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DATA EVALUATION REPORT

STUDY TYPE: mechanistic

TOX. CHEM. NO.: 470A

MRID NO.: 435350-02

PC Code: 109801

TEST MATERIAL: Iprodione

CAS No.: 36734-19-7

CHEMICAL: [3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide] (IUPAC); 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide (CA); 1-isopropylcarbamoyl-3-(3,5-dichlorophenyl)hydantoin; 3-(3,5-dichlorophenyl)-hydantoin-1-carboxylic acid isopropylamide; **Empirical Formula:** $C_{13}H_{13}Cl_2N_3O_3$

SYNONYMS: Rovral®; 26019 RP; Chipco 26019; Kidan; Verisan;

STUDY NUMBER: RTI Identification # 65C-5703

SPONSOR: Rhône-Poulenc Ag Company/RTP, NC

TESTING FACILITY: Laboratory of Reproductive Endocrinology
Center of Life Sciences and Toxicology
Chemistry and Life Sciences
Research Triangle Institute

TITLE OF REPORT: Toxicity Testing of a Fungicide, Iprodione, in Adult Male CD® Sprague-Dawley Rats. (Part I): Chemistry Binding and Dose-Range Finding in Adult Male CD® Sprague-Dawley Rats Exposed to Oral Iprodione; (Part II): 30-Day Endocrine Toxicology Screen in Adult Male CD® Sprague-Dawley Rats Exposed to Oral Iprodione

AUTHORS: PA Fail, SA Anderson, and SW Pearce

REPORT ISSUED: October 24, 1994

QUALITY ASSURANCE: A quality assurance statement and a Good Laboratory Practice Compliance Statement were provided.

EXECUTIVE SUMMARY: There are several pieces of evidence that suggest that Iprodione may have similarities to Flutamide, while other information strongly suggests that Iprodione is not the same type of antiandrogen. Poor binding affinity to the androgen receptor was found following Iprodione exposure at very high levels. Two metabolites of Iprodione displayed affinities for the androgen receptor comparable to that found for Flutamide. While Flutamide caused marked effects on all male sex-related organs [↓ organ weight, microscopic lesions], Iprodione caused no effect on the prostate and showed a slight effect on the seminal vesicles and

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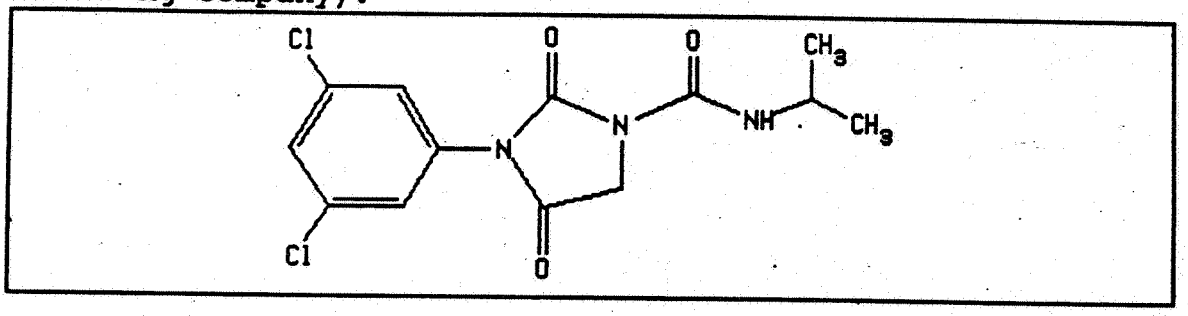
epididymidis [↓ organ weight, microscopic lesions]. Increased concentrations of LH, FSH, testosterone, and estradiol were found following 15 and 30 days of exposure to Flutamide compared to vehicle control values. Following Iprodione treatment for 15 but not 30 days, only LH and FSH concentrations were increased relative to the vehicle control. Testosterone concentrations at necropsy were comparable between the Iprodione and pair-fed control rats, but there were subtle changes in the secretion pattern of testosterone and LH between these two groups. Estradiol concentrations were increased in the Iprodione rats at necropsy following 30 days of exposure compared to both the vehicle and pair-fed control groups. A marked effect [increase] on adrenal weight associated with histopathologic lesions [vacuolation] indicative of an alteration of steroidogenesis was observed following 30 days of exposure to Iprodione but not to Flutamide. Although there is some evidence to suggest that Iprodione interferes with sex/steroid hormone regulation, the difference in the spectrum of effects observed between Iprodione and Flutamide in the current study suggests that the two compounds share only certain parts of a mechanism of toxicity/ carcinogenicity. Compared to Flutamide, Iprodione appears to be a much less active/potent endocrine toxicant.

Classification:This study is Acceptable. The study is a non-guideline [mechanistic] study, which provides information as to Iprodione's mode of action with respect to its effects on male sex organs.

A. MATERIAL

1. Test Compound: Iprodione; Description: off-white, granular powder; Batch #: synthesis batch # [SBN] 8906201; Purity: 97.3%; Structure: See below; Source: Rhône-Poulenc, Research Triangle Park, NC. Other compounds: Flutamide [Lot # IRQ-BTA-7-D-76D (Experiment 1); Lot # FTT2B (Experiments 2 and 3); CAS No. 13311-84-7], Hydroxyflutamide [Lot # 26492-79 (Experiment 1)], Testosterone [Lot # 11H0756 (Experiment 1), analytical grade], Dihydroxy-testosterone (DHT) [Batch # G225], Methyltrienolone/R1881 [Lot # 2876-074 (Experiment 1)], tritiated Methyltrienolone/^{3}H-R1881 [Lot # 3043-207 (Experiment 1)], Iprodione metabolites: RO36119 [SBN GD8002], RP32490 [SBN GD8309], RP36114 [SBN GD7995], RP36118 [SBN GD6842], RP25040 [TV2840/F], RP36112 [SBN BES1526], RP36115 [SBN BESS129] (supplied by Rhône-Poulenc Ag Company).



2. Test Animals Species: rat; Strain: VAF/Plus® CD® Sprague-Dawley Crl:CD[SD] BR; Age: Experiment 1: ≈7 weeks, Experiment 2: ≈11 weeks, Experiment 3: 9-10 weeks old; Weight: ♂♂ 336-427 grams; Source: Charles River Laboratories, Inc. (crl), Raleigh, NC.

3. Statistics: **Parametric data:** Appropriate General Linear Models [GLM] procedures for the Analyses of Variance [ANOVA] were employed. Prior to GLM analysis, a log 10 transformation was performed on all hormone data [Snedecor and Cochran, 1967] to allow use of parametric methods. Bartlett's test for homogeneity of variance was performed on all data prior to ANOVA analysis. GLM analysis was used to determine whether significant dose effects had occurred for selected measures [ANOVA, LSMEANS]. When a significant main effect for dose occurred, Dunnett's Multiple Comparison Test [1955, 1964], LSMEANS for unbalanced data, or orthogonal contrasts were used to compare each chemical exposed group to the vehicle control group for that measure. A 2-tailed test [Dunnett's] was used for all pairwise comparisons, body, and body-weight parameters, and feed consumption. **Nonparametric data:** These data were analyzed using Kruskal-Wallis test and Mann-Whitney U for pairwise comparisons.

