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

005882

MAY 18 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Re-review of the 1-year dog study

To: Robert Forrest, PM-21
Registration Division, TS-767C

From: Marcia van Gemert, Ph.D.
Head, Section III
Toxicology Branch, HED

M. van Gemert 5/14/87

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

Theodore M. Farber 5/14/87

Chemical: Iprodione

Company: Rhone-Poulenc

Caswell No: 470A

Project No: 7-0608

Rhone-Poulenc has submitted arguments on several points concerning their 1-year dog study on Iprodione. They stated that in fact the low dose of 100 ppm was actually measured in the feed of the dogs and as measured was 4.2 mg/kg rather than the theoretical amount of 2.5 mg/kg stated in the original Tox. Branch memo. Tox Branch agrees with Rhone-Poulenc and the ADI has been changed to reflect the larger amount.

Rhone-Poulenc has also presented arguments concerning the toxicological effects of Iprodione seen at the mid dose in the previous tox review of this study by A. Arce. This study was re-reviewed by M. van Gemert and the DER of this re-evaluation is attached. The new review supports for the most part the conclusions made in the old review and has restated that the NOEL is 100 ppm based on decreased prostate weights and Heinz bodies in male erythrocytes seen at the mid dose.

Rhone Poulenc might submit historical control data from this laboratory and strain of dog on Heinz bodies and prostate weights to support their argument that these are not toxicological effects. A new study might also be initiated looking primarily at the hematological, liver and prostate endpoints using doses between and including the 100 and 600 ppm to clarify the NOEL for this compound.

This reviewer did not feel that a re-review of the subchronic dog study was necessary.

Reviewed by: Marcia van Gemert, Ph.D. *M van Gemert 5/14/87*
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: Re-evaluation of the 1-year dog study TOX. CHEM. NO.: 470A

ACCESSION NUMBER: none MRID NO.: .

TEST MATERIAL: Iprodione

SYNONYMS:

STUDY NUMBER(S): 84/RH002/179

SPONSOR: Rhone-Poulenc Agrochimie

TESTING FACILITY: Life Sciences Research, Suffolk, England

TITLE OF REPORT: Iprodione: 52-week toxicity study in Dietary administration to Beagle dogs

AUTHOR(S): P. Lee, R. Ashby, et al

REPORT ISSUED: Sept. 28, 1984

CONCLUSIONS: 6 dogs/sex/group were treated with 0, 100, 600 or 3600 ppm for 52 weeks. Effects were seen at the high dose in the liver, with increased absolute and relative weights, alkaline phosphatase, SGOT, SGPT, LDH enzyme levels. Heinz bodies were seen in both mid and high dose males and high dose female erythrocytes, with RBCs, PCV, Hb and MCHC down at the high dose. There was a slight increase in hyperreflexion in the eyes seen at the high dose. Adrenal relative and absolute weights were increased at the high dose with microscopic changes seen. Prostate weights were decreased at the mid and high dose with statistical significance seen in absolute weights at the mid and high dose and relative weights at the high dose.

NOEL = 100 ppm (4.2 mg/kg)

LEL = 600 ppm

Classification: core-minimum

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: Iprodione, Description white, slightly lumpy powder.

Batch # DA-237-OP-81-461

Purity 86,5%, contaminants: list in CBI appendix

2. Test animals: Species: Dog, Strain: Beagle, Age: 15-17 weeks
Weight: 2.7-6.8 kg. Source: Balbeggie Kennels, Fife, Scotland

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study	
		52 weeks male	52 weeks female
1 Cont.	0	6	6
2 Low (LDT)	100	6	6
3 Mid (MDT)	600	6	6
4 High(HDT)	3600	6	6

2. Diet preparation

Diet was prepared twice per week and stored at 4°C before use. Samples of treated food were analyzed for stability and homogeneity before treatment and for concentration during weeks 1, 13, 26, 29, and 52. Stability and homogeneity sampling methods are on appended pages 1 and 2.

Results -

Test compound was found to be stable in bulk for for the entire experimental period.

3. Animals received food (500 gms dry food (650 gms wet) Laboratory Diet A, Special diet services Ltd. Witham England and water ad libitum were given. Formulation of diet and quality control of dosage are on appended page 1.

4. Statistics - The statistical procedures used are on appended page 3,4 and 5.

5. Quality assurance statement was given in the text and signed Oct. 3, 1984.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected for signs of toxicity and mortality before start of the experiment and after weeks 3, 8, 11, 16, 19, 24, 27, 31, 35, 39, 43, 47 and 51 weeks.

