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

APR 27 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea].

CASRN. 35367-38-5
EPA Chem. Code: 108201
Caswell No. 346A

FROM: George Z. Ghali, Ph.D. *G. Ghali*
Manager, RfD/QA Peer Review Committee
Health Effects Division (7509C)

THRU: William Burnam *W. Burnam*
Chairman, RfD/QA Peer Review Committee
Health Effects Division (7509C)

TO: Dennis Edwards, PM 19
Insecticide-Rodenticide Branch
Registration Division (7505C)

Esther Saito, Chief
Reregistration Branch
Special Review and Reregistration Division (7508W)

The Health Effects Division-RfD/Peer Review Committee met on March 16, 1995 to discuss and evaluate the existing and recently submitted toxicology data in support of Diflubenzuron (Dimilin) reregistration and to reassess the Reference Dose (RfD) for this chemical.

Material available for review consisted of data evaluation, records (DERs) for two combined chronic toxicity/carcinogenicity studies in rats (83-1a and -2a or 83-5), a carcinogenicity study in mice (83-2b), a one-year oral toxicity study in dogs (83-1b), a three-generation reproductive toxicity study in rats (83-4), developmental toxicity studies in rats and rabbits (83-3a and -3b), subchronic feeding toxicity studies in rats, mice and dogs (82-1a and 1b), and a battery of mutagenicity studies in the categories of gene mutation (84-2a), structural chromosomal aberrations (84-2b), and other genotoxic effects (84-4), in addition to a 1989 NTP report on p-chloroaniline (a diflubenzuron metabolite) which included carcinogenicity studies in rats and mice and a battery of mutagenicity studies.



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A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity phase of the combined chronic toxicity/carcinogenicity study in rats (1984, 83-1a, MRID No. 00145467) to be acceptable and the data evaluation record (HED Doc. No. 003607, 003950, 004292) to be adequate. This study was initially classified as Core-supplementary data in part because no NOEL was established for the chronic phase of the study. The study was subsequently upgraded to a Core-minimum status because it was determined that a NOEL for methemoglobinemia and sulfhemoglobinemia had already been established in an older chronic feeding study in rats (1975, MRID No. 00044329, 00099712, HED Doc. No. 000964, 004292). The Committee agreed with the evaluation and interpretation of data and classification of the chronic toxicity phase of the study.

The Committee considered the chronic toxicity study in dogs (83-1b, MRID No. 00146174) to be acceptable and the data evaluation record of this study (HED Doc. No. 004777) to be adequate. The Committee generally agreed with the reviewer's evaluation and interpretation of data and classification of the study.

The Committee considered the subchronic toxicity studies in rats, mice (82-1a, MRID No. 00064550, 00074534; 00114330) and dogs (82-1b, MRID No. 00038706) to be acceptable and the data evaluation records of these studies (HED Doc. No. 004360; 000993, 001770; 000963) to be adequate.

B. Carcinogenicity:

The Committee considered the carcinogenicity phase (1984, 83-2a, MRID No. 00145467) of the combined chronic toxicity/carcinogenicity study in rats to be acceptable and the data evaluation record (HED Doc. No. 003607, 003950, 004292) to be adequate. The Committee considered the dose levels tested in this study to be adequate for carcinogenicity testing based upon statistically significant increases in methemoglobinemia and sulfhemoglobinemia in both males and females at all dose levels and signs of hemolytic anemia, erythrocyte destruction and compensatory regeneration of erythrocytes at the highest dose level of 10,000 ppm (500 mg/kg/day). Furthermore, the high dose level tested was at half of the limit dose for carcinogenicity testing in this species (20,000 ppm). The dose selection was also based on the results of a subchronic toxicity/range finding study in rats. The Committee concluded that the treatment did not alter the spontaneous tumor profile in this strain of rat under the testing conditions.