B. STUDY DESIGN

The results from three studies are contained in this report. The objectives of the studies were to: Experiment 1: assess competitive binding affinity of Iprodione and related compounds to the androgen receptor; Experiment 2: establish an effective dose and dosing regimen and quantify testosterone, luteinizing hormone [LH], follicle-

stimulating hormone [FSH], and estradiol concentrations in a single plasma sample; and Experiment 3: describe testosterone, LH and FSH profiles during a 4-hour baseline occurring after 30 days of Iprodione exposure. One-hundred and sixty-four male rats were utilized in these studies [Experiment 1 (51), Experiment 2 (37), and Experiment 3 (72 + 4 for viral titer checks)]. The rats were randomly assigned to cages during the 7-day acclimation period, and in Experiments 2 and 3 were housed singly while those in Experiment 1 were housed 3 per cage. The diet [Purina Certified Rodent Chow #5002] was available ad libitum except for the pair-fed group in Experiment 3], and water was available ad libitum. For Experiments 2 and 3, the rats were observed for clinical signs at least once daily [morbidity/mortality (twice daily) and cageside observations for changes in skin, fur, eyes, and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, somatomotor activity, and behavior]. Body weights were recorded once during acclimation, daily [Experiment 2]/weekly [Experiment 3] during dosing, and at termination. Feed consumption was monitored daily [Experiment 2 and for Iprodione group in Experiment 3]/weekly on days 1, 8, 15, 22, and 29 for the vehicle and Flutamide control groups in Experiment 3. In both Experiments 2 and 3, the rats were administered one-half the total assigned dose [Table 1] of test material or control twice daily [Experiment 2 (for 15 days)/Experiment 3 (for 30 days)]. At study termination, a blood sample was collected from the heart [cardiac puncture] after sacrifice via CO_2 asphyxiation, and each rat was necropsied. The hormones LH, FSH, testosterone, and estradiol were determined in a single necropsy blood sample. After 25 days of dosing [Experiment 3], the rats were fitted with intra-atrial cannulae, and on day 30 blood samples were collected every 10 minutes during a 4-hour window [see below]. Experiment 3 was conducted in 2 replicates, which began within one week of each other, and the rats were the same weight at initiation of the replicate. Cannulation and Blood Sampling Regimen [Experiment 3]: From the 18 rats/dose group, 16 were randomly chosen and were fitted with indwelling jugular cannulae on Day 25 of dosing. For basal secretion studies following 30 days exposure to Iprodione, blood samples were drawn every 2 hours for 8-10 hours and, within that time frame, every 10 minutes for a 4-hour window to determine baseline variability of LH, FSH, and testosterone. Before necropsy, an additional blood plasma sample was collected after CO_2 anesthesia from all rats, both cannulated and noncannulated. Limited necropsies were performed on day 31, and the following organs were examined microscopically: testes, epididymis, liver, seminal vesicles,ventral prostate, and adrenals.

Table 1. Experimental Design for Experiments 2 and 3		
Treatment Groups	Dose(mg/kg/day♦)/Frequency♥	# rats
Experiment 2		
Vehicle control♦	0/2	6
Iprodione	120/2	6
Iprodione	300/2	6
Iprodione♣	300/1	6
Iprodione	600/2	7
Flutamide	150/2	6
Experiment 3		
Vehicle control♦	0/2	9♪
Iprodione	600/2	9♪
Pair-fed to Iprodione♦	0/2	9♪
Flutamide	150/2	9♪

♦ 0.4% Methylcellulose in deionized, distilled H_2O administered at a volume of 12 mL/kg (treated groups with appropriate concentration of compound to deliver desired dose); ♦ rats fed an amount of feed equal to the average feed consumption of Iprodione group from the previous day; ♥ doses administered twice daily at ≈12-hour intervals; ♣ administered as a single dose when other groups received p.m. dose; ♪ 9 rats/replicate

Hormone Measurements: Radioimmunoassays [RIA] validated for male rats at the testing facility were utilized in determining hormone concentrations in the plasma. RIA for LH and FSH were miniassays designed to use ≤50 µL/duplicate determination, using reagents purchased from Dr. AF Parlow [Torrance, CA]. RIA for estradiol [Experiment 2] was an extraction method using antiserum from Endocrine Sciences Products [Tarzana, CA], tritiated estradiol from DuPont NEN [Wilmington, DE], and estradiol standard from Sigma Chemical Co. [St. Louis, MO]. The estradiol RIA used for analyzing Experiment 3 samples was a double antibody kit purchased from Diagnostic Products Corporation [DPC; Los Angeles, CA], and the testosterone RIA utilized an antibody-coated tube from DPC. Blood samples were drawn via heparinized syringes,,injected into heparinized tubes, and centrifuged to collect plasma [stored at -20°C until analyzed]. Hormones were analyzed in plasma, with a priority given to testosterone and LH in all samples and FSH as volume permitted. Estradiol was analyzed in necropsy samples only.

EXPERIMENT 1: **Relative binding affinity [RBA]** was assessed using ventral prostates of the male rats [procedure reported by Reel, et al., (1979), as modified by Kelce et al., (1994)]. Modifications to the standard protocol were the use of: (1) intact, not castrated, rats; (2) all androgen (cytosol + nuclear) receptors; (3) and R1881 (methyltrienolone) and its tritiated form as the standard ligand. The nuclear androgen receptor extract was prepared from the ventral prostates of rats killed with CO_2; all prostates were pooled for each procedure to minimize variation due to animal. Four rats were used for the Scatchard analysis, 38 for the RBA assays, and 9 for validation of the androgen receptor binding studies. The prostates were placed in TEDGMP buffer [Tris, EDTA, dithiotheitol, glycerol, sodium molybdate, and phenylmethyl-sulfonyl fluoride] and homogenized, and the homogenate was centrifuged and the pellet was resuspended in a high salt TEDGMP [KCl] for Scatchard assay. The pellet was homogenized and centrifuged and the supernatant was saved as the nuclear androgen receptor extract. Protein analysis was performed on the nuclear