During these examinations, attention was paid to:

Teeth and gums

Mucous membrane and skin

Ears (external auditory canal)

Superficial lymph nodes

Abdomen- including palpation

External genitalia and mammary glands

Chest- including auscultation of heart and lungs

Gait and stance, including palpation of limbs

General behaviour and appearance

Toxicity - Results:

The study text claims that there were no treatment-related signs of toxicity with Iprodione.

Mortality- results:

One female in the 600 ppm group was killed in extremis with convulsions, which did not appear to be treatment-related.

2. Body weight

Animals were weighed weekly throughout the test period.

Results: No treatment-related changes in body weight were evident throughout the study.

3. Food consumption and compound intake

Each animal was given 500 gms dry or 650 gms wet food per day. Any uneaten portion of the food was removed. Food consumption was not affected by treatment.

Compound Intake, Results: The achieved doses are on appended pages 6 and 7.

4. Ophthalmological examinations

Performed before start of the study and after 3, 8, 12, 18, 35 and 50 weeks of treatment, both eyes of all dogs were examined using a Fison Binocular Indirect Ophthalmoscope after infusing Mydriacyl (tropicamide, 1%).

Results:

There was a slightly increased incidence of hyperreflection in groups 3 and 4 males and females. However, the results in the individual animal data indicate that the hyperreflection seen was for the most part slight, and wasn't seen consistently in the same animal over the seven observation periods, and was also seen on occasion in controls. For example at week 19 hyperreflection was seen in one control male and four control females. One would assume that if this were a true toxicological phenomenon it would appear in the same animals consistently, and the severity would increase. Neither of these two happened, and it is therefore difficult to call hyperreflection in this situation a toxicological consequence of Iprodione Administration. See page 8 for details. However, electron microscopy could have been done on the tissues of the animals with hyperreflection, to determine if any treatment-related effects were evident.

Two male dogs in group 4 showed finely scattered opalescent particles (asteroid bodies) in the vitreous humor. This unilateral for both animals. This may be a treatment-related phenomenon since no control dogs exhibited this and it was not seen until 13 weeks after the start of the experiment. (see appended page 8 for details)

5. Blood was collected before treatment and at 4, 8, 13, 17, 21, 25, 38 and 51 weeks, after an overnight fast for hematology and clinical chemistry analyses from each animal. The checked (X) parameters were examined. Those with an (A) preceding were examined pre-treatment. 005882

a. Hematology

X	X	Hematocrit*	X	X	Leukocyte differential count*
A	X	Hemoglobin *	A	X	Mean corpuscular HGB
A	X	Leukocyte count *	A		Mean corpuscular HGB conc.
A	X	Erythrocyte count *			Mean corpuscular volume
A	X	Platelet count *		X	Reticulocyte count
		Blood clotting measurements	A	X	Packed cell volume
	X	Thromboplastin time	A	X	Erythrocyte sedimentation rate
		clotting time	A	X	Mean cell volume
A	X	Prothrombin time (PTTK)			

* required for subchronic and chronic studies

Heinz bodies were examined after 4 weeks using the film prepared for reticulocyte count. These were graded as follows below (using the number of erythrocytes containing Heinz bodies):

- 0 = None seen
- 1 = Very occasional
- 2 = Up to two per field
- 3 = Two to five per field
- 4 = Six to 50 per field
- 5 = Over 50 per field.

Bone marrow samples were taken from the femur under local anaesthetic several days before necropsy. In addition a costal bone marrow smear was prepared at necropsy for the dog which died in extremis.

Results:

Week 4

PCV, Hb and RBC were decreased in group 4 males, Platelets and PTTK were increased in group 4 males. Hb and MCHC were decreased in group 4 females and PTTK was increased in group 4 females.

Week 8

PCV, Hb, RBC and MCHC were decreased in group 4 males. MCV and PTTK were increased in group 4 males and platelets were increased in groups 2 and 4 in males. MCHC was decreased in group 1 and 4 females. MCV was increased in group 3 and 4 females, and platelets and PTTK were increased in group 4 females.

Week 13

PCV, Hb, and RCB were decreased in group 4 males, platelets were increased in groups 2 and 4 males.

Platelets and PTTK were increased in group 4 females.

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Week 17

MCHC was decreased in group 4 males, platelets were increased in groups 2 and 4 males.
PTTK was increased in group 4 females.

Week 21

MCHC was decreased in group 4 males and platelets were increased in groups 2 and 4 males.
MCH was decreased, and neutrophils and PTTK were increased in group 4 females.

