DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

004678

Date: March 25, 1970

Reply to Attn of:

File: PP# OFO 9

Subject: Benomyl ("Benlate;" methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), requested tolerances: At 1 ppm on bananas, of which not more than 0.2 ppm shall be in pulp after peel is removed and discarded (PP No. OFO-906); temporary tolerances at 10 ppm in or on peaches, nectarines, apricots, cherries, prunes, plums, and grapes; at 2 ppm in or on beans; and at 0.2 ppm (negligible residue) in or on pecans, peanuts, and sugar beet roots (PP No. OGO-936).

PESTICIDE PETITION NO. OFO-906
PESTICIDE PETITION NO. OGO-936

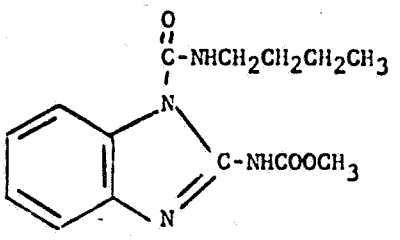
E. I. du Pont de Nemours & C
Wilmington, Delaware 19898
(AF 4-408)

TO: Mr. Drew Baker, DRPC (BF-320)

Petitioner wants tolerances for benomyl, a fungicide, as listed in title.

Formulated as a 50% wettable powder (Benlate), it is to be used at ½ to 1 lb per 100 gallons water (= 300 to 600 ppm) for single post-harvest treatment as dip or spray of bananas for control of crown rot and surface molds, (PP No. OFO-906). Recommended rates vary from 3/8 to 2 lbs per acre for nut crops and from 3/8 to 1 lb per 100 gallons of water for fruit and nut crops for control of certain diseases (PP No. OGO-936).

Benomyl is methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.



Its formula is shown here.

A white crystalline solid, benomyl is decreasingly soluble in chloroform dimethylformamide, acetone, xylene, ethanol, heptane, and (negligibly soluble in) water.

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Benomyl is formulated, as follows:

PP No. OFO-906. "Benlate," Benomyl, 50% wettable powder

[redacted]

Samples of benomyl used for toxicity studies were, as follows:

Haskell No.	Dates of Use	Sample	% Active Ingredients (Technical)
5043	3/22/67 - 5/17/67	INT-1991-30	72.2
5167-1	5/17/67 - 11/6/67	INT-1991-54	51.5
5167-2	11/6/67 - 1/3/68	INT-1991-75	51.5
5167-2	1/3/68 - 4/3/68	INT-1991-90	52.0
5167-5	4/3/68 - present	INT-1991	52.0

[redacted]

[redacted]

In diet preparation, formulations were used as if they consisted of 70% or 50% active ingredient, 1-butylcarbamoyl-2-benzimidazolecarbamic acid, methy ester.

No tolerances presently exist for this material (benomyl).

TOXICITY:*

A. Acute toxicity, oral.

Rat LD_{50} > 9,590 mg/kg

Acute toxicity, dermal.

Rabbit LD_{50} > 10,000 mg/kg; no deaths.**

Acute toxicity, inhalation.

Rat LC_{50} > 1.37 mg/liter; no deaths.***

Skin and Eye Tests

No skin irritation and moderate sensitization, seen in guinea pigs, and "mild transitory conjunctivitis, with minor corneal effects and slight iritic congestion," seen in rabbits.

B. Subacute toxicity studies

Rat, M. Cnk-CD strain. Six rats each were intubated with peanut oil suspension of benomyl as unformulated chemical 5 times/wk for 2 weeks at 200 and 3,400 mg/kg body wt. Former caused no deaths, latter 4/6. Lethal dose caused weight loss, diarrhea, and histologic changes in stomach, liver, and, especially, the testis; effects in former two organs were erosion and thickening of squamous mucosa of the stomach

* All studies were done at Haskell Laboratory of E. I. du Pont de Nemours & Co. unless otherwise indicated.

** Except for dermal irritation, no signs of toxicity. Test involved 24-h exposure of intact and abraded abdominal skin (wrapped).

*** Four-hour exposure to atmospheric dust.

with submucosal inflammation and a decrease in the large globular-shaped vacuoles located centrolobularly in the liver. Low dose caused no clinical signs of toxicity and only very minor changes in testis of 2 of 6 rats, as seen on histological examination.

90-day feeding in rat (ChR-CD).