extract using the methods of Bradford with bovine gamma-globulin as the standard. The RBA assay was performed by adding increasing concentrations of the competitor [R1881, DHT, Testosterone, Flutamide, Hydroxyflutamide, Iprodione, and seven Iprodione metabolites] to a fixed concentration of ^{3}H-R1881. The competitors were assayed at 6-7 concentrations in duplicate with the nuclear extract, following initial test to determine solubility of the compounds in solvents other than ethanol, since Iprodione undergoes hydrolysis in ethanol. The compounds were prepared by serial dilution in organic solvents from the stock solution. R1881, DHT, Testosterone, Flutamide and Hydroxyflutamide were diluted in ethanol, RP32490 and RP36114 were diluted in dimethylformamide, and Iprodione and the 5 remaining metabolites were diluted in acetonitrile. Test concentrations for Testosterone, DHT, and Methyltrienolone were in the range: 0.032, 0.16, 0.8, 4, 20 100 nM; those for Hydroxyflutamide and Flutamide in the range: 100, 1000, 5000, 10000, 100000, 500000 nM; those for Iprodione and the metabolites were in the range: 1000, 5000, 10000, 50000, 100000, 500000, 1000000 nM. The test concentrations were added to assay tubes, the solvent was evaporated, and the compound was reconstituted in TEDGMP buffer. All tubes were sonicated, vortexed, and chilled prior to adding the ^{3}H-R1881 and nuclear extract. The assay tubes were incubated overnight and free ^{3}H-R1881 was separated from bound ^{3}H-R1881 by adding a 60% slurry of hydroxylapatite in Tris buffer. Following incubation with stirring, the samples were centrifuged and the supernatant was discarded and the pellet was washed 3 times with Tris. The bound ^{3}H-R1881 was then extracted from the HAP with ethanol, incubated, and centrifuged. The supernatant was combined with scintillation fluid and counted on a Packard Tricarb scintillation counter. The % of bound ^{3}H-R1881 was calculated from the DPM data. From the data, curves were plotted [SigmaPlot for Windows, Version 1.0]. The concentration of ligand that caused 50% displacement of ^{3}H-R1881 was calculated, and the relative binding affinities of the compounds compared to R1881 were determined. Three standard curves for R1881 were prepared because of the use of 3 different solvents [ethanol, dimethylformamide, and acetonitrile]. Since the standard curves for ethanol and acetonitrile were identical, they are presented as one curve. **Scatchard Analysis** The androgen standard chosen for use in the Scatchard was R1881 and its tritiated form also, which bind to prostate androgen receptors with affinities equivalent to DHT. The Scatchard analysis for Iprodione was performed using intact male rats with receptors isolated from the ventral prostate as for the RBA assay. The competitive inhibition Scatchard was performed using procedures outlined in Steroid Hormone Receptors: Basic Principles and Measurement, JH Clark and others, as modified in Kelce et al. [1994]. An aliquot of the nuclear androgen receptor extract was incubated overnight with ^{3}H-R1881 at concentrations of 0.5-20.0 nM alone to measure total binding, or with ^{3}H-R1881 at concentrations of 0.5-20.0 nM and 100-fold molar excess of unlabeled R1881 at concentrations of 50-2000 nM to measure nonspecific binding. The specific binding was calculated by subtracting the nonspecific binding from the total binding. Iprodione solutions in acetonitrile were tested at 4 concentrations [2000, 10000, 20000, and 100000 nM]. Each concentration was incubated overnight with increasing amounts of ^{3}H-R1881 [0.5-20.0

nM] and nuclear extract. After incubation, duplicate 100 µL aliquots of each extract were pipetted, and free ^{3}H-R1881 was separated from bound ^{3}H-R1881.

2. Dose Preparation: The dosing solution was prepared by mixing Iprodione in 0.4% methylcellulose at a given concentration and homogenizing. The concentration was determined by the following formula: concentration [mg/mL]=dosage rate [mg/kg] + dose volume [12 mL/kg]. Dilutions of Iprodione in 0.4% methylcellulose were formulated independently for each concentration and prepared in a quantity sufficient for use for 16 days. An aliquot of each formulation of Iprodione and vehicle was analyzed to verify the concentration of test material from one out of two mixes [Experiment 2] and 2 out of 4 mixes [Experiment 3]. Additionally, prior to study initiation, dosage formulations in the range of concentrations to be used in the study were tested for homogeneity and dose concentration accuracy. Flutamide [positive control] was mixed with 0.4% methylcellulose similarly and an amount sufficient for one day's dosing was prepared at a concentration of 150 mg/kg/day [equivalent to 6.25 mg/mL given in 2 equal doses at 12-hour intervals]; however, it was not tested for stability in the vehicle, and the dosing solutions were not tested for homogeneity or dose concentration accuracy.

RESULTS

The mixing procedures were found to be adequate and to result in uniform suspensions. The percentage variation in dose concentration among the 3 sample locations was 9.0% at the low dose [5 mg/mL] and 1.0% at the high dose [25 mg/mL]. The storage stability study showed Iprodione to be stable in 0.4% methylcellulose for a period 16 days. The % of nominal concentration ranged from ≈93 to 110.

EXPERIMENT 1 Androgen Receptor Binding Activity

RESULTS

Iprodione and most of its metabolites did not bind with high affinity to the prostate androgen receptor [Figures 1-1 , 1-2, and 1-3, copies appended]. The relative binding affinity [RBA] is listed in Table 2. Testosterone and dihydroxytestosterone bind with 35% and 100% relative affinity to the prostatic tissue, respectively, while Flutamide and hydroxyflutamide are less potent [<1% relative to R1881]. The metabolite RP25040 was the only Iprodione-compound that bound with an affinity of ≈half that of Flutamide [39%-49% that of Flutamide]. The relative affinities of Iprodione and metabolites were RP25040 >> RP36112 > RP36115 > RP36118 > RP32490 and RP36114 > RP36119 and Iprodione. Scatchard: It appears that the curves all overlap [Figure 1-6, copy appended], with the possible exception of the highest concentration of Iprodione. The lines appear parallel rather than converging upon the same point in the X axis, indicating a noncompetitive inhibition. The saturation curves [Figure 1-5, copy appended] also suggest a minor amount of displacement by the highest concentration of Iprodione [Table 3]. Iprodione does not appear to

inhibit R1881 binding to the androgen receptor.

Table 2. Relative Binding Affinity

Compound	C_{50}♦	RBA♥ [% bound at 50% intercept]
PLOT 1		
Methyltrienolone [R1881]♦	3.5 nM	100
Dihydroxytestosterone [DHT]	3.5 nM	100
Testosterone [T]	10 nM	35
Flutamide [FLU]	27000 nM	0.013
Hydroxyflutamide [FLU-OH]	2200 nM	0.16
Iprodione	>1000000nM	<0.00035
Metabolites		
RP36118	1600000 nM□	0.00026
RP25040	55000 nM	0.0064
RP36119	>1000000 nM■	<0.00035
PLOT 2		
R1881-DMF♢	2.7 nM	100
RP32490	>1000000nM■	<0.00027
RP36114	>1000000nM■	<0.00027
PLOT 3		
R1881♦	4.2 nM	100
DHT	4.5 nM	93.3
FLU	30000 nM	0.014
FLU-OH	2500 nM	0.17
RP25040	77000 nM	0.0055
RP36112	150000 nM□■	0.0028
RP36115	350000 nM□■	0.0012

♦ concentration at 50% displacement [or 50% ^{3}H-R1881 bound]; ♥ relative binding affinity, defined as % of competitor vs standard concentrations at 50% displacement on standard curve; ♦ R1881 curve prepared in ethanol or acetonitrile; ♢ R1881 curve and samples prepared in dimethylformamide solvent; □data are extrapolated relative binding affinities based on projection of curve from points in the curve above 50% bound; ■ 3 highest points [100000, 500000, and 1000000 nM] appeared to be increasingly insoluble in buffer solution used in assay despite sonication. Lack of solubility may mask physiological activity.

