ENVIRONMENTAL PROTECTION AGENCY

REPLY TO ATTN OF:

DATE: April 15, 1971

SUBJECT: Request for tolerances of Daconil (2,4,5,6-tetrachloroisophthalonitrile) and its metabolite 4-hydroxy-trichloroisophthalonitrile, on sugar beets, peanuts and numerous vegetables

TO: Mr. Frank J. McFarland, Director
Pesticides Tolerances Division

File: PP# 1F1024

PESTICIDE PETITION NO. 1F-1024

Diamond Shamrock Corporation
Cleveland, Ohio 44115
(AF 25-202)

The proposed use of Daconil is as a fungicide on various crops. The evaluation of the toxicological data submitted for Daconil can be found in (1) memo of E. C. Hagan, BF-147, DT, 2-16-71 and (2) memo of H. Blumenthal, BF-148, DT, 3-1-71.

The oral LD_{50} in dogs is > 5 g/kg and in rats > 10 g/kg. The 2-year chronic studies in rats and dogs raised questions as to pathological changes in the kidneys and this is explained at length in Dr. Blumenthal's memo cited above. He concludes that the no-effect levels for 2-year feeding studies in the dog are 60 ppm with questionable effect at 120 ppm, and in the rat the no-effect level is 60 ppm.

Dr. Blumenthal's memo recommended that no final decisions on Daconil should be taken until a complete evaluation of the reproduction study was made. This data for this study was included in PP # 7F0-599 (April 1969) and my evaluation follows:

Two reproduction studies in rats were conducted:

(1) Three-generation reproduction study, started June 1964, completed January 1966 after weaning of the second litters (F_{3b}).

(2) Three-generation reproduction study to supplement the previous study. Initiated December 1964, completed July 1966.

In the first study DAC-2787 was used for the first seven weeks and then a blend of tetrachloroisophthalonitrile DAC-2787, [redacted] was used for the remainder of the test.

The studies were performed by Hazelton Laboratories, Falls Church, Virginia. Charles River Caesarian-derived albino rats, 10 males and 20 females per group were fed 0, 0.15, 1.5, and 2.0% Daconil, 0, 1500, 15,000, and 20,000 ppm, respectively. Mixture fed contained approximately [redacted] and 93.6% DAC-2787. The 1.5 and 2.0% level of feeding resulted in food refusal and poor weight gain so rats were removed from compound from days 3-14 and gradually

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

APR 19 [illegible]

brought back-up to the level by the eighth week. Rats were mated after 11 or 12 weeks on the diet. Twenty-four hours after birth of pups, litter size was reduced to eight pups. After 21-day nursing period, all F_{1a} pups in the control, 0.15 and 1.5% test groups were discarded. The high level (2.0%) test group was discontinued with the casting of the F_{1a} litter and male and female parents sacrificed and necropsies performed. When the F_{3b} litter was cast, the P_3 parents and the F_{3b} weanlings were sacrificed and the following tissues preserved in formalin: brain, pituitary, thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon (ascending, descending, transverse), urinary bladder, gonad, sternum, and rib junction. Weights of kidneys of P_3 and brain and kidney for F_{3b} weanlings were obtained prior to fixation. Microscopic examination of preserved tissues from 10 male and 10 female F_{3b} weanlings from control and 1.5% test group, and target organs from 10 F_{3b} rats per sex from the 0.15% test group was made.

With the F_{2a} and F_{3a} litters the nursing procedure was modified so that several litters from the control group (3 from F_{2a} and 4 from F_{3a}) were exchanged with the 1.5% test group. No exchange of F_{2b} or F_{3b} litters was made.

The supplementary 3-generation reproduction study used the blend of DAC-2787, 2020, 3200, 2397 as in the first study. A control group and a test group at 0.5% (5000 ppm) were carried through P_3 and F_{3b} litters as before. During the first cycle of the second and third breeding phase (F_{2a} and F_{3a}), four litters from control group were exchanged with litters from the test group.

RESULTS

Parents

The percent survival in P_1, P_2, and P_3 was the same in test and control rats. The body weight was decreased in both sexes in all three parental sets of both studies in a dose-related fashion.

At the 0.5, 1.5, and 2% levels a number of males and females of P_1, P_2, and P_3 parents exhibited a hunched position, yellow color of the ears, rough fur, and swollen eyelids. Urine was amber and feces were soft in consistency.

At necropsy the P_1 parents at the 2% level had pale brown kidneys and enlarged cecum in the females. At the 0.5% and 1.5% level of the P_1, P_2, and P_3 there was enlargement of the kidneys and a green discoloration of the renal cortex (not in the P_2 females at 0.5%). This discoloration was also noted in six out of ten males and seven out of 20 females at the 0.15% (1500 ppm) level in the P_3 parents. There was no microscopic pathology on the parental animals in either P_1, P2, or P_3.

Offspring

The lactation indices for F_{1a} test groups at the 0.15 and 2% levels were lower than those for controls. The mean body weights at weaning for the F_{1a} male pups at 0.5, 1.5, and 2% levels and for the F_{1b} males and females at the 0.5 and 1.5% levels were lower than those of controls.

The F_{2a} litters at the 1.5% level had a lower lactation index than controls, but at the 0.15 and 0.5% level the lactation indices were the same as controls. The lactation indices were comparable among all the other F_1 and F_2 offspring of the three-generation test and control groups. The fertility, gestation, and live birth indices were not affected in any of the F_1, F_2 or F_3 generations, indicating no compound-related effects on reproductive performance.

