-	-	-	50	49	50	50
cystic endometrial hyperplasia	-	-	-	-	32	17	28	17
endometrial hyperplasia	-	-	-	-	3	8	3	5
CERVIX - N=	-	-	-	-	49	50	49	50
epithelial thick & keratinized	-	-	-	-	12	4	4	7
epithelium thick & non-keratinized	-	-	-	-	19	11	13	12
epithelium thin & non- keratinized	-	-	-	-	9	22	20	27
amyloid deposits	-	-	-	-	0	1	2	5
VAGINA - N=	-	-	-	-	50	50	49	49
epithelial thick & keratinized	-	-	-	-	12	4	4	7
epithelium thick & non-keratinized	-	-	-	-	19	12	13	11
epithelium thin & keratinized	-	-	-	-	10	22	21	28

Group/Lesion	MALES				FEMALES			
	0	160	800	4000	0	160	800	4000
KIDNEY - N=	50	50	50	50	50	50	50	59
amyloidosis	11	12	7	17	9	12	15	19
amyloid deposits	2	1	3	4	4	2	1	1
cortical scarring	6	8	7	8	5	2	4	14
minimal	3	4	8	5	4	0	2	5
moderate	3	4	0	2	0	2	2	8
marked	0	0	0	1	1	0	0	1
Spleen - N=	50	28	22	50	50	50	50	50
extramedullary hematopoiesis	22	13	8	25	32	31	27	38

2) Neoplastic - **LIVER:** At the high-dose level, there was a significant increase in the incidence of benign and malignant liver cell tumors in both sexes compared to the control. The adenomas found at the control, low-, and mid-dose levels in males were all in terminally-sacrificed mice, while 4 of the 11 adenomas in the high-dose males, 1 of 2 in the mid-dose females and 5 of 15 in the high-dose females were found in mice dying on test. No historical control data were provided.

Group Dose (ppm) Lesion	Liver Tumor Incidence			
	0	160	800	4000
MALES - N=	50	50	50	50
adenoma	3	3	4	11**
carcinoma	4	3	6	15**
# bearing liver cell tumor	7	6	10	26**
FEMALES - N=	50	50	50	50
adenoma	1	1	2	15**
carcinoma	1	1	0	6*
# bearing liver cell tumor	2	2	2	21**

* $p<0.05$; ** $p<0.01$

OVARIES: The incidence of luteoma of the ovaries was significantly increased at the high-dose level in females compared to the controls, and there was a significant trend when all dose groups were considered. All tumors were found at terminal sacrifice. No historical control data were provided.

Group Dose (ppm) Lesion	Ovarian Tumor Incidence			
	0	160	800	4000
FEMALES - N=	50	49	50	50
luteoma	0	2	1	5*

KIDNEY: Clear cell carcinoma (malignant) was observed in the kidney of one high-dose male at termination.

D. DISCUSSION

The administration of Iprodione in the diet of mice at dose levels up to 4000 ppm for a two-year period resulted in a slight decrease in the survival of the high-dose mice of both sexes, compared to the controls, but adequate numbers of mice survived to study termination. The majority of the deaths were attributed to amyloidosis, with the high-dose males and females at each dose level (dose-related) displaying a greater incidence than their respective control groups. Body-weight gain during the first 18 weeks of the study was comparable among the groups (both sexes), but during weeks 18 to 45, both sexes at the high dose displayed a significant decrease compared to the control values. Overall (weeks 0-99), body-weight gain was decreased at the high-dose level in both sexes compared to the controls. With the exception of the first week when a statistically significant decrease in food consumption and a greater food efficiency were observed at the high dose for both sexes, food consumption and food efficiency were comparable among the groups. The liver is a target organ for Iprodione. There was a significant increase in the incidence of benign and malignant liver cell tumors in both sexes compared to the controls, and numerous other findings in the liver correlated with this increase. At necropsy, the number of high-dose mice with enlarged livers and/or liver masses was greater than in the controls. There were microscopic lesions (centrilobular hepatocyte enlargement/vacuolation) in the livers of both sexes at the high-dose level at the interim sacrifice, as well as at the terminal sacrifice. Centrilobular hepatocyte enlargement was also observed in the mid-dose females at the terminal sacrifice. Other liver lesions included area(s) of enlarged eosinophilic hepatocytes, pigmented macrophages, centrilobular necrosis, and amyloid deposits, which were increased at the high-dose level in both sexes. Additionally, statistically significant increases were observed in GPT and GOT levels relative to control values at the high-dose level (♂♂ & ♀♀) at week 52 (only interval examined), and liver weight was increased in the high-dose mice (both sexes) at both the 12-month and terminal sacrifices.

