5/5/71

004678

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), a fungicide, -- requests for tolera[illegible] as follow: PP No. 0F0906, 1 ppm on bananas, of which [illegible] than 0.5 ppm will be in pulp; PP No. 0F1000, 15 ppm in or on plums, prunes, nectarines, apricots, cherries, and prunes; PP No. 1F1016, 15 ppm in bean forage and hay, peanut hulls, forage, and hay, and sugarbeet tops; 2 ppm in beans; 0.2 ppm in peanuts and sugarbeets (roots); and 0.05 ppm in milk and meat, fat, and meat byproducts of cattle, goats, hogs, horses, and sheep; PP No. 1F1033, 7 ppm in or on apples, crab-apples, and pears; and PP No. 1F1045, 1 ppm on cucumbers, melons pumpkins, summer squash, and winter squash. (Toxicity data are from the Haskell Laboratory, Wilmington, Delaware or the Hazleton Laboratory, Falls Church, Virginia.)

File: PP# 0F0906

TO: Mr. Drew Baker
Pesticides Tolerances Division

Pesticide Petition Nos. 0F0906, 0F1000, 1F1016, 1F1033, and 1F1045

E. I. DuPont De Nemours & Co.
Wilmington, Delaware

Petitioner requests tolerances for benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, on commodities listed in title of this memo.

TB (JW) So far, OT has approved tolerances for benomyl, as follows: temporary, negligible ones at 0.2 ppm on pecans, peanuts, and sugarbeet roots (PP No. 9F0936) and temporary ones at 15 ppm in or on peaches, nectarines, apricots, cherries, prunes, and plums; at 10 ppm on grapes; and at 7 ppm in or on apples, pears, crabapples, and quinces (PP No. 1F1033)--Cf. OT memo of March 1, 1971, in latter petition and that of March 25, 1971, in PP No. 0F0906.

TOXICITY:

Petitioner herewith submits report of completed chronic dog feeding study and new material in response to OT's request (in earlier memo, above) for satisfactory teratologic findings in two animal species (including the rat); subacute data in one animal species for the chief plant metabolite of benomyl, methyl 2-benzimidazolecarbamate; and information on whether benomyl, a carbamate, exerts cholinergic effects.

One chronic feeding study on benomyl, final report (Haskell Laboratory Report No. 48-70), dated March 17, 1970, submitted with petition.

(For our review of interim report, Cf. earlier OT memo, above.)

No. of Animals. 4M and 4F/group.

Feeding Levels.* 0, 100, 500, and 2,500 ppm.

Duration. 2 years.

* See next page for footnote.

Mortality. No deaths, but one male dog at 2,500 ppm was killed at 47 weeks after showing weight loss, passage of black stools, and ascites.

A replacement dog was put on test for one year. One male and one female each from control and 2,500-ppm groups were killed at 1 year for histopathologic study.

Body Weight and Food Consumption. Both decreased at intervals in 2 males and one female at 2,500 ppm. When dogs were temporarily put back on control diet, they gained weight but lost it again on resuming test regimen.

General Behavior. Toxic effects, as noted above, in the male dog at 2,500 ppm which was killed at 47 weeks.

Clinical Laboratory Tests. Apparent elevated blood glutamic-pyruvic transaminase, cholesterol, and alkaline phosphatase and apparent decreased blood total protein and albumin-globulin (A/G) ratio in male dogs at 2,500 ppm. In females at 2,500 ppm, apparent increase in blood cholesterol level and decrease in both total protein and A/G ratio. No effect on hematological or urinary values.*2 No effect on blood glucose or urea nitrogen values.

Organ Weight and Histopathology.*3 In the male 2,500-ppm dog, killed at 47 weeks, severe liver damage (cirrhosis) plus mild arrest of spermatogenesis and reduced testis weight (said by Petitioner, probably due to failure of damaged liver to inactivate estrogen).

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* Test material, an approximately 50% wettable powder, was of composition given in previous DI memo (March 25, 1970, PP No. 0F0990, p. 2), starting with Haskell No. 5167-8, 11/20/68 - 3/23/69, INT-1991, 51.5% active and Haskell No. 5167-C, 3/23/69 - End, INT-1991-190, 53.0% active ingredient.

*2 RBC, WBC (total and differential), hemoglobin level, and hematocrit. Urinary volume, concentration, protein, pH, sugar, urobilogen, acetone, bilirubin, occult blood, and sediment, microscopic appearance.