Table 3. IPRODIONE Scatchard Analysis [Capacity and Dissociation Constants]

Compound	Capacity [# of receptors (fmol)]	Capacity (fmol)/mg protein	Kd♦
R1881	172	135	4.33
2000 nM Iprodione	189	148	5.01
10000 nM Iprodione	174	137	4.49
20000 nM Iprodione	159	125	3.81
100000 nM Iprodione	152	120	4.29

♦ Kd=concentration [nM] of R1881 at 50% bound

EXPERIMENT 2 Endocrine Toxicity of Iprodione [15-day Dose Range-Finding and Regimen Pilot Study]

RESULTS

Survival, Clinical Signs, Body-Weight Gain, and Feed Consumption: All rats survived to study termination, and there were no adverse clinical signs observed. Body-weight gains were decreased following Flutamide exposure throughout the study, and decreases were observed following Iprodione exposure [Table 4] at dose levels of 300 and 600 mg/kg/day from Day 6 or 7 on, but there was no dose response. Feed consumption was decreased following Flutamide and Iprodione at dose levels of 300

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and 600 mg/kg/day [dose-related].

Table 4. Body-Weight Gains and Feed Consumption [% of vehicle]

Time/Group	Flutamide	120 mg/kg/day I♦	300 mg/kg/day I	300 mg/kg/day I♦	600 mg/kg/day I
BWG [day]					
-3	99	99	100	99	100
1	100	101	100	96	101
2	95*	100	99	100	99
3	93*	98	97	98	97
5	93*	98	95	98	96
6	93*	99	95*	96	95
7	92*	99	93*	96	95*
10	91*	100	91*	93*	92*
13	90*	98	90*	94*	90*
15	89*	98	86*	92*	89*
Feed [week]					
1	70*	98	79*	84*	72*
2	79*	97	81*	84*	76*

♦ I=Iprodione; ♦ dosed once in pm; * $p<0.05$

Organ Weights: Decreased body weight at necropsy relative to the vehicle control group was observed in the Flutamide and Iprodione groups at 300 and 600 mg/kg/day dose levels. Following **Flutamide** exposure, significant increases in absolute and relative liver weights were observed [Table 5]. With regard to the sex organs, significant decreases were observed in the absolute testes, epididymides, prostate, seminal vesicles, and total accessory sex organ [TASO] weights. Significant decreases in the relative weights of the epididymides, prostate, seminal vesicles, and total accessory sex organ weights were observed also. Following **Iprodione** exposure, an apparent dose-related decrease in absolute epididymis weight was observed at dose levels of 300 and 600 mg/kg/day. Absolute prostate weights were decreased significantly at all dose levels [dose-related]. There was a dose-related decrease in both the absolute seminal vesicle and total accessory sex organ weights compared to the vehicle control value, but only the 600 mg/kg/day dose level attained statistical significance. The relative prostate, seminal vesicle, and TASO weights were decreased significantly compared to the vehicle control values [Table 5].

Table 5. Organ Weights [Absolute & Relative; (% of vehicle)]

Organ/Group	vehicle	Flutamide	120 mg/kg/day I♦	300 mg/kg/day I♦	300 mg/kg/day I♦	600 mg/kg/day I♦
ABSOLUTE						
Liver	19.5	24.9*(128)	19.2	19.7	18.5	21.8(112)
Rt. Testis	1.83	1.66**(91)	1.79	1.84	1.84	1.74(95)
Lf. Testis	1.83	1.64**(90)	1.81	1.68(92)	1.67(91)	1.61(88)
Rt. Epididymis	0.66	0.38*(58)	0.61(92)	0.57*(86)	0.61(92)	0.54*(82)
Lf. Epididymis	0.62	0.37**(60)	0.62	0.52(84)	0.55**(89)	0.49**(79)
TASO	3.09	0.71*(23)	2.82	2.63(85)	2.57(83)	2.06*(67)
Prostate	1.04	0.14*(13)	0.77*(74)	0.76*(73)	0.73*(70)	0.61*(59)
Seminal Vesicle	1.86	0.49*(26)	1.94	1.79	1.70(91)	1.27*(68)
RELATIVE						
Liver	40.4	58.1*(144)	40.4	46.5*(115)	42.0	50.6*(125)
Rt. Testis	3.8	3.8	3.8	4.3(113)	4.2	4.1
Lf. Testis	3.8	3.8	3.8	4.0	3.8	3.7
Rt. Epididymis	1.4	0.9*(64)	1.3	1.3	1.4	1.3
Lf. Epididymis	1.3	0.9*(69)	1.3	1.2	1.3	1.1(85)
TASO	6.4	1.7*(27)	5.9	6.2	5.9(92)	4.8*(75)
Prostate	2.2	0.3*(14)	1.6(73)	1.8(82)	1.7(77)	1.4*(64)
Seminal Vesicle	3.9	1.1*(28)	4.1	4.2	3.9	3.0*(77)
Terminal Body Weight	482.1	428.8(89)	474.3	424.0*(88)	439.0*(91)	431.0*(89)

♦ I=Iprodione [dosed twice/day]; ♦ dosed once in pm; * $p<0.05$; ** $p<0.01$

Plasma Hormones: Following exposure to both Flutamide and Iprodione, an increase in peripheral plasma hormones was observed at study termination [Table 6]. Following Flutamide exposure, all measured hormone levels were increased significantly. Following Iprodione exposure [2-dose administration], there was a dose-related increase in LH and FSH, but estradiol values were decreased [non-significantly] relative to the control.

