UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

MAY 21 1976

SUBJECT: Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, tolerances at 0.2 ppm each on wheat, oats barley, and rye - grain form; at 0.2 ppm each on oats, barley, and rye - straw; and at 15 ppm on wheat straw, requested,-human safety evaluation.

DATE:

FROM: TB/RD

TO: Product Manager

PP No. 6F1748

E. I. du Pont de Nemours & Co.
Wilmington, Delaware 19898

CONCLUSIONS:

1) Available toxicity data are adequate to support safety of requested benomyl tolerances (memo title, above). TB recommends they be granted, if considerations of CB and EEEB permit.

2) Petitioner should be informed that TB recommends that Petitioner initiate mammalian mutagenicity test for heritable effects on benomyl and its metabolite, methyl 2-benzimidazolecarbamate (MBC), to support safety of future tolerances which may be requested. (The basis of this recommendation and specific type of test(s) suggested are given on last page, last paragraph of this memo.)

3) (We note, a lifetime oncogenicity test in mammalian species other than rat is lacking to complete Sec. 3 requirements for registration and reregistration.)

INTRODUCTION:

Petitioner requests tolerances for residues of benomyl and its metabolites containing the benzimidazole moiety (calculated as benomyl) listed in memo title (above).

Benomyl is to be used as a seed treatment on small grains to control Powdery Mildew, Septoria Glume Blotch, and Cercosporella Foot Rot. The formulation of concern is a 50% one, EPA Reg. No. 352-354.

Analytical method to be used is a high-speed cation exchange liquid chromatographic one, which determines MBC, preformed plus that converted from benomyl, and 2-aminobenzimidazole separately.

Petitioner claims (Sec. D, this PP) that proposed use causes no residues in the grains or straws concerned (memo title, above) except that a finite residue occurs in wheat straw.

NEW TOX DATA, SUBMITTED BY PETITIONER.

New toxicity data consist only of a reprint, "Reproduction, Teratogenic and Mutagenic Studies with Benomyl," by H. Herman, R. Culif, and R. A. Jackson, Toxicol. Appl. Pharmacol. 32, 305-15 (1975). Only two experiments therein have not been previously reviewed in benomyl PP's.

One study, in which 2-C^{14}-benomyl was given p.o. to a male ChR-CD rat at 900 mg/kg, provided (1 hour later) C^{14} in the blood, 68% as 2-C^{14}-methyl benzimidazolecarbamate and less than 15% as intact benomyl.

The second study found dietary benomyl at highest level tested to be negative for dominant lethal mutation in the rat. Four groups of 10 M ChR-CD rats each received for 7 days 0, 250, 1,250, or 2,500 ppm benomyl in the diet. Each rat was then mated with three untreated F rats, same strain, per week, for 6 weeks of the ten thought required for the spermatogenic process. Dominant lethality and mutation rates, based on increase in frequency of pre-implantation losses of eggs and early resorptions of embryos in treated and control animals, were calculated according to Röhrborn (1970). The controls showed lower mating indices (during test weeks 1, 2, and 3) and slightly higher pre-implantation losses and markedly higher post-implantation losses (during all test weeks) than did rats in all test groups or historical controls. Calculated mutation rates, therefore, are negative; benomyl did not show positive dominant lethal mutation activity in this study.

NEW TOX DATA IN OPEN LITERATURE (NOT SUBMITTED BY PETITIONER).

Discussion of genetic effects of benomyl and its major metabolite, methyl 2-benzimidazolecarbamate (MBC) - partial abstract of review article, "Toxicology and Genetic Effects of Benzimidazole Compounds," by J. P. Seiler, Swiss Federal Research Station, Waedenswil (Switzerland), Mutation Research 32, 151-67 (1975).

MBC, a major plant metabolite of benomyl, is absorbed better from the gastro-intestinal tract than is benomyl. Both are about equally distributed throughout the mammalian organism, and no organ concentrates or accumulates them especially; however, no barriers seem to prevent their penetration into the genital organs.