*3 Organs weighed: brain, heart, lung, liver, spleen, kidney, testis, stomach, thyroid, adrenal, and pituitary. These and following, examined microscopically: Pancreas, prostate, urinary bladder, epididymis, fallopian tube, uterus, ovary, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, mammary gland, esophagus, gall bladder, spinal cord, trachea, thymus, salivary gland, and tonsil.

No effect in male or female killed at 1 year. In dogs on 2,500 ppm for 2 years, liver cirrhosis in 3 (of 6)--a female and 2 male; in 3rd male, marked diffuse testicular degeneration with reduced testis weight, absence of spermatozoa, and partial lossoef primary spermatocytes. In 3 (of 3) male 500-ppm dogs (only), chronic orchitis; in one or two males at all test levels (not in controls), focal testicular degeneration--each of these conditions called "spontaneous" by Petitioner.

"No-Effect Level." 500 ppm, says Petitioner; Cf. "Addendum," below.

"Effect Level." 2,500 ppm, "effects" being liver damage (cirrhosis) and apparent adverse effect on testis, says Petitioner. Cf. "Addendum."

Addendum to evaluation of chronic dog study (above).

"No-Effect Level." Above estimation of this is based on Petitioner's account of the study; however, for confirmation, our pathologists checked slides from Petitioner's study (Cf. report of L. Davis, DVM, dated April 30, 1970). Based on his findings that "testicular lesions....are of little significance and do not appear to be attributable to benomyl ingestion," and that liver pathology in dogs at 2,500 ppm is considerably less severe than reported by Petitioner, we estimate "no-effect level" for this study to be at least as high as 100 ppm. To establish whether it is higher, e.g., 500 ppm or more, we will need the further histopathological examination of dog tissues recommended by Dr. Davis in the report.

Acute oral toxicity study of methyl 2-benzimidazolecarbamate, Haskell Laboratory Report No. 99-66, dated July 15, 1966, submitted with PP No. 1F1011.

Test material was given by stomach tube (as a 5-to-30% suspension of methyl 2-benzimidazolecarbamate (sample of Code No. DMC-965-27) in peanut oil to young adult Charles River-CD male rats in single doses. Survivors were killed after 14 days; testis and epididymis were studied grossly and microscopically.

The acute lethal dose is greater than 17 g/kg body weight.

One g/kg body weight and above causes adverse effect on testis, with reduction in, or absence of, sperm.

PP #s 0F0994, 0F1033, 1F1010, 1F1033 and 1F1045

004678

Ten-day subacute oral toxicity of methyl 2-benzimidazolecarbamate, above report.

Details of study, as above, except five daily doses each per week, at 0.2 and 3.4 g/kg/body weight/day, given during 2 weeks, and other tissues examined microscopically.*

Two (of 6) rats died after 7 or 10 doses on high level; survivors lost weight and had mild diarrhea. No toxic symptoms in low-level rats.

In high-level rats, severe testicular damage and absence of sperm plus edema and focal necrosis of duodenum; reduction of blood-forming elements in bone marrow; and decrease in large globular-shaped vacuoles located centrolobularly in the liver.

In low-dose animals, slight adverse effect on testis, only tissue checked histologically.

Acute oral and subacute oral toxicity of methyl 2-benzimidazolecarbamate, Haskell Laboratory Report No. 125-65, dated August 23, 1965, in PP No. 1F1010

Acute lethal dose of test substance to young adult Charles River rats of the Wistar strain is greater than 11 g/kg/body weight. Dose of 1 g/kg body weight, or higher, depresses spermatogenesis.

Rats (6/6) survived ten daily doses of 5 g/kg/body weight/day of methyl 2-benzimidazolecarbamate. They lost weight, showed weakness, hair loss, and polyuria. The testes were soft, small, and aspermatogenic both at 3 hours after, and at 10 days after, last dose.

Ninety-day rat feeding study on methyl 2-benzimidazolecarbamate, Haskell Laboratory Report No. 95-66, dated May 15, 1966, submitted with PP No. 1F1[illegible].

No. of Animals. 16 M and 16 F/group.

Feeding Levels.** 0, 100, 500, and 2,500 ppm.

Duration. 90 days.

* In addition to testis--liver, kidney, spleen, bone marrow, thyroid, lung, G.I. tract, brain, thymus, and pancreas.

