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To: Larry Schnaubelt and Patricia Cohn
Product Manager 72
Special Review and Reregistration Division (H7508W)

From: Norman Cook, Acting Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : benomyl, MBC
Type Product : fungicide
Product Name : Benlate
Company Name : DuPont
Purpose : RED Science Chapter

Action Code : 623 Date Due : 01/05/97
Reviewer : R. Petrie Date In EEB: 01/17/96

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)	42817002	P
71-2(B)			72-3(B)			122-1(B)	42817002 43363401	N P
71-3			72-3(C)			122-2	42817802 42854802	Y Y
71-4(A)	44103001	S	72-3(D)			123-1(A)		
71-4(B)	44103002	Y	72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)	43872801	Y	124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 30 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

D221854

MEMORANDUM

SUBJECT: Ecological Effects Branch Science Chapter for benomyl Reregistration Eligibility Document (RED)

FROM: Norman Cook, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Kathy Monk, Acting Chief
Science Analysis and Coordination Staff
Environmental Fate and Effects Division (7507C)

Attached is the Ecological Effects Branch (EEB) contribution to the RED for the fungicide benomyl. Also attached are the following DER reviews (see RED bibliography for full references):

1.) D221857 - Channel catfish early life stage study (72-4) MRID43872801,

2.) D193402 - Aquatic plant algal tests (4), (122-2) MRID42854801,

3.) D193402 - Aquatic plant macrophyte (1), (122-2) MRID42854802,

4.) D208069 - Terrestrial plant phytotoxicity, vegetative vigor (122-1), MRID43363401,

5.) D193153 - Terrestrial plant phytotoxicity, seed germination - seedling emergence - vegetative vigor, (122-1), MRID42817002.

6.) D230010 - Avian reproduction studies (71-4) - Bobwhite quail, MRID44103001 and Mallard duck, MRID44103002.

Data Requirements

The ecotoxicity data bases for benomyl and it's primary degradate MBC are essentially complete (refer to data tables attached to the RED chapter).

Toxicity Profile

Based on the results of acceptable ecotoxicity studies using TGAI benomyl, TGAI MBC (carbendazim), benomyl TEP 50%WP, and benomyl TEP 50%DF, benomyl/MBC are categorized as very highly toxic to freshwater fish, freshwater aquatic invertebrates, and marine/estuarine aquatic invertebrates. Benomyl/MBC are also very highly toxic to terrestrial invertebrates (earthworms). For these organisms, acute high risk LOC's, acute restricted use LOC's, acute endangered species LOC's and chronic risk LOC's are exceeded.

Acute risk LOC's for birds and mammals are not exceeded. Chronic risk to birds is a concern. While a risk assessment was not conducted for plants, available data indicates that green algae may be adversely affected by over-sprays, drift, and runoff.

Use Patterns and Exposure Potential

Rice grown in the South-eastern U.S. receives the largest amount of benomyl. The movement of water from rice fields to adjacent streams or other water bodies either intentionally or unintentionally is a significant acute and chronic concern for freshwater and estuarine aquatic organisms, whether nontargets or endangered species. Over-sprays and drift during aerial applications of benomyl to labeled crops are also a significant acute and chronic concern for freshwater and estuarine aquatic organisms. Terrestrial invertebrates (earthworms) are expected to be adversely affected on an acute and chronic basis from multiple applications of benomyl.

Risk Mitigation

Some risk mitigation proposals might include:

1.) restricted use classification,

2.) within the field buffer zones for aerial and chemigation uses,

3.) application - release interval for rice field drainage.

Other Labeling

Revise label statements in the "Environmental Hazards" and rice use directions sections to read: This pesticide is toxic to fish, aquatic invertebrates, terrestrial invertebrates (earthworms), and aquatic plants. Drift and runoff from treated areas may be hazardous to fish, aquatic invertebrates, terrestrial invertebrates, and aquatic plants in adjacent areas. Keep all other existing "Environmental Hazards" and crop use restrictions.

Add appropriate endangered species label statements.

If you have any questions regarding this RED chapter, please direct them to Richard Petrie @ 305-7358, Room 1004-B, or Ann Stavola @ 305-5354.