The Committee considered the mouse carcinogenicity study (83-2b, MRID No. 00142490) to be acceptable and the data evaluation record (HED Doc. No. 003159, 004075, 004292) to be adequate. The dose selection was based on the results of a subchronic

toxicity study in mice (MRID No. 00114330, 00074534). The high dose of 10,000 ppm was considered to be higher than the limit dose for carcinogenicity testing in this species (7,000 ppm). The treatment did not alter the spontaneous tumor profile in this strain of mouse.

In view of the above, the Committee concluded that Diflubenzuron per se should be classified as a "Group E", evidence of non-carcinogenicity for humans.

The Committee also considered the carcinogenicity of p-chloroaniline (PCA), a known diflubenzuron metabolite, that was tested by the National Toxicology Program (NTP) in 1989 for carcinogenicity in rats and mice as a hydrochloride form (see memo from Stephanie Irene to Peter Caulkins and Stephen Johnson, dated March 8, 1995; attached).

In rats treated with p-chloroaniline, a treatment-related increased incidence of uncommon sarcomas of the spleen was observed in males and included fibrosarcomas, hemangiosarcomas, and osteosarcomas, many of which metastasized to other sites. In addition, in treated females, one fibrosarcoma and one osteosarcoma were also observed. Furthermore, there was a marginally increased incidence of pheochromocytomas in the adrenal glands in both males and females at the highest dose tested.

In mice treated with p-chloroaniline, a treatment-related increased incidence of combined hepatocellular adenomas/carcinomas was observed in males. The increase in combined tumors was primarily due to a dose-related increase in hepatocellular carcinomas. Many of these tumors metastasized to the lungs. An increased incidence of hemangiosarcomas in the spleen and/or liver of the male mice was also observed at the highest dose tested. The incidence was higher than the historical control mean for male mice. There was no evidence of a carcinogenic response in female mice.

On this basis p-chloroaniline was classified by the Committee as a "Group B2", probable human carcinogen.

The Committee also concluded that it will not be necessary to convene a Carcinogenicity Peer Review Committee meeting to confirm these classifications.

C. Reproductive and Developmental Toxicity:

The Committee considered the three-generation reproductive toxicity study in rats (83-4, MRID No. 00044330, 00099687) to be unacceptable and the data evaluation record (HED Doc. No. 000964, 004360) to be inadequate. The Committee recommended down-grading the study from Core-Guideline to Core-supplementary status. The Committee was informed that a new reproductive toxicity study has

been conducted and was recently submitted to the Agency and is currently under review. There were no reproductive effects identified in the old reproductive toxicity study under the testing conditions. However, the study was deficient and did not provide a reliable assessment of potential reproductive toxicity in this species. Therefore, the Committee recommended that assessment of reproductive toxicity be based on the new study.

The Committee considered the developmental toxicity study in rats (83-3a, MRID No. 41703504) to be acceptable and the data evaluation record (HED Doc. No. 008501) to be adequate. The Committee generally agreed with the reviewer's evaluation and interpretation of data and classification of the study.

The Committee considered the developmental toxicity study in rabbits (83-3b, MRID No. 41703505) to be acceptable and the data evaluation record (HED Doc. No. 008502) to be adequate.

Overall, the Committee concluded that there was no indication of developmental toxicity effects in the developmental toxicity studies in rats and rabbits, up to the limit dose of 1000 mg/kg/day, and that a developmental neurotoxicity study would not be required.

D. Mutagenicity:

Several mutagenicity studies were submitted to the Office of Pesticide Programs on the parent compound. Diflubenzuron was negative for gene mutations in *Salmonella* and cultured mouse lymphoma cells, for unscheduled DNA synthesis (UDS) in primary rat hepatocytes and human WI-38 cells, and for micronuclei in mouse bone marrow.

On the other hand, the NTP tested the metabolite, p-chloroaniline (PCA), in several mutagenicity assays. PCA was positive in all tests. PCA was mutagenic in salmonella strains TA98 and TA100 with metabolic activation. Gene mutations were induced by PCA in cultured mouse lymphoma cells with and without metabolic activation. In cultured Chinese hamster ovary (CHO) cells, treatment produced significant increases in sister chromatid exchanges (SCEs) with and without metabolic activation. Chromosomal aberrations were also significantly increased in CHO cells in the presence of metabolic activation.