Table 6. Hormone Levels Following 15 Days of Exposure

Hormone/Group	Vehicle	Flutamide	120 mg/kg/day I♦	300 mg/kg/day I	300♥ mg/kg/day I	600 mg/kg/day I
LH	1.06	8.92*(842)♦	1.84(174)	2.22(209)	1.51(142)	4.54*(428)
FSH	10.66	22.98*(216)	12.86(121)	13.82*(130)	15.80*(148)	15.53*(146)
Estradiol	3.40	6.55*(193)	3.15	2.67(79)	2.27(67)	2.29(67)
Testosterone	4.76	32.96*(692)	4.28	8.05(169)	7.98(168)	4.80

♦ I=Iprodione; ♥ single dose; * $p<0.05$; ♦ (% of control)

EXPERIMENT 3 Endocrine Toxicity of Iprodione [30-day Test of Basal Secretion of LH, FSH, and Testosterone]

RESULTS

Survival, Clinical Signs, Body-Weight Gain and Feed Consumption: Three treatment-related deaths occurred during the experiment, and there were two non-treatment related deaths [one due to gavage error and one occurred during surgery]. Two of the three deaths related to treatment occurred during surgery also, but these rats were debilitated at the time of surgery. The remaining death occurred during week 3 when, due to debilitation, he was euthanized. The deaths were said to be due to dehydration, defined as loss of skin elasticity along with weight loss, rough coat, hunched back, and/or lethargy. During weeks 3 to 6,

five rats in the Iprodione group appeared unthrifty and were given subcutaneous 0.9% NaCl solution once or twice a day for 1-3 days. It was stated that, since the purpose of the study was to obtain endocrine data, these rats were retained on the study. Two of these latter rats recovered after this treatment. Body-weight gains were decreased following Flutamide, Iprodione, and pair-fed treatment throughout the study relative to the control values [Table 7]. Feed consumption was decreased relative to the control for all groups also. There were no significant differences in feed consumption reported between the Iprodione and the pair-fed groups.

Table 7. Body-Weight Gains and Feed Consumption [% of vehicle]

Time/Group	Iprodione [600 mg/kg/day]	Flutamide	Pair Fed
BWG [day]			
1	100	100	100
8	92*	91*	90*
15	90*	90*	89*
22	86*	88*	86*
25	84*	87*	85*
Necropsy	78*	85*	86*
Feed [week]			
1	71*	65*	70*
2	83*	84*	83
3	77*	81*	75*
4	66*	79*	64*

* $p<0.05$

Organ Weights: Decreased body weight at necropsy relative to the vehicle control group was observed in all groups. **FLUTAMIDE:** Following Flutamide exposure, significant increases in absolute and relative liver weights were observed [Table 8], and both the absolute and relative adrenal weights were increased relative to the control, but only the relative weight attained statistical significance. With regard to the sex organs, both the absolute and relative weights of the epididymides, prostate, seminal vesicles and total accessory sex organ [TASO] weights were significantly decreased relative to the control. A significant decrease in testis weight was observed, but the relative testis weight was comparable to the control and likely due to the decreased body weight. **PAIR-FED:** Decreased absolute and relative prostate, seminal vesicles, and total accessory sex organ weights were observed. The testes weights were comparable to the control values. **IPRODIONE:** Following Iprodione exposure, body weight was decreased relative to the vehicle control [78% of control], as well as the pair-fed group [91% of pair-fed]. Absolute and relative liver and adrenal weights were increased relative to the vehicle and/or the pair-fed groups. Both the absolute and relative seminal vesicle and TASO weights were decreased relative to the vehicle and pair-fed groups. The decreased epididymis and prostate weights and the increased relative testes weights are most likely due to the decrease in body weight [Table 8].

Table 8. Organ Weights [Absolute & Relative; (% of vehicle); (of pair-fed group)]

Organ/Group	vehicle	Iprodione	Flutamide	Pair-Fed
ABSOLUTE				
Liver	16.92	17.88♦(106)(127)	22.19*(131)	14.08*(83)
Adrenal	71.1	150.5*♦(212)(210)	78.8	71.5
Rt. Testis	1.80	1.78	1.54*(86)	1.70
Lf. Testis	1.78	1.79	1.58*(89)	1.69
Rt. Epididymis	0.63	0.48*♦(76)(86)	0.31*(49)	0.56*(89)
Lf. Epididymis	0.61	0.47*♦(77)(84)	0.31*(51)	0.56*(92)
TASO	2.66	1.37*♦(52)(76)	0.53*(20)	1.80*(68)
Prostate	0.89	0.63*(71)(124)	0.24*(27)	0.51*(57)
Seminal Vesicle	1.62	0.71*♦(44)(60)	0.29*(18)	1.19*(73)
RELATIVE				
Liver	37.70	51.62*♦(137)(142)	58.34*(155)	36.47
Adrenal	0.16	0.43*♦(269)(226)	0.21*(131)	0.19(119)
Rt. Testis	4.04	5.11*♦(126)(116)	4.05	4.42*(109)
Lf. Testis	4.01	5.13*♦(128)(117)	4.16	4.38(109)
Rt. Epididymis	1.40	1.37	0.82*(59)	1.45
Lf. Epididymis	1.38	1.35♦(92)	0.81*(59)	1.46
TASO	5.93	3.84*♦(65)(82)	1.40*(24)	4.69*(79)
Prostate	1.98	1.75♦(88)(135)	0.62*(31)	1.30*(66)
Seminal Vesicle	3.61	2.03*♦(56)(65)	0.76*(21)	3.11*(86)
Terminal Body Weight	447.71	350.81*(79)♦(91)	380.74*(85)	385.90*(86)

* significantly different from vehicle control; ♦ significantly different from pair-fed rats; (% of vehicle control); (% of pair-fed rats)

Plasma Hormones: **PLASMA TESTOSTERONE** - Compared to the vehicle control, both the Iprodione and pair-fed rats displayed lower testosterone concentrations throughout the 10-hour sampling period [Table 9]. There were no significant differences between these two groups. During the first 240 minutes of sampling, the values for Iprodione [0.24 to 0.48 ng/mL] were comparable to those of the pair-fed rats [0.21-0.48 ng/mL]. Thereafter, the values gradually increased until 490 hours for both groups [from 0.51 to 0.72 ng/mL for Iprodione and 0.54 to 0.84 ng/mL for the pair-fed rats]. The last two time points were similar to the highest values observed during the first 240 hours of sampling following the last dose. Iprodione displayed the larger decrease in both the total mean testosterone concentration [31% of control] and the maximum testosterone concentration [26% of control], and both parameters were significantly different from control for both groups. From the individual testosterone profiles it appears that both Iprodione and pair-feeding reduced the baseline testosterone values, the maximum values, pulse amplitude, and, in the piar-fed rats the number of pulses [Table 10]. The author stated that Iprodione did not depress the number of pulses, but the maximum values and probably pulse amplitude were greatly reduced. The percent of samples greater than 0.25, 0.5, and 1.0 ng/mL was markedly fewer for both the Iprodione and pair-fed rats, and the Iprodione rats showed fewer samples at these values than the pair-fed rats. Flutamide displayed a large increase over vehicle control values throughout the sampling period but statistical significance was usually not attained due to the wide variation among the rats. With time, the testosterone values in the Flutamide rats decreased and the lowest values were observed at the 490- and 610-hour samples, although the values were still greater than the control values. Baseline testosterone was increased, and the maximum values were much larger than the controls. NOTE: The 0-hour sample was collected ≈30 minutes after dosing and the