EXECUTIVE SUMMARY FOR BENOMYL FUNGICIDE

Benomyl rapidly converts to carbendazim (MBC) in the presence of water. The 1984 "Registration Standard" report for benomyl called for MBC tests for terrestrial and aquatic organisms. Overall, benomyl TGAI, MBC TGAI, and the benomyl formulations 50%WP (wettable powder) and 50%DF (dry flowable) were all very similar in their levels of acute and chronic toxicity to tested terrestrial and aquatic animal species (MBC-benomyl comparisons were not available for mammals and plants). There is evidence in the literature that benomyl conversion to MBC while still on plant foliage is slower than it's conversion upon contact with the soil. Therefore, use of benomyl forms are more appropriate in bee and terrestrial plant phytotoxicity tests. The movement of water from rice fields to adjacent streams or rivers either intentional or unintentional is a significant acute and chronic concern for freshwater and estuarine aquatic organisms. Oversprays and drift during aerial applications of benomyl to labeled crops are also a significant acute and chronic concern for freshwater and estuarine aquatic organisms. Terrestrial invertebrates (earthworms) are expected to be adversely affected from multiple applications of benomyl.

ACUTE TOXICITY DATA SUMMARY:

Benomyl and it's primary metabolite carbendazim (MBC) are "Practically non-toxic" to birds using TGAI/MBC (LD50= >2100mg/Kg,LC50= >5,000ppm); "Practically non-toxic" to small mammals using TGAI/benomyl(75%)- LD50= >5,000mg/Kg); "Relatively non-toxic" to bees using TGAI/benomyl - (>10.0 ug/bee); "Very highly toxic" to channel catfish using TGAI/MBC, TGAI/benomyl, or 50%WP - (LC50=0.0074 ppm); "Very highly toxic" to *Daphnia magna* using TGAI/benomyl, 50%WP, and 50%DF, "Highly toxic using TGAI/MBC - (0.0390 ppm); "Moderately toxic" to sheepshead minnow using TGAI/benomyl and 50%WP; "Moderately to slightly toxic using 50%DF - (3.7920); "Very highly toxic" to Mysid shrimp using TGAI/MBC; "Highly toxic" using TGAI/benomyl, 50%WP, and 50%DF - (0.0980); and the 50DF formulation resulted in "100% inhibition" of the green algae *Kirchneria subcapitata* (*Selenastrum capricornutum*) in Tier I tests at the maximum label dosage of 1.5 pounds active ingredient per acre. Benomyl and MBC are "very highly toxic" to earthworms.

CHRONIC DATA TOXICITY SUMMARY:

Adverse avian chronic effects are expected to occur (Mallard NOEC=322 ppm). Chronic effects in freshwater fish, freshwater invertebrates and marine/estuarine organisms occur at very low levels: Channel catfish MATC = 0.00144 ppm; *Daphnia magna* MATC = 0.0040 ppm; Mysid shrimp MATC = 0.0354 ppm. Benomyl and MBC are chronic toxicity concerns for earthworms.

Risk Assessment:

Birds: Acute risk LOC's not exceeded. Avian reproduction studies indicate that adverse chronic effects may occur.

Mammals: Acute risk LOC's not exceeded. Refer to Hazard Evaluation section for chronic mammalian effects.

Aquatic organisms:

Freshwater fish - Acute high risk LOC's exceeded, acute restricted use LOC's exceeded, acute endangered species LOC's exceeded, chronic risk LOC's exceeded;

Freshwater invertebrates - Acute high risk LOC's exceeded, acute restricted use LOC's exceeded, acute endangered species LOC's exceeded, chronic risk LOC's exceeded;

Marine/estuarine fish - Acute endangered species expected for rice only.

Marine/estuarine invertebrates - Acute high risk LOC exceeded, acute restricted use LOC's exceeded, acute endangered species LOC's exceeded, chronic risk LOC exceeded.

Plants: A risk assessment was not conducted for plants. Data indicate potential for adverse effects to green algae. The widespread phytotoxicity that occurred from use of benomyl DF formulation has not occurred with benomyl 50% wettable powder formulation. The DF formulation is not currently registered. All greenhouse/ornamental uses of benomyl have been cancelled.

HISTORY/INCIDENTS:

No avian or mammalian benomyl related field incidents have been reported to the EFED to date. In 1976 a catfish kill following the drainage of water from a 90 acre rice field in Arkansas into catfish ponds and a goldfish kill in Georgia in 1978 resulted in heightened toxicity testing and a rice monitoring study. The rice drainage occurred 90 days after benomyl application. Shortly following this incident, the registrant added label warning statements relating to the release of rice drainage/irrigation water into areas where catfish or crayfish farming is practiced. More recently, the registrant has completed a chronic channel catfish study and a rice monitoring field study in Arkansas and Louisiana that measured MBC sediment and water residues in the rice paddy, drainage ditches, and streams in southern rice growing states. One catfish kill occurred during the conduct of this monitoring study. In 1990, DuPont Chem. Co. reported the kill of 38 black bullhead catfish following the aerial application of the maximum use rate of benomyl 50%WP to rice during their rice monitoring study. The catfish were located in a ditch 15 feet distant from the treated field. The registrant reported that sufficient drift occurred to kill the catfish.

