February 16, 1971

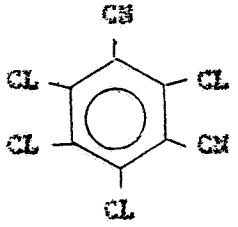
Daconil 2787; Petition for the Establishment of Tolerances for the Pesticide Chemical (tetrachloroisophthalonitrile) on Raw Agricultural Commodities (see attached table of commodities and levels involved).

Division of Regulations and Petitions Control (BF-320)

Thru: Dr. H. Blumenthal
Chief, Petitions Review Branch, DT (BF-148)

PESTICIDE PETITION No. 1F1024

Diamond Shamrock Corporation
Painesville, Ohio
(AF 25-202)



BACKGROUND

1. Acute Studies

The oral LD_{50} in dogs is greater than 5000 mg/kg; in male and female rats greater than 10,000 mg/kg (Haxleton and Hilltop Research). Rabbits, dermal, an LD_{50} greater than 10,000; rabbit, eye, 3 mg produced transient conjunctivitis; rabbits, inhalation, LD_{50} greater than 4.7 mg/liter.

2. Rat and Dog Subacute and Chronic Feeding Studies

Dr. Long previously reviewed data (memoranda of 1/31/69 and 8/8/69) and concluded kidney and growth effects in dogs fed daconil at 1500, 15,000 and 30,000 ppm for two years, and effects in rats at 15,000 and 30,000 ppm, but doubtful effects at 1500 ppm. However, in an additional study (reviewed by Dr. Long on September 16, 1969) rats were fed for 18 months at 0.5, 0.1, 0.05 and 0 ppm of daconil and effects concentrated in kidney and liver were noted at all treatment levels.

3. Reproduction Studies (Summary of Dr. Long's comments)

Rabbits:

Eight does each were dosed at 0, 180, or 375 mg/kg on days 8-9 of pregnancy; then at days 10-16 with doses of 0, 31.25 and 62.5 mg/kg.

Maternal deaths were observed at ascending doses in ratios of 0/8, 2/8 and 3/8. Decreased food intake was considered as responsible for weakness, of the maternal deaths, and increased foetal deaths. No anatomical abnormalities were noted in the fetti.

RATS

10 male and 20 female rats per group were fed 0, 0.15, 1.5 and 3% of daconil in a three generation study. Decreased growth occurred at all levels of treatment. The offspring showed depression of growth at all levels of treatment, but no increase in malformations. In a second rat study a level of daconil of 0.5% was fed with an additional group of rats receiving the basal ration alone. No malformations were observed in the offspring.

CONCLUSIONS:

From Dr. Long's memoranda of 1/31/69, 8/3/69 and 9/16/69 the following was concluded: In all completed studies of two years duration at levels of above 120 ppm in dogs and above 60 ppm in rats, effects were noted with the possible exception of 1300 ppm in rats. Consequently, the 120 ppm level in dogs and 60 ppm level in rats were selected as the levels fed in subsequent two year studies. Results of these studies are described in the following pages.

DOGS: Experiment No. 200-206

Groups of 8 male and 8 female dogs were fed daconil at 120, 60, and 0 ppm for 1-2 years. The animals were observed daily for appearance, behavior, appetite, elimination and signs of compound effects, and bi-weekly for body weight, food and drug consumption. Clinical examinations were performed initially and at 6, 12, and 24 months and covered erythrocyte counts, total and differential leukocyte counts, coagulation times, and hematocrit and hemoglobin determinations. Biochemical studies included determination of fasting blood sugar, blood urea nitrogen, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. Urine analysis included appearance at 0 and 6 months and quantitation of non-protein nitrogen, specific gravity, protein, glucose, acetone, bilirubin, and microscopic sediment examination.

At 52 weeks, 4 of each sex were sacrificed per treated group and the control (3 males at the high dose group). The remainder was sacrificed after 24 months experimentation. Gross necropsy was performed on all dogs including the one male which died at the high dose level after 13 weeks. Brain, thyroid, heart, liver, spleen, kidneys, adrenals and testes were weighed.

The tissues preserved in formalin were brain, pituitary, eyes, thyroids, lungs, heart, liver, gall bladder, kidneys, adrenals, stomach (fundus and

(ascending, transverse, and descending colon), pancreas, mesenteric lymph node, urinary bladder, ovaries, nerve with muscle, bone (costochondral junction) and bone marrow (femur) and testes. Microscopic examinations were performed on sections of the liver, thyroid and kidneys of all dogs. All wet tissues and paraffin blocks are in storage at Hazleton Laboratories for possible future reference. Sections from liver, kidneys, thigh muscle and body fat of each dog sacrificed at the 52 week interval were taken, frozen, and shipped to the sponsor for chemical analysis.

RESULTS:

There were instances of diarrhea, emesis, weight loss, occurrence of worms, skin eruption, a few elevations in SGOT, urea nitrogen, eosinophils, bilirubin elevation in male dogs at 12 months and elevation of urine specific gravity. One showed significance insofar as differences between the control and treated dogs were concerned, except the elevation of the bilirubin levels in the seven male dogs surviving at the high level at 12 months. However, this was not confirmed at 24 months when assays were performed on 4 of the same dogs.