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10-hour sample was collected ≈11 hours after dosing. **PLASMA LH** - During the first 2 hours post dose, the levels for the Iprodione rats were decreased relative to the vehicle control, but thereafter alternatively were greater than or less than/equal to control values [statistical significance was not attained relative to the vehicle control]. At the 370-hour sampling, Iprodione displayed a statistically significant increase in LH relative to the pair-fed rats. Pair-fed rats displayed decreased values throughout the first 150 minutes and then alternately displayed increases or decreases relative to the control. The total mean LH was comparable among the vehicle control, pair-fed, and Iprodione rats. The author stated that analyses of the individual LH profiles [Table 10] suggest no marked differences between the pair-fed and Iprodione rats with respect to basal LH values, maximum value, and pulse height, but the LH pulses may have been more frequent in the Iprodione rats compared to the pair-fed rats. The increase in the frequency of LH pulse with Iprodione was considered by the author as possibly biologically significant although not statistically. Iprodione appeared to prevent the depression of LH caused by pair feeding, since the percentage of samples less than 0.1 ng/mL was markedly lower in the pair-fed rats compared to those in the Iprodione group. Statistically significant increases in plasma LH were observed throughout most of the sampling period following Flutamide exposure for 30 days, and the total mean and maximum LH concentration were significantly elevated relative to the vehicle control [Table 9]. The individual profiles indicate that release of LH was pulsatile in all dose groups, but Flutamide elevated the baseline, increased the pulse height, and may have caused more pulses. **PLASMA FSH** - At several time points, the FSH values for the Iprodione rats were significantly increased relative to the pair-fed rats but comparable to those of the vehicle control. Statistically-significant increases in FSH were observed in the Iprodione rats only for the 0-, 140-, and 610-hour sampling periods. The total mean and maximum concentrations in the Iprodione rats were comparable to the vehicle control. For the pair-fed rats, statistically-significant decreases were observed relative to the vehicle control at 180 and 490 hours post dose. The total mean and maximum concentrations were slightly lower than the vehicle control values, but statistical significance was not attained. The following is the author's assessment of the results with respect to FSH concentrations: it was stated that the FSH concnetrations were not consistently influenced by either Iprodione treatment or decreased food consumption. Flutamide caused increased FSH compared to the vehicle controls at all time points. Characteristics of FSH secretion for individual rats indicated a pulsatile release in all treatment groups, but the number of pulses were lower after Flutamide compared to the vehicle control [Table 10]. Flutamide increased basal values, and Iprodione may have increased basal values, which were not statistically significant. It also appears that a larger percentage of the samples were greater than 10 ng/mL in the Iprodione group, suggesting that pulse duration was extended or amplitude was greater in this group. In all groups, many FSH pulses did not meet the 2-fold baseline criteria set for LH and testosterone. These individual data need further definition to characterize pulses, pulse height, and pulse amplitude. **NECROPSY HORMONE LEVELS** - Statistically significant increases were observed in all of the hormone levels relative to the vehicle control following Flutamide exposure [Table 11]. Only Estradiol levels were

significantly different [increased] relative to the vehicle control following Iprodione exposure. Testosterone levels were significantly decreased relative to the vehicle control in the pair-fed rats.

Table 9. Plasma Hormone Concentrations [ng/ml]

Organ/Group	vehicle	Iprodione	Flutamide	Pair-Fed
TESTOSTERONE				
minutes				
0	1.56	0.46(29)♦	8.02(514)	0.42(27)
120	0.93	0.24(26)	9.11(980)	0.21(23)
130	0.98	0.30(31)	7.26(741)	0.24(24)
140	1.14	0.35(31)	8.77(769)	0.35(31)
150	1.20	0.42*(35)	7.82*(652)	0.31*(26)
200	1.48	0.45(30)	7.13(482)	0.34*(23)
210	1.57	0.33*(21)	6.29(401)	0.37*(24)
230	2.09	0.41*(20)	6.27(300)	0.45*(22)
240	2.02	0.48*(24)	6.19(306)	0.48*(24)
280	1.85	0.55*(30)	5.69(308)	0.75(41)
320	1.30	0.62*(48)	4.70(362)	0.83(64)
360	1.18	0.66*(56)	4.10(347)	0.70(59)
490	0.93	0.48*(52)	1.98(213)	0.45(48)
610	1.23	0.41(33)	2.70(220)	0.43(35)
total mean T	1.41	0.44*(31)	6.95*(493)	0.54*(38)
max. T conc.	3.26	0.86*(26)	11.74(360)	1.09*(33)
LH				
minutes				
0	0.23	0.15(65)	1.47*(639)	0.16(70)
120	0.24	0.18(75)	1.46*(608)	0.20(83)
130	0.28	0.35(125)	1.48*(529)	0.11(39)
140	0.35	0.26(74)	1.58*(451)	0.13(37)
150	0.35	0.26(74)	1.25*(357)	0.12(34)
160	0.18	0.32(178)	1.27*(706)	0.11(61)
170	0.24	0.16(67)	1.37*(571)	0.27(113)
180	0.18	0.24(133)	1.11*(117)	0.25(139)
190	0.24	0.25(104)	0.81*(338)	0.18(75)
200	0.20	0.26(130)	1.18*(590)	0.36(180)
210	0.28	0.17(61)	0.94*(336)	0.26(93)
320	0.18	0.30(167)	0.46(256)	0.20(111)
340	0.20	0.17(85)	0.42(210)	0.14(70)
370	0.13	0.30*(231)	0.37(285)	0.11*(85)
490	0.20	0.19(95)	0.40(200)	0.14(70)
610	0.30	0.27(90)	0.43*(143)	0.16(53)
total mean LH	0.21	0.22(105)	1.06*(505)	0.19(90)
max. LH conc.	0.70	0.75(107)	2.57*(367)	0.72(103)
FSH				
minutes				
0	7.16	9.17*(128)	16.46*(230)	8.79(123)
120	8.37	8.97(107)	16.20(194)	6.49(77)
130	6.41	7.93(124)	15.61*(244)	6.59(103)
140	6.75	7.08*(105)	15.58*(231)	7.27(108)
150	8.29	7.50(90)	18.46*(223)	7.28(88)
160	8.23	6.61(80)	16.30*(198)	6.53(79)
180	9.39	8.63♦(92)(144)	14.34*(153)	5.99*(64)
200	7.38	6.30(85)	15.55*(211)	6.95(94)
230	6.97	8.15(117)	13.86*(199)	6.01(86)
240	6.26	9.23(147)	14.59*(233)	6.69(107)
280	7.29	8.28♦(114)(121)	14.80*(203)	6.83(94)
330	8.14	8.11♦(100)(116)	13.16*(162)	7.02(86)
360	7.39	8.29♦(112)(130)	13.57*(184)	6.39(86)
490	8.68	8.83♦(102)(125)	14.75(170)	7.07*(81)
610	7.00	8.72*♦(125)(121)	15.17*(217)	7.22(103)
total mean FSH	7.38	8.02(109)	15.31*(207)	6.82(92)
max. FSH conc.	11.79	11.78	19.49*(165)	10.61(90)