The only other increase in tumor incidence was seen in the ovaries where an increase in the incidence of luteoma at the high-dose level was observed, which was slightly outside that of the historical control range [no historical control data presented; statement in Discussion Document MRID # 428250-01]. Decreased uterine and ovarian weights were observed in the high-dose females at both sacrifices, although enlarged ovaries were noted at the terminal sacrifice. Other findings in the ovaries included an increase in luteinization of the interstitial cells, corpora lutea absent, and prominent granulosa cells.

Other apparent treatment-related findings include an increase in the incidence of (1) generalized vacuolation/hypertrophy of the interstitial cells of the testes in the mid- and high-dose mice and (2) amyloid deposits in the various organs at the high-dose level in both sexes, and a decrease in the incidence of thickened uteri in the high-dose females compared to the controls.

The Registrant presented a discussion of the possible mechanisms of action of Iprodione with respect to tumor formation and their relevance to human risk. Because of the negative results from various genetic toxicity studies on Iprodione, the Registrant concludes that Iprodione is a nongenotoxic carcinogen. Additionally, because each tumor type observed in the rat and mouse studies was detected in only one species and each occurred only at MTD dose levels, it is proposed that the non-neoplastic lesions observed in the reproductive system of both sexes suggests a perturbation of sex hormone regulation. The Registrant argues that the tumor formation [Leydig cell tumors in testes of rat; luteoma in ovaries of mice] "is likely to be secondary to prolonged and profound hormonal imbalance at the target organ level" only at high dietary levels. Since such "a mechanism would be expected to occur above a threshold that would need to be exceeded to overcome the powerful normal hormonal homeostasis", the Registrant contends that quantitative carcinogen risk assessment based on "linearized" multistage model is inappropriate in the case of Iprodione; safety factors are considered to be an appropriate and adequate method of risk assessment of threshold carcinogens. The Registrant intends to conduct mechanistic studies to demonstrate the effects of Iprodione on sex hormone regulation and to establish a link between this hormonal perturbation and the increased incidence of tumor formation. Also discussed is the fact that compounds lacking genotoxic potential produce tumors primarily in the rodent liver after prolonged periods at high dose levels, a group to which the Registrant contends Iprodione belongs. These issues, as well as all of the available data on Iprodione, will be presented to the HED Carcinogenicity Peer Review Committee in the near future for their consideration.

E. CONCLUSION

Treatment of mice with Iprodione at dose levels in the diet of 160, 800, and 4000 ppm for 99 weeks did not have an adverse effect on survival or food consumption, but body-weight gain at the high dose was decreased overall in both sexes. There was an increase in the incidence of liver tumors in both sexes at the high-dose level, which was accompanied by increases in several liver lesions (centrilobular hepatocyte enlargement/

vacuolation, area(s) of enlarged eosinophilic hepatocytes, pigmented macrophages, centrilobular necrosis, and amyloid deposits). Additionally, statistically significant increases were observed in GPT and GOT levels relative to control values at the high-dose level (♂♂ & ♀♀) at week 52 (only interval examined), and liver weight was increased in the high-dose mice (both sexes) at both the 12-month and terminal sacrifices. There was also an increase in the incidence of benign ovarian tumors (luteoma) at the high dose compared to the control incidence, which was accompanied by an increase in luteinization of the interstitial cells, corpora lutea absent, and prominent granulosa cells. Also increased was the incidence of generalized vacuolation/hypertrophy of the interstitial cells of the testes in the mid- and high-dose mice. The NOEL can be set at 160 ppm, the LEL at 800 ppm, based on the increased incidence of centrilobular hepatocyte enlargement in females and the increased incidence of generalized vacuolation/ hypertrophy of the interstitial cells in the testes. This study is classified Core Minimum, and it satisfies the guideline requirement (83-2) for a carcinogenicity study in mice.

Discrepancies: On page 1892 of the report [Addendum 6], the summary states that Iprodione was administered to mice for 97 weeks. All other references to the study's duration list 99 weeks.