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

SEP 14 1996

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP 7F03530, Imazalil, Adequacy of Submitted Data with Regard to Second Carcinogenicity Peer Review

DP Barcode D225180
Case 260384
Submission S493632

Tox. Chem. No. 497AB
PC Code 111901

FROM: Edwin R. Budd, Acting Section Head
Review Section III, Toxicology Branch I
Health Effects Division (7509C)

Budd 9/6/96

TO: Steve Robbins
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

THRU: Karl Baetcke, Ph.D., Chief
Toxicology Branch I
Health Effects Division (7509C)

9/6/96

cc: James Stone/Cynthia Giles-Parker
Product Manager Team 22
Registration Division (7505C)

Action Requested

Examine information and data submitted by Janssen Pharmaceutica since the first carcinogenicity peer review meeting on Imazalil and advise Registration Division as to its adequacy with regard to scheduling a second carcinogenicity peer review meeting.

Conclusions

Following a thorough consideration of the information and data submitted by Janssen Pharmaceutica, it has been determined that there is insufficient justification for scheduling a second carcinogenicity peer review meeting at this time. This decision was reached at an ad hoc meeting of HED staff members on August

13, 1996 attended by Karl Baetcke, Ph.D. and Edwin Budd of Toxicology Branch I/HED and by William Burnam and Esther Rinde, Ph.D. of Science Analysis Branch/HED. This decision was based on the following conclusions.

1) It was concluded by the meeting participants that the Pathology Working Group (PWG), which met on May 23, 1995 to re-evaluate selected liver slides from the 1993 mouse carcinogenicity study on Imazalil (MRID 429720-01) and to evaluate selected liver slides from a historical control study, did not adhere to the provisions of Pesticide Regulation (PR) Notice 94-5, which sets forth procedural requirements to be followed by registrants for submission of re-evaluations of pathology readings to the Agency. Hence, the re-evaluation of liver slides by the PWG is not acceptable. The rationale for this decision is provided later in this memorandum. Consequently, the prior evaluation of the 1993 mouse carcinogenicity study by the HED Carcinogenicity Peer Review Committee (CPRC) will remain unchanged.

2) It was also concluded by the meeting participants that Janssen Pharmaceutica did not adequately justify the dose levels used in the 1985 rat carcinogenicity study (MRID 00162413) in which the highest dose level was 400 ppm. This was suggested by the CPRC as a possible alternative to the requirement to conduct a new 2-year chronic feeding/ carcinogenicity study in rats at higher dose levels. Since the carcinogenicity peer review meeting, Janssen Pharmaceutica submitted two new 3-month dose-range finding studies in rats. One study (MRID 437350-05, 439657-04) used dose levels up to 800 ppm. The other study (MRID 439657-05) used dose levels up to 3200 ppm in a different substrain of rat (Hannover) that was subsequently proposed to be used in a new 2-year chronic feeding/carcinogenicity in rats. Effects observed in the first study at the highest dose level (800 ppm) were considered to be of insufficient seriousness to justify the highest dose level (400 ppm) used in the previously conducted 1985 rat carcinogenicity study. More detailed information on this study is provided later in this memorandum. Therefore, a new 2-year chronic feeding/ carcinogenicity study in rats at higher dose levels will be required to support the continued registration and reregistration of Imazalil. Based on effects observed in the second study, Toxicology Branch I concurs with Janssen Pharmaceutica that an appropriate highest dose level for a new 2-year rat carcinogenicity study would be 2400 ppm. This decision was reached in response to a separate submission (DP Barcode D225939) in which Janssen Pharmaceutica requested Agency comments on protocols for a new 2-year chronic feeding/carcinogenicity study on rats of the Hannover substrain and for a "historical control" study

to be conducted concurrently. Toxicology Branch I will comment in more detail on these proposed protocols in a separate memorandum.

3) The meeting participants further concluded that a full data base, including results of a new 2-year chronic feeding/ carcinogenicity study in rats, would be required before convening a second carcinogenicity peer review meeting on Imazalil. The lack of an acceptable rat carcinogenicity study was considered by the meeting participants to be a critical data gap with respect to the evaluation of the carcinogenic potential of Imazalil, particularly since positive findings had already been observed in mice and significant toxicological concerns had arisen in the evaluation of long-term rat studies for certain other structurally related chemicals.