* significantly different from vehicle control; ♦ significantly different from pair-fed rats; (% of vehicle control); (% of pair-fed value)

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Table 10. Hormone Secretion Characteristics [mean]

Hormone/Group	vehicle	Iprodione	Flutamide	Pair-Fed
TESTOSTERONE				
# pulses	2.4[0-8]♥	2.0[0-6]	2.8	1.7[0-4]
% ≥0.1 ng/mL	100	89.1*	99.7	97.7
% ≥0.25 ng/mL	99.6	72.9*	97.8	81.1*
% ≥ 0.5 ng/mL	87.1	38.5*	92.7	35.9*
% ≥ 1.0 ng/mL	47.4	9.1*	74.0*	13.0*
maximum value [ng/mL]	3.26[1.11-9.68]	0.86*[0.06-1.90]	11.74[0.80-35.21]	1.09[0.43-2.62]
estimated basal value [ng/mL]	0.6[0.3-1.2]	0.3*[0.1-0.6]	3.03[0.07-10.19]	0.3*[0.1-0.6]
LUTEINIZING HORMONE				
# pulses	3.6[0-7]	4.2[2-7]	4.8[2-7]	3.5[0-8]
% ≥0.1 ng/mL	79.3	80.5♦	96.6*	57.9*
% ≥0.25 ng/mL	27.4	30.8	79.2*	21.2
% ≥ 0.5 ng/mL	8.2	8.5	51.2*	6.8
% ≥ 1.0 ng/mL	0.8	1.1	26.3*	1.0
maximum value [ng/mL]	0.70[0.15-1.69]	0.75[0.08-1.48]	2.57*[0.73-5.40]	0.74[0.12-1.58]
estimated basal value [ng/mL]	0.09[0.08-0.10]	0.11[0.10-0.15]	0.3[0.1-0.7]	0.10[0.08-.010
FOLLICLE-STIMULATING HORMONE				
# pulses	5.0/2.9♦	4.2/1.2	1.5*/0.1*	3.9/1.3
% ≥0.1 ng/mL	100	100	100	100
% ≥0.25 ng/mL	96.4	96.6	100	98.7
% ≥ 0.5 ng/mL	82.8	75.3	99.3*	76.2
% ≥ 1.0 ng/mL	19.0	30.7	80.3*	6.9
maximum value [ng/mL]	11.79[7.69-20.08]	11.78[5.53-19.04]	19.49*[8.74-33.38]	10.61[7.00-18.91]
estimated basal value [ng/mL]	4.5[1.2-8.9]	5.9[1.6-9.9]	12.7*[4.9-19.5]	4.7[1.6-8.0]

* p≤0.05 [different from vehicle control]; Individual values were used to determine %, maximum, and baseline [basal] values, and number of pulses. The basal value [estimated baseline] for each rat was defined as the lowest value that occurred 1 to 3 times during the sampling period or the average of 2 to 3 of the lowest values. The # of samples analyzed varied between 15-26/rat. A pulse was defined as excursions above the basal value of at least one sample that increased in value by greater than 2-fold basal value. The % are the percentage of samples in the cohort of samples for each rat that have values [in ng/mL] that are greater than or equal to the value indicated. ♥ [range]; ♦ significantly different than pair-fed rats; ♦ # of pulses 30% ↑ above basal/# of pulses >200% above baseline]

Table 11. Hormone Concentrations [ng/mL] - Necropsy Sample

Hormone/Group	vehicle	Iprodione	Flutamide	Pair-Fed
LUTEINIZING HORMONE	0.56	0.73(130)♦	1.41*(252)	0.45(80)
FOLLICLE-STIMULATING HORMONE	10.47	10.27	17.64*(168)	9.05(86)
TESTOSTERONE	1.60	1.03(64)	10.47*(654)	0.44*(28)
ESTRADIOL	2.71	3.86*(142)	12.27*(453)	2.93(108)

* p≤0.05; ♦ (% of vehicle control)

Microscopic Examination: **IPRODIONE** - Centrilobular hepatocellular hypertrophy [liver] and vacuolization of the zona fasciculata [adrenals] were observed at termination. Glandular atrophy in a few of the rats was observed in the prostate and seminal vesicles, but the incidence of both was similar to that in the pair-fed rats [Table 12]. The severity of the atrophy [Table 13] in both the seminal vesicles and prostate was slightly greater in the Iprodione-treated rats than in the pair-fed rats. Focal bilateral degeneration and unilateral atrophy of the seminiferous tubules were observed once only [2 different rats]. **FLUTAMIDE** - Testicular, liver, seminal vesicle, and prostate gland lesions were observed in the majority of the rats following Flutamide exposure for 30 days, and atypical cells were observed in the lumen of the epididymis. Leydig cell hyperplasia was observed in ≈88% of the

rats, bilateral focal seminiferous tubular degeneration was seen in 50% of the rats, and one rat displayed focal unilateral seminiferous tubular atrophy.

Table 12. Microscopic Findings

Organ/Lesion/Group	Vehicle	Pair-fed	Flutamide	Iprodione
TESTES N=	16	16	16	13
atrophy, seminiferous tubular., unilat.	0	0	1	1
degeneration, seminiferous tubular, bilat.	0	0	8	1
hyperplasia, interstitial cell	0	0	14	0
EPIDIDYMIS N=	16	16	17	13
atypical cells, lumen	0	0	7	1
hypospermia, lumen	0	0	2	0
SEMINAL VESICLES N=	16	16	16	13
inflammation, subacute	1	0	0	1
atrophy, glandular	0	4	16	4
PROSTATE N=	16	16	16	13
inflammation, chronic	2	2	0	0
atrophy, glandular	0	4	16	3
inflammation, acute	0	0	0	1
LIVER N=	16	16	16	13
hypertrophy, hepatocellular, centrilobular	0	0	0	11
hypertrophy, hepatocellular, diffuse	0	0	15	2
ADRENALS N=	16	16	16	13
vacuolization, zona fasciculata	1	2	1	13

Table 13. Severity of Atrophy in Prostate and Seminal Vesicles

Organ/Severity	Iprodione	Flutamide	Pair Fed
Prostate	13	16	16
normal limits	9	0	12
minimal	2	2	3
mild	1	14	1
moderate	1	0	0
Seminal Vesicles	13	16	16
normal limits	9	0	12
minimal	1	0	3
mild	3	1	1
moderate	0	15	0