** Test substance: 70% wettable powder formulation of technical methyl 2-benzimidazolecarbamate, dietary levels being of active ingredient: for details of formulation, Cf. under reproduction portion of study (below).

Mortality. None.

Body Weight and Food Consumption. No effect.

General Behavior. No effect.

Clinical Laboratory Tests. No effect on routine hematologic or urinary test values except for hematuria in one male rat each at 500 and 2,500 ppm; no effect on plasma alkaline phosphatase or glutamicpyruvic transaminase (clinical laboratory tests, conducted at monthly intervals).

Organ Weight and Histopathology. No effect on various organ weights, including that of testis, or on tissues examined histopathologically.*

"No-Effect Level." 2,500 ppm (however, we note no higher "effect" level was tested).

Reproduction study portion of above report on methyl 2-benzimidazolecarbamate

No. of Animals. 6M and 6F/group.

Feeding Levels. 0, 100, 500, and 2,500 ppm.**

Duration. Time needed to produce one generation, two litters of offspring.

* Brain, heart, lungs, liver, spleen, kidney, testis, stomach, thymus, adrenal, pituitary, ovary, epididymus, fallopian tube, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, spinal cord, trachea, pancreas, thyroid, parathyroid, salivary gland, and exorbital lacrimal gland from 2,500-ppm rats (and controls).

** [redacted] Dietary levels are of active ingredient, methyl 2-benzimidazolecarbamate.

Reproductive Performance. See table, below.

Feeding Level (ppm)	Av. No. of Pups/Litter (Born)	(Born Alive)	Indices* (%) F.I.	G.I.	Viab.	L.I.	Av. Weight of Pups (g)
	---F_{1a} Litter---						
0	12.9	12.3	50	100	100	100	67
100	----	----	0	---	---	---	--
500	12.2	11.9	67	100	92	100	6
2,500	10.5	10.5	67	100	100	100	61
	---F_{1b} Litter---						
0	14.0	14.0	33	100	100	85	66
100	----	----	0	---	---	--	--
500	8.0	7.0	67	100	84	96	62
2,500	11.0	10.5	33	100	95	100	61

"No-Effect Level." 2,500 ppm (however, we note, a higher "effect" level was not tested).

Benomyl--in vitro test for effect on activity of cholinesterase, in PP No. 1F1010.

Test was modified assay of Hobbiger et al. (J. Econ. Ent. 51, 326-9 (1958)) in which acetylcholine is added to test system after candidate inhibitor has been incubated with acetylcholinesterase of bovine erythrocytes for a specified period of time.

Test determines "acetylcholinesterase inhibition constant," K_I (concentration of inhibitor necessary to give 50% inhibition of enzyme).

$K_I = 4.5 \times 10^{-3}$ was found; whereas K_I's, determined by same procedure, for methomyl and carbaryl = 3.2×10^{-7} and 1.4×10^{-6}, respectively.

* Indices are, respectively: Fertility Index, per cent pregnancies of matings; Gestation Index, per cent live litters of pregnancies; Viability Index, per cent 4-day survivors of births; and Lactation Index, per cent 21-day survivors of 4-day survivors.

In vitro, Enca, benomyl inhibits cholinesterase very little. Compared to such standard carbamate-, cholinesterase-inhibiting pesticides as methomyl and carbaryl, it inhibits only 1/10,000th or 1/1,000th as much.

Teratological study on benomyl in rats, Haskell Laboratory Report No. 495-75, dated July 9, 1975. Submitted in PP #s [illegible], received July 15, 1975.

No. of Animals. 25 to 28 (pregnant Charles River-CD) F rats/group.

Feeding Levels. 0, 100, 500, 2,500, and 5,000 ppm* in diet of rats from 6th through 15th days of gestation.

Duration. Twenty days of gestation. All rats killed then.

Body Weight and Food Consumption. No effect (on maternal rats) except for slightly lower food consumption in 5,000-ppm rats.

Clinical Signs. No effect (on maternal rats).

Gross Pathology. No effect (on maternal rats).

Teratologic Data. No effect on: Number of implantation sites,*** number and location of live fetuses,**** dead fetuses,***** and resorption sites****** (in respective uterine horns); body weight, crown-rump length, and sex of fetuses; type or incidence of gross external, gross visceral,** or skeletal anomalies** in the fetuses.

