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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM (3rd)

SUBJECT: Iprodione - Report of the Cancer Assessment Review Committee

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Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

THROUGH: William Burnam
Chairman
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Health Effects Division (7509C)

TO: Mary Waller
Acting Product Manager #21
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The Cancer Assessment Review Committee met on November 19, 1997, to evaluate the carcinogenic potential of Iprodione. Attached please find the Cancer Assessment Document.

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012523

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

IPRODIONE (3rd)

Final Report

February 26, 1998

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Linda Taylor, Toxicologist

DOCUMENT PREPARATION: Jess Rowland, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the assessment unless otherwise stated).

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Marion Copley

Kerry Dearfield

Virginia Dobozy

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Pamela Hurley

Mike Ioannou

Esther Rinde

Joycelyn Stewart

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NON-COMMITTEE MEMBERS IN ATTENDANCE

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EXECUTIVE SUMMARY

On February 23, 1994, the Health Effects Division's (HED's) Cancer Peer Review Committee (CPRC) classified Iprodione as a **Group B2 Carcinogen**; Probable Human Carcinogen based on statistically significant increases in hepatocellular tumors in both sexes of CD-1 mice and ovarian luteomas in female CD1 mice as well as a statistically significant increase in testicular Leydig cell tumors in male Sprague-Dawley rats. The CPRC recommended that a Q_1 * be determined for the combined hepatocellular adenoma/carcinoma for both sexes of mice and also separately for the testicular tumors in the male rats.

On April 1, 1997, the CPRC evaluated the mechanistic data submitted by the Registrant to support their theory of a hormonal mode of action for the development of the male Leydig cell tumors and the hepatocellular tumors in both sexes of mice. The CPRC concluded that the available data did not provide a definitive mode of action with respect to either the Leydig cell or the liver tumors.

In October, 1997, the Agency requested an assessment by an external peer review panel of the available data on Iprodione with specific emphasis on: 1) whether a mechanism or mode of action for the development of Leydig cell and liver tumors had been demonstrated; 2) what additional data would be needed if the available data were not adequate; 3) the relevance to humans of hormonally mediated rat Leydig cell hyperplasia/adenoma and mouse liver tumors; and 4), in characterizing the cancer risk, which method is supported by the data (linear or non-linear). Similarly, the Registrant submitted their own external peer review panel conclusions to analogous questions for consideration by the Agency.

On November 11, 1997, HED's Cancer Assessment Review Committee (CARC) met to evaluate the reports of two external peer review panels (the Agency's and the Registrant's) and their assessments of the Registrant's mechanistic data for both tumor types and to determine if the present human risk characterization approach of a linear low dose extrapolation (Q1*) should be retained or changed to a Margin-of-Exposure (MOE) approach. The CARC concluded that there was no reason to deviate from the previous conclusion of the CPRC that the mechanistic data available do not provide a definitive mode of action with respect to either the Leydig cell tumors or the liver tumors and the Q_1*should be retained. This decision was based on the Committee's evaluation of the conclusions of the Agency's external peer review panel regarding the mechanistic data available on Iprodione for both tumor types, as well as those of the Registrant's external peer review panel. The CARC also agrees with both external peer review panels that more information/data are necessary before a definitive mode of action for either tumor type can be delineated.

Therefore, in accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the CARC classified Iprodione as a **"likely"** human carcinogen and re-affirmed the current linear low-dose extrapolation approach for human risk characterization based on both tumor types. For the combined hepatocellular adenomas/ carcinomas, the Q_1*s are 8.7×10^{-3} for the male mouse and 5.07×10^{-3} for the female mouse. For the Leydig cell tumors in male rats, the Q_1* is 4.39×10^{-2}. The CARC determined that of these, the most potent Q_1* of 4.39×10^{-2} should be used for cancer risk assessments.

I. INTRODUCTION

On November 11, 1997, the Health Effects Division's Cancer Assessment Review Committee met to determine whether the present human risk characterization for Iprodione, using a low dose extrapolation model (Q_1 *) should be retained or changed to a Margin-of-Exposure (MOE) approach. This was a result of the submission of the Registrant's external peer review panel conclusions and the conclusions of the Agency's external peer review panel regarding the mechanistic data from studies designed to demonstrate modes of action for the induction of Leydig cell tumor formation in male Sprague-Dawley rats and hepatocellular tumors in both sexes of CD-1 mice submitted by the Registrant.

II. BACKGROUND INFORMATION

On February 23, 1994, the HED Cancer Peer Review Committee (CPRC) classified Iprodione as a **Group B2 Carcinogen**; Probable Human Carcinogen based on the evidence of carcinogenicity in two species, mice and rats. Dietary administration of Iprodione to Charles River CD-1 mice for 99 weeks resulted in statistically significant increases in hepatocellular tumors in both sexes and ovarian luteomas in females. When fed to Sprague-Dawley rats for 104 weeks, Iprodione induced a statistically significant increase in testicular Leydig cell tumors in males. Based on these tumor types and the lack of data to support a hormonal mode of action, the CPRC recommended low-dose extrapolation (Q_1 *) for human risk characterization. The CPRC recommended that a Q_1 * be determined for the combined hepatocellular adenoma/ carcinoma for both sexes of the mouse and also separately for the testicular tumors in the male rat *(Memorandum: E. Rinde, HED to S. Robbins, RD, dated June 23, 1994).*

The following Q_1*s were calculated for both tumor types: 8.7×10^{-3} for the male mouse and 5.07×10^{-3} for the female mouse, combined hepatocellular adenomas/carcinomas and 4.39×10^{-2} for the testicular tumors in male rats *(Memorandum: B. Fisher, SAB to L. Taylor, Toxicology Branch II, dated July 19, 1994)*).

In response to the classification of Iprodione as a Group B2 Carcinogen, the Registrant submitted mechanistic studies to support their theory of a hormonal mode of action for the development of the male Leydig cell tumors, as well as the male mouse liver tumors.

On April 1, 1997, the CPRC evaluated the mechanistic data and concluded that the available data do not provide a definitive mode of action with respect to either the Leydig cell or the hepatocellular tumors. This was based on the following factors *(Memorandum: L. Taylor, HED to C. Scheltema, RCAB, HED, dated April 24, 1997).*

- With regard to the Leydig cell tumors in male Sprague-Dawley rats, it was concluded that there are inconsistencies between the *in vivo* and *in vitro* data and organ weight data that have not been fully addressed, and there is a lack of knowledge of target concentrations *in vivo*. Although the Registrant contends that the mechanistic research has determined that tumor formation is linked to a

prolonged hormonal perturbation, the CPRC concluded that prolonged perturbation has not been demonstrated.

- With respect to the hepatocellular adenomas/carcinomas (combined) in male and female CD-1 mice, Iprodione was shown to exhibit similar effects as observed following Phenobarbital exposure. Although the Registrant concluded that the large number of non-genotoxic chemicals that induce mouse liver tumors strongly suggests that cell proliferation plays an important role in mouse liver tumorigenesis, cell proliferation following Iprodione exposure has been demonstrated only after a 14-day exposure period. Based on the endpoints investigated in the liver mechanism study, the Registrant concluded that Iprodione most closely resembled Phenobarbital.

- The Agency noted that Phenobarbital is a mitogen, and cell proliferation is observed following acute, but not chronic, exposure to Phenobarbital. In the mouse carcinogenicity study on Iprodione, hepatocellular hyperplasia was not observed, but there was an increased incidence of (1) generalized vacuolation/ hypertrophy of the interstitial cells of the testes, (2) luteinization of the interstitial cells of the ovary, and (3) luteoma of the ovaries. Although many of these endpoints display no-effect levels, a definitive mode of action for Iprodione with respect to liver tumorigenesis was not identified by the Registrant.