4) To fulfill a data gap in the mutagenicity battery, the CPRC previously concluded that "an acceptable in vivo/in vitro unscheduled DNA synthesis (UDS) study with liver accompanied with a cell proliferation (S-phase) study" should be submitted. The UDS study was required, but the S-phase cell proliferation assay was only recommended. Both studies have now been received by the Agency and are acceptable. DERs for these studies will be provided in a separate memorandum.

Concurrence

Individuals present at the ad hoc HED staff meeting on August 13, 1996. Signature indicates concurrence with the above Conclusions.

Karl Baetcke, Ph.D.
Chief, Toxicology Branch I

William Burnam
Chief, Science Analysis Branch

Esther Rinde, Ph.D.
Manager, Carcinogenicity Peer Review Committee

Edwin Budd
Toxicology Branch I

Edwin Budd 9/6/96

DETAILED REVIEW

Background

The CPRC, which met on August 24, 1994 (first meeting), classified Imazalil as a Group C--possible human carcinogen--with a Q_1^* (see memo of meeting dated December 27, 1994). This classification was based primarily on results in a 1993 mouse carcinogenicity study (MRID 429720-01) in which statistically significant increases in hepatocellular adenomas and combined adenomas/carcinomas were observed in male mice. In addition, an increase in hepatocellular carcinomas in male mice, although not statistically significant, was considerered nevertheless to be biologically significant. Further, the incidence of carcinomas in male mice exceeded that of the historical control data submitted by the registrant. Positive trends for adenomas, carcinomas and combined adenomas/carcinomas were also observed in male mice. The presence of positive trends for hepatocellular adenomas and combined adenomas/carcinomas in female mice and SAR data provided additional support for the classification.

Treatment-related tumors were not observed in rats, but in the most relevant study (1985, MRID 00162413), the CPRC concluded that the highest dose level tested in that study (400 ppm) was inadequate and required the registrant to submit a new 2-year chronic feeding/carcinogenicity study in rats at higher dose levels "unless the registrant could properly justify the doses which have been used in the previous rat studies."

Mutagenicicty studies were negative for genotoxic effects, but the CPRC noted a data gap in the battery and concluded that the registrant should submit "an acceptable in vivo/in vitro unscheduled DNA synthesis (UDS) study with liver accompanied with a cell proliferation (S-phase) study." The UDS study was required, but the S-phase cell proliferation assay was only recommended.

The CPRC stated that when the additional data are submitted and reviewed, the classification of imazalil would be re-evaluated.

Note: $Q_1^* = 6.20 \times 10^{-2}$ $(mg/kg/day)^{-1}$ (B. Fisher; SACB/HED; March 7, 1995)

New Information and Data

Following the first carcinogenicity peer review of Imazalil, Janssen Pharmaceutica submitted the information and data listed below:

Regarding the 1993 carcinogenicity study in mice (MRID 429720-01)

Pathology Working Group (PWG) Report on Imazalil Liver Histopathology in Mice: Supplement No. 2 to MRID 429720-01, Report No. 2194, June 1995 (DP Barcode D218208, MRID 437350-02).

Regarding the adequacy of the dose levels in the 1985 carcinogenicity study in rats (MRID 00162413)

Justification of the Doses Used in the Rat Carcinogenicity Studies With Imazalil: Supplement to MRID 00162413, April 19, 1995 (DP Barcode D218208, MRID 437350-04).

Three-Month Dose Range Finding (DRF) Toxicity Study in SPF Wistar Rats, Interim Report After 1 Month, Experiment No. 3514, June 6, 1995 (DP Barcode D218208, MRID 437350-05)

Study on the Possible Induction and/or Inhibition of Hepatic Drug Metabolizing Enzymes by Imazalil in Male and Female SPF Wistar Rats After Oral Administration Through the Diet for One Month at Imazalil Levels of 200, 400 and 800 ppm, Interim Report, Report No. R023979/FK1960, May 1995 (DP Barcode D218208, MRID 437350-06).

Three-Month Oral Dose Range Finding & Mechanistic Toxicity Study with One Month Interim Sacrifice in SPF Wistar Rats, Final Report, Report No. 3514, March 13, 1996 (DP Barcode D224886, MRID 439657-04).