No. of Animals. 16 M and 16 F/group.
Feeding Levels.* 0, 100, 500, and 2,500 ppm.
Duration. 90 days.
Mortality. One male at 100 ppm died after 39 days; death not related to feeding of benomyl, according to Petitioner.
Body Weight. No effect. (No effect, also, on food consumption or on food efficiency.)
Organ Weight. Livers of females at 2,500 ppm, significantly heavier ($p < 0.001$) than those of corresponding controls. Otherwise, no effect on weights of following: Brain, heart, lungs, liver, spleen, kidney, testis, stomach, thymus, adrenal, or pituitary.
Clinical Laboratory Tests. No effect on hematological values (on 6 male and 6 female rats, done pre-test and at 30, 60, and 90 days), including RBC, WBC (total and differential), hemoglobin, and hematocrit. No effect on alkaline phosphatase in 2,500-ppm rats, but increased serum glutamic-pyruvic transaminase (34 vs. 23 for control of males at 2,500 ppm. No effect on results of urinalysis, including determinations of volume and concentration; tests for protein, sugar and ketone bodies; determinations of pH and color; test for presence of occult blood; or macroscopic examination of sediment.
Histopathology. No significant effects on following tissues of control and of 2,500-ppm rats: Brain, heart, lung, 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; mammary gland, not examined.
"No-Effect Level." 500 ppm.
("Effect-Level," 2,500 ppm. caused increased liver weight in female rats and slightly increased serum glutamic-pyruvic transaminase level (SGPT) in males.)

90-day feeding in dog (beagle).

No. of Animals. 4 M and 4F/group.
Feeding Levels. 0, 100, 500, and 2,500 ppm.**
Duration. 90 days.
Mortality. No deaths.
Body Weight. No effect. (Nor any on food consumption.)

*Test material was 70% wettable powder, formulated as given above (for INT-1930).
** Test material was INT-1991-54, formulated as described (above) for "other samples of INT-1991."

Organ Weight. No effect on weights of brain, heart, lungs, liver, spleen, pancreas, kidney, testis, prostate, stomach, thyroid, adrenal, or pituitary.

Clinical Laboratory Tests. No effect on hematological values (determined pre-test and at 1, 2, and 3 months), including RBC, WBC (total and differential), hemoglobin, and hematocrit. No effect on values for blood glucose and urea nitrogen (determined at 1, 2, and 3 months); however, 2,500-ppm dogs had slightly increased cholesterol alkaline phosphatase, and glutamic-pyruvic transaminase; slightly decreased total protein; and consistently increased (about 40%) albumin-globulin ratio. No effect on results of urinalysis, including determinations of volume and concentration; tests for protein, sugar, urobilogen, presence of occult blood, acetone, and bilirubin; determination of pH; and microscopic examination of sediment.

Histopathology. No effect on brain, heart, lungs, liver, spleen, pancreas, kidney, testis, prostate, stomach, thyroid, adrenal, pituitary, epididymus, fallopian tube, uterus, ovary, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, mammary gland, esophagus, gall bladder, urinary bladder, trachea, thymus, salivary gland, and tonsil.

"No-Effect Level." 500 ppm.

"Effect Level " is 2,500 ppm, and effect consists of slightly increased cholesterol, alkaline phosphatase, and serum glutamic-pyruvic transaminase and significantly decreased A/G ratio.

C. Chronic toxicity studies

Rat, 2-year feeding.

No. of Animals. 36 M and 36 F/group.

Feeding Levels.* 0, 0 (second control group), 100, 500, and 2,500 ppm

Duration. 2 years.

Mortality. No effect.

Body Weight. No significant effect. (None on food consumption or food efficiency, either.)

General Behavior. No effect. No clinical signs of toxicity attributed to effect of benomyl.

Organ Weight. No effect on weights (either absolute or relative to body weight) of brain, heart, lungs, liver, spleen, kidneys, testes, stomach, adrenals, and pituitary.

Clinical Laboratory Tests. No effect on alkaline phosphatase or serum glutamic-pyruvic transaminase determined in rats of both control

* Test substance was 70% or 50% wettable powder formulated as given, above for either INT-1991-30 or INT-1991. Dietary levels based on active ingredient.

groups and those at 500 or 2,500 ppm at 0, 1, 3, 6, 9, 12, 18, and 24 months on test. No effect on hematologic values (same ones as determined in rat 90-day study at time intervals given in preceding sentence). No effect on results of urinalysis (also done at same time intervals): Volume; solute concentration; levels of sugar, protein, and ketone bodies; color; pH; presence of occult blood; and microscopic appearance of urinary sediment.