The CPRC recommended, since the Agency at present has no set policy for regulating such chemicals as threshold carcinogens, that the Registrant might consider putting together a possible workshop on liver cell tumorigenesis to determine what data/aspects would be necessary for a determination of a linear vs a non-linear outcome; i.e., what data would be required in order to conclude that a particular chemical that produces liver tumors in the mouse is likely to be acting through a threshold mechanism and, when a MOE approach may be appropriate for human risk characterization

III. EXTERNAL PEER REVIEWS

In September 1997, the Registrant convened an external peer review panel of independent experts to consider the carcinogenic potential of Iprodione and to assess the appropriate methodology for human risk characterization. The panel members were asked to provide answers to the following questions: 1) Are the chronic/carcinogenicity studies adequate to define the carcinogenicity of Iprodione ? Were the dose levels studied appropriate?; 2) Does Iprodione present a genotoxic hazard?; 3) What is the most appropriate method to characterize the carcinogenic potential (risk) of Iprodione ? Do the mechanistic studies support a MOE, rather than a linear dose extrapolation risk assessment mode?; 4) What is the relevance to humans of hormonally-mediated rat Leydig cell hyperplasia and adenoma formation?; 5) What is the relevance to humans of a mouse liver carcinogen acting through a Cytochrome P-450 induction mechanism?; and 6) What is the relevance to humans of mouse liver tumors induced by hepatic injury?

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In October, 1997, the Agency requested a similar assessment of the data by an external peer review panel with specific emphasis on: 1) whether a mechanism or mode of action for the development of Leydig cell tumors in male rats and liver tumors in both sexes of mice had been demonstrated; 2) what additional data would be needed if the available data were not adequate; 3) the relevance to humans of hormonally mediated rat Leydig cell hyperplasia/adenoma and mouse liver tumors; and 4), in characterizing the cancer risk, which method is supported by the data (linear or non-linear).

IV. RESPONSES OF THE REGISTRANT'S AND THE AGENCY'S EXTERNAL PEER REVIEWS.

The responses provided by the Registrant's external peer review are provided in Attachment 1. In summary, this panel concluded that the mouse and rat studies were adequate and the dose levels are appropriate and that Iprodione does not present a genotoxic hazard. The panel determined that a MOE approach would be the most appropriate method for risk assessment, because it concluded that the mode of action for the process of enhancement of the two tumor types (rat Leydig cell and mouse liver) does not involve a mechanism that is linear.

With regard to the Leydig cell tumors, the Registrant's expert panel concluded that the mechanistic data indicate that testosterone production is blocked by Iprodione due to an interference with cholesterol transport into the mitochondria of the Leydig cells. Luteinizing hormone levels were slightly and transiently increased in rats exposed to high levels of Iprodione, most likely due to a reduction in circulating levels of testosterone, thereby leading to Leydig cell hyperplasia and in the chronic studies to subsequent increased incidence of Leydig cell adenoma formation. However, the panel noted that an alteration in testosterone biosynthesis *in vivo* has not been demonstrated. Stimulation of the Leydig cells by the elevated serum levels of LH and consequent proliferation was considered to be the first step in tumor formation of the testes. The panel found no indication in the data base to warrant the use of a linear extrapolation to assess tumorigenic risk and recommended regulation with the present data using an MOE approach, although further data were considered desirable. The panel also concluded that human risk of induction of Leydig cell tumors would be minimal to absent, especially when consideration is given to the mechanism of action and potential levels of exposure.

With regard to liver tumors, the Registrant's expert panel concluded that although it is conceivable that liver tumors could be induced in humans by sustained hepatic injury, the dose would have to be high, and the administration long-term. The panel concluded that mouse liver tumor induction was most likely the result of combined effects consisting of liver cell injury, indicated by the marked increases in SGOT and SGPT, potential for oxidative stress, expressed by the presence of pigmented macrophages, and liver cell hypertrophy associated with the induction of P450 isozymes that are not found in humans. The panel found no evidence that this mechanism is applicable to humans and indicated that the risk would not be linear. Using the available data, the panel concluded that Iprodione should be evaluated using the MOE approach.

The Registrant's expert panel also indicated several areas where there are missing data; e.g., a lack of mechanistic data for the female mouse liver; the need to demonstrate that testosterone biosynthesis is altered *in vivo*; the need to establish that the Leydig cell hyperplasia/adenoma had been interpreted histopathologically in a uniform manner.

The responses provided by the external peer review panel to the Agency's questions are provided in Attachment 2 A-C. In summary, all the panel members agreed that: 1) the mouse and the rat studies were adequate to assess the carcinogenic potential of Iprodione and the dose levels tested in these studies were appropriate, 2) Iprodione does not poses a genotoxic hazard, 3) that more information/data are necessary before a definitive mode of action for either tumor type can be delineated and 4) the data suggest a non-linear mode of action (one member, however, declined to respond to the question on linear or nonlinear mode of action for cancer risk assessment).

V. COMMITTEE'S CONCLUSIONS

The Committee concluded that there was no reason to deviate from the previous conclusion of the CPRC on April 1, 1997 that the mechanistic data available do not provide a definitive mode of action with respect to either the Leydig cell tumors or the liver tumors. This decision was based on an evaluation of the two external peer review panels and their assessments of the Registrant's mechanistic data for both tumor types (Leydig cell and liver).

Both expert panels concluded that more information/data are necessary before a definitive mode of action for either tumor type can be delineated, which is consistent with the previous CPRC conclusion. Direct evidence of an effect *in vivo* on testosterone biosynthesis has not been demonstrated, information on the concentration of Iprodione at target tissues is lacking, the relationship between the *in vitro* doses attained in the mechanistic studies to those attained *in vivo* in the bioassays is unknown, information on cell proliferation both beyond 14 days and in the female mouse liver is lacking, and a No Observable Adverse Effect Level (NOAEL) for liver cell proliferation has not been demonstrated.

Both expert panels agreed that the data suggested a non-linear mode of action. The Committee did not concur with the external peer review members recommendation for a non-linear approach because a definitive mode of action has not been demonstrated for either tumor type in order to take the non-linear approach for human risk characterization. Therefore, the Committee concluded that there was no reason to deviate from the previous decision of a linear low-dose approach for human risk characterization and the use of the Q_1* for Leydig cell tumors, which gives the most conservative estimate.

With respect to the issue of relevance to humans, the Committee concluded that Iprodione has not been demonstrated to function as an GnRH agonist or a dopamine agonist, both of which are considered not relevant to humans. If the Registrant's hypothesis that inhibition of testosterone biosynthesis is the initial event in the production of rat Leydig cell tumors, then this particular mechanism is considered of relevance to humans. Additionally, since no mode of induction of these tumors in rats has been identified, their relevance to humans cannot be negated. These

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conclusions are in agreement with the principles stated by the Agency-sponsored workshop on rodent Leydig cell adenomas and human relevance.

The Registrant should be encouraged to explore further the mode of action of Iprodione for both tumor types.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified Iprodione as a **"likely"** human carcinogen (or a **Group B2 Carcinogen** in accordance with the 1986 Guidelines for Carcinogen Risk Assessment). The weight-of-the-evidence for this classification are as follows:

(i) Statistically significant increases in hepatocellular tumors in both sexes of CD-1 mice. Female mice also had a statistically significant increase in ovarian luteomas.

(ii) Statistically significant increase in testicular interstitial cell tumors (Leydig cell) in male Sprague-Dawley rats.

(iii) Tumors seen at the same sites with two structurally related compounds; Procymidone and Vinclozolin. Both compounds were associated with testicular tumors in the rat and liver tumors in the mouse (as well as other tumor types).

(iv) The available data do not provide a definitive mode of action with respect to either the Leydig cell or the liver tumors.