Study on the Possible Induction and/or Inhibition of Hepatic Drug Metabolizing Enzymes by Imazalil in Male and Female SPF Wistar Rats After Oral Administration Through the Diet for One or Three Consecutive Months at Levels of 200, 400 and 800 ppm, Final Report, Report No. R023979/FK1960, October 19, 1995 (DP Barcode D218208, MRID 437350-06).

Dose-Range Finding Study for Proposed New 2-Year Chronic Feeding/Carcinogenicity Study in Rats (at Higher Dose Levels)

Three (3) Month Dose Range Finding and Mechanistic Toxicity Study in SPF Wistar Rats, Report No. 3672, March 13, 1996 (DP Barcode D224886, MRID 439657-05).

Study on the Possible Induction and/or Inhibition of Hepatic Drug Metabolizing Enzymes by Imazalil in Male and Female SPF Wistar Rats After Oral Administration Through the Diet for Three Months at Levels of 800, 1,600 and 2,400 and 3,200 ppm, Final Report, Report No. R023979/FK2060, March 12, 1996 (DP Barcode D224886, MRID 439657-05).

Toxicokinetics of Imazalil (R023979)in SPF Wistar Rats at the End of a Three-Month Oral Dose-Range-Finding and Mechanistic Toxicity Study (Exp. No. 3672) with an Imazalil-Medicated Diet at Intended Dose Levels of 80, 160, 240 and 320 mg/kg/day, Final Report, Report No. R023979/FK2057, March 13, 1996 (DP Barcode D224886, MRID 439657-05).

Regarding the required/recommended mutagenicity studies

Measurement of Unscheduled DNA Synthesis and Replicative DNA Synthesis in Mouse Liver Using an in vivo/in vitro Procedure, Corning Hazleton Report No. 1073/3-1052, February, 1996 (DP Barcode D224886, MRID 439657-02).

Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro with Imazalil. CCR Project 192600, Janssen Report No. 920, August 20, 1990 (DP Barcode D219216, MRID 437802-01).

In vitro Mammalian Gene Mutation Assay, Report No. 3470; April 27, 1995 (DP Barcode D218208, MRID 437350-03).

Additional information submitted by Janssen Pharmaceutica

Relevance of Liver Tumor Formation in Mice, Report No. PPD-4, March 30, 1994 (DP Barcode D203268, MRID 432024-01)
Note--This document was submitted and reviewed prior to the first carcinogenicity peer review meeting on Imazalil.

Relevance of Liver Tumor Formation in Mice: Supplement to MRID 432024-01, Report No. PPD-4, July 5, 1995 (DP Barcode D218208, MRID 437350-01).

Relevance of Liver Tumor Formation in Mice: Supplement to MRID 432024-01 & Related MRID 437350-01, Report No. PPD-4, February 20, 1996 (DP Barcode D224886, MRID 439657-03).

An examination of the information and data submitted by Janssen Pharmaceutica indicates that it is complete with respect to the additional data required by the CPRC at its first meeting on August 24, 1994. A review and evaluation of the submitted information and data will be included in separate memoranda which will be prepared for each of the DP Barcodes under which the new data was submitted.

Discussion

Regarding the Acceptability of the PWG Report on Imazalil (MRID 437350-02)

Chronology and Description of Events

The initial reading of the liver slides in the 1993 mouse carcinogenicity study was made by Dr. Vandenberghe of the Janssen Research Foundation. He classified the neoplastic liver lesions in this study as hepatic neoplastic nodules or as hepatic carcinomas. He, therefore, utilized nomenclature and diagnostic criteria that are different than that used by the Agency. Subsequently, at the request of the study sponsor, Dr. Sparrow of Pharmaco LSR, made a second independent reading of all the liver slides in the study. Dr. Sparrow, however, utilized nomenclature and diagnostic criteria that are consistent with that used by the Agency. The neoplastic liver lesions in the study were classified by Dr. Sparrow as hepatic adenomas or as hepatic carcinomas. The pathology reports of both Dr. Vandenberghe and Dr. Sparrow were included in the initial study report (MRID 429720-01).