Examination of liver, thyroid and kidneys of dogs sacrificed after 12 months showed compound related changes only in the kidneys of male dogs at the 120 ppm level. The changes are described as increased vacuolation of the epithelium in both the convoluted and collecting tubules and increased pigment in the convoluted tubular epithelium. These changes were not observed at 24 months. This conclusion of effects at 12 but not at 24 months was made by Hazleton Laboratories and confirmed for the 12 month feeding period by FDA in collaborative studies of slides. FDA did not study the 24 month slides. The opinions of a second consulting laboratory (KETTERING; Dr. Hans Stemmler) were sought by the petitioner. After slide examination he concluded no effects different from the controls at either treatment levels at 12 and 24 months (see attached table of summary of laboratory involvement in evaluation of daconil safety).

Gross observations which included examination for weight gain, food intake, and determination of organ weights at sacrifice reveals nothing attributable to compound effects. Chemical, biochemical, and urine examination were negative for effects of treatment.

RATS: Experiment No. 200-205

Groups of 100 Charles River caesarean-derived rats the males weighing 80-132 grams, females 80-128 grams and numbering 50 per sex were fed daconil 2787 for 2 years with interim sacrifice at 13 and 52 weeks. Levels fed were 0 (basal diet of Purina Laboratory Chow) and 4, 10, 20, 30, 40 and 60 ppm. Body weight, food consumption and gross signs of effects were recorded bi-weekly for the first 52 weeks and then monthly. Any mortality was recorded daily. Palpable growths noted throughout the study were recorded as to incidence, location, size and change in character.

The following examinations were made of 7 animals of each sex at 13, 52 and 104 weeks. Hematological examinations involving erythrocyte counts, total and differential leukocyte counts, and hematocrit and hemoglobin determinations. Biochemical examinations covering urea nitrogen, blood glucose and SGPT. Sodium, potassium, chloride and carbon dioxide were determined in serum at the conclusion of the experiment. Urine examination included appearance, pH, specific gravity, sugar, albumin, acetone, protein, bilirubin, occult blood and microscopic examination of sediment.

Fifteen rats of each sex were sacrificed at 13 and 52 weeks. Organ weight and organ to body weight ratios were determined for thyroid, heart, liver, kidney, spleen, adrenals, and testes. Histopathological examinations were made of (1) brain, eye, heart, lung, spleen, liver, stomach, small intestine, large intestine, urinary bladder, testes, ovary, bone and bone marrow of 5 males and 5 females from the 0 and 60 ppm groups. (2) Livers of 5 males and 5 females at 4 and 40 ppm. (3) Kidneys, pituitary, thyroid, and adrenal glands and tissues masses of terminally sacrificed animals.

RESULTS:

1. Physical appearance, behavior, mean body weight, weight range, food consumption and survival were normal at all levels (food consumption and growth were evaluated statistically only for the first year).

2. Hematological: At 2 years, 2 of 7 males and 1 of 7 females receiving Daconil at 60 ppm showed low hematocrit, hemoglobin and red blood cells. One of 7 male controls showed the same picture. There was a lymphopenia (increased percent of segmented cells and decrease in lymphocytes) in the three test animals which was absent in the one control. However, the percent of abnormalities is too low to support significance, though the lymphopenia could be indicative of stress from treatment.

3. Biochemical

   The only variations in these tests are not considered treatment related. Blood sugar (fasting) was depressed at 104 weeks but in all groups including the controls. Slight to moderate proteinuria (common to Charles River rats) was present at 52 and 104 weeks, but again was present in the controls as well as in the treated animals.

4. Gross pathological changes were spotty and considered unrelated to treatment.

Inspection of tabulated significant differences between test and control animals in organ weights reveals a spotty occurrence and appears unrelated to treatment. Occurrence was not dose related and unconfirmed by the histological studies according to the examiner.

Microscopic Pathology

To summarize, at 104 weeks, changes attributable to treatment were absent in rats at doses of 4-60 ppm. There was mention of certain changes not considered accountable to treatment. These are as follows:

(1) Slight increase in mammary tumors in treated female rats over the controls.

(2) Tumors of other tissues and chronic inflammatory lesions of a spotty nature.

(3) Kidney lesions considered related to occurrence of murine nephropathy.

(4) In the 60 ppm females there were nephropathic changes in kidneys considered insufficient for significance.

Kidney changes noted in rats sacrificed at 3 and 12 months of the 2 year experiment (experiment no. 200-205).

There were histological kidney changes described as follows which occurred principally in the groups fed 40 and 60 ppm of Daconil: fine vacuolation and swelling of cells lining the proximal tubules in the renal cortex. Normal appearing cells bordered such abnormal cells. The nuclei of these abnormal cells were segregated from the remainder of the cytoplasm of these vacuoles. (Note: Examiner states a similar but lesser vacuolation in control kidneys. We did not find this mentioned in the description and summary of microscopic pathology.)