(v) The relevance of the observed tumors to human exposure can not be negated since no mode of action for these tumors in animals has been identified.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee did not see the need to deviate from the previous decision of a linear low-dose approach for human risk characterization. The Committee re-affirmed that the current linear low-dose extrapolation should be based on the liver tumors in both sexes of mice and the Leydig cell tumor in male rats. For the combined hepatocellular adenomas/carcinomas, the Q_1*s are 8.7 x 10^{-3} for the male mouse and 5.07 x 10^{-3} for the female mouse. For the Leydig cell tumors in male rats, the Q_1*is 4.39 x 10^{-2}. The Committee concluded that of these, the most potent Q_1* of 4.39 x 10^{-2} should be used for cancer risk assessments.

ATTACHMENT 1

REGISTRANT'S EXTERNAL PEER REVIEW

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Executive Summary of the External Peer Review Panel on Iprodione

The peer review panel was asked to provide answers to the following questions:

1. Are the chronic/carcinogenicity studies adequate to define the carcinogenicity of Iprodione? Were the dose levels studied appropriate?

The two long-term studies in rat and mouse are adequate. The dose levels are appropriate, but there is evidence that the high dose that was administered to mice (4,000 ppm) exerted chronic toxicity.

2. Does Iprodione present a genotoxic hazard?

No. This compound has been studied in a typical battery of short-term tests and except for one bacterial system it has been shown to be completely negative. There are no structural alerts for genotoxicity in the chemical.

3. What is the most appropriate method to characterize the carcinogenic potential (risk) of Iprodione? Do the mechanistic studies support a MOE, rather than a linear dose extrapolation risk assessment model for: (i) rat Leydig cell tumors and (ii) mouse liver tumors?

The panel agreed that the MOE would be the most appropriate method of risk assessment, because the mode of action for the process of enhancement of these two tumor types does not involve a mechanism that is linear.

4. What is the relevance to humans of hormonally-mediated rat Leydig cell hyperplasia and adenoma formation?

There is no established relevance for human cancer risk assessment for induction of Leydig cell hyperplasia or adenoma in rats. A closely related species, mice, did not respond similarly.

4A. Are there mechanistic data to support the induction of Leydig cell hyperplasia?

The rat chronic study provided suggestive evidence for chronic deficient testosterone production manifested as atrophy of the seminiferous tubules and secondary sex organs at 1600 ppm. Several mechanistic studies demonstrated a compensatory increase in circulating levels of LH from the pituitary gland, which could lead to the increased Leydig cell proliferation in the 30-day study. In chronic studies at high doses Iprodione predisposes Leydig cell hyperplasia to the increased incidence of adenomas. This mechanism of hormonal imbalance is well established for a large number of chemicals and marketed drugs (Clegg et al., 1997). The *in vitro* data supported the finding that testosterone production is disrupted by Iprodione at the stage of active transport

of cholesterol (substrate for steroid hormone synthesis) into mitochondria of Leydig cells. The decreased ovarian and uterine weights observed in female mice at the high dose level in the carcinogenicity study would support a similar disruption in steroid hormone (estrogen) biosynthesis occurring in the ovary.

4B. Is the possible mode of action relevant to humans?

There is no established relevance of the mechanism for the development of Leydig cell adenomas in rats for human risk assessment. Two reasons can be cited: 1) The mechanism has never been demonstrated in humans, and 2) even if the hormonal perturbations (i.e. elevations in serum LH) did occur in humans there is no evidence that they would lead to the development of Leydig cell tumors.

5. What is the relevance to humans of a mouse liver carcinogen acting through a Cytochrome P450 induction mechanism?

P450 induction is not related linearly to dose for risk assessment purposes. Induction of P450 enzymes is very species dependent, and to date there has not been xenobiotic-mediated induction of CYP 2A and CYP 3A established in humans.

6. What is the relevance to humans of mouse liver tumors induced by hepatic injury?

It is conceivable that liver tumors could be induced in humans by sustained hepatic injury; moreover, the dose would have to be high and the administration long-term such as with ethanol. It would not be scientifically sound to quantify the risk from such a sustained chemical insult by using linear extrapolation. A margin-of-exposure approach would be more suitable. Furthermore, the toxic injury seen in the mouse liver with Iprodione may be species-specific since it did not occur in the rat.

EXTERNAL PEER REVIEW OF IPRODIONE CARCINOGENICITY

A panel of independent experts was convened on September 15th, 1997 to consider the carcinogenic potential of Iprodione and to assess the appropriate methodology for characterizing the carcinogenic risk to humans.

The panel was composed of:

Charles C. Capen, D.V.M., Ph.D.
Professor and Chairman
Department of Veterinary Biosciences
The Ohio State University
Columbus, Ohio

David Clegg, Panel Chairperson
Clegg and Assoc.
Carp, Ontario, Canada

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Genotoxicity

The panel initially addressed the potential for Iprodione genotoxicity. The panel considered the available extensive mutagenicity data, based on weight of evidence, did not indicate Iprodione to be genotoxic. It was noted, however, that a *Bacillus subtilis* assay for DNA damage showed selective growth inhibition in DNA-repair deficient strains at dose levels of 21 µg/ml and above in a number of strains both with and without metabolic activation suggesting DNA damaging activity (Felkner, 1985). A second *B. subtilis* study (Sharisu et al., 1976) which was negative, although the strains used (M45 and H17) were either negative or not tested in the first study. The *B. subtilis* assay is only a screening assay and by itself does not demonstrate DNA damage per se. Agents can evoke positive responses in this assay as a result of direct or indirect effects. All other measures of genotoxicity were clearly negative. Neither vinclozolin, nor procymidone, which are closely related compounds, show evidence of genotoxicity.

Rat Studies

The high dose (1600 ppm) in the rat chronic/carcinogenicity study was deemed to be adequate by the panel, confirming the EPA evaluation. The panel was of the opinion that the MTD was achieved and possibly slightly exceeded since, at 1600 ppm, a decrease in body weight gain in males between weeks 0-22 was 14-16% and in females between weeks 0-12 was 19%. Food intake was consistently reduced in males (5-8%) but was reduced in females (7%) only during the 13-22 week period. It was noted that survival was highest at 1600 ppm. The panel noted an increase in the severity but not incidence of testicular interstitial cell hyperplasia (dose-related) at 300 ppm and above in male rats at terminal sacrifice. Lesions in the Leydig cells (hyperplasia 2/10 rats) at 1 year appeared to be preceded by lesions in the adrenal (generalized or focal enlargement of zona glomerulosa cells, [10/10 rats]; generalized fine vacuolation or generalized rarefaction and fine vacuolation of the zona fasciculata [8/10 rats] and generalized fine vacuolation of the zona reticularis [5/10 rats]). Histopathology of the adrenal gland was characterized by an accumulation of lipid stores which may be the result of an interference in cholesterol mobilization.

The incidence of Leydig cell tumors was increased at 1600 ppm (29/60 vs. 3/60 controls). The panel noted that the Leydig cell lesions were classified using STP criteria, but that the historical control data were not based on the same criteria. The criteria were not specified for defining hyperplasia. However, the hyperplasia at 300 ppm was one of the criteria (based on an increase in severity from trace to minimal rather than an increase in number of animals affected) utilized in setting the NOEL of 150 ppm (other effects at 300 ppm were reduction of epididymal spermatozoa, reduced secretion of seminal vesicles [both indicative of possible compromised testosterone availability], adrenal vacuolation of the zona reticularis [indicative of possible upsets in steroidogenesis], and increased splenic hemosiderosis). It is important to note that both reduced numbers of epididymal sperm and decreased seminal vesicle weight are

indices of compromised testosterone availability.

The studies by Fail et al., (1994, 1996a and 1996b) and Benhamed, (1995 and 1996) were evaluated in considering the mechanism of action of Iprodione. Androgen- receptor binding assays were performed on Iprodione and on seven of the Iprodione metabolites (see proposed metabolic pathway in Appendix A for metabolite identities) (Fail, et al., 1994). Iprodione, RP36118, RP36119, RP32490, RP36114 had low affinity for prostate androgen receptors *in vitro*. However, RP25040 showed about half the androgen receptor binding capacity of flutamide with metabolites RP36112 and RP35115 having less than 20% of the binding capacity of flutamide. RP35115 and RP 35112 are major metabolites of Iprodione. RP25040 is a minor metabolite.