Histopathology. Tissues examined histologically, in addition to those listed under "organ weight," above, are: Ovary, epididymus, fallop tube, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, lumbar spinal cord, trachea, thymus, pancreas, thyroid, parathyroid salivary gland, lymph node, and exorbital lacrimal gland. These tissues from control groups and from 2,500-ppm group examined at both 1 and 2 years. At 2 years, liver, kidney, and testis of 100- and 500-ppm groups also studied. No significant findings believed related to intake of test compound, benomyl, were made. Validity of this opinion is verified by K. Davis, DVM, Pathologist (told to M. Quaife on January 20, 1970). We note that only certain tissues of animals in one of the control groups were studied. In male rats of the other control group (1A), there was a very high incidence of pituitary tumors and chronic nephritis (> 85% each); such incidence of pituitary tumors is not matched in the 2,500-ppm male rats. Liv changes were of frequent occurrence but about equally spread betwee control and test groups. Likewise, for testicular degeneration in male rats.

Neoplasms. No effect.

"No-Effect Level," 2,500 ppm.

Dog, 1-year feeding.

A study with 4 M and 4 F dogs/group and with same feeding-levels as used in the chronic rat study has been conducted for 1 year. Results are said to show no nutritional, clinical, hematologic, urinary, or pathologic evidence of toxicity due to benomyl except some aberrant biochemical values in 2,500-ppm dogs. The latter include decreased total serum proteins and albumin/globulin ratios and increased levels of cholesterol, plasma alkaline phosphatase, and serum glutamic-pyruvi transaminase.

E. Reproduction study

Rat, 3-generation, 7-litter.

No. of Animals. 6 M and 6 F/group, F_0 parents (animals left from 90-d study); 12 M and 12 F/group, F_{1b} parents; and 20 M and 20 F/group, F_{2b} parents.

Feeding Levels.* 0, 100, 500, and 2,500 ppm.
Duration. Time to produce 3 generations, 7 litters in all.
Mortality. No effect on numbers of stillborn or on survival to 4 days or to weaning.
Body Weight. Pups from parents at 500 and 2,500 ppm weighed less, at weaning, than control or "100-ppm" pups in the F_{2b}, F_{3a}, F_{3b}, F_{3c} litters. (See Table, below.) However, the various groups of F_{3c} pups kept on test for 9 weeks post-weaning and for a further weeks on control diets had growth curves of similar slope.
Histopathology. No effect on F_{3b} weanlings. Tissues studied were: Pituitary, thyroid, parathyroid, adrenal, skeletal muscle, sciatic nerve, brain, spinal cord, eye, exorbital lacrimal gland, mammary gland, bone marrow, spleen, thymus, lung, upper trachea, heart, stomach, duodenum, cecum, salivary gland, pancreas, liver, testis or ovary, epididymus or fallopian tube, uterus or prostate, urinary bladder, and kidney.
"No-Effect Level." Conservatively, 100 ppm; since average weanling weights in F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters are low for "500-ppm" "2,500-ppm" pups, as compared to corresponding control and "100-ppm" values.

* Test substance was 70% or 50% wettable powder prepared from technical benomyl and formulated as described in introduction to this memo for INT-1991-30 or INT-1991. (However, in place of INT-1991-30, a sample numbered INT-1991-9 was used.) Dietary levels are of active ingredient.

Parameters in this reproduction study are tabulated:

ppm Benomyl	Average Litter Size	Average No. Born Alive	F.I. (%)	G.I. (%)	V.I. (%)	L.I. (%)	Average Weanling Weight (g)
			F_{1a} Litter				
0	11.7	11.2	100	100	94	98	48
100	11.2	11.2	67	100	100	100	54
500	10.2	10.0	83	100	98	98	59
2,500	13.0	12.8	100	100	97	93	57
			F_{1b} Litter				
0	12.5	10.8	100	100	87	98	57
100	13.6	13.2	83	100	97	100	58
500	11.6	10.6	83	100	91	93	62
2,500	13.2	12.1	100	100	91	100	54
			F_{2a} Litter				
0	10.8	10.4	83	100	95	96	51
100	11.8	11.6	92	100	97	95	56
500	10.6	10.5	83	100	98	97	52
2,500	11.3	11.2	91	100	96	98	48
			F_{2b} Litter				
0	10.8	10.0	92	91	90	99	60
100	13.6	13.6	92	100	100	100	59
500	11.1	10.6	67	100	89	97	52
2,500	12.9	12.6	91	90	96	100	51
			F_{3a} Litter				
0	9.5	8.9	85	100	93	99	56
100	11.3	10.7	75	93	90	98	57
500	9.6	9.5	70	100	98	100	52
2,500	11.9	11.7	80	100	98	99	51
			F_{3b} Litter				
0	13.1	12.6	80	100	95	99	58
100	13.5	13.3	68	92	97	100	59
500	11.1	10.7	70	100	94	99	52
2,500	11.9	10.4	85	100	84	98	54
			F_{3c} Litter				
0	11.6	10.0	65	92	87	100	60
100	11.9	10.5	67	100	87	100	62
500	9.5	8.5	55	100	88	93	52
2,500	13.0	10.6	75	93	79	96	51