A comparison of the numbers of hepatic carcinomas reported by both pathologists in the treatment and control groups indicated only minor differences. Dr. Sparrow, however, reported considerably fewer hepatic adenomas than Dr. Vandenberghe reported hepatic neoplastic nodules. The reason for this difference was attributed to the inclusion of some lesions by Dr. Vandenberghe as neoplastic nodules that were classified by Dr. Sparrow as "hepatocellular hyperplasia" or as "foci of alteration (basophilic or eosinophilic)". Since the classification scheme used by Dr. Sparrow was consistent with that used by the Agency, his results were used by the CPRC when it met on August 24, 1994 (at the recommendation of Dr. Lucas Brennecke, pathology consultant to HED).

Subsequent to the meeting of the CPRC, Janssen Research Foundation, on its own initiative, convened a Pathology Working Group (PWG) on May 23, 1995 in Philadelphia, PA "to evaluate the hepatic carcinomas [underlining added] reported in the 2 year mouse carcinogenicity study (Exp. 2194) on the fungicide Imazalil" (PWG Report, p. 5). Further, it was also stated in the PWG report that "Janssen Research Foundation decided to hold a PWG in order to confirm the diagnosis of hepatocarcinoma [underlining added] which showed an apparent increase in the male high dose group and of concern to the EPA. The incidence of adenomas showed a compound related increase" (PWG Report, p. 6).

The PWG was composed of the following five pathologists: Dr. W. H. Butler (Chairman, BIBRA International, Surrey, England), Dr.

W. R. Brown (Research Pathology Services Inc.), Dr. R. A. Squire (Baltimore, MD), Dr. S. Sparrow (Pharmaco LSR, Eye, England), and Dr. J. J. K. Vandenberghe (Janssen Research Foundation).

The selection of specific liver sections to be examined by the PWG was described in the PWG report as shown below.

For the male mice--"All sections from male mice in which a diagnosis of hepatocarcinoma [underlining added] had been made by either Dr. Vandenberghe or Dr. Sparrow in any group, and an additional control mouse reported as adenoma but considered uncertain, were blinded and randomly numbered. All the blinded slides were examined individually by the Group and the individual diagnosis recorded. The consensus opinion was then recorded" (PWG Report, p. 6).

For the female mice--"The livers of female mice in which a diagnosis of hepatic neoplastic nodule (HNN) by Janssen Research Foundation, adenoma (Dr. Sparrow, peer review) or carcinoma had been made were examined without blinding by the PWG but the members of the PWG were not aware of the previous diagnoses" (PWG Report, p. 6). The consensus incidence of carcinomas and adenomas was recorded.

Regarding historical control data--"Only 1 comparable study was available from Janssen Research Foundation (Exp. 2166). The male and female control mice from this study in which a diagnosis of neoplasia (adenomas or carcinomas) had been made were examined by the Group. These slides were not blinded as only 1 group was examined but the PWG was not aware of the individual diagnoses. In the male control group 8 carcinomas and 5 adenomas were recorded" (PWG Report, p. 7). The consensus incidence of carcinomas and adenomas was recorded.

Non-Compliance with Pesticide Regulation (PR) Notice 94-5

PR Notice 94-5 describes the procedural requirements to be followed by registrants for submission of pathology re-evaluations to the Agency. A copy of PR Notice 94-5 is attached to this memorandum (see Attachment #1). The notice makes reference to a study pathologist (Dr. Vandenberghe), a peer review pathologist (Dr. Sparrow) and the specific procedures to be followed by the PWG to resolve significant differences in diagnoses between them. Regarding the selection of slides to be examined by the PWG, PR Notice 94-5 states that "the PWG will review, as a minimum, all [underlining added] slides about which there were significantly differing diagnoses between the study and peer review pathologists."

For the male mice in this study, the PWG did not review, as required by the notice, all slides for which there were

significantly differing diagnoses between the two pathologists, but rather only those slides in which a diagnosis of hepatocarcinoma had been made by either Dr. Vandenberghe or Dr. Sparrow in any group. Slides with lesions described as hepatic neoplastic nodules (by Dr. Vandenberghe) or as hepatic adenomas, hepatocellular hyperplasia, or foci of alteration (basophilic or eosinophilic) (by Dr. Sparrow) were not reviewed by the PWG unless the other pathologist diagnosed the same slide as hepatic carcinoma. The participants of the ad hoc HED meeting (on August 13, 1996) concluded that this failure to examine all the slides with "neoplastic" or "pre-neoplastic" lesions in the male mice in this study, as required by PR Notice 94-5, was a sufficiently serious breach of the procedural requirements to justify not accepting the PWG report.