Week 25

PCV, Hb, RBC were decreased, total WBC, neutrophils, leukocytes and eosinophils and platelets were increased in group 4 males.
Neutrophils, platelets and PTTK were increased in group 4 females.

Week 38

PCV, Hb, and RBC were decreased and platelets were increased in group 4 males.
PCV, Hb, and RBC were decreased and platelets and PTTK were increased in group 4 females.

PCV, Hb, and RBC were decreased in groups 2 and 4 males and platelets and PTTK were increased in group 4 males.
PTTK was increased in group 4 females.

Bone Marrow Smears

No significant treatment-related effects were seen in the bone marrow smears.

TABLE I

HEMATOLOGY

<u>Week 4:</u>								
males		females						
Groups	PCV	Hb	RBC	Plt	PTTK	Hb	MCHC	PTTK
1	42	14.1	6.23	220	12.8	14.8	35	12.8
SD	4	1.2	0.49	55	0.9	1.8	1	1.7
2	42	14.2	6.21	294 ^a	12.1	15.2	34	12.2
SD	3	1.1	0.42	29	0.7	1.1	0	1.4
3	42	14.1	6.26	245	12.4	14.2	34	12.4
SD	5	1.7	0.90	38	1.3	1.0	1	0.2
4	35 ^b	11.7 ^b	5.06 ^b	377 ^c	15.9 ^c	12.8 ^a	33 ^a	22.5 ^c
SD	3	0.9	0.38	72	1.9	2.2	1	3.1

SD- Standard deviation

a- Significantly different from controls, P < 0.05

b- Significantly different from controls, P < 0.01

c- Significantly different from controls, P < 0.001

TABLE-1 cont.

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Week 8								females		
males										
Groups	PCV	Hb	RBC	MCHC	MCV	Pltlt	PTTK	MCV	Pltlt	PTTK
1	46	15.4	6.80	34	67	292	11.7	66	315	11.5
SD	3	1.1	0.41	1	2	35	0.9	1	67	1.2
2	44	14.7	6.54	33	67	367 ^a	11.3	67	373	11.3
SD	2	0.5	0.29	1	2	33	0.6	2	50	1.1
3	45	15.1	6.75	33	67	299	11.7	69 ^a	340	11.4
SD	4	1.5	0.63	1	3	70	1.3	1	34	0.6
4	40 ^b	12.9 ^c	5.68 ^c	33 ^b	70 ^a	474 ^c	14.7 ^a	69 ^b	450 ^b	15.6 ^c
SD	2	0.9	0.38	1	1	53	4.4	2	96	1.5

Week 13					females	
males						
groups	PCV	Hb	RBC	Pltlt	Pltlt	PTTK
1	45	16.7	6.64	261	282	12.7
SD	3	1.4	0.47	46	57	1.0
2	44	16.3	6.47	318 ^a	342	11.4
SD	2	0.9	0.32	31	35	0.7
3	47	17.2	6.88	296	306	12.2
SD	5	1.8	0.64	24	28	0.7
4	41 ^a	14.9 ^a	5.89 ^b	422 ^a	390 ^b	18.8 ^c
SD	3	1.1	0.35	61	103	4.2

Week 17				Week 21				
males				females				
Groups	MCHC	Pltlt	PTTK	males	females	MCH	Neut	PTTK
1	35	277	13.2	35	266	23	5.1	13.1
SD	1	21	1.2	1	40	1	0.6	1.8
2	34	339 ^b	12.6	35	322 ^a	23	5.2	11.7
SD	1	42	0.7	0	43	1	1.5	1.1
3	35	286	12.6	35	274	23	5.7	12.2
SD	1	33	0.7	1	9	1	0.8	1.1
4	34 ^a	357 ^c	16.5 ^b	34 ^a	350 ^b	22 ^a	7.6 ^a	27.5 ^c
SD	1	33	2.6	1	50	0	3.2	6.8

Week 25											
males					females						
groups	PCV	Hb	RBC	Total WBC	Neut.	Leuk.	Eosin	Pltlt	Neut	Pltlt	PTTK
1	50	17.1	7.23	11.1	4.5	5.2	0.9	240	4.6	271	12.8
SD	4	1.4	0.60	1.5	1.1	1.1	0.3	52	1.0	53	1.4
2	46	16.0	6.84	11.0	4.1	4.8	1.3	284	3.8	285	14.0
SD	4	1.1	0.51	2.0	1.1	0.9	0.6	34	1.4	45	4.2
3	49	17.1	7.16	10.8	5.5	3.5	1.1	250	4.1	264	13.0
SD	5	1.7	0.61	2.1	1.3	0.9	0.6	25	0.8	29	1.4
4	39 ^c	13.6 ^c	5.67 ^c	14.6 ^a	6.0 ^a	6.1 ^a	1.9 ^b	358	7.0 ^b	410 ^c	17.6 ^a
SD	3	1.2	0.39	3.2	1.0	1.9	0.6	100	0.8	95	4.6