D. Biochemical studies

"Isolation and Identification of a Metabolite of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate in Rat Urine." J. A. Gardiner, R. K. Brantley, and H. Sherman, J. Ag. Food Chem. 16, 1050 (1968).

Methyl 5-hydroxy-2-benzimidazolecarbamate was the major metabolite of benomyl isolated from rat urine. Rats had received 2,500 ppm benomyl in the diet for 6 months. The metabolite occurred in urine as 40 ppm free and 1,200 ppm conjugated (as sulfate and/or glucosu benomyl.

"Metabolism of Methyl 1-(Butylcarbamoyl)-2-C^{14}-benzimidazolecarbam in the Rat." J. A. Gardiner, H. Sherman, and R. W. Reiser.

This study is summarized in DOP/PEB memo of February 19, 1970, PP OGO-936, p. 4. Again, methyl 5-hydroxy-2-benzimidazolecarbamate w virtually the only urinary metabolite of benomyl, and no parent co pound appeared in urine.

"Studies with 2-C^{14}-Labeled Methyl 1-(Butylcarbamoyl)-2-benzimidaz carbamate (Benomyl) in Rats." I. J. Belasco, J.J. Kirkland, H.L. and H. Sherman.

Residues of benomyl (I) and/or methyl 2-benzimidazolecarbamate (II methyl 5-hydroxy-2-benzimidazolecarbamate (III), and methyl 4-hydr 2-benzimidazolecarbamate (IV) were simultaneously determined in ra blood and testis. Acid hydrolysis of sample converted I to II and freed metabolites from conjugates. An organic solvent extract of hydrolyzed sample was cleaned up by solvent-solvent partition and subjected to a single scan by cation exchange liquid chromatograph

After rats took 1,000 mg/kg benomyl by mouth, once, C^{14}-residues (calculated as benomyl) were 3 to 13 ppm in blood and 2 to 4 ppm testis. The metabolite, III, appeared in both tissues within an h after dosage. Concentration of I and/or II decreased with time, a that of III increased, in both tissues. No (< 0.1 ppm) IV occurre either tissue.

At 1 hour after taking last of 10 repeat doses of 200 mg/kg/day o benomyl by mouth, rats showed no (< 0.1 ppm) I/II or IV and only levels of III (1.5 ppm in blood and 0.3 ppm in testis). At 24 ho no (< 0.1 ppm) I/II, III, or IV was found. (Only very minor histo pathologic changes in testis occurred in these treated rats.)

No I/II or IV occurred in blood or testis of rats on 2,500 ppm benomyl for 1 year. Only 0.2 ppm III was found in blood and none (< 0.1 ppm) in testis.

F. "Toxicity-to-wildlife" studies

Species	Time	LC_{50} (ppm)
goldfish	96 hrs	4.2*
bluegill sunfish	96 hrs	2.6*
duckling	8 days	> 10,000 **
quail	8 days	> 10,000 **

DISCUSSION:

Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, a fungicide, seems to be relatively non-toxic; acute toxicity values (oral and dermal) are in excess of 10 g/kg body wt/day. Lethal doses affect principally the testis but, also, the liver and stomach.

We consider Petitioner is remiss in not supplying enough details of acute toxic effects. In particular, does benomyl, a carbamate, produce cholinergic effects?

Further, no data on its effects, if any, on blood and/or brain cholinesterase are included: although such data is routinely requested for a carbamate.

Petitioner states that 2,500 ppm benomyl in the diet caused no overt effect in long-term feeding of rats and dogs (2 years and 1 year, respectively). Neither weight nor histologic appearance of the testis was affected in either species. Liver may have been affected adversely in dogs, as shown by decreases in total protein and in A/G ratio and by increase in blood values for GPT, alkaline phosphatase, and cholesterol. Livers of female rats were significantly heavier than those of controls.

In rat reproduction, the only adverse effect noted was lower weanling weights of pups on 500 or 2,500 ppm benomyl; these occurred in F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters.