In discussing the PWG report, the ad hoc meeting participants noted that for the male mice in this study, six mice (Nos. 102M, 126M, 167H, 62L, 129M, 165H) were previously diagnosed by both Dr. Vandenberghe and Dr. Sparrow as having hepatic carcinoma, whereas the PWG later reclassified these same mice as have hepatic adenoma (PWG Report, pp. 8-10). Considering the demonstrated capacity of the PWG to reach a consensus classification different from that of both the study pathologist and the peer review pathologist, the HED meeting participants felt that if the PWG had the opportunity to review the additional "neoplastic" and "pre-neoplastic" slides not diagnosed as hepatic carcinomas by either pathologist that some of these slides might possibly have been classified as hepatic carcinomas by the PWG.

The ad hoc meeting participants also considered the comments of Dr. Lucas Brennecke, pathology consultant to HED, on the acceptability of the PWG Report. His comments are presented in two memoranda, one dated July 2, 1996 and the other August 14, 1996. Both of his memoranda are attached to this memorandum (see Attachments #2 and #3). He stated in the August 14, 1996 memorandum that "it ... appears that the provisions of PR Notice 94 were not met, since the PWG did not review '...as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists'" , and later in the same memorandum, "with regard to the male mice, it is ... my recommendation that the CPRC should not accept the PWG's findings until it is documented that a properly conducted PWG was conducted."

It is noted for the female mice in this study that the PWG apparently did adhere to the provisions of PR Notice 94-5 in that the livers of all female mice in which a diagnosis of hepatic neoplastic nodule (by Dr Vandenberghe), adenoma (by Dr. Sparrow) or carcinoma (by either pathologist) was made were, in fact, examined by the PWG.

Regarding the review of the historical control data by the PWG, Dr Brennecke stated the following in his memorandum of July 2, 1996: "With regard to the review of the control data, I believe that that review was flawed as well. It appears that the study had not been peer reviewed, and only the animals having neoplastic diagnoses were reviewed. It is my opinion that if significant weight is to be placed on the results of that single study, then it should have been subjected to the same review process as is outlined in PR Notice 94. The incidences of carcinomas and adenomas in the control group appear to be significantly higher than published data from Charles River. I recommend that little weight be given to the data from the single control study, at least until it has been properly reviewed." The ad hoc HED meeting participants concurred with the recommendation of Dr. Brennecke.

Regarding the 3-Month Oral Dose Range Finding Study in Rats, Final Report (MRID 439657-04)

Citation

Van Deun, K. (1996) Imazalil:R023979: Three-month oral dose range finding and mechanistic toxicity study with one month interim sacrifice in SPF Wistar rats, Final Report. Janssen Research Foundation, Beerse, Belgium. Experiment No. 3514. March 13, 1996. MRID 439657-04. Unpublished. (Sponsor: Janssen Pharmaceutica N.V.)

Summary of Study

In a three-month feeding study, technical grade Imazalil base was administered in the diet to groups of 20 male and 20 female SPF Wistar rats (supplied by Charles River, Germany) at dose levels of 0, 200, 400 or 800 ppm (equivalent to 0, 15.8, 32.1 or 63.9 mg/kg/day in males and to 0, 18.7, 37.9 or 76.4 mg/kg/day in females). Ten rats/sex/group were sacrificed at 1 month and the remainder sacrificed at 3 months. Mortality, clinical signs of toxicity, body weights and food consumption were monitored at appropriate times during the study. Ophthalmologic examinations were conducted. Hematological examinations, clinical chemistries and urinalyses were made on all rats prior to sacrifice. Gross pathologic examinations were performed on all animals and organ weight determinations were made on all animals at both the 1 month and 3 month sacrifices. Histopathological examinations were performed on a very limited set of tissues at the scheduled sacrifices--at 1 month on liver, thymus, thyroid (and parathyroid) and all abnormal tissues (10/sex/group) and at 3 months on liver, kidney, adrenals (females only) and all abnormal tissues (10/sex/group).