In certain bacteria, benomyl and MBC induce point mutations. (Benzimidazole is incorporated into nucleic acids instead of guanine. In translation, however, benzimidazole is read preferentially as adenine, thus effecting a guanine..adenine transition).

Benomyl and MBC are toxic for fungi, worms, and weeds. MBC is the main fungitoxic metabolite of benomyl, as well as of the thiophanates.

It inhibits DNA synthesis or some closely related process.[a] It does[a] this, not, apparently, by anti-metabolite activity. Rather, in certain fungal cells and higher organisms, MBC interferes somehow with spindle formation.[b, c]

For example, benomyl and MBC act as spindle poisons with A. nidulans. The evidence is for a non-disjunction mechanism of mutation.

Mutagenicity of benzimidazole compounds in mammals. MBC, given to male mice at 1,280 mg/kg i.p., failed to show dominant lethal mutation; nor did thiophanate-methyl, of which MBC is a chief metabolite, show dominant lethal activity at up to 500 mg/kg in similar testing.[d,e]

One possible reason for this may be MBC's relatively weaker activity as spindle poison, compared to colchicine, for example. Since the latter shows dominant lethal activity only at a toxic dose (4/9 males died), it is not to be expected that the much weaker mitotic poison, MBC, will show positive dominant lethal activity.[f]

Secondly, MBC, given i.p., was very poorly absorbed into the blood; it left solid deposits in the ip. cavity.[d] Therefore, neither MBC nor thiophanate-methyl can have been present at high enough concentration in the blood to induce chromosomal aberrations in germ cells, which would lead to dominant lethals.

This possibility was substantiated with a micro-nucleus test in mouse bone marrow.[g] MBC, given i.p. to mice, caused it to be deposited in the intraperitoneal cavity, and the micro-nucleus count in RBC's was unaffected; whereas the micro-nucleus count rose more than five-fold after oral administration of MBC. This latter test was positive for mutagenic effects caused by MBC.

However, results with MBC in rats differed somewhat; since MBC, given to rats produced similar values for cytotoxic activity in the serum against Chang cells - whether given p.o. or i.p.[h]

Mutagenic effects were shown in these experiments, too. Both in cultures of Chang cells in vitro and in rat bone marrow cells in vivo, MBC and benomyl caused mitotic arrest or at least mitotic delay; a low incidence of chromosome breakage; and some bridge formation.[h] Results from these and other experiments are summarized in the following table (Table 1).

Table 1. MUTAGENICITY TESTS WITH BENZIMIDAZOLE COMPOUNDS

Compound	Test System					
	Bacteria	Fungi	Plants	Mammalian cells		
				in vitro	in vivo	dom. lethal
Benomyl	+	+		+		
MBC	+	+	±	+	+	-
Fuberidazole	+	+				
Thiabendazole						
Mebendazole					-	-
Thiophanate-methyl					-	-

The following table details results of the micronucleus test with mice with MBC.

Table 2. MICRONUCLEUS TEST IN MICE WITH MBC (Reference g)

4 animals at each dose level were treated twice 24 h apart. 6 h after the second dose, the mice were killed and bone marrow smears were prepared. 2000 polychromatic erythrocytes were scored per animal, and the range of results is given in the table.

Compound	dose	erythrocytes with micronuclei (per 1000 polychromatic erythrocytes)	
		Polychromatic	Normochromatic
MBC	400 mg/kg i.p.	4-6	3-4
	1000 mg/kg p.o.	24-28	16-22
	500 mg/kg p.o.	15-23	9-16
	100 mg/kg p.o.	12-18	0-10
	50 mg/kg p.o.	4-7	2-4
saline	0.5 ml i.p.)		
gum arabic 2%,	0.5 ml p.o.)	3-5	2-4

With MBC, two mutational mechanisms seem to operate. In bacteria, the compound is a base-substituting agent producing point mutations.[i] In higher organisms, this mechanism is concealed by the much more pronounced effects on the mitotic process. This latter can be seen especially well in the micro-nucleus test, where not only are part of the micronuclei much larger than usual, but, also, the proportion of micronucleated normochromatic erythrocytes rises (Table 2).