Growth depression was noted at weaning in the first and second litters at the 0.15, 0.5, and 1.5% test groups through all three filial generations in a dose-related fashion. In the exchange nursing study, the control pups nursed by the 0.5 and 1.5% level mothers had a depressed weanling weight, but when the 0.5 and 1.5% level pups were nursed on control mothers their weanling weights were comparable to those of the controls. Since it has been shown that residues of Daconil per se were not detected in the milk of lactating cows (PP No. 1F-1024, memo W. S. Cox, 1-6-71), the weanlings must have obtained the compound from the mother's food. The exchange nursing experiment supports this assumtion. Further evidence is found in the weights of the offspring which were recorded at 24 hours, and at 7, 14, and 21 days (weaning) after birth. At 7 days the weights of control and test pups were comparable, but at 14 and 21 days the weights were depressed. It would appear that the effect noted arose from the Daconil in the diet and not from the mother's milk since the young are known to eat chow while still nursing.

Microscopic pathology on the offspring on the F_{3b} rats showed slight to moderate thickening of squamous mucosa of esophagus and forestomach and a covering layer of keratin. In the kidney tubules there was focal vacuolation in the glomerular region at the 0.15 and 1.5% test levels.

CONCLUSIONS

Data submitted on a 3-generation reproduction study in rats in PP No. 7F0-599 shows:

(a) A no-effect level of Daconil for reproductive performance is 15,000 ppm.

(b) A no-effect level for the growth of nursing pups has not been demonstrated, but exchange nursing at the 1.5% level indicates that toxic effects on growth were not due to Daconil in the milk.

(c) A no-effect level for lactation index is 1500 ppm for all filial generations except F_{1a}.

(d) No malformations were observed in the offspring.

(e) Renal and stomach mucosa effects in the offspring were present at all test levels (1500, 5000, 15,000 ppm) so that a no-effect level cannot be established for this parameter. However, from the 2-year rat feeding study the no-effect level based upon kidney pathology has been determined as 60 ppm.

(f) In terms of Daconil per se the lack of effects upon reproductive capacity of rats supports the safety of the requested tolerance.

Petition Evaluation Section (telephone conversation with W. S. Cox, 4-9-71) states that the residues of Daconil will be small, and the petitioner has altered the request for tolerances so that there will be no danger that Daconil per se will appear as a residue in milk and meat. It has not been proved that the 4-hydroxy 2,4,6-trichloroisophthalonitrile is absent from meat and milk, but the petitioner has restricted feeding of treated plants to livestock, and has withdrawn the request for a tolerance on plants used as feeds and for which restrictions against feeding are not practical.

Clara H. Williams

Clara H. Williams, Ph.D.
Toxicology Branch
Pesticide Tolerances Division

cc: BF-216
BF-148
BF-203
BF-140
VM-300
BF-218
CA-227
PP # 1F1024; 7F0599

4/15/71

Our review clearly stated that the 4-hydroxy metabolite transfers to meat and milk

LITTER OBSERVATIONS IN 3-GENERATION RAT REPRODUCTION STUDY
DACONIL

Litter	Fertility Index (# preg/ # mated)	Gestation Index (# litter born)	Live Birth Index (# alive/# born	Lactation Index (# left to nurse /# weaned)	Mean Litter Size	Weight at Weaning M	F
			Control				
F-1a	80.0	93.8	100.0	67.5	11	62	61
F-1b	94.8	100.0	100.0	99.3	12	59	55
F-2a	85.0	100.0	98.0	100.0	13	54	52
F-2b	95.0	100.0	99.1	95.3	12	59	57
F-3a	90.0	100.0	99.2	98.2	14	54	51
F-3b	95.0	100.0	100.0	95.3	14	54	53
			Dietary Level 1500 ppm (0.15%)				
F-1a	80.0	100.0	100.0	46.9^{S}	11	54^{S}	52
F-1b	100.0	100.0	99.6	86.8	12	55	52
F-2a	100.0	100.0	100.0	96.9	13	47	45
F-2b	100.0	95.0	100.0	97.4	13	51^{S}	49^{S}
F-3a	100.0	100.0	100.0	99.4	13	51	49
F-3b	100.0	100.0	100.0	95.0	14	51	49^{S}
			Dietary Level 15,000 ppm (1.5%)				
F-1a	80.0	100	100.0	61.4	10	40^{S}	39^{S}
F-1b	90.0	100	100.0	90.6	14	38	35
F-2a	100.0	100	99.0	86.1	10	31^{S}	31^{S}
F-2b	100.0	100	100.0	89.0	10	36^{S}	34^{S}
F-3a	100.0	100	99.6	96.9	11	34	34
F-3b	95.0	100	100.0	95.3	12	34^{S}	33^{S}
			Dietary Level 20,000 ppm (2.0%) Discontinued after 1st litter F_{1a}				
F-1a	65	100	98.3	47.5^{S}	9	32^{S}	31^{S}
			Control (2nd study)				
F-1a	95	100	100.0	88.0	11	58	55
F-1b	100	100	100.0	89.5	13	58	57
F-2a	90	100	99.6	100.0	13	59	55
F-2b	100	100	99.7	97.4	14	58	56
F-3a	100	100	99.6	100.0	13	54	53
F-3b	100	95	99.6	94.1	13	56	54
			Dietary Level 5000 ppm (0.5%) (2nd study)				
F-1a	100	100	100.0	82.8	10	48^{S}	47^{S}
F-1b	95	100	99.6	91.4	13	47^{S}	46^{S}
F-2a	100	100	98.7	99.2	12	49	47
F-2b	100	100	100.0	99.3	13	50^{S}	50^{S}
F-3a	95	100	100.0	99.0	10	48^{S}	47^{S}
F-3b	85	100	100.0	98.5	12	49	48

^{S} Significant difference from controls.