SD = Standard Deviation

a- Significantly different from controls, P < 0.05

b- Significantly different from controls, P < 0.01

c- Significantly different from controls, P < 0.001

Week 38

males					females				
groups	PCV	Hb	RBC	Plt/lts	PCV	Hb	RBC	Plt/lts	PTTK
1	51	17.8	7.14	227	52	17.9	7.32	260	13.6
SD	4	1.7	0.56	37	6	2.2	0.80	44	2.5
2	49	16.5	6.79	287 ^a	51	17.7	7.25	279	12.3
SD	3	0.8	0.37	45	4	1.3	0.60	45	0.6
3	52	18.1	7.25	257	51	17.5	7.29	265	13.5
SD	6	2.3	0.80	36	1	0.2	0.29	30	1.0
4	45 ^b	15.3 ^a	6.27 ^a	338 ^c	45 ^a	15.3 ^a	6.38 ^a	359 ^b	19.2 ^c
SD	3	0.9	0.36	48	5	1.8	0.58	67	1.9

Week 51

males						females
groups	PCV	Hb	RBC	Plt/lts	PTTK	PTTK
1	50	17.9	7.14	208	11.1	11.5
SD	5	2.0	0.72	42	0.6	1.4
2	43 ^a	15.4 ^a	6.29 ^a	255	11.2	11.3
SD	3	1.1	0.43	37	0.6	0.2
3	48	17.1	6.86	230	11.0	12.1
SD	6	2.3	0.93	19	1.6	0.8
4	43 ^a	15.3 ^a	6.19 ^a	333 ^c	12.8 ^a	14.9 ^c
SD	3	1.3	0.42	54	1.8	1.6

SD = Standard Deviation

a- Significantly different from controls P < 0.05

b- Significantly different from controls P < 0.01

c- Significantly different from controls P < 0.001

Appended pages 11 and 12 contain tables of the hematology data in the various weeks compared to the pre-treatment levels. The results are essentially the same as those seen comparing the treated results with concurrent control data. Group 4 platelets PTTK, PCV, Hb, and RBCs in both males and females were effected by treatment with Iprodione.

The study text states that "at each examination Heinz bodies were observed in moderate numbers of the erythrocytes of male and female dogs receiving 3600 ppm. A small number of erythrocytes were also affected up to Week 18 in dogs receiving 600 ppm. The incidence of Heinz bodies in the erythrocytes of dogs receiving 100 ppm was similar to that of the controls."

Appended pages 16 and 17 have the tabulated numbers and severity of Heinz bodies seen in all doses at all the time period tested. There was some indication of an effect in the 600 ppm male animals, especially in the earlier time periods, as well as a definite effect seen in the high dose groups, in terms of severity and increased incidence. The high dose increase in Heinz bodies definitely correlates with the other effects seen in hematological parameters, such as decreased hemoglobin, red blood cells, PCV, and MCHC.

b. Clinical Chemistry

<p><u>X</u></p> <p><u>Electrolytes:</u></p> <p>X Calcium*</p> <p>Chloride*</p> <p>Magnesium*</p> <p>Phosphorous*</p> <p>X Potassium*</p> <p>X Sodium*</p> <p><u>Enzymes</u></p> <p>X Alkaline phosphatase</p> <p>Cholinesterase#</p> <p>Creatinine phosphokinase*°</p> <p>X Lactic acid dehydrogenase</p> <p>X Serum alanine aminotransferase (also SGPT)*</p> <p>X Serum aspartate aminotransferase (also SGOT)*</p> <p>gamma glutamyl transferase</p> <p>glutamate dehydrogenase</p> <p>X Electrophoretic protein fractions</p>	<p><u>X</u></p> <p><u>Other:</u></p> <p>Albumin*</p> <p>Blood creatinine*</p> <p>X Blood urea nitrogen*</p> <p>X Cholesterol*</p> <p>Globulins</p> <p>X Glucose*</p> <p>X Total Bilirubin*</p> <p>X Total Serum Protein*</p> <p>Triglycerides</p> <p>Serum protein electrophoresis</p> <p>X Direct bilirubin</p>
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- * Required for subchronic and chronic studies
- # Should be required for OP
- ° Not required for subchronic studies

Results:

Week 4

Alkaline phosphatase, and potassium were increased in group 4 males, glucose and alpha globulins were decreased in group 4 males.