(The molecular basis of action of MBC as a spindle poison is thought to be the absorption of the carbamate side groups to micro-tubular proteins and the resultant disturbance in formation of the micro-fibrillar spindle apparatus. Mitotic delay, non-disjunction, and other mitotic disturbances follow.)

(We reiterate, the fungitoxicity of benzimidazoles operates with the same mechanism. Resistance mutations in fungi would also be favored by the selective pressure exerted by fungicidal spraying with these substances. Induction of point mutations by benzimidazoles could even produce resistants, additionally favored by the selection pressure against sensitive fungi. In the field, rapid development of resistant strains of pathogenic fungi has already occurred, and this begins to pose severe problems.)

The genetic risk for man. Induction of point mutations by benzimidazoles can't be shown, at present, in mammalian test systems as easily as in bacteria. Therefore, we can't evaluate such risks, although negative dominant lethal data show that, at least, no extensive damage through point mutations has been caused by these substances.

In higher organisms - from fungi upwards - the antimitotic activity of some benzimidazole derivatives seems to be more important. So we evaluate risk due only to the latter effect and, herein, only to fungicides.

For benzimidazoles (as fungicides), acceptable daily intakes (ADI's) have been estimated, based on chronic toxicity. Are these values sufficiently low to protect man from genetic consequences of benzimidazole ingestion?

The (WHO/FAO) ADI for benomyl is 9 mg/day; that for MBC is 10 mg/day or 0.16 mg/kg/day. [For benomyl, EPA/FDA set an ADI at 0.125 mg/kg/day or 7.5 mg/day.]

To produce an effect on spindle formation and on chromosome distribution, colchicine and colcemid must be present in vitro at about 10^{-9}M; whereas MBC arrests mitoses only at more than 10^{-5}M.[h] Obviously, MBC will be less dangerous in this respect than colcemid or colchicine.

Based on micronuclei data (Table 2), MBC acts at a lowest dose of 100 mg/kg, equivalent to a mean concentration of 5×10^{-4}M throughout the body of the mouse; whereas the lowest micronuclei-producing dose of colchicine is reported to be 1.25 mg/kg or 3×10^{-6}M.[j]

Based on available data, one would not expect to observe any effects below a concentration of 10^{-5}M or 2 mg MBC/kg. The (FAO/WHO) ADI for MBC is 0.16 mg/kg, a value which is less than one-tenth this lower limit, and this body burden in humans will seldom be actually reached.

Even if the body burden of MBC should be less than one-hundredth of the concentration able to inhibit mitoses, some precautionary remarks seem advisable. Till now, no experiments have been reported which could be used for an estimation of non-disjunction caused by MBC. Just as slight disturbances in spindle formation need not necessarily inhibit the whole mitosis, such events could nevertheless affect the distribution of chromosomes, and lower concentrations could produce such

effects. However, as the mutagenic action of MBC proceeds indirectly via an inhibition of the spindel apparatus, a threshold value could certainly be defined, and, as a starting point, micro-nuclei or other mammalian mutagenicity data could be used. From present data (Table 2), such a threshold level would be set at 0.5 mg/kg, taking into account the safety factor of 100 that is normally used in pesticide evaluation.

[Another approach is possible. Recently, 10^4 atoms or molecules per cell have been proposed as a possible absolute threshold for most specific reactions, e.g., enzyme inhibition or mechanism involving trace elements or to produce any effect at all. [k,l,m]

[If MBC concentration were to be the same in every cell, then the ADI (of 0.16 mg/kg) would provide 2×10^5 molecules per human cell. This is a maximal theoretical figure; actual body burden would surely be less than 10^5 molecules per cell; whereas the threshold level, calculated, as above, from the micronuclei data, would be 6×10^5 molecules per cell. Based on metabolic data for benzimidazoles in several animal species, an equal distribution throughout the organism seems to be not far from the truth. So, we may suppose safely that levels of MBC in the human body will be near or below the proposed absolute threshold.]