Repeated consecutive doses of Iprodione at 120, 300 and 600 mg/kg bw/day over 15 days to male rats with terminal measurements of LH, FSH, testosterone and estradiol (Fail, et al., 1994) revealed increased LH (4X) at 600 mg/kg bw/day and increased FSH (1.3-1.4X) at 300 mg/kg bw/day and above. No changes were seen in testosterone or estradiol levels at the intervals sampled. Levels of all four hormones were increased in a separate group of animals administered flutamide (150 mg/kg bw/day for the same period. Body weight was reduced at 300 mg/kg bw/day and above and changes were observed in organ weights. Absolute prostate weights were reduced at 120 mg/kg bw/day and above, the absolute weights of epididymides were reduced at 300 mg/kg bw/day and above, and seminal vesicles were reduced at 600 mg/kg bw/day and relative liver weight was increased at 600 mg/kg bw/day. However, the relative epididymal weights were not affected at any dose, and relative prostate weight was only affected at 600 mg/kg bw/day.

In another experiment, male rats were administered Iprodione orally 600 mg/kg bw/day and intra-atrial cannulae were inserted on day 25 (Fail, et al., 1994). On day 30, blood samples were taken every 2 hours for 8-10 hours and, within that time frame, every 10 minutes for a 4-hour window. Hormonal assays were performed on all blood samples.

Baseline testosterone levels were reduced in pair fed (75%) and Iprodione-treated (70%) rats compared to controls. During the 4 hour window plasma testosterone levels were determined and shown to follow a pulse-release pattern. Plasma LH levels were unaffected, however, pulse frequency of LH was increased in Iprodione-treated rats. Iprodione appeared to counter the baseline depression in LH caused by pair-feeding. FSH levels were unaffected. Estradiol (measured at necropsy only) was significantly increased in Iprodione-treated rats.

Comparison of 15-day (single sample) and 30-day treated rats showed that at 15 days there were increased LH and FSH levels, but at 30 days there was only increased estradiol levels. The increase in LH was also observed in rats that had received Iprodione in the diet at 3000 ppm for 14 days in the Fail, et al., 1996a study.

The data in these mechanistic studies indicated:

1) In contrast to other cyclic imide fungicides, such as vinclozolin, Iprodione is not an androgen-receptor antagonist, although three of its metabolites show some level of binding,

2) The hormonal imbalances seen at 14/15 days were greater than at 30 days, indicating a possible compensatory mechanism with return of levels toward normal, and

3) There was an absence of effects on the testosterone levels in plasma.

Further investigation of testosterone levels in male rats were made after dietary feeding of 3000 ppm Iprodione for 2, 7 or 14 days. No changes were detected in levels of either plasma or testicular testosterone, although body weight, food intake and organ weights were all affected (Fail, et al., 1996b).

In *in vitro* studies on incubated porcine Leydig cells, Iprodione, RP36112 and RP36115, caused about 50% inhibition of testosterone production at 1 μg/ml and maximum inhibition occurred between 3 and 10 μg/ml. The metabolites RP25040, RP32490 or RP36118 revealed no inhibition of testosterone production (Benhamed, 1995).

Reversibility of the inhibitory effects on steroidogenesis was also investigated. In one group of cultures, a dose-response inhibition was demonstrated. A second group of cultures were similarly treated, and were then washed after incubation and re-incubated in fresh medium. Regardless of Iprodione dose, the inhibitory effects on steroidogenesis were no longer present. The time course of inhibition was also investigated over the 3-72 hour incubation periods. Inhibition was seen at all time intervals, i.e. onset of inhibition is less than 3 hours. Positive control data using ketoconazole showed similar inhibition of testosterone secretion to that seen with Iprodione.

The demonstration of testosterone inhibition *in vitro* in this study raised the question of why inhibition of testosterone has not been detected in *in vivo* studies. The most logical explanation is that the timing of sampling in the *in vivo* studies was inappropriate due to the rapid compensatory mechanisms in the hypothalamic-pituitary-gonad axis.

A number of studies were conducted to identify the biochemical steps at which Iprodione and the metabolites RP36112 and RP36115 interfere with gonadotrophin-stimulated testosterone secretion from cultures of porcine Leydig cells (Benhamed, 1996). Inhibition of testosterone secretion was observed when Leydig cells were stimulated by hCG or agents which enhanced cAMP production. However, incubation with 22R hydroxycholesterol blocked the inhibiting effect of Iprodione and RP36115. Similarly, further studies indicated that inhibition of 17α-

hydroxylase/17,20-lyase was the probable site of disruption of testosterone production by RP36112. Thus Iprodione and RP36115 most likely act by blocking cholesterol mobilization and the movement of cholesterol into the mitochondria, while RP36112 acts by prevention of conversion of 17α-hydroxypregnenolone to dehydroepiandrosterone. RP36112, thus, appears to act in a similar manner to ketoconazole.

One mechanism considered for tumor induction was the induction of cell proliferation in the testes with subsequent tumor formation. Leydig cell proliferation was detected in the testes following 600 mg/kg bw/day oral administration of Iprodione for 30 days (Fail, et al., 1994; Eldridge, 1996). This was quantified using proliferating cell nuclear antigen (PCNA). The proliferating index (as a percentage of total Leydig cells) was increased by Iprodione 2-9.5-fold (i.e. Iprodione-induced cell proliferation). The Leydig cell proliferation was diffuse rather than focal.

The most important hormonal parameter to correlate with the Iprodione-induced Leydig cell proliferation would be increased levels of serum LH. The panel noted the increase in LH observed in several studies in vivo (Fail, et al., 1994; Fail, et al., 1996a) would be the expected finding in response to an interference in testosterone production following exposure to Iprodione. An interference in testosterone production was clearly demonstrated in well controlled *in vitro* studies. The failure to detect changes in circulating levels of testosterone in *in vivo* studies most likely was related to the sampling intervals.

The major routes of metabolism of Iprodione result in metabolites such as RP36115 and RP36114. RP25040 is a relatively minor metabolite. There are no data on the distribution or persistence of any of the metabolites of Iprodione in the reproductive organs. The panel, after some discussion, concluded that although such data would be interesting, it was not essential for the risk assessment of Iprodione. Regardless of the site of the block that resulted in a reduction in testosterone production, the elevated circulating levels of LH was considered to be the most important event that preceded Leydig cell tumor promotion.

The available data on LH *in vivo* and testosterone decreases at least *in vitro* indicate that perturbation in the hypothalamus-pituitary-gonadal axis is likely occurring in males. The degree or perturbation required to lead to tumor formation is unknown, but may be relatively short due to the rapid compensatory action to correct hormonal imbalances.

The panel concluded that Iprodione and vinclozolin have a different mechanism of action, since vinclozolin is a demonstrable anti-androgenic agent while Iprodione tumor induction and toxicity appear to be driven largely by a compensatory Leydig cell hyperplasia to maintain testosterone levels.

The panel also noted that there are 20-30 medicinal drugs that have produced Leydig cell tumors in rats, but there is no evidence that any of these drugs have caused any disruptions in Leydig cell function in humans even though pharmacologic doses, rather than lower occupational/environmental exposures are used. Some of these drugs have been on the market for over 20 years. There is considerable evidence in the literature that the rat is extremely sensitive to Leydig cell tumor induction compared to other species (Clegg et al., 1997).