E. Reference Dose (RfD):

The Committee recommended that the existing Reference Dose remain unchanged. The Reference Dose for this chemical was established by the RfD Committee in their meeting of July 11, 1986 and was verified by the Agency RfD Work Group on August 05, 1986. The RfD was set at 0.02 mg/kg/day based upon the one-year oral toxicity study in dogs with a NOEL of 2.0 mg/kg/day (MRID No.

00146174). Increases in methemoglobin and sulfhemoglobin were observed at the next higher dose level of 10.0 mg/kg/day. An uncertainty factor (UF) of 100 was applied to account for the interspecies extrapolation and intraspecies variability.

It should be noted that this chemical has been reviewed by the FAO/WHO joint committee on pesticide residues (JMPR) and an Acceptable Daily Intake (ADI) of 0.02 mg/kg/day was established in 1985. The ADI was based upon the one-year oral toxicity study in dogs with a NOEL of 2.0 mg/kg/day. A Safety Factor of 100 was applied to account for the interspecies extrapolation and intraspecies variability.

F. Individuals in Attendance:

Peer Review Committee members and associates present were William Burnam (Chief, SAB; Chairman, RfD/Peer Review Committee), George Ghali (Manager, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Marcia Van Gemert (Chief, TB II), David Anderson, Kerry Dearfield, Susan Makris, Melba Morrow, Esther Rinde, Henry Spencer, William Sette, and Rick Whiting. In attendance also was Linda Kutney of RCAB, HED as an observer.

Scientific reviewer (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report):

Edwin Budd

Edwin Budd

Respective branch chief (Committee member; Signature indicates concurrence with the peer review unless otherwise stated)

Karl Baetcke

Karl Baetcke

CC: Stephanie Irene
Karl Baetcke
Edwin Budd
Debra Edwards
Albin Kocialski
Beth Doyle
Kerry Dearfield
RfD File
Caswell File

G. Material Reviewed:

1. Burdock, G. A. (1984). Oncogenicity study in rats; Final report. MRID No. 00145467, HED Doc. No. 003607, 003950, 004292. Classification: Core-minimum data. This study together with certain additional chronic feeding data from MRID No. 00044329 (see below) satisfies data requirements 83-1a and -2a (or 83-5) of Subpart F of the Pesticide Assessment Guideline for chronic toxicity/ carcinogenicity testing in rats.
2. Hunter, B. et al. (1973). Effects of Du-112307 in dietary administration to rats for 104 weeks. MRID No. 00044329, HED Doc. No. 000964, 004292. Classification: Core-supplementary for chronic toxicity and invalid for carcinogenicity. This study (alone) does not satisfy data requirements 83-1a or -2a (or 83-5) of Subpart F of the Pesticide Assessment Guideline for chronic toxicity/ carcinogenicity testing in rats (see above).
3. Colley, J. et al. (1984). The effect of Diflubenzuron given by oral administration with the feed on toxicity and tumor development in male and female HC/CFLP mice; Final report. MRID No. 00142490, HED Doc. No. 003159, 004075, 004292. Classification: Guideline data. This study satisfies data requirement 83-2b of Subpart F of the Pesticide Assessment Guideline for carcinogenicity testing in mice.
4. National Toxicology Program (1989). Toxicology and carcinogenesis studies of para-chloroaniline hydrochloride in F344/N rats and B6C3F1 mice. Technical Report Series 351, NIH Publication No. 89-2806. HED Doc. No. 000000. Classification: N/A. This study was conducted using p-Chloroaniline, a metabolite of Diflubenzuron.
5. Greenough, R. et al. (1985). Diflubenzuron: 52-week oral toxicity study in dogs. MRID No. 00146174, HED No. 004777. Classification: Guideline data. This study satisfies data requirement 83-1b of Subpart F of the Pesticide Assessment Guideline for chronic toxicity testing in dogs.
6. Palmer, A. K. and Hill, P. A. (1975). Effect of Du 112307 on reproductive function of multiple generations in the rat. MRID No. 00044330, 00099687, HED Doc. No. 000964, 004360. Classification: Core-supplementary. This study does not satisfy data requirement 83-4 of Subpart F of the Pesticide Assessment Guideline for reproductive toxicity testing in rats.
7. Kavanagh, P. (1988). Diflubenzuron: Oral (gavage) rat teratology limit study. MRID 41703504, HED Doc. No. 008501. Classification: Core-minimum data. This study satisfies data

requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

8. Kavanagh, P. (1988). Diflubenzuron: Oral (gavage) rabbit teratology limit study. MRID 41703505, HED Doc. No. 008502. Classification: Core-minimum data. This study satisfies data requirement 83-3b of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

9. Burdock, G. A. et al. (1980). Diflubenzuron: Subchronic dietary toxicity study in rats. MRID No. 00064550, HED Doc. No. 004360. Classification: Guideline data. This study satisfies data requirement 82-1a of Subpart F of the Pesticide Assessment Guideline for subchronic oral toxicity testing in rats.

10. Chesterman, H. et al. (1974). Du 112307: Toxicity in repeated dietary administration to beagle dogs. MRID No. 00038706, HED Doc. No. 000963, 004360. Classification: Guideline data. This study satisfies data requirement 82-1b of Subpart F of the Pesticide Assessment Guideline for subchronic oral toxicity testing in dogs.

11. Colley, J. et al. (1981). The effects of dietary administration of Diflubenzuron to male and female HC/CFLP mice for 14 weeks. MRID No. 00114330, HED Doc. No. 000993, 001770, 004360. Classification: Guideline data. This study satisfies data requirement 82-1a of Subpart F of the Pesticide Assessment Guideline for subchronic oral toxicity testing in mice.

Attachment

D04/11-13
F04/20



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

MAR 8 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Diflubenzuron - Carcinogenicity in Animals

FROM: Stephanie Irene, Ph.D. *Stephanie Irene*
Acting Director
Health Effects Division (7509C) 3-8-95

TO: Peter Caulkins
Acting Director
Special Review & Reregistration Division (7508W)

Stephen Johnson
Director
Registration Division (7505C)

Diflubenzuron; CAS Registry No. 35367-38-5; Chemical No. 108201

The Health Effects Division (HED) Carcinogenicity Peer Review Committee has not met to evaluate the carcinogenicity data for diflubenzuron. The carcinogenicity studies submitted to the Office of Pesticide Programs (OPP) have been reviewed by HED's Toxicology Branch I and will be presented to the HED's Reference Dose (RfD) Peer Review Committee in the near future.

A. Carcinogenicity in Animals

After a full evaluation of all the data and supporting information regarding animal carcinogenicity, it is concluded that administration of diflubenzuron to Sprague-Dawley rats and CH/CFLP mice did not induce a significant increase of tumors. However, it is also concluded that the diflubenzuron metabolite, para-chloroaniline (PCA), when tested in rats and mice induced cancer. Exposure to PCA results in the induction of malignant uncommon sarcomas in the spleen of male F344/N rats. The sarcomas included fibrosarcomas, hemangiosarcomas, and osteosarcomas, many of which metastasized to other sites. A suggestive increase in sarcomas of the spleen of female rats was also observed. In addition, a marginally increased incidence of pheochromocytomas observed in the adrenal gland of male and female rats. PCA increased the incidence of combined hepatocellular adenomas/carcinomas in male B6C3F1 mice. The increase in combined tumors was primarily due to an increase in malignant carcinomas; many of these carcinomas metastasized to the lungs. Also, PCA increased the incidence of hemangiosarcomas in



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the spleen and/or liver of male mice.