Conclusion of review article. "The situation is gradually improving, as with development of resistant strains of fungi, benzimidazole compounds are less and less likely to be used. The theoretical problem of whether 10^5 molecules of MBC per cell might be sufficient to produce numerical chromosome aberrations nevertheless merits attention. Since such disturbances, e.g., Down's syndrome, are very common, a few more cases of mongolism could escape notice in population survey studies. The social costs and the pyschic stress accompanying such cases of genetic illness obviously warrant further investigations on this point, even if the genetic risk for man is considered very small or negligible. Also, a restrictive usage pattern of such pesticides will further help to decrease this risk, finally, to the point of practical non-existence."

OLD TOX DATA

Toxicity data submitted previously in support of benomyl tolerances are reviewed by us in earlier PP's. Cf., for example, our memo of 12/17/75 in PP No. 6E1760. These data are incorporated by reference in Sec. C of this pp. They fulfull previous TOX requirements for tolerances.

EVALUATION.

Results cited above appear to show that benomyl/MBC exhibits multitest evidence of causing mutation, including in rat and mouse in vivo by peroral route. Therefore, benomyl may be a condidate for presumptive refusal of reregistration. In fact (per Mr. Ron Drear, 4/20/76), it is now so classified, with final decision to be made later. (C. and E. has concluded that results do not now trigger a presumption against benomyl; because cytotoxic, not heritable, effects of mutagenicity are shown (memo, L. B. Dale, Dr., to R. D. Schmitt, 1/14/76.))

These actions, technically, relate to registration - not to benomyl tolerance safety. However, we need to consider all available data in evaluating the latter.

Petitioner's new data show benomyl negative for dominant-lethal activity, given p.o. to rats at 2,500 ppm in diet (1 wk). However MBC (methyl 2-benzimidazolecarbamate) - not benomyl - is chief residue component on benomyl-treated r.a.c.'s and, thus, in human diet.

Benomyl is converted in vivo to MBC in rats (see under new TOX, above) and in mice, sheep, and rabbits given it p.o. (or by their liver or blood in vitro), according to Douch.[n] However, both "ten-fold lower" cytotoxicity (to Chang cells) in blood serum and decreased mitotic abnormalities (in bone marrow) were observed for rats given benomyl orally, in contrast to those given equivalent MBC orally (Styles and Garner).[h] This suggests that benomyl by mouth may provide only one-tenth as much MBC to mammalian target cells or tissues as MBC per se. If so, MBC derived from benomyl would have been tested for dominant lethal activity in the rat, effectively, at only 250 ppm in the diet.

We note that if thiophanates, which give rise to MBC on treated raw agricultural commodities, are granted the numerous tolerances pending in the U. S., then the maximum human dietary content of MBC may increase considerably.

To this reviewer, nevertheless, considerations set forth by the Seiler paper (above) plus the fact that theoretical maximum residues in an average U. S. daily diet (1.5-kg diet/60-kg adult) due to present and requested tolerances are much less than our ADI for benomyl - 1.7 vs. 7.5 mg/day - plus the observed negative rat dominant lethal activity of benomyl support safety of these and present tolerances with regard to genetic risk to U. S. human consumers.

We do believe that, because of the evidence of mutation/cytoxicity by benomyl/MBC (including by oral route in two mammalian species), as well as the relative insensitivity of the dominant lethal test,* that - to support future requested tolerance(s) for benomyl - Petitioner should promptly initiate other mammalian mutagenicity test for heritable effects; those cited in Sec. 3 appendicies are the heritable translocation test and, especially, the specific locus test. These tests are also mentioned in the so-called "tier" approach to mutagenicity testing (Flamm, 1975).

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*The dominant lethal test .... "does not supply the type of information for evaluation (background) of long-term genetic effects on the population due to high spontaneous of dominant lethality in most strains of rats and mice....dominant lethal test negative results cannot be regarded as proof that the agent is non-mutagenic; the converse is true," Principles for Evaluating Chemicals in the Environment, NAS-NRC, 1975, p. 172.

M.L.Q., 5/10/76

Mary L. Quaife, Ph.D., TB/RD
April 28, 1976
MLQ, 5/10/76

R for OEP 5/11/76

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