The general consensus of the panel was that the formation of Leydig cell tumors in Sprague Dawley rats by Iprodione involves sustained increases in Leydig cell proliferation that are hormonally mediated and nongenotoxic. These hormonal perturbations have been less prominent and more transient than those induced by flutamide and vinclozolin. In addition, Iprodione nor several of its metabolites have shown significant binding to the androgen receptor. While decreases in circulating testosterone have not been demonstrated *in vivo*, it was demonstrated *in vitro* using porcine Leydig cells under controlled conditions. The lack of demonstrable decreases in testosterone may reflect compensatory responses of the hypothalamic-pituitary-gonadal axis. A sustained increase in Leydig cell proliferation was demonstrated in Sprague Dawley rats dosed with 600 mg/kg Iprodione for 30 days. This sustained increase in cell proliferation is considered the primary mode of action for the induction of Leydig cell tumors by Iprodione. This mode of action will not be linearly related to dose. Therefore, the panel recommends that a MOE approach be used for extrapolation of risk to humans. This MOE should recognize that rats are much more sensitive to the induction of Leydig cell tumors than other species, including man.

Mouse Studies

Two studies performed on mice were examined by the panel. The 99-week carcinogenicity study (Chambers, et al., 1993) was deemed adequate. The high dose level (4,000 ppm) caused a decrease of almost 50% in body weight gain during weeks 19-44 of the study in both sexes. This effect on body weight was compounded by increased liver weights. The effect could not be attributed to an alteration in food intake which was reduced only in the first week of the study and generally exceeded control intake thereafter. This lead to discussions on whether the top dose exceeded the maximal tolerated dose (MTD). However, it was noted that survival was highest in the top dose level. The panel concluded that although 4,000 ppm was a toxic dose, it was acceptable in the context of the study. The mid-dose level (800 ppm) was considered to be below the MTD.

The long-term study showed an increase in benign (3/50 in control males vs. 11/50 high dose males; 1/50 control females vs. 15/50 high dose females) and malignant (4/50 control males and 15/50 high dose males; and 1/50 control females vs. 6/50 high dose females) liver tumors in both sexes at 4,000 ppm. The livers at this dose level were markedly enlarged (absolute male weight, 143%, and females 146% of controls at 53 weeks at 4,000 ppm).

The clinical chemistry in this study also reflected changes in the liver function since both SGOT (at one year; males, 43%, females 47%) and SGPT (at one year; males, 207%, females 156%) were increased at 4,000 ppm . In the histopathological examination of the liver, the incidence of enlarged eosinophilic hepatocytes and fat-containing hepatocytes, centrilobular hepatocyte enlargement (also seen at 53 week sacrifice) and augmented macrophages (male mice only) was increased at 4,000 ppm over the course of the study.

In addition, changes were observed in weights of reproductive organs and in the adrenal glands. A slight increase in the incidence of luteomas was also observed (0/50 controls; 5/50 at 4,000 ppm). The panel members were of the opinion that, since the cells involved are granulosa/theca cells, disruption of the same hormonal mechanism (hypothalamic-pituitary-gonadal axis) may be involved in their formation.

The second study reviewed by the Panel (Bigot, 1996) was a 14-day dietary study at 4,000 and 12,000 ppm. The data indicated that Iprodione increased cytochrome P450 activity by 35 and 48% at 4,000 and 12,000 ppm, respectively, after 14 days of exposure. Isoforms CYP 2B and CYP 3A showed dose-related increased staining in Western blots. Enzymatic activity correlated with Western blot staining with dose-related increases in PROD (2.7 and 6.8-fold at 4,000 and 12,000 ppm, respectively) and BROD (8 and 17-fold at 4,000 and 12,000 ppm, respectively). EROD increased 2.4-fold at 12,000 ppm and lauric acid hydroxylation increased 34 and 39% at 4,000 and 12,000 ppm, respectively.

Clinical chemistry evaluation at day 14 revealed an increased ALAT (78% at 4,000 ppm and 208% at 12,000 ppm). ASAT was increased only at 12,000 ppm (72%).
Hepatic cell proliferation was increased at 3 and 14 days at both dose levels (3 days; 4,000 ppm-90%, 12,000 ppm- 450%; 14 days; 4,000 ppm- 200%; at 12,000 ppm- 320%). Phenobarbital also produced an increased P450 (54%) and increased isoforms CYP 2B and CYP 3A.

The closest corollary to these data can be seen with phenobarbital which has been well studied with pharmacologic doses in human patients. This eosinophilic centrilobular hypertrophy characteristic of phenobarbital was also demonstrated in both male and female mice with Iprodione, and increases in cell proliferation demonstrated in male mice were also similar to that of phenobarbital. This non-genotoxic mechanism is not relevant to humans and these effects are not expected to occur at any dose in humans based on data with pharmacologic doses of phenobarbital in humans for greater than 50 years (McClain, 1995).

The panel noted that liver weight increases occurred and that cell proliferation (dose related) was present, but at a higher dose level with Iprodione than with phenobarbital. Increases in the same isoforms occurred with both Iprodione and phenobarbital. There is no data to suggest that these isoforms have been found in humans and are relevant to liver tumor induction in human subjects.

It was noted by the panel that the mechanistic data were available only in male mice. It was also pointed out that the data on cell proliferation should have been extended beyond 14 days, since there is an indication that the degree of proliferation is decreasing with time, at least at the 12,000 ppm Iprodione dose level. However, it is clear that the dose response for liver tumors in females is nonlinear.

The panel concluded that Iprodione was a non-genotoxic enhancer of mouse liver tumors. The exact mechanism for tumor induction by CYP P450 is not known for either phenobarbital or Iprodione. However, it is clear that phenobarbital is not a human carcinogen. The panel questioned whether the amount of cell proliferation was sufficient to account for the difference between 9 tumor-bearing animals (in male and female controls) and 47 tumor-bearing animals (males and females at 4,000 ppm) reported in the long-term feeding study with Iprodione. They also noted the increases in circulating levels of liver enzymes (SGOT, SGPT) and the indications of potential oxidative stress suggested by the increased incidence and severity of pigmented macrophages. Therefore, there is evidence of liver injury and potential oxidative stress, which may be an additional factor contributing to tumor formation in the liver.

The compound Iprodione is a typical P450 enzyme inducer and it is probable that increased oxidative activity also is present at high doses. The literature indicates that this type of activity is not associated with liver tumor induction in human subjects (McClain, 1995).

In summary, the panel was of the opinion that the Iprodione-induced mouse liver tumors, occurring only at 4,000 ppm in a life-time exposure study were unlikely to indicate a human cancer hazard, because increased hepatic cell proliferation as the endpoint for risk assessment purposes is not a likely outcome with Iprodione exposure to humans. However, they noted that there is a major data gap in that results on cell proliferation are available only in the male. Further, pigmented macrophages were observed at a lower incidence in females compared to males at 4,000 ppm. The data gap could be bridged by doing PCNA studies in females. Studies on apoptosis would be interesting, but it was noted that the investigations on apoptosis has been performed mainly in rats, not mice. Iprodione appears to be acting as a promoter, as reflected in increased cell proliferation and, if so, this would account for the development of tumors in the mouse liver, which is well known to be very "promotable". Clear evidence of hepatic toxicity was evident at doses associated with increased liver tumors. This included increased circulating levels of SGOT and SGPT in both sexes at 4,000 ppm as well as pigment in macrophages that is likely to be lipofuscin, an indicator of oxidative stress. None of this toxicity will occur at occupational or environmental exposures. Therefore, the panel concludes that the MOE method should be used for extrapolating the risk of Iprodione associated with mouse liver tumors.

Conclusions

1. In the 2-year rat feeding study at dietary concentrations of 1600 ppm (equal to a mean daily intake of 69 mg/kg bw/day in males) Iprodione induced benign Leydig cell tumors of the testes.

2. In a 99-week mouse feeding study at dietary concentrations of 4,000 ppm (equal to a mean daily intake of 604 mg/kg bw/day in males and 793 mg/kg bw/day in females) Iprodione induces liver tumors in both sexes of CD-1 mice These doses were clearly associated with toxicity in the liver. In addition, a slight increase in the incidence of ovarian luteomas was observed in females at 4,000 ppm.