Mortality rates, clinical signs of toxicity, ophthalmologic examinations and food consumption were not affected by treatment with Imazalil at any dose level. Slightly decreased body weights (6.1% at 3 months) and decreased body weight gains (9.3% at 3 months) were observed in 800 ppm males between 1 and 3 months. These decreases were not statistically significant and their relationship to treatment with Imazalil is equivocal. Body weights of females were not affected at any dose level. Although decreased mean cell volumes (MCV) were observed at 3 months in both males and females at 800 ppm, these observations were not considered to be treatment-related because no other meaningful effects were observed in any other red blood cell parameter. At 800 ppm, decreased alanine aminotransferase was observed at 1 and 3 months in both males and females and decreased aspartate aminotransferase at 1 and 3 months in males. The toxicological significance of decreased levels of these enzymes, if any, is uncertain. Decreased blood urea nitrogen (BUN), observed in 800 ppm females at 3 months, was of little concern. Urinalyses examinations were negative as were gross pathology examinations at 1 and 3 months. At 1 month only, statistically significant dose-related increased liver weights were observed in both males and females at 400 and 800 ppm. In males, the increases were 13.6% and 15.2% at 400 and 800 ppm respectively. In females, the increases were 9.0% and 13.0% at 400 and 800 ppm respectively. Similar increases in liver weights were not observed, however, at the 3 month sacrifice. Increased adrenal weights were observed at 3 months in females at 400 ppm (14.5%) and at 800 ppm (23.2%). At 1 month, but not at 3 months, histopathologic examination revealed in the livers of males at 400 and 800 ppm, a slightly increased incidence of centrilobular hepatocytic swelling. Incidences were 5/10, 5/10, 8/10 and 8/10 for the control, low, mid and high dose level groups respectively. At 1 month, but not at 3 months, in the livers of females at 400 and 800 ppm, slightly increased incidences of hepatic large vacuoles were observed. Incidences were 3/10, 3/10, 6/10 and 7/10 for the control, low, mid and high dose level groups respectively. The histopathologic effects in the livers of both males and females correlated with the increased liver weights at 1 month. In addition, in 800 ppm females at 3 months, swelling of adrenocortical cells in the adrenal gland was observed in 1/10 females at 400 ppm and in 2/10 females at 800 ppm. The swelling correlated with the increased adrenal weights at 3 months.

Discussion of Study Results

At the highest dose level tested in this study (800 ppm), the following effects were considered to be possibly treatment-related.

In the male rats

1) Slightly decreased body weights (6.1% at 3 months) and body weight gains (9.3% at 3 months) between 1 and 3 months. Not statistically significant.

2) Increased liver weights (15.2%) at 1 month only. Statistically significant.

3) Increased incidence of centrilobular hepatocytic swelling in liver at 1 month only (8/10 vs. 5/10 in control group).

In the female rats

1) Increased liver weights (13.0%) at 1 month only. Statistically significant.

2) Increased incidence of hepatic large vacuoles in liver at 1 month only (7/10 vs. 3/10 in control group).

3) Increased adrenal gland weights (23.2%) at 3 months only.

4) Increased incidence of swelling of adrenocortical cells in adrenal gland at 3 months only (2/10 vs. 0/10 in control group).

The results at 800 ppm in this study are similar to and consistent with results observed at 800 ppm in several other subchronic and chronic rat feeding studies on Imazalil that were previously submitted to the Agency by Janssen Pharmaceutica. It is evident from these studies, and from several associated studies on the induction and/or inhibition of hepatic drug metabolizing enzymes, that Imazalil stimulates the liver microsomal enzyme system (LMES) of rodents following repeated administration in the diet. Thus, increased contents of microsomal protein and of cytochrome P-450 have regularly been demonstrated in the liver of treated rodents, as well as stimulation and/or inhibition of a variety of hepatic drug metabolizing P-450 isoenzymes. In addition, electron microscopy of liver sections, testosterone metabolism studies and toxicokinetic (serum concentration) data have also provided evidence that Imazalil stimulates the LMES. The HED Carcinogenicity Peer Review Committee does not consider stimulation of the LMES, however, <u>in the absence of other significant toxicity</u>, to be a sufficient basis for establishing the highest dose level to be tested in carcinogenicity studies. In the case of Imazalil, it has been determined that other significant toxicity of a sufficiently serious or potentially life-threatening nature was <u>not</u> observed in any of the submitted subchronic or chronic rat studies on Imazalil at dose levels up to and including 800 ppm and that these studies either individually or in their totality do <u>not</u> justify acceptance of a

highest dose level of 400 ppm in the 1985 rat carcinogenicity study. This determination includes consideration of the recently submitted 3-month range finding study (MRID 439657-04) described above.