After the introduction of benomyl 50% dry flowable formulation (DF) in 1987, the first complaints of plant phytotoxicity were received by the Registration Division of OPP. In 1989, atrazine herbicide residues were identified in benomyl 50%DF formulation. DuPont Chem. Co. was fined by the EPA for atrazine contamination and all contaminated lots were withdrawn from commerce. In the time period 1990-1991, the agency received more than 1800 additional reports of plant phytotoxicity from use of the 2 pound package of the 50%DF formulation. The benomyl 50%DF formulation was voluntarily canceled in 1994, however, concerns still remain regarding potential for 50%WP formulation phytotoxicity.

ENVIRONMENTAL ASSESSMENT FOR BENOMYL FUNGICIDE

1. Ecological Toxicity Data

a. Toxicity to Terrestrial Animals

(1) Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient is required to establish the toxicity of a pesticide to birds. The preferred test species is either mallard duck or bobwhite quail. While data using technical benomyl are not available, it was determined that avian acute oral acute toxicity data using the benomyl metabolite carbendazim (MBC) is acceptable. Results of the MBC test follow:

Table: Avian Acute Oral Toxicity

Species	%A.I.	LD_{50} mg/Kg	Toxicity Category	MRID No. Author/Year	Study Classification
Northern Bobwhite Quail (*Colinus virginianus*)	99MBC	>2100	Practically non-toxic	43129604 Grimes, 93	Core
Northern Bobwhite Quail	98MBC	> 2250	Practically non-toxic	00260572 Beavers, 85	Core
Northern Bobwhite Quail	99MBC	> 2250	Practically non-toxic	00073596 Fink, 76	Core
Starling	99	>100	Moderately toxic	00020560 Schaefer, 72	Supplemental*
Redwing Blackbird	99	100.0	Moderately toxic	00020560 Schafer, 72	Supplemental*

* Not a guideline test species.

These results indicate that the benomyl metabolite (MBC) technical is practically non-toxic to avian species on an acute oral basis. Practically non-toxic is defined by Brooks (1973) as falling in the LD50 range of >2000 mg/Kg toxicity. The guideline requirement (71-1) is fulfilled (MRID # 43129604).

Two subacute dietary studies using the technical grade of the active ingredient are required to establish the toxicity of a pesticide to birds. The preferred test species are mallard duck (a waterfowl) and bobwhite quail (an upland gamebird). Subacute dietary toxicity studies using technical grade MBC and Benlate 50WP formulation are available. The preferred test substance is TGAI/MBC.

Table : Avian Subacute Dietary Toxicity

Species	% A.I.	LC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Northern Bobwhite Quail (Colinus virginianus)	98MBC	> 10,000	Practically nontoxic	00073598 Fink, 76	Core
Northern Bobwhite Quail	99MBC	> 5,000	Slightly to Practically non-toxic	43129605 Grimes, 93	Core
Mallard Duck (Anas platyrhynchus)	98MBC	> 10,000	Practically nontoxic	00073597 Fink, 76	Core
Mallard duck	99MBC	2278	Slightly toxic	43205501 Ebert, 87	Suppl.*
Northern Bobwhite Quail	50% ai	> 10,000	Practically nontoxic	00066783 Busey, 68	Suppl.**
Mallard Duck	50% ai	> 10,000	Practically nontoxic	00066783 Busey, 68	Suppl.**

* Dietary concentrations of 1205 or greater induced anorexia. The NOEL was 578 ppm.
**Formulated Product test, not TGAI.

These results indicate that the primary benomyl metabolite MBC and the 50%WP formulation are practically non-toxic to avian species on a subacute dietary basis. Practically non-toxic is defined by Brooks (1973) as falling in the LC50 range of > 5000 ppm toxicity. The guideline requirement (71-2) is fulfilled (MRID#'s 00073598, 00073597).

(2) Birds, Chronic

Avian reproduction studies using the technical grade of the active ingredient were requested because: (1) birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season and (2) MBC is stable in the environment to the extent that potentially toxic amounts may persist in animal feed. The preferred test species are mallard duck and bobwhite quail. The preferred test substance is TGAI/MBC.