Alkaline phosphatase, total bilirubin and ALT were increased in group 4 females, urea and albumin were increased in groups 2 and 4 females and gamma globulins were decreased in group 4 females.

Week 8

Alkaline phosphatase was increased in group 4 males. Alkaline phosphatase, ALT, urea, total bilirubin, alpha-2 globulins, and potassium were increased in group 4 females. LDH was decreased in group 2 and 4 females, and sodium was increased in group 2 and 4 females.

Week 13

Alkaline phosphatase, total bilirubin, and albumin were increased in group 4 males, alpha-2 globulins were decreased in group 4 males and potassium was increased in both group 3 and 4 males. Alkaline phosphatase, ALT, AST, total bilirubin, total cholesterol total proteins, B-globulins were all increased in group 4 females, and LDH was increased in both group 3 and 4 females, while calcium was decreased in groups 3 and 4.

Week 17

Alkaline phosphatase, and ALT were increased in group 4 males, calcium was decreased in the same group.

Alkaline phosphatase, total proteins, and albumin were increased in group 4 females, while total bilirubin was increased in both groups 2 and 4 females.

Week 21

Alkaline phosphatase, LDH, total and total bilirubin were increased in group 4 males, gamma globulins were decreased slightly in group 4 males.

Alkaline phosphatase was increased in group 4 females and albumin levels were increased at all dose levels.

Week 25

Alkaline phosphatase and total bilirubin were increased in group 4 males, glucose and calcium were decreased in group 4 males and alpha 2 globulins were decreased in group 3 and 4 males.

Alkaline phosphatase, LDH and total bilirubin were increased in group 4 females, Albumin was increased in both groups 3 and 4 females, and alpha-2 globulins were decreased in group 4 females.

Week 38

Alkaline phosphatase and gamma globulins were increased in group 4 males. Glucose and albumin were decreased in group 4 males.

Alkaline phosphatase, LDH and total bilirubins were increased in group 4 females, urea was increased in both group 2 and 4 females.

Week 51

Alkaline phosphatase and total cholesterol were increased in group 4 males, and alpha-2 globulins were decreased in group 3 and 4 males.

Alkaline phosphatase and albumin were increased in group 4 females.

TABLE II

Clinical Chemistry

<u>Week 4</u>		globulins				females			Total		
males		Glu	A-1	K	AP	ALT	Urea	Bili	Alb	g-glob	
Group	AP										
1	109	121	0.3	4.4	101	42	27	0.2	2.7	0.5	
SD	26	5	0.1	0.2	18	8	6	0.0	0.2	0.1	
2	101	122	0.3	4.3	88	37	38 ^b	0.3	3.0 ^a	0.4	
SD	21	7	0.0	0.3	17	7	8	0.1	0.2	0.1	
3	112	128	0.4	4.5	80	38	30	0.2	2.9	0.4	
SD	17	5	0.1	0.3	13	7	3	0.0	0.2	0.1	
4	145 ^a	113 ^a	0.3 ^a	4.7 ^a	164 ^c	103 ^a	37 ^b	0.5 ^c	3.2 ^c	0.3 ^a	
SD	41	7	0.1	0.4	40	82	5	0.1	0.2	0.1	

SD = Standard Deviation

a- Significantly different from controls P < 0.05

b- Significantly different from controls P < 0.01

c- Significantly different from controls P < 0.001

Week 8

males		females				Total globulins			
Group	AP	AP	ALT	LDH	Urea	Bili	a-2	Na	K
1	99	91	46	143	31	0.3	0.7	141	4.2
SD	22	19	12	62	4	0.1	0.1	1	0.2
2	83	91	38	80 ^a	32	0.3	0.7	142 ^a	4.0
SD	21	18	7	27	4	0.1	0.1	1	0.1
3	97	72	43	122	33	0.4	0.6	141	4.1
SD	26	12	12	59	4	0.1	0.0	1	0.1
4	133 ^a	164 ^c	160 ^c	86 ^a	37 ^a	0.5 ^c	0.8 ^a	143 ^b	4.5 ^a
SD	33	26	155	25	6	0.1	0.1	1	0.2