No-Effect Level. 5,000 ppm (but no higher level tested).

[redacted]

** Studied, respectively by Wilson's method (fixation of fetuses in Bouin's fluid, followed by free-hand razor blade sectioning) and staining of skeleton with Alizarin, followed by clearing.

*** Implantation sites/pregnant female = 9.3, 9.2, [illegible], and 10.0, respectively in order of dosage = 0, 100, 500, and 2,500 ppm.

**** Live fetuses/litter= 8.8, 9.3, 9.0, 9.1, and 10.0, same order.

***** There were no dead fetuses.

****** Total resorption sites, % of implantation sites. = 5.1, 5.8, 2.7, 6.9, and 1.6, same order.

PP #s 0F[illegible], 0F[illegible], 1F1[illegible], - 6 - -
1F1033 and 1F1045

Teratologic study on Lenamyl in albino rabbits, Hazleton Laboratory Report dated July 15, 1983, submitted with PP No. 1F1010.

We judge following summary to be a fair appraisal of results of study. We add selected values, below (in footnotes), to illustrate findings.

"The purpose of this study was to evaluate the potential of Fungicide 1991 (Lenamyl, Code No. ISI-1991-95, powder, approximately 50% active ingredient) for embryotoxic and/or teratogenic effects in (New Zealand White) albino rabbits. The test material was administered in the diet (Purina Rabbit Chow available ad lib.) at dose levels of 0, 100, and 500 ppm (to 15 each artificially inseminated does/group on days 6-18, inclusive, of gestation).

"There were no maternal deaths during the study. One abortion occurred in the low level group. Tissue masses which were apparent fetuses and dead pups were found in the cage pans of one low-level doe and one high-level doe prior to initiation of the treatment period. Both of these animals were sacrificed on Day 6 and were excluded from the study. A total of 34 of 43 does used in this study (excluding the two does which were sacrificed because pregnant (12 control, 13 low level, and nine high level).

"The appearance, behavior, body weight gain,* and food consumption of the test animals were, in general, comparable to the controls. No evidence of a compound-related effect was noted in the following criteria: Findings from gross necropsies performed on the does, the number and placement of implantation sites,** resorption sites,*** or live or dead fetuses**** from Caesarean deliveries; weight and length of fetuses, fetal external appearance, and gross visceral anatomy; the number of live and dead pups from full-term litters,***** pup weight and length, external appearance, and visceral anatomy. The development and structure of test fetal and pup skeletons (studied after alizarin staining and clearing) were comparable with the control animals and with accumulated control data.

"Dietary administration of Fungicide 1991 (Lenamyl) to female albino rabbits from Day 6 through Day 18 of gestation (at 100 or 500 ppm in the diet) had no discernible effect on fetal development."

* Mean weight gain during 3-week period for controls, 100-, and 500-ppm females is 413, 421, and 3[illegible] g, respectively.

** Implantation sites, 7.3, 7.3, and 8.0/maternal rabbit--control to high level groups.

*** Resorption sites, 0.3, 0.3, and 0.3/maternal rabbit (0- to 500-ppm).

**** Live fetuses, 6.5, 6.0, and 6.0/maternal rabbit and dead fetuses, 0.2, 0.4, and 1.0/maternal rabbit--same progression.

***** Live pups, 6.2, 6.0, and 5.5/maternal rabbit and dead pups, 2.5, 0.7, and 0.3/maternal rabbit--same progression as above.

EVALUATION:

To our previous estimates of "no-effect levels" for benomyl and/or metabolites (in our memo of March 25, 1970, PP No. 0F0900, p. 11) can be added these new ones:

Study	"No-Effect Level"
Dog chronic, benomyl	At least 100 ppm (tentative)
Rat subacute, metabolite*	2,500 ppm**
Rat reproduction, 1-generation, 2-litter, metabolite*	2,500 ppm**
Rat teratologic, benomyl	5,000 ppm*
Rabbit teratologic benomyl	500 ppm**

* Metabolite is methyl 2-benzimidazolecarbamate.

** Value is highest level tested in study.

Also, acute lethal dose to rat of methyl 2-benzimidazolecarbamate exceeds 11 g/kg body wt. A 1-g/kg dose damages testis.