Further, as mentioned on page 2 of this memorandum, Toxicology Branch I concurs with Janssen Pharmaceutica that an appropriate highest dose level for a new 2-year rat carcinogenicity study would be 2400 ppm based on effects observed in another 3-month dose-range finding study in rats that used dose levels up to 3200 ppm in a different substrain (Hannover) of rats (MRID 439657-05). Recall that this decision was reached in response to a separate submission (DP Barcode D225939) in which Janssen Pharmaceutica requested Agency comments on protocols for a new 2-year chronic feeding/carcinogenicity study on rats of the Hannover substrain and for a "historical control" study to be conducted concurrently.

Attachment #1

PESTICIDE REGULATION (PR) NOTICE 94-5
NOTICE TO REGISTRANTS OF PESTICIDE PRODUCTS

ATTENTION: Persons Responsible For Registration of Pesticide Products

SUBJECT: Requests for Re-considerations of Carcinogenicity Peer Review Decisions Based on Changes in Pathology Diagnoses.

This notice sets forth a procedure to be followed for submission of pathology re-reads to the Agency.

I. BACKGROUND

From time to time the Office of Pesticide Programs receives requests for re-consideration of Peer Review decisions based on re-evaluations of the pathology readings. These re-evaluations reflect voluntary activity on the part of the registrants, and are not the result of a requirement imposed by the Agency. The Agency is then asked to disregard the original readings and base its evaluation on the most recent ones. As a result the Agency may have two (or at times even more) pathological diagnoses for the same study.

Since this situation is occurring more and more frequently, the Agency is instituting a procedural requirement for any voluntary submissions of revised pathology diagnoses. This procedure will require a comprehensive peer review process, similar to the one used by the National Toxicology Program (NTP).

The National Toxicology Program (NTP) has a protocol for quality assurance in pathology, involving a quality assessment (peer review) pathologist and a Pathology Working Group (PWG) which is used to resolve differences in diagnoses between the laboratory (study) pathologist and the peer review pathologist. The PWG consists of a chair, the peer review pathologist and other pathologists (to include the study pathologist), all of whom are experienced in rodent toxicologic pathology. This group examines the tissues without knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differs from the opinion of the study pathologist, the diagnosis is changed. Thus, the final diagnoses represent a consensus of study, peer review, and consultant pathologists on the PWG. This procedure is described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." EPA believes that the use of a PWG, similar to one used by NTP, should be part of every pathology re-evaluation.

II. POLICY AND RATIONALE

The Agency believes that a procedure for obtaining consensus in pathology re-reads will improve the quality of decision-making in classifying pesticide chemicals having carcinogenic potential. The Agency has determined that unless the re-reads have been conducted using a Peer Review procedure, the Agency will base its evaluations upon the original readings.

The following will be required:

For any target tissue which is being re-evaluated, all slides containing that tissue in all dose groups, as well as the controls, must be re-read by the peer review pathologist. This is to include slides previously classified by the study pathologist as within normal limits, in addition to those having tumors, hyperplasia, hypertrophy, foci of cellular alteration or other non-neoplastic lesions.

The pathology reports from both the study and peer review pathologist and the original slides are to be submitted to a Pathology Working Group (PWG) similar to that described in the NTP Technical Reports under the section: "Clinical Examinations and Pathology." The PWG will review, as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists.

Finally, the Agency should be provided with a detailed pathology report, which presents the PWG findings and includes the original diagnosis and the new diagnosis for each slide read, and a comment column to note any discrepancies, missing slides, etc.

The Agency also is considering including the requirement for review by a PWG for all original submissions in the future. This present Notice deals only with re-reads.

III. EFFECTIVE DATE

This policy notice is effective immediately. If you have questions, contact Esther Rinde at (703) 305-7492.