Week 13

Males		Total Globulins				females				Total Total tot.			B- Ca	
Gr	AP	Bili	Alb	a-2	K	AP	ALT	AST	LDH	Bili	Chol	Prot.	Glob.	Ca
1	82	0.4	3.1	0.8	4.1	87	66	32	124	0.2	127	6.0	1.4	5.9
SD	26	0.1	0.3	0.1	0.2	15	68	4	15	0.1	15	0.3	0.2	0.2
2	81	0.4	3.1	0.9	4.1	73	38	29	134	0.2	118	6.3	1.5	5.7
SD	29	0.1	0.1	0.1	0.2	20	9	3	30	0.1	9	0.2	0.2	0.3
3	103	0.3	3.1	0.7	4.4 ^a	66	42	31	191 ^b	0.2	127	6.1	1.4	5.5 ^b
SD	33	0.1	0.3	0.1	0.1	16	8	3	46	0.1	7	0.1	0.1	0.1
4	153 ^b	0.6 ^a	3.4 ^b	0.6 ^a	4.4 ^a	169 ^c	285 ^a	48 ^a	191 ^b	0.6 ^c	154 ^a	6.5 ^c	1.6 ^a	5.5 ^b
SD	46	0.1	0.1	0.1	0.2	26	287	26	48	0.1	32	0.2	0.1	0.2

Week 17

males				females				total			
Gr.	AP	ALT	Ca	AP	Bili	prot	Alb.	AP	Bili	prot	Alb.
1	86	29	5.4	80	0.3	5.8	2.7	80	0.3	5.8	2.7
SD	30	4	0.2	14	0.1	0.2	0.2	14	0.1	0.2	0.2
2	83	27	5.1 ^a	79	0.5 ^a	5.9	3.0	79	0.5 ^a	5.9	3.0
SD	19	6	0.2	30	0.1	0.3	0.2	30	0.1	0.3	0.2
3	90	34	5.4	68	0.4	5.8	3.0	68	0.4	5.8	3.0
SD	25	8	0.2	10	0.1	0.3	0.3	10	0.1	0.3	0.3
4	140 ^b	47 ^a	5.1 ^b	127 ^c	0.7 ^c	6.2 ^b	3.2 ^b	127 ^c	0.7 ^c	6.2 ^b	3.2 ^b
SD	32	28	0.1	13	0.1	0.2	0.3	13	0.1	0.2	0.3

Week 21

males					females	
Gr	AP	LDH	Total Bili	G-glob.	AP	Alb.
1	77	100	0.1	0.5	94	3.0
SD	25	28	0.0	0.1	58	0.2
2	89	102	0.1	0.5	64	3.4 ^b
SD	28	32	0.0	0.1	25	0.1
3	95	128	0.1	0.5	60	3.3 ^a
SD	31	67	0.0	0.1	11	0.2
4	177 ^c	180 ^a	0.2 ^a	0.4 ^a	149 ^a	3.4 ^b
SD	41	60	0.1	0.0	29	0.3

SD = standard deviation

a- Significantly different from controls P < 0.05

b- Significantly different from controls P < 0.01

c- Significantly different from controls P < 0.001

Week 25

males						females				
Gr	AP	Gluc.	Bili	A-2	Ca	AP	LDH	Tot. Bili.	Alb.	Glob. A-2
1	64	103	0.1	0.7	5.8	64	137	0.1	3.0	0.9
SD	29	7	0.0	0.1	0.3	22	35	0.1	0.4	0.2
2	69	103	0.1	0.7	5.6	50	127	0.2	3.1	0.7
SD	26	4	0.0	0.1	0.2	25	33	0.1	0.2	0.1
3	79	105	0.1	0.5 ^c	5.6	52	152	0.2	3.5 ^a	0.9
SD	21	4	0.0	0.1	0.1	16	53	0.0	0.6	0.1
4	147 ^c	94 ^a	0.2 ^c	0.5 ^b	5.5 ^b	111 ^b	235 ^b	0.3 ^c	3.7 ^b	0.4 ^c
SD	44	7	0.0	0.1	0.2	22	81	0.1	0.2	0.1

Week 38

males					females			Total
Gr	AP	Gluc.	Alb.	Glob. Gamma	AP	LDH	Urea	Bilir.
1	62	107	3.1	0.5	63	140	31	0.2
SD	22	6	0.2	0.1	11	28	4	0.1
2	60	101	3.1	0.5	46	148	38 ^a	0.3
SD	22	6	0.2	0.1	12	65	7	0.1
3	92	108	3.3	0.6	51	150	31	0.2
SD	58	8	0.4	0.1	17	45	4	0.0
4	149 ^b	97 ^a	2.7 ^a	0.8 ^c	129 ^c	231 ^b	37 ^a	0.4 ^c
SD	46	10	0.2	0.1	31	59	4	0.1