While the parent compound, diflubenzuron, had no evidence of tumor induction, primary sites of non-neoplastic treatment related effects were the spleen and liver. These are the major sites of cancer induction by the diflubenzuron metabolite, para-chloroaniline. It is noted that different strains of rat and mouse were used for the metabolite versus the parent compound. The mutagenicity data for diflubenzuron itself does not support much of a concern for both mutagenicity and carcinogenicity. However, there is clear evidence of mutagenic activity by PCA. This supports the carcinogenicity concern presented by PCA.

B. Animal Carcinogenicity Studies

Male and female CH/CFLP mice were fed 0, 16, 80, 400, 2000, or 10,000 ppm (equivalent to 0, 2.4, 12, 60, 300, or 1500 mg/kg/day, respectively) of diflubenzuron for 91 weeks. Mortality, body weights, and food consumption were not affected by treatment. Methemoglobin and sulfhemoglobin were seen in both sexes at dose levels of 80 ppm and higher. Throughout the study, a blue/gray discoloration of the skin and extremities and dark eyes accompanied the increased methemoglobin and sulfhemoglobin in males at 80 ppm and in both sexes at 400 ppm and higher. Dosing was adequate since the highest dose tested (HDT) exceeded the limit dose of 1000 mg/kg/day for carcinogenicity studies.

Treatment with diflubenzuron was not associated with an increased incidence of tumors in either male or female mice. It is noted that treatment-related histomorphologic lesions were observed in the spleen, skin, livers, and lungs of treated mice of both sexes. In particular, the incidences of splenic enlargement, and enlarged livers were detected at 2000 and 10,000 ppm. These results were correlated well with the significant increases of incidence in the higher spleen and liver weights of the same treated groups. Evidence of definite histological effects are demonstrated by treatment-related lesions in the spleen (extramedullary haemopoiesis and siderocytes) and the liver (hepatocyte enlargement, hepatocyte vacuolation, extramedullary haemopoiesis, congested/dilated contrilobular sinusoids, brown pigmented Kupffer cells, and fat deposition).

Male and female Sprague-Dawley rats were fed 0, 156, 625, 2500, or 10,000 ppm (equivalent to 0, 7.8, 31, 125, or 500 mg/kg/day, respectively) of diflubenzuron for 104 weeks. Mortality, clinical signs, body weights, and food consumption were not affected by treatment. Statistically significant increases in methemoglobin and sulfhemoglobin were observed in both sexes at all treatment levels. Dosing was considered adequate due to significant toxicity, particularly methemoglobin, sulfhemoglobin,

hemolytic anemia, erythrocyte destruction, and secondary regeneration of erythrocytes at the HDT.

Treatment with diflubenzuron was not associated with an increased incidence of tumors in either male or female rats. It is noted that increased pigmented macrophages were found in the spleen and liver of treated rats of both sexes at 156 to 10,000 ppm. In addition, the increased incidences of pathological changes in the spleen and liver in both sexes treated with 2500 and 10,000 ppm diflubenzuron correlated well with the statistically significant increases in the spleen and liver weights of the same male and female groups.

Male and female Sprague-Dawley rats were fed 0, 10, 20, 40, or 160 ppm (equivalent to 0, 0.35, 0.70, 1.43, or 5.83 mg/kg/day in males, respectively and to 0, 0.43, 0.88, 1.73, or 7.05 mg/kg/day in females, respectively) of diflubenzuron for 104 weeks. Mortality, clinical signs, body weights, food consumption, organ weights, and histopathological examinations were not affected by treatment with diflubenzuron.

There was no evidence of tumor induction, but this study is considered inadequate to assess carcinogenic potential. This is based on the lack of significant toxicity at these low dose levels. Also, limited histopathological examinations were performed.

C. Additional Information

p-Chloroaniline Carcinogenicity

A known diflubenzuron metabolite, para-chloroaniline (p-chloroaniline or 4-chloroaniline; PCA), has been tested by the National Toxicology Program (NTP) for carcinogenicity in rats and mice. The compound actually tested was p-chloroaniline hydrochloride. Following are the findings from these two studies.