Incidence of pathological changes described in the previous paragraph was as follows: 11/14 (60 ppm); 5/14 (40 ppm); 2/14 (30 ppm); 1/14 (10 ppm) and 1/14 (4 ppm). Neither necrosis of the epithelial lining tubules nor dilatation of tubules was observed which had occurred when Daconil was fed previously at higher levels. Regenerative kidney processes were increased in the current studies in the 40 and 60 ppm groups. The examiner considers the adverse changes as significant at 40 and 60 ppm.

At 12 months (the previous description was of animals fed for three months) kidney changes were observed in male and female rats at 40 and 60 ppm and 3/7 at 30 ppm. Changes are described as follows: increased vacuolation of epithelial cells together with swelling or hypertrophy of the individually affected cells often with a deposition of an eosinophilic droplet material in the cytoplasm and vacuoles occurring primarily in the deeper cortical tubules.

The degree of this described change was considered increased in females at the 40 and 60 ppm levels.

Question: What if any importance can be given to interim kidney effects which are reversed after two years feeding? Further, one evaluator did

not feel the observed changes were attributable to treatment. While there is no evidence of functional changes resulting from these structural changes, the latter are a matter of record. From our observation these changes were completely absent in the controls at three months. However there were such changes in the kidneys of the control rats at 12 months. It might then be speculated that treatment may have potentiated the onset of occurrence of these changes. However, after 24 months the animals compensated by reversal of adverse effects despite continued Daconil treatment. Are these kidney changes generally characteristic of Charles River rats fed only their maintenance diet?

CONCLUSIONS:

1. The no effect level in dogs is 60 ppm at 12 months; probably 120 ppm at 24 months.

2. After 24 months feeding the no effect level in rats is 60 ppm. The no effect level in rat at 12 months is less than 30 ppm.

The following is a reproduction of the table constructed by Dr. Lone on the vacuolation of proximal tubules of rats treated for 3 months.

VACUOLATION OF PROXIMAL TUBULES OF RATS TREATED THREE MONTHS

Daconil

PPM Diet	Micro. Sec'd. M	F	MALES Hazleton No.	Grade	Long No.	Grade	FEMALES Hazleton No.	Grade	Long No.	Grade
0	7	7	0	0	0	0	0	0	0	0
4	7	7	0	0	0	0	3	V.Sl.	3	Slight
10	7	7	2	Min.	0	0	4	V.Sl.	5	Mod.
20	7	7	2	Min.	1	Min.	7	V.Sl.	5	Slight
30	7	7	3	Min.	2	Min.	6	Slight	4	Slight
40	7	7	6	Slight	1	Min.	6	Slight	6	Sl.-Mod.
60	7	7	6	Slight	1	Min.	7	Mod.	7	Sl.-Mod.

Abbreviations

Min. - minimal
V.Sl. - very slight
Mod. - moderate
Sl. - slight

3. Based on structural changes noted in the 3 months table, the no-effect level estimates to less than 4 ppm in female rats at 3 months.

The intake of Daconil if all proposed and regulated food items were ingested would be about 1 milligram a day - See Attached Table.

DACOHIL

Experiment No.	Animal	Duration	Level PPM	FDA	Hazleton	When Evaluated	Kettering*	Conference
200-205	rat	3 mos.	4-60**	pos: at 4 ppm & above	pos: at 4 ppm & above	1969	no exam	
"	"	12 mos.	4-60**	pos: at 40 & 60 ppm; possibly at 30 ppm	pos	1969	neg. at all levels	
"	"	24 mos.	4-60**	no exam.	neg.	1970	neg. at all levels	
200-206	dog	12 mos.	0 60 120	pos	pos	1969	neg. at all levels	Yes: FDA & Hazleton confirmed negative finding at 60 ppm by collaborative examination of slides
"	"	24 mos.	0 60 120	no exam.	+ - at 120 ppm	1970	neg. at all levels	

*Became involved in mid 1970

**4, 10, 20, 30, 40 and 60 ppm

Pesticide Petition No. 1F1024 (Daconil)

Status	Product	Diet*	Level PPM	Microgram Ingested per day
Regulated	Potatoes	15%	0.1	22.5
Proposal	Peanut	F	20	none
	Vine	E		"
	Hay	T		"
	Sugar Beet	F	20	"
	Tops	B		"
	Sweet Corn	G	20	"
	Forage	D		
	Celery	0.75	15	171
	Snap Beans	0.74	5	35
	Broccoli	0.19	5	13
	Brussels Sprouts	0.16	5	3
	Cabbage	0.49	5	37
	Carrots	0.56	5	42
	Cauliflower	0.43	5	32
	Cucumbers	0.73	5	53
	Melons	1.98	5	150
	Pumpkins Squash (Summer&Winter)	0.19	5	3
	Tomatoes	3.34	5	250
	Lima Beans	0.74	1	11
	Sweet Corn	1.19	1	17

*Lehman: The annual per capita consumption of selected food items in the U.S.