Week 51

males				females	
Gr	AP	Total Chol.	Glob A-2	AP	Alb
1	77	114	0.8	66	3.0
SD	41	10	0.1	17	0.2
2	72	129	0.7	53	3.1
SD	36	9	0.1	24	0.2
3	93	118	0.6 ^c	63	3.2
SD	31	9	0.1	30	0.3
4	207 ^c	143 ^b	0.6 ^b	138 ^c	3.3 ^a
SD	89	21	0.1	19	0.2

SD = Standard Deviation

a- Significantly different from controls P < 0.05

b- Significantly different from controls P < 0.01

c- Significantly different from controls P < 0.001

6. Urinalysis^o

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Urine was collected from fasted animals at 3, 7, 12, 16, 20, 24, 37 and 50 weeks. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen
X	Total reducing substances		

* Required for chronic studies

^o Not required for subchronic studies

Results: No treatment-related changes in urinalysis were evident.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination and examined. . The (XX) organs in addition were weighed.

<u>X</u>	<u>X</u>	<u>X</u>
Digestive system	Cardiovasc./Hemat.	Neurologic
X Tongue	X .Aorta*	XX. Brain*† ^d
X .Salivary glands*	XX. Heart*	X Periph. nerve*#
X .Esophagus*	. Bone marrow*	X Spinal cord (3 levels)*#
X .Stomach* ^c	X .Lymph nodes* ^a	XX. Pituitary*
X .Duodenum*	XX. Spleen*	X Eyes (optic n.)*#
X .Jejunum*	X .Thymus*	Glandular
X .Ileum*	Urogenital	XX. Adrenals*
X .Cecum*	XX. Kidneys*†	Lacrimal gland#
X .Colon*	X .Urinary bladder*	X Mammary gland*# ^b
. Rectum*	XX. Testes*†	. Parathyroids*††
XX. Liver*†	X Epididymides	XX. Thyroids*††
X Gall bladder*#	XX Prostate	Other
X .Pancreas*	Seminal vesicle	X Bone*# (sternum)
Respiratory	XX Ovaries*†	X Skeletal muscle*#
X .Trachea*	XX. Uterus*	X Skin*#
XX. Lung*	X Cervix	X All gross lesions and masses*
Nose°		
X Bronchi		
Larynx°		

* Required for subchronic and chronic studies

° Required for chronic inhalation

In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement

† Organ weights required in subchronic and chronic studies

†† Organ weight required for non-rodent studies

a- cervical, mesenteric and peribronchial

b- caudal, cranial

c- fundus, pylorus

d- sectioned to include cerebellum, cerebral cortex, thalamic nuclei, mid-brain and medulla, and the spinal cord was prepared in transverse section at the cervical and thoraco-lumbar levels.

a. Organ weight

Absolute weights:

Liver and adrenal weights were increased in the high dose males and prostate weights were decreased in mid and high dose males. Adrenal weights were increased in high dose females.

Relative weights:

Relative liver and adrenal weights were increased in high dose males and relative prostate weights were decreased in this group. In females, relative heart, liver and adrenal weights were increased in the high dose.

b. Gross pathology

Very little pathology was seen on gross examination. One high dose male had a swollen and pale liver. 2/6 high dose females had enlarged adrenals. One high dose female had crystalline bile.

c. Microscopic pathology

1) Non-neoplastic

Treatment-related effects were seen in the adrenals, gall bladder, kidneys, urinary bladder and liver. The study text states that "the changes seen in the adrenal glands, kidneys, and gall bladder were exacerbations of physiological changes. The changes seen in the urinary bladder and the more serious lesion in the liver were of a distinctly pathological nature."

Table III
Histopathological Changes

Group/Sex N	1m	2m	3m	4m	1f	2f	3f	4f
<u>Adrenals</u>								
Deep pallid Zona fasciculata	0	0	0	6	0	0	0	5
fat vacuolation of zona fasciculata	0	0	0	1	1	0	1	6
pallid zona glomerulosa	2	0	3	6	0	0	1	6
<u>Gall Bladder-enlarged</u>	0	0	0	2	0	0	0	1
<u>Kidney</u>								
Lipofuscinosis in proximal tubular epithelium	2	1	2	4	2	0	4	4
<u>Liver</u>								
Occasional centrilobular Hepatic cord atrophy	0	0	0	3	0	0	0	4
<u>Urinary Bladder</u>								
Submucosal granuloma	0	0	0	4	0	0	0	5
Submucosal crystals within giant cells	0	0	0	4	0	0	0	4