Table : Avian Reproduction

Species	% A.I.	NOEC/LOEC (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Northern Bobwhite Quail (Colinus virginianus)	97MBC	NOEC=3600 LOEC=>3600	NONE	441103001 Frey, L.T. et.al. 1996	Supplemental*
Mallard Duck (Anas platyrhynchus)	97MBC	NOEC=322 LOEC=720	Viable embryos of eggs set, normal hatchlings of eggs set, 14 day old survivors of eggs set.	44103002 Frey, L.T. et. al. 1996	Core

* Is highest dosage level at or near maximum nominal field residue level? Need registrant response.

44103001 - This study is supplemental. When compared to the control, there were no significant treatment related effects on any of the endpoints measured at any concentration tested (322, 720, 1610, and 3600 ppm ai nominal carbendazim). The test report did not state whether the test was conducted with the highest dosage level at or above the maximum field residue level. This must be resolved with the registrant. Data were reported by pen for the following endpoints: eggs laid, eggs cracked, eggs set, viable embryos, normal hatchlings, 14 day-old survivors, weights of 14 day-old survivors, egg shell thickness, total food consumption, initial and final body weights by sex, hatchling weights, maximum number of eggs laid, and maximum number of eggs set.

44103002 - This study is core. There were significant reductions in the percentage of viable embryos, normal hatchlings, and 14 day-old survivors of eggs set at 720 ppm ai nominal carbendazim. Other endpoints measured included: eggs laid, eggs cracked, eggs set, live 3-week embryos, weights of 14 day-old survivors, egg shell thickness, total food consumption, initial and final body weights by sex, hatchling weights, and maximum number of eggs set and eggs laid.

The guideline requirement (71-4) is fulfilled for the mallard duck (MRID 44103002). The guideline requirement (71-4) is not fulfilled for the bobwhite quail.

(3) Mammals, Acute and Chronic

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In most cases, rat or mouse toxicity values obtained from the Agency's Health Effects Division (HED) substitute for wild mammal testing. These toxicity values are reported in the Table below.

Table : Mammalian Toxicity

species	% A.I.	Test Type	Endpoint	MRID No.
laboratory rat	75% ai	LD50	>5000 mg/Kg	000863

The results indicate that benomyl is practically nontoxic to small mammals on an acute oral basis. Testicular alterations were noted at dosage levels of 500 mg/Kg and greater (Doc. No. 004678, and 000863). No MBC studies are available.

(4) Nontarget Insects

A honey bee acute contact study using the technical grade of the active ingredient is required if the proposed use will result in honey bee exposure. A honey bee acute contact study is required for benomyl due to its use as a foliar spray on insect pollinated crops.

Table : Nontarget Insect Acute Contact Toxicity

Species	% A.I.	LD_{50} (µg/bee)	Toxicity Category	MRID No. Author/Year	Study Classification
Honey Bee (Apis mellifera)	Tech	>10.0 ug/bee	Relatively Non-toxic	05001991 Stevenson, 78	Core

The results indicate that benomyl fungicide is relatively non-toxic to bees on an acute contact basis. The guideline requirement (141-1) is fulfilled (MRID # 05001991).

Other nontarget arthropods tested were the mite *(Amblyseius fallacis)* (05009345), syrphid fly (*Syrphus corollae*) (42854901), midge *(Chironomus plumosus)* (40098001), and ladybird beetle *(Stethorus punctatus)* (00059461). Benomyl was determined relatively non-toxic to the ladybird beetle, predaceous mite, and midge; and slightly harmful to the syrphid fly in laboratory tests.

(5) Terrestrial Field Testing

Based on a limited review of the literature, benomyl is very highly toxic to earthworms on an acute and chronic basis; and has been used as a standard reference toxicant in earthworm studies. [WHO Environmental Health Criteria 148, 149. (1993)]

"Under certain conditions benomyl may have an effect on populations of earthworms. In apple orchards where foliage has been treated repeatedly at a rate of 0.28 Kg/ha and has fallen to the ground, earthworms may be eliminated after two years of benomyl use (up to 13 sprayings). The earthworms *Lumbricus terrestris* and *Allolobophora chlorotica* were most affected. Populations of other species recovered within two years of the termination of spraying. Orchard yields were unaffected, as were earthworm populations adjacent to the

orchards, because of the immobility of benomyl in the soil (Stringer and Wright (1973); Stringer and Lyons (1974) from WHO Environmental Health Criteria 148 - Benomyl (1993).

b. Toxicity to Aquatic Animals

(1) Freshwater Fish, Acute

Benlate 50WP degrades rapidly to MBC in the presence of water, therefore aquatic animal MBC studies have been included in this report. Two freshwater fish toxicity studies using the technical grade of the active ingredient are required to establish the toxicity of a pesticide to fish. The preferred test species are rainbow trout (a cold-water fish) and bluegill sunfish (a warm water fish). Results of these and other tests are tabulated below.