Regarding effect of benomyl on cholinesterinase when tested in vitro, Petitioner has shown inhibition is very slight, e.g., 1/1,000th (or less) of that caused by carbaryl or methomyl--each a standard carbamate-, cholinesterase-inhibiting pesticide. (Nor are in vivo cholinergic effects of benomyl or metabolite noted anywhere in toxicity studies described in various benomyl petitions.)

Petitioner has now satisfactorily fulfilled requests we made for additional toxicity data on benomyl and metabolite, methyl 2-benzimidazolecarbamate (DI memo of March 25, 1970, PP No. 0F0900).

To date (May 3, 1971), dog chronic study shows lowest "no-effect level" for benomyl; it equals at least 100 ppm or 2.5 mg/kg body wt/day. (Since "no-effect levels" for methyl 2-benzimidazolecarbamate--in subacute rat or rat reproduction study--exceed this, on either ppm- or mg/kg/day-basis, the ADI, based on it applies to this metabolite as well.)

Based on dog "no-effect level," and on 1 : 1 safety factor, allowable daily intake (ADI) for humans is 0.025 mg/kg body wt/day (1 ppm in diet).

Maximum residues of benomyl to be expected in an average human daily diet, if all tolerances requested in petitions listed in title of this memo are granted (cf. accompanying table) total 0.1 ppm, whole-diet basis.

Since total residues to be expected in the average daily diet of a human in the U. S., should all presently requested tolerances for benomyl be granted, are less than the ADI calculated from available toxicity data, these tolerances will be safe.

For consideration of safety of any tolerances on benomyl which may be asked for in the future, however, we will need the studies asked for by Dr. Davis (report of April 30, 1971, PP No. OF1000), i.e., "a detailed histopathology examination (and report) including fat stains on liver and iron stains on bone marrow....on the 500-ppm and 100-ppm dogs."

CONCLUSIONS:

1) Tolerances on benomyl asked for in PP's Nos. OF0936, OF1000, IF1013, IF1033, and IF1045 (listed in title of this memo) are safe.

2) For consideration of safety of any tolerances on benomyl which may be asked for in the future, we require further studies on tissues from the dogs fed benomyl for 2 years. They are specified in Dr. K. J. Davis' memo of April 30, 1971, in PP No. OF1000, viz. "a detailed histopathology examination (and report) including fat stains on liver and iron stains on bone marrow.....on the 500- and 100-ppm dogs.

Mary L. Qualfe, Ph.D.
Toxicology Branch
Pesticides Tolerances Division

cc:
WGFitzhugh
JCCummings
PRD/EPA
Perrine Br.
Atlanta Br. (CLewis)
PP Nos. OF0936, OF1000,
IF1010, IF1033 & IF1045

MLQualfe/cc
5/5/71
RD/Init: GEWhitmore
5/4/71

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TABLE

Benomyl tolerances, calculations of diet coverage, to date*

Tolerance at	Commodity	Fraction in diet**	Whole-diet basis (ppm)
15 ppm	apricots	0.0012	
	bean forage & hay	------	
	cherries	0.0017	
	nectarines	0.0001	
	peaches	0.0103	
	peanut hulls, forage, & hay	------	
	plums)	0.0023	
	prunes)		
	sugarbeet tops	------	
		0.0161	0.2415
10 ppm	grapes ***	0.0032	
		0.0032	0.0320
7 ppm	apples	0.0181	
	crabapples	?	
	pears	0.0032	
	quinces***	?	
		0.0213	0.1492
2 ppm	beans	0.0124	
		0.0124	0.0248
1 ppm	cucumbers	0.0073	
	melons	0.0128	
	pumpkins, winter squash, and summer squash	0.2419	
		0.0290	0.0290
1 ppm (0.2 ppm in pulp)	bananas	0.0151	
		0.0151	0.0030
0.2 ppm	peanuts	0.0060	
	sugarbeets	?	
	pecans	?	
		0.0060	0.0012

(Table continued on next page)

* See next page for footnotes

TABLE

0.05 ppm	milk	0.2600	
	meat, fat, and meat byproducts of cattle, goats, hogs, horses, and sheep	0.0902	
		0.3500	0.0175
	TOTAL		0.4062

a These are tolerances requested in PP's nos. 5G6, 3E, 1505, 1513, 1536, and 1045.

aa Lehman's tables in Assoc. Fd & Drug Officials Quart. Bull. 26, 149 (1962).

aaa Temporary tolerance only.