Penelope A. Fenner-Crisp,
Deputy Director (Acting)
Office of Pesticide Programs

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Attachment #2

MEMORANDUM

SUBJECT: Comments regarding the Pathology Working Group (PWG) on Imazalil

TO: Esther Rinde, Ph.D.
Carcinogenicity Peer Review Manager
Science Analysis Branch
Health Effects Division (7509C)

and

Edwin R. Budd
Toxicology Branch.I
Health Effects Division (7509C)

FROM: Lucas H. Brennecke, DVM, DACVP
Pathology Consultant

DATE: 2 July 1996

Action Requested: Provide comments with regard to the interpretation and methodology of the review process used in the Pathology Working Group (PWG) for Imazalil

A PWG was convened "to evaluate the hepatic carcinomas reported in the 2 year mouse carcinogenicity study (Exp. 2194) on the fungicide Imazalil." It was the consensus opinion of the PWG that there was "no significant pairwise difference in the incidence of hepatocarcinomas in any treated group compared to the controls and no significant trend." In arriving at this conclusion, the PWG reclassified seven carcinomas (diagnosed by the study pathologist) as adenomas. The PWG report was ambiguous in that it did not indicate whether the reviewing pathologist had reviewed all of the liver slides (as required by PR Notice 94) or merely the carcinomas. Notice 94 states that, "The PWG will review, as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists." There were indications that more than just those livers having the original diagnosis of carcinoma were reviewed by the reviewing pathologist, but there is no indication of how many (study pathologist) diagnoses of benign neoplasms, non-neoplastic proliferations, or 'normal' with which the peer review pathologist may have disagreed. It is possible that some of those could have been classified as carcinomas by the PWG had they been reviewed. The result could also have dramatically changed the significance of the adenomas as well. It is clear that the PWG did not adhere to the provisions of PR Notice 94, and it is my recommendation that the Carcinogenicity Peer Review Committee should not accept its findings.

With regard to the review of the control data, I believe that that review was flawed as well. It appears that the study had not been peer reviewed, and only the animals having neoplastic diagnoses were reviewed. It is my opinion that if significant weight is to be placed on the results of that single study, then it should have been subjected to the same review process as is outlined in PR Notice 94. The incidences of carcinomas and adenomas in the control group appear to be significantly higher than published data from Charles River. I recommend that little weight be given to the data from the single control study, at least until it has been properly reviewed.

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Attachment #3

MEMORANDUM

SUBJECT: Comments regarding the Pathology Working Group (PWG) on Imazalil

TO: Esther Rinde, Ph.D.
Carcinogenicity Peer Review Manager
Science Analysis Branch
Health Effects Division (7509C)

and

Edwin R. Budd
Toxicology Branch I
Health Effects Division (7509C)

FROM: Lucas H. Brennecke, DVM, DACVP
Pathology Consultant

DATE: 14 August 1996

Action Requested: Provide additional comments with regard to the interpretation and methodology of the review process used in the Pathology Working Group (PWG) for Imazalil

In a 7/2/96 memo to you , subject as above, I recommended that the Carcinogenicity Peer Review Committee (CPRC) not accept the findings of the PWG because it did not adhere to the provisions of PR Notice 94. During two elephone conversations with Mr. Budd on 8/13/96, he noted that the reviewing pathologist (RP) did, in fact, review all of the liver slides. This, by no means, was clear from the RP's report or PWG report. Any animals for which hepatic carcinoma was diagnosed by either the study pathologist (SP) or the RP were included in the PWG review. However, it still appears that the provisions of PR Notice 94 were not met, since the PWG did not review ". . .as a minimum, all slides about which there were significantly differing diagnoses between the study and peer review pathologists."

I have chaired and participated in enough PWGs to know that in dealing with many tumors, particularly mouse liver neoplasms, the consensus diagnoses of the PWG may or may not agree with either the SP or the RP. Any disagreements relative to any of the proliferative liver lesions should have been included in the PWG review. Realistically, a properly conducted PWG should include a complete discussion of the criteria used. In addition, examples of various proliferative lesions (non-neoplastic, benign, and malignant) should be reviewed to insure that all of the PWG participants are "reading from the same sheet of music." With regard to the male mice, it is still my recommendation that the CPRC should not accept the PWG's findings until it is documented that a properly conducted PWG was conducted.

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