3. Mechanistic data indicate that testosterone production is blocked by Iprodione due to an interference with cholesterol transport into the mitochondria of Leydig cells. This mechanism also is observed with the active metabolite RP 36115. A second metabolite, RP 36112 probably blocks 17α-hydroxylase/ 17,20 lyase activity. Luteinizing hormone levels were slightly and transiently increased in rats exposed to high levels of Iprodione, most likely due to a reduction in circulating levels of testosterone, thereby leading to a Leydig cell hyperplasia and in chronic (2-year) studies with subsequent increased incidence of Leydig cell adenoma formation. The decrease in testosterone production has been demonstrated only *in vitro*, possibly due to a lack of early sampling to measure the circulating hormonal levels following exposure to Iprodione *in vivo*. Stimulation of the Leydig cells by the elevated serum levels of LH and consequent proliferation was considered to be the first step in tumor formation of the testes. This sustained increase in cell proliferation was demonstrated in rats treated with 600 mg/kg bw/day Iprodione for 30 days.

4. There are no indications in the data base to warrant the use of a linear extrapolation to assess tumorigenic risk.

5. The panel recommended the compound should be regulated with the present data using an MOE approach. Further data are desirable for the continuing evaluation of the compound.

6. Human risk of induction of Leydig cell tumors was concluded to be minimal to absent, especially when consideration is given to the mechanism of action and potential levels of exposure.

7. The panel concluded that mouse liver tumor induction was most likely the result of combined effects consisting of liver cell injury, indicated by the marked increases in SGOT and SGPT, potential for oxidative stress (expressed by the presence of pigmented macrophages), and liver cell hypertrophy associated with the induction of P450 isozymes that are not found in humans.

8. There was no evidence that the above mechanism is applicable to human subjects although it is conceivable that liver tumors could be induced in humans by sustained hepatic injury. However, the dose would have to be extremely high and the administration long-term. The risk would not be linear.

9. Iprodione should be evaluated using the MOE approach with the available data.

Further Data Desirable for the Continuing Evaluation of Iprodione

1. Re-evaluate the mouse liver slides in the 1-year interim sacrifice in the chronic study using PCNA in both sexes at the high and mid dose.

2. Re-read testes slides from the 90-day, 1 and 2 year studies to establish that the Leydig cell hyperplasia/adenoma has been interpreted histopathologically in a uniform manner. In addition, evaluate PCNA staining in Leydig cells.

3. Investigate circulating levels of hormones at earlier time periods (eg. 12, 24, 36 hours) in short-term endocrinologic studies to determine if high doses of Iprodione result in a significant reduction of testosterone levels and an elevation in serum LH.

4. Conduct a 15- and 30- day study in female mice with Iprodione to determine liver cell proliferation, CYP P450 induction, and circulating levels of selected liver enzymes to evaluate the degree of liver injury. Use two dose levels, 4,000 and 800 ppm, and perhaps a lower dose.

5. Investigate whether Iprodione produces a demonstrable reduction in the testicular interstitial fluid concentration of testosterone at shorter sampling times than those previously measured following the administration (by gavage) of the compound (eg. at doses of 600 and 120 mg/kg) with appropriate controls (e.g. lansoprazole or ketoconazole- known inhibitors of testosterone biosynthesis).
(One member stated that this study should only be performed if #3 above does not show a definitive decrease in testosterone levels, and an increased serum LH).

Another member of the panel commented that it is NOT necessary to demonstrate an "elevation in LH", but it is necessary to demonstrate that testosterone biosynthesis is altered in vivo (as was demonstrated in vitro). For this, an ex vivo (animals exposed to in vivo, tissue evaluated after necropsy) approach is recommended. Short term sampling of both testicular interstitial fluid testosterone and LH-stimulated parenchymal testosterone production will determine whether: 1) there is less testosterone in the interstitial compartment of treated animals, and 2) whether such a reduction, if observed, is concomitant with decreased responsivity of Leydig cells to LH."

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ATTACHEMENT 2

AGENCY'S EXTERNAL PEER REVIEWS

ATTACHMENT 2.A

Evaluation of Iprodione - Risk Assessment

Question # 1 **Adequacy of the carcinogenicity studies**

The studies in both rats and mice identified carcinogenic effects at the dose levels utilized. Dose levels were adequate for male and female rats and male mice. A no effect level for non-neoplastic findings was not achieved for female mice

Question # 2 **Does Iprodione represent a genotoxic hazard.**

Mutagenicity studies and the carcinogenicity studies favor a non-genotoxic mechanism for both rats and mice.

Question #3 **Mechanistic Considerations**

The data presented favors the modulation of steroidogenesis resulting in the inhibition of testosterone biosynthesis and increased LH levels. Determination of LH levels at mid dose levels (300 ppm or lower) for rats would contribute to the information base.

Liver Tumors in Mice

The demonstration of centri-lobular hepatic hypertrophy associated with increased levels of p450 (rats) suggest growth enzyme promotion. The generalized vacuolization hypertrophy of the interstitial cells of the testes at mid and high dose levels (mice) and the increased luteinization of interstitial cells of the ovary at all dose levels of the chronic study strongly suggests a disruption of hormonal hemostasis. Persistent growth stimulation would be expected to induce hepatic neoplasia in mice at high (over threshold) doses.

Question # 4 **Relevance to Humans**

In addition to the consideration of carcinogenicity, possible effects of endocrine disruption agents on human (and animal) reproduction should be another point of consideration. The EPA evaluates each chemical on its merits without considering potential consequences or impact of naturally occurring hormone disrupting agents (example, phytoestrogens) and other already approved pesticides. In review of the data package there was no evidence of a multigeneration reproductive study. These data would also be important prior to a final decision on this chemical.

Question # 5 **Linear or nonlinear cancer risks**

The data strongly favors a nonlinear risk. The toxicity endpoints would correspond to the dose level without morphologic evidence of Leydig cell hyperplasia (rats and mice) or luteinization of interstitial cells of the ovary (female mice).

ATTACHMENT 2B (Dr. B. Zirkin)

JOHNS HOPKINS
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Department of Population Dynamics
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Division of Reproductive Biology

November 4, 1997

Dr. Henry Spencer
SAB/HED/OPP (CM-2) (H7509C)
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Dr. Spencer:

The following summarizes my review of the data that I was sent on the fungicide Iprodione. As you know, my expertise is in male reproduction. I have focused my comments in areas in which I can be of help.

Question #1.

For chronic studies in the rat, Iprodione was fed to male Sprague-Dawley rats at levels from 6.1 to 69 mg/kg/day for two years, A two-year study of Sprague-Dawley rats represents an adequate chronic study for the detection of carcinogenicity in this strain, At 69 mg/kg/day, the highest dose, decreases were observed in body weight gain and food consumption Data resulting from the use of even higher doses, administered long-term, could not easily have been interpreted given the generalized toxicity that would be expected.

Interstitial poll tumors were reported at the high dose, and interstitial cell hyperplasia, along with reduced spermatozoa in the epididymides, reduced secretion of the seminal vesicles, and increased liver weight, were seen at lower doses. However, these data are difficult to relate to human disease for several- reasons: first, the doses used resulted in systemic toxicity; second among the doses used, none was without effect; third, the rat is well known to be far more sensitive to Leydig cell tumorigenic agents than is the human; and fourth, it is not possible, given the data, to relate the doses that had effect on Leydig cells to human exposure.

Question #2

With respect to the Leydig cell there is insufficient data to conclude that a hormonal mechanism causes interstitial cell tumors in response to Iprodione. In part for this reason, the possibility that Iprodione presents a genotoxic. hazard remains an open one. I saw no compelling evidence to indicate that this is the case, however.

Question 3.