DISCUSSION

In Sprague-Dawley rats fed Iprodione for 2 years [MRID # 426378-01; Chronic Toxicity/Carcinogenicity study], interstitial cell hyperplasia in the testes, reduced spermatozoa in the epididymides, and absent/empty secretory colloid cells or reduced secretion in the seminal vesicles were observed in males at the 300 and 1600 ppm dose levels. Atrophy of the seminiferous tubules in the testes, with atrophy of the prostate and absence of spermatozoa in the epididymides were observed at 1600 ppm. There was an increase in the incidence of both unilateral and bilateral benign interstitial cell tumors in the testes of males at the 1600 ppm dose level. The current study was undertaken to determine whether Iprodione had any antiandrogen activity. The study author pointed out that interpretation of the Scatchard analysis and relative binding affinity must be made with caution in that very high concentrations of Iprodione [10000 nM] were used before any binding or displacement

occurred [circulating concentrations of steroids ≈0.1 to 10 nM]. Additionally, no specific tissue response of the prostate to Iprodione was observed in this study other than what was observed [decreased prostate weight] in the pair-fed controls also. In the relative binding affinity study, Iprodione was not found to compete strongly for the androgen receptor, compared to Flutamide, dihydroxyflutamide, and 3 ~~Iprodione metabolites~~ [~~RP25040 and to a lesser extent RP36112 and~~ RP36115 had an affinity for the androgen receptor close to that of Flutamide]. Following 15 days of exposure to Iprodione [dose levels of 120, 300, and 600 mg/kg/day], minimal antiandrogen activity was observed as evidenced by the dose-related effect on plasma LH and especially FSH, which were intermediate to those of Flutamide, but since no pair-fed group was included in this study, effects due to body-weight loss could not be determined. Following 30 days of exposure at a dose level of 600 mg/kg/day, an effect on mineralocorticoid secretion was suggested in light of a 2-fold increase in adrenal weight, along with the clinical signs indicative of dehydration. Comparing hormone data at necropsy, Flutamide showed consistent changes [increases] in all hormones measured following 15 and 30 days of exposure, but only LH and FSH were increased after 15 days and only estradiol was increased after 30 days exposure to Iprodione. It was stated that the differences between the 15- and 30-day exposures may be due to treatment duration, sample size, or Type I errors inherent in single-sample endocrine data. Serial sampling of hormones following 30 days of exposure indicated that Iprodione rats had prolonged periods of decreased basal concentrations of testosterone, but no obvious changes in average LH concentrations were observed. The author indicated that the individual LH profiles suggest that Iprodione increased the frequency of samples in most of the concentration ranges and the pulse frequency when compared to the pair-fed controls, which may be biologically significant.

CONCLUSION

Although several pieces of evidence suggest that Iprodione may have similarities to Flutamide, an antiandrogen that induces Leydig cell hyperplasia and tumorigenesis due to hypersecretion of LH, other information strongly suggests that Iprodione is not the same type of antiandrogen. Poor binding affinity to the androgen receptor was found following Iprodione exposure at very high levels, but due to solubility in the buffers at higher concentrations, a definitive statement is not possible. Two metabolites of Iprodione displayed an affinity for the androgen receptor close to that found for Flutamide. While Flutamide caused marked effects [↓ organ weight, microscopic lesions] on all male sex-related organs, Iprodione caused no effect on the prostate, but showed a slight effect on the seminal vesicles and epididymidis [↓ organ weight, microscopic lesions]. The author stated that this differential effect on the prostate and seminal vesicle following Iprodione exposure "is intriguing since these organs are known to be androgen dependent (prostate is under dihydrotestosterone regulation and epididymides and seminal vesicles are under testosterone regulation). Increased concentrations of LH, FSH, testosterone, and estradiol were found following 15 and 30 days of exposure to Flutamide compared to vehicle control values. Following Iprodione treatment for 15 but not 30 days, only LH and FSH concentrations were increased relative to the vehicle control. Testosterone concentrations at necropsy were comparable between the Iprodione and pair-fed control rats, but there were subtle changes

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in the secretion pattern of testosterone and LH between these two groups. Estradiol concentrations were increased in the Iprodione rats at necropsy following 30 days of exposure compared to both the vehicle and pair-fed control groups. A marked effect [increase] on adrenal weight associated with histopathologic lesions [vacuolation] indicative of an alteration of steroidogenesis was observed following 30 days of exposure to Iprodione but not to Flutamide. Although there is some evidence to suggest that Iprodione interferes with sex/steroid hormone regulation, the difference in the spectrum of effects observed between Iprodione and Flutamide in the current study suggests that the two compounds share only certain parts of a mechanism of toxicity/carcinogenicity. Compared to Flutamide, Iprodione appears to be a much less active/potent endocrine toxicant.

This study is Acceptable. The study is a non-guideline [mechanistic] study, which provides information as to Iprodione's mode of action with respect to its effects on male sex organs.

Table 14. Summary of Findings With Respect to Antiandrogenic Activity

Parameter/Group	Iprodione	Flutamide	Pair-fed
affinity for androgen receptor	low only at extremely high concentrations	low	-
organ weight effects A=absolute R=relative	↑ R liver, testes; ↑ A&R adrenal; ↓ A epididymides; ↓ A&R prostate, seminal vesicle, TASO	↑ A&R liver; ↓ A&R epididumides, prostate, seminal vesicles, TASO; ↓ A testes	↓ A liver; ↓ A epididumides; ↓ A&R prostate, seminal vesicles, TASO;
plasma hormone concentrations	↓ plasma testosterone♥; ↑ estradiol♥; ↑ LH♦; ↑ FSH♦	↑ plasma testosterone, ↑ plasma LH, ↑ plasma FSH; ↑ estradiol	↓ plasma testosterone
<u>microscopic lesions</u> liver-hypertrophy/centrilobular liver-hypertrophy/diffuse adrenals-vacuolation zona fasciculata prostate-grandular atrophy prostate-acute inflammation seminal vesicles-glandular atrophy epididymides-atypical cells/lumen epididymides-hypospermia/lumen testes-atrophy/semineferoustubular/uni. testes-atrophy/semineferous tubular/bi. testes-hyperplasia/interstitial cell	 11/13 2/13 13/13 3/13 1/13 4/13 1/13 0/13 1/13 1/13 0/13	 0/16 15/16 1/16 16/16 0/16 16/16 7/16 2/16 1/16 8/16 14/16	 0/16 0/16 2/16 4/16 0/16 4/16 0/16 0/16 0/16 0/16 0/16
<u>severity of atrophy in prostate</u> normal limits minimal mild moderate	 9 2 1 1	 0 2 14 0	 12 3 1 0
<u>severity of atrophy in seminal vesicles</u> normal limits minimal mild moderate	 9 1 3 0	 0 0 1 15	 12 3 1 0

♦ only after 15 days of dosing; ♥ only after 30 days of dosing

IPRODIONE

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