Adrenal Cortex

The study text described changes seen in the zona glomerulosa and zona fasciculata as zones with increased depth and the cells as being large, with pale "watery" cytoplasm. In table 3 these are described as "pallid zona glomerulosa" and "deep pallid zona fasciculata". The zona fasciculata changes in the high dose were considered "occasionally marked." These changes according to the study text were associated with foci of cells which were largely represented by vacuoles, tabulated in table 3 as "fatty vacuolization, zona fasciculata. The increased pallor of the zona glomerulosa were associated with high dose animals. Mid dose group males had 3/6, however, there were two animals seen in control group males.

Urinary Bladder

high dose males and females showed treatment-related granulomata, considered small, in the immediate submucosa of the urinary bladder. These were "virtually all" associated with bladders containing crystals. The crystals were described as having a constant shape which was described as tall pyramidal with a circular base. The crystals were only present within the cytoplasm of phagocytic giant cells. The study text states that two of the six male dogs in the high dose had small foci of polymorphonuclear leucocyte infiltration within the transitional epithelium.

Liver

The study text stated that there was an increase in the size and frequency of agglomerates of pigmented macrophages in the liver of animals receiving high dose iprodione. They stated that these phagocytic cell associations are a usual feature of canine liver, representing a record of minor tissue damage. 9/12 high dose animals showed these changes as compared to none seen at these

levels of severity in the control animals. High dose male and female livers also had "hepatic cord atrophy" or a "rounding" of hepatocytes seen in occasional centriacinar zones. They state that this is probable evidence of previous hepatocyte necrosis in the liver sections seen at termination.

Kidney

In kidneys there was a slight trend towards increased frequency of slight or moderate lipofuscinosis of the epithelium of the proximal convoluted tubules. This is considered a fairly common occurrence.

Discussion:

Iprodione produced a number of changes such as in the eye, liver prostate, kidneys, red blood cells, adrenals, gall bladder and urinary bladder.

In the red blood cells, Heinz bodies were evident at 600 ppm and 3600 ppm in males with increased severity seen at 3600 ppm. The appearance of Heinz bodies correlates with some of the other hematological changes seen, such as a decrease in the high dose of hemoglobin, MCHC, PCV and RBCs. Heinz bodies are the produce of the degradation of unstable hemoglobin. The erythrocytes containing Heinz bodies are cleaned from the system by the spleen, and this might account for the increased plasma protein concentrations seen.

In the liver, both male and female high dose absolute and relative weights were increased, with an increase in the size and frequency of agglomerates of pigmented macrophages. High dose males and females also showed "hepatic cord atrophy", indicative of hepatic necrosis. This hepatic damage correlates with the blood enzyme changes seen at the high dose, such as increased alkaline phosphatase alanine amino transferase, aspartate aminotransferase, and lactate dehydrogenase activities; prolonged partial prothrombin times, and raised platelet counts.

In the eye, there was a slight increase in hyperreflection seen mostly in the high dose animals. However, this phenom was seen very erratically, and it would be hard to call this occurrence a toxicological consequence of iprodione administration. 2/6 male high dose dogs shoed finely scattered opalescent particles (asteroid bodies) in the vitreous humor. This phenom may be treatment-related since no controls showed these asteroid bodies. In the prostate, high dose males had both decreased absolute and relative weights. Mid dose absolute weights were significantly decreased with a definite trend seen in relative weights, although the mid dose was not quite significantly different from controls. In the urinary bladder of high dose males and females, small granulomata were seen in the immediate submucosa with foci of polymorphonuclear leucocyte infiltration seen in the transitional epithelium. The granulomata contained tall pyramidal crystals within the cytoplasm of phagocytic giant cells according to the study text.

In the adrenals both absolute and relative high dose males and females were increased. Microscopically the changes seen were described as zones in the zona glomerulosa and zona fasciculata with increased depth and the cells were large with pale "watery"

cytoplasm. Fatty vacuolization of the zona fasciculata was also seen, along with increased pallor of the zona glomerulosa. In the kidneys, there was a slight trend towards increased frequency of slight or moderate lipofuscinosis of the epithelium of the proximal convoluted tubules. 005882

NOEL = 100 ppm (4.2 mg/kg)

LEL = 600 ppm based on prostate weight reductions and Heinz bodies seen in the erythrocytes.

Core classification = minimum