A number of mechanistic studies have been performed, both in vivo and in vitro. For *in vivo* studies, rats were fed a relatively high dose of Iprodione for 2, 7 or 14 days, and then challenged with a tail vein injection of hCG. The data indicate no effect of Iprodione on plasma or testicular homogenate testosterone concentrations; and consistent with this, minimal effects on accessory sex organ weights. (It should be noted that the reported control plasma, testosterone level, about 3 ng/ml, [p. 19, Final Report, "Endocrine Toxicology Studies...... In Vivo] is what others have reported, but that the reported testicular homogenate concentration of testosterone about 15 ng/ml, is very low compared to what others report: (Most workers report 50-70 ng/ml or higher, depending upon the methods used and the intratesticular fluids that are sampled. The reason for the low values is not apparent). Additionally, receptor binding studies with prostate tissue suggest that Iprodione's actions are not mediated via androgen receptor antagonism; and indeed, Iprodione has been shown to have low affinity for the AR. LH levels did rise, however, for reasons that apparently cannot be explained by reduced serum levels of testosterone. This is important, as chronic elevation of LH can lead to hyperplasia/adenoma.

In contrast to the lack of *in vivo* effects, Leydig cell culture in the presence of Iprodione apparently has an effect on testosterone production *in vitro.* Studies have demonstrated Iprodione inhibition of testosterone production by hCG-stimulated porcine Leydig cells cultured for 72 hours, and that removal of Iprodione from the culture medium resulted in recovery of the ability of the cells to produce testosterone. (Note: The amount of testosterone produced by these cells is extremely low compared to what rat Leydig cells produce, by about two orders of magnitude. My suspicion is that this has far more to do with the culture methods than to species differences.) These *in vitro* results using Leydig cells were consistent with the results of in vitro exposure of rats testis sections to Iprodione. The in vitro results were in striking contrast to the failure of Iprodione to produce effects on serum or intratesticular testosterone levels when administered *in vivo* or on the ability of testis sections to produce testosterone in response to hCG when Iprodione was administered *in vivo. Unfortunately, it is very difficult to relate the in vivo and in vitro results because the in vivo studies did not measure Iprodione in interstitial fluid the site of the Leydig cells.*

There have been a number of studies that have examined the possible mechanism by which Iprodione effects reduced testosterone production by Leydig cells in vitro. The major finding such studies is that the inhibition is downstream from CAMP production, as no effects were seen of Iprodione on hCG stimulated CAMP production . When porcine Leydig cells were incubated with 220h---cholesterol, inhibition by Iprodione was not seen, suggesting that the Iprodione-induced defect was in the ability of exposed Leydig cells to transport cholesterol to the inner mitochondrial membrane. This provides a plausible explanation for reduced testosterone production, but, of, course, does not explain Leydig cell tumor formation.

The issue of how Iprodione might cause tumor development remains open. Most Leydig cell tumorigenic agents that have relevance to the human act by increasing LB levels by one mechanism or another, including antagonism of the androgen receptor, inhibition of testosterone production via direct effects on the Leydig cell or indirect effects via paracrine factors from the test, inhibition of 5a-reductase, inhibition of aromatase, or estrogen agonism. LH levels are reported to increase following Iprodione, but there apparently is no *in vivo* effect of Iprodione on

testosterone production. This suggests an *in* vivo compensatory mechanism of some kind with respect to testosterone production. Based upon the studies of a number Of chemicals, Leydig cell proliferation in response to Iprodione might well be caused by the mitogenic effects of elevated LH. To demonstrate whether or not this is the case, greater attention might be paid to measuring LH more rigorously (serial sampling with more sensitive measurements such as (imunofluorometry), and perhaps to determining Leydig cell numbers in elevated LH (by stereological methods). Intratesticular testosterone measurement which indicated above, seem very low, also might be made more rigorously. Finally, it might be worthwhile to provide data that distinguishes between Leydig cell hyperplasia and nodules. The reason I say this is that the diagnosis of Leydig cell hyperplasia is difficult if there is simultaneous shrinkage of the seminiferous tubules; when the latter occurs, Leydig cells occupy a greater volume percent of the testis, and this can easily be mistaken as hyperplasia. Stereological analyses at the level of the light microscope might well be required for the unequivocal identification of hyperplasia. Nodules, on the other hand, can be distinguished readily, particularly when they become extensive.

Question #4.

This question is related to Question #3, and in particular to the issue of whether or not additional data are necessary to perform a risk assessment on Iprodione. Chronic administration of Iprodione apparently resulted in hyperplasia and, depending on dose, in benign tumors. With the knowledge that the human is far less sensitive than the rat to agents that induce Leydig cell adenomas . This raises the question of whether Leydig cell hyperplasia or adenomas in the rat are of concern: the human. If the potential exists for high exposure in the human (I don t know whether or not this is the case), it makes sense to be cautious, increased tumor incidence in the rat, even if benign, should be considered a potential adverse reproductive effect for the human given that there is little knowledge of the mode of induction of these tumors in rat and therefore of their relevance to the human.

Question #5.

If induced hormonally, there should be an identifiable relationship between elevated LH and the progressive occurrence of hyperplasia/adenomas over tim. As I am not an expert in risk assessment, however, I an unable to provide insight on how the data at hand should be analyzed for this purpose.

I hope this external review proves of some value to you and your colleagues. Thank you for the opportunity to review the data.

Sincerely,

Barry K Zirkin, Ph.D
Professor and Head
Division of Reproductive Biology

ATTACHMENT 2.C (Dr. Foster)

RESPONSE TO QUESTIONS POSED BY, HEALTH EFFECTS DIVISION (OPP)

Question # 1

Are the chronic/carcinogenicity, studies adequate to define the carcinogenicity - of Iprodione? Where the dose levels studied appropriate?

Response

The answer to both these questions is YES. Dose levels employed on these studies did produce the requisite degree of systemic toxicity at the highest dose tested and the Agency has agreed that a NOAEL for carcinogenesis was achieved. Clearly the presence of non-neoplastic lesions in the rat study at the lowest dose level tested is another issue outside the scope of this review.

One major factor that could warrant a re-examination of some of the data by the Agency is with regard to the testicular Leydig cell tumor incidence. It is that the incidence of tumors in the low and mid dose levels in the rat bioassay was higher than both the concurrent and historical control values, although this did not achieve statistical significance. There has been considerable debate over the appropriate criteria used for the diagnosis of a Leydig cell tumor (is this equivalent to 1- or 3 seminiferous tubule diameters). I would assume that the 3 seminiferous tubule diameter criterion was applied here.

It is clearly apparent that morphologically, the only difference between a Leydig cell hyperplastic nodule and a tumor is one of **SIZE.** It could therefore be argued, that for risk assessment purposes it would be appropriate to examine the Leydig call tumor and hyperplasia data together. Normally, only one section is examined per testis on a study of this type and the diagnosis of tumor, or hyperplastic nodule, is totally dependent on where the section was taken from the blocked tissue (i. e. a section through the "tip" of a tumor that was 1-2 seminiferous tubules in diameter, would be classified as a hyperplastic nodule).

Question # 2.

Does Iprodione represent a genotoxic hazard ?

Response

The answer to this question is **NO.** A weakness in the, database was the lack of a valid, in vivo genotoxicity assessment. This has now been rectified. with the provision of a mouse micronucleus assay. This valid study, clearly indicated a lack of genotoxic response with Iprodione even at high, systemically toxic, dose levels. The one weakly positive response in B. Subtilis, in a poor study should be ignored for, risk assessment purposes. The database now reliably indicates that this compound does not represent a genotoxic hazards,

Question #3.

Do the mechanistic data, demonstrate a mechanism mode of action for the development of the benign male rat Leydig cell tumors, and the male and female mouse liver tumors (benign and malignant)?

If the data are not adequate, what further data would be needed to demonstrated a mechanism or mode of action for such tumor development?

Are these additional data necessary to perform a risk assessment on Iprodione?

Response

The data supplied do yield information regarding mechanism and mode of action, but in both cases the degree of information supplied is inadequate.

Leydig cell tumor mechanism

The registrants provide in vitro data to support the notion that inhibition of testosterone production. is the initial event in the process of disturbing sex steroid metabolism and producing the ensuing Leydig cell tumor response. This is a plausible mechanism but the data for the compound supporting this mechanism are not adequate.