These results recall the lower growth rates in 2-year rat study and lower weanling weights in mouse reproduction study with Thiabendazole which, like benomyl, is also a benzimidazole derivative.

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* Determined at Woodard Research Corporation.
** Determined at Hazleton Laboratories.

Plant metabolites consist of, primarily, methyl 2-benzimidazolecarbamate and, also, 2-aminobenzimidazole, the former having comprised 40% or more of total residue in a study on apples and oranges, according to DOP/PEB (memo of February 19, 1970, PP No. OGO-936). The compounds were not found as mammalian metabolites. No toxicity studies on either of them are included in either petition.

Various inert ingredients of the benomyl formulation are not of concern toxicologically; they are exempt under various sections except for the [redacted] (DOP memo cited in preceding paragraph).

EVALUATION:

Tentative "no-effect levels" of benomyl are listed:

Rat, 2-year feeding	2,500 ppm	250 mg/kg body wt
Dog, 1-year (interim)	500 ppm	12.5 mg/kg body wt
Rat reproduction	100 ppm*	10 mg/kg body wt*
Rat, 90-day	500 ppm	50 mg/kg body wt
Dog, 90-day	500 ppm	12.5 mg/kg body wt

*Since the only adverse effect in rat reproduction is borderline, consisting of somewhat lower weanling weights in "500-" and "2,500-ppm" pups of the last 4 (of 7) litters, we can assign the tentative "no-effect level" for the dog, 12.5 mg/kg body wt, as that of the most sensitive species.

The latter is equivalent to 500 ppm in the human diet. A corresponding ADI would be 5 ppm with 100-fold safety factor, but this applies to parent compound, only.

We lack information on possible cholinergic effects, including effects on blood and/or brain cholinesterase of benomyl and of its plant metabolite methyl 2-benzimidazolecarbamate. We, also, lack toxicity data (acute or subacute) on either of the plant metabolites, methyl 2-benzimidazolecarbamate and 2-aminobenzimidazole.

Although we conclude there is an ample margin of safety if the requested "negligible residue" and temporary tolerances of PP Nos. OFO-906 and OGO-936 (listed in title of this memo) are granted, before we can evaluate safety of any permanent, finite tolerances for benomyl, we will need information (as given below) in "Conclusions."

CONCLUSIONS:

1) Requested "negligible residues" tolerance for benomyl on bananas is safe (PP No. OFO-906; see title of this memo).

2) Requested temporary tolerances of PP No. OGO-936 are safe (see title of this memo).

3) To evaluate safety of any finite, permanent tolerances for benomyl which may be requested in the future, we are asking Petitioner to supply information on:

   a) Any information relative to possible cholinergic effects which benomyl may have and

   b) Subacute (90-day) toxicity data on at least one animal species for the principal plant metabolite of benomyl, methyl 2-benzimidazolecarbamate.

Dr. M. Quaife
Division of Toxicology
Petitions Review Branch (BF-148)

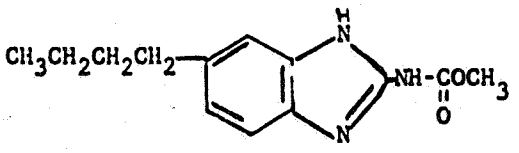
INIT:HBlumenthal

cc: BF-148
BF-140
SC-330
SC-310
VM-100
PP Nos. OFO-906 & OGO-936

MQuaife:dps 3-25-70

ADDENDUM to DT memo of March 25, 1970, Pesticide Petition Nos. OFO-906 and OGO-936.

Privileged data on a compound (which is related to benomyl), 2-carbomet oxyamino-5-n-butylbenzimidazole (structure shown), tell us



it is a teratogen in both shee and rats (Cf. VID 221 or NDA N D40-167V in our files.)

This being so, we will require studies to determine whether benomyl is teratogen in at least two animal species before we can establish safety of any tolerances other than temporary, negligible ones.

NEW CONCLUSIONS:

1) Of requested tolerances for benomyl in PP Nos. OFO-906 and OGO-936 (Cf. title of memo for list), we judge only those temporary ones at 0.2 ppm (negligible residue) in or on pecans, peanuts, and sugar beet roots are safe.

2) To establish safety of any finite or permanent tolerance for benomy will require satisfactory findings in studies for teratogenicity in at least two animal species in addition to studies listed under conclusion 3a and b", in our memo (of March 25, 1970).

M. Quaife, Ph.D.

MQuaife:dps 4-7-70