Male and female F344/N rats were administered by gavage 0, 2, 6, or 18 mg/kg/day of PCA for 163 weeks. Some increased survival in male rats was observed. Only minimal changes in body weight were observed for both sexes. There were dose-related increases in methemoglobin and mild hemolytic anemia at dose levels of 6 and 18 mg/kg/day. Histopathological examinations indicated nonneoplastic treatment-related effects in the spleen (fibrosis, increased fatty metaplasia) and liver (hepatic hemosiderosis).

A treatment related increased incidence of uncommon sarcomas of the spleen was observed in the male rats. The sarcomas included fibrosarcomas, hemangiosarcomas, and osteosarcomas, many of which metastasized to other sites. The combined incidence of sarcomas in male rats was 0/49, 1/50, 3/50, and

38/50 at doses of 0, 2, 6, and 18 mg/kg/day, respectively. In addition, in female rats, 1 fibrosarcoma at 6 mg/kg/day and 1 osteosarcoma at 18 mg/kg/day were observed. The historical control incidence of splenic connective tissue sarcomas (all types) in water gavage studies was 0.3% for males and 0% for females and of hemangiosarcomas in water gavage studies was 0% for males and 0.3% for females. There was also a marginally increased incidence of pheochromocytomas observed in the adrenal gland of male and female rats at the HDT. For male rats, the incidence was 13/49, 14/48, 15/48, and 26/49 and for female rats, 2/50, 3/50, 1/50, and 6/50 at dose levels of 0, 2, 6, and 18 mg/kg/day, respectively. Historical control incidence in water gavage studies was 40% ($\pm 16\%$) in males and 7% ($\pm 2\%$) in females.

Male and female B6C3F1 mice were administered by gavage 0, 3, 10, or 30 mg/kg/day of PCA for 103 weeks. Increased mortality was observed in male mice at 10 mg/kg/day after 99 weeks, but not at the HDT; treatment did not affect mortality in female mice. Mean body weights for both sexes were not affected by treatment. Pigmentation (hemosiderin) of the Kupffer cells was observed in the livers of both sexes at the HDT.

A treatment related increased incidence of combined hepatocellular adenomas/carcinomas was observed in male mice (incidences were 11/50, 21/49, 20/50, and 21/50 at 0, 3, 10, and 30 mg/kg/day, respectively). The increase in combined tumors was primarily due to a dose related increase in hepatocellular carcinomas (incidences were 3/50, 7/49, 11/50, and 17/50 at 0, 3, 10, and 30 mg/kg/day, respectively). Many of these carcinomas metastasized to the lungs. The historical control incidence for combined hepatocellular tumor in water gavage studies in male mice was 31% ($\pm 6\%$). An increased incidence of hemangiosarcomas in the spleen and/or liver of male mice was observed at the HDT (4/50, 4/49, 1/50, and 10/50 at 0, 3, 10, and 30 mg/kg/day, respectively). The historical control incidence for hemangiomas or hemangiosarcomas at all sites (combined) in water gavage studies in male mice was 3% ($\pm 3\%$). There was no evidence of carcinogenic response in female mice.

Mutagenicity

In submitted studies to the OPP, diflubenzuron was negative for gene mutations in *Salmonella* and cultured mouse lymphoma cells, for unscheduled DNA synthesis (UDS) in primary rat hepatocytes and human WI-38 cells, and for micronuclei in mouse bone marrow.

The NTP tested PCA in several mutagenicity assays. PCA was positive in all tests. PCA was mutagenic in *Salmonella* strains TA98 and TA100 with metabolic activation. Gene mutations were induced by PCA in cultured mouse lymphoma cells with and without

metabolic activation. In cultured Chinese hamster ovary (CHO) cells, treatment produced significant increases in sister chromatid exchanges (SCEs) with and without metabolic activation. Chromosomal aberrations were also significantly increased in CHO cells only in the presence of metabolic activation.

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