The primary issue is, where is the effect on testosterone *in vivo* ? And where is the sustained elevation in "LH challenge" proposed by the registrant, when hyperplasia was not noted at 1 year ?
The data that the registrants have supplied may still be consistent with their hypothesis, but there is a requirement for more information. For example, intratesticular concentrations of testosterone are significantly higher than those found in the peripheral circulation of rats (some estimates indicate up to 1 0 fold higher). It is therefore not unreasonable to suggest that a significant effect on intratesticular levels (as measured in interstitial fluid, IF) of testosterone could be produced by compound administration: (as indicated by the in vitro data), but have no effect on those levels noted in the periphery (as noted in *the-in vivo* studies). The, effects on Leydig cell function and -proliferation could therefore be due to a paracrine instead of, or in addition to, an endocrine pathway, via. LH, since a sustained effect on LH levels *in vivo* was not noted. Measurement of IF levels after compound treatment, at different dose levels (including those used in the bioassay) for different time periods would then provide direct evidence of an *in vivo* effect on testosterone biosynthesis.

one problem with all of the *in vitro* studies reported is that the reviewer cannot "place"the in vitro concentrations used into context with *in vivo* exposure, What are the concentrations of the test article (± metabolites) at the target tissues the appropriate dose levels producing an *in vivo* response? Are any of the metabolites present at sufficient concentrations to elicit a response *in*

vivo ? The registrants should be clear in the understanding that the use of testicular homogenate, or testicular sections *in vitro* evaluates both the biosynthesis of testosterone (by Leydig cells) and utilization (by peritubular and germ cells and sequestration by androgen binding protein). The use, of isolated Leydig cells reflects a "cleaner"' system for evaluation of potential effects on biosynthesis.

Why did the registrant undertake such elegant work in pig Leydig cells, when the Leydig cell tumors are in rats? Purified rat Leydig cells are easy to obtain an undertake the exact same studies. Moreover, the registrants, should be aware that, in the rat, steroidogenesis proceeds through the delta-four pathway, via progesterone and hydroxyprogesterone, and not the delta-five pathway used in the pig, via pregnenolone and hydroxypregneneolone. Although the same enzyme are involved in the process, the differences in the substrate may be important in ascribing the precise site of action within the Leydig cell.

If rat *in vitro* data (with relevance of concentrations used to *in vivo* exposure) were available, it would be much easier to relate this to potential effects in this species *in vivo*. The registrants may also wish to consider going back to the interim kill on the rat study and examining more testicular sections to determine if hyperplasia was evident earlier than the terminal, kill on the chronic bioassay.

Some laboratories (albeit very few) are able to undertake in vitro studies with human testicular material, or isolated Leydig cells. If these data were available, then the parallelogram approach could be undertaken i.e. compare rat *in vivo* with rat *in vitro* data and then if there is concordance, compare rat *in vitro* With human in vitro data. If the rat in vivo data did reveal a response on testosterone biosynthesis, it would be interesting to understand the relationship to the adrenal and ovarian effects noted (although this is outside of the response requested by the Agency).

Liver tumor mechanism

Similar flaws could also be. ascribed to the mechanistic studies regarding liver growth and tumors. The data available are consistent with the hypothesis proposed by the registrant, but they are insufficient. In particular, there is a need to equate the dose levels used in the 14 day mechanistic study, with those used on the mouse bioassay. An extended period of exposure would also be of benefit to examine whether short term increases in hepatocyte proliferation are sustained over a longer term and whether there is (or not) a second wave of proliferation related to tumor formation. The registrants should consider addressing a number of questions including: What would be the NOAEL for hepatocyte proliferation? Is cell proliferation observable at the dose levels used in the mouse bioassay ?

Necessity of additional mechanistic data

The Agency has, in the past and can continue to, undertaken risk assessments for carcinogenesis without mechanistic information. It could therefore be argued, that these data are **NOT** strictly necessary to undertake the carcinogenesis risk assessment for Iprodione. However, the major

thrust of the Agency's proposed risk assessment guidelines is to incorporate the APPROPRIATE mechanistic information to provide a more scientifically based risk assessment process. To meet these goals, I believe that the additional data, indicated above, would allow the Agency to place the mechanistic data into a proper perspective for risk assessment purposes.

The Agency has used the structural similarities of Iprodione to Flutamide. and Vinclozolin as part of its risk assessment weight of evidence. Based on the data made available, this is inappropriate, since both of flutamide and vinclozolin (and its metabolites) produce their effects by androgen receptor (AR) mediate mechanisms. This is, not the case with Iprodione, where AR binding was not observed. This could be further clarified by knowing testicular concentrations of Iprodione (and its relevant metabolites) at dose levels used in the bioassay and making direct comparisons with AR binding affinity. At this time, these "structure-activity" relationships should not be used in the risk assessment process.

Question # 4.

What is the relevance to humans of hormonally mediated rat Leydig cell hyperplasia/adenoma formation and the mouse liver tumors?

Response

Leydig, cell **tumors,**

If the registrant's hypothesis that inhibition of testosterone biosynthesis is the initial event in the production of rat Leydig cell tumors, then this particular mechanism is of relevance to humans (see arguments made in Clegg *at al,* 1997, Reprod Tox 1 1 107121). Ketoconazole, the positive control used in the *in vitro* studies with Iprodione, has been clinically used as an anti-androgen and does produce hormonal changes in testosterone biosynthesis in humans, although there have been no reports of Leydig cell tumors with this agent. The major consideration then becomes as to whether humans are ever exposed to levels of the agent sufficient to produce such an effect in humans. Epidemiological evidence (although very poor) would' suggest that rats are more sensitive than humans and that as a high dose level was required to produce such effects in rats, then the risk to humans should be low. A major point that should be noted with regard to human Leydig cell hyperplasia and tumors are that these rare conditions are very frequently associated with a hormonal imbalance that provides other symptoms (e.g.. gynecomastia) or can be malignant. We do not have information on potential human Leydig cell hyperplasia and benign tumors where such changes are not evident. Thus there is likely to be a significant under reporting of Leydig cell hyperplasia and tumors in humans, as the same diagnostic criteria used in rat studies are not applied. The data supplied for Iprodione in the rat are consistent with a number of other agents where significant hormonal change's *in vivo* are difficult to detect and reliance is made on pathological descriptions. The likelihood of a benign Leydig cell adenoma, which does not produce other hormonal symptoms, appearing in a cancer or death registry is remote. Similarly malignant rat Leydig cell tumors are also very rare as is the case for humans.

Liver tumors

The argument to be made here is that the liver tumors due to Iprodione exposure would only occur at high dose levels sufficient to produce hepatomegaly as a precursor response. Liver tumors are relevant to man *per se,* but serious question marks would be indicated with regard to relevance, if unrealistic (to human exposure), high, dose levels (at the MTD) are required to induce such effects in rodents. Thus, the liver tumors observed should be considered a high dose phenomenon particularly (as is the case with Leydig cell tumors) when accompanied by information that indicate that a genotoxic mechanism is not involved in tumor production.

Question #:5.

Hypothetically, if one were to characterize the cancer risk for Iprodione given, the present information available on this chemical, which of the two methods (linear or non-linear) would be supported by the data?

If non-linear risk assessment were used, what would be the appropriate, toxicity end point?

Response-

I would characterize the cancer risk based on the available data as non-linear for both tumor types. Both tumor types indicate high dose level responses in rodents that show clear NOAEL's and would be more appropriately described by non-linear. dose responses.

For Leydig cell tumors it could hypothetically be argued that data on hyperplasia + tumors would be an appropriate toxicity end point (if *in vivo* testosterone data were available, this might also. be considered)

For liver tumors, it could hypothetically be argued that data on Cell proliferation rates (if over a wider dose and time range) could be used in addition to the tumor incidence data.