Table : Freshwater Fish
Acute Toxicity

Species	% A.I.	LC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout (Oncorhynchus mykiss)	99	0.120	Highly Toxic	40098001 Mayer, 86	Core
	99	0.160	Highly Toxic	40098001 Mayer, 86	Core
	99	0.170	Highly Toxic	40098001 Mayer, 86	Core
	99	0.190	Highly Toxic	40098001 Mayer, 86	Core
	99	0.200	Highly Toxic	40098001 Mayer, 86	Core
	99	0.230	Highly Toxic	40098001 Mayer, 86	Core
	99	0.280	Highly Toxic	40098001 Mayer, 86	Core
	99	0.600	Highly Toxic	40098001 Mayer, 86	Core
	99	0.880	Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.145	Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.320	Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.370	Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.370	Highly Toxic	00003535 Johnson, 80	Core
	99MBC	0.650	Highly Toxic	40098001 Mayer, 86	Core
	99MBC	1.320	Moderately Toxic	43129606 Bowman, 89	Core
	50WP MBC	0.230	Highly Toxic	00223400 ____, 71	Suppl. *
	50WP	0.456	Highly Toxic	00097615 Pitcher, 72	Suppl.*
	50WP	0.310	Highly Toxic	40098001 Mayer, 86	Core
	50WP	0.460	Highly Toxic	FAOBEN02 McCann, 76	Core

Species	% A.I.	LC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
	50WP	0.410	Highly Toxic	00070426 Heinemann, 71	Core
Bluegill sunfish (Lepomis macrochirus)	99	0.310	Highly Toxic	FAOBEN05 McCann, 77	Core
	99	0.560	Highly Toxic	FAOBEN01 Finley, 77	Core
	99	0.750	Highly Toxic	40098001 Mayer, 86	Core
	99	0.850	Highly Toxic	00003505 Johnson, 80	Core
	98	0.920	Highly Toxic	FAOBEN05 McCann, 77	Core
	99	1.200	Moderately Toxic	40098001 Mayer, 86	Core
	99	1.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	1.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	1.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	1.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	2.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	6.400	Moderately Toxic	40098001 Mayer, 86	Core
	99MBC	> 1.8500	Moderately Toxic	43129607 Bowman, 89	Core
	50WP	0.265	Highly Toxic	FAOBEN05 McCann, 77	Core
	50	0.380	Highly Toxic	FAOBEN05 McCann, 77	Supplemental*
	50WP	1.200	Moderately Toxic	00003505 Johnson, 80	Core
	50WP	1.800	Moderately Toxic	FAOBEN05 McCann, 77	Core
	50WP	2.400	Moderately Toxic	FAOBEN01 Finley, 77	Supplemental**
	50WP	2.600	Moderately Toxic	00066782 Knott, 68	Core
Fathead minnow (Pimephales promelus)	99	1.300	Moderately Toxic	40098001 Mayer, 86	Core
	99	2.200	Moderately Toxic	40098001 Mayer, 86	Core
	99	2.200	Moderately Toxic	00003505 Johnson, 80	Core
	50WP	1.900	Moderately Toxic	0003505 Johnson, 80	Supplemental**
Channel catfish (Ictalurus punctatus)	99	0.006	Very Highly Toxic	40098001 Mayer, 86	Core***
	99	0.012	Very Highly Toxic	FAOBEN01 Finley, 77	Supplemental ****
	99	0.013	Very Highly Toxic	40098001 Mayer, 86	Core
	99	0.029	Very Highly Toxic	40098001 Mayer, 86	Core

Species	% A.I.	LC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Channel catfish	99MBC	0.0074	Very Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.0100	Very Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.0120	Very Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.0160	Very Highly Toxic	40098001 Mayer, 86	Core
	99MBC	0.0160	Very Highly Toxic	00003505 Johnson, 80	Core
	99MBC	0.0190	Very Highly Toxic	40098001 Mayer, 86	Core
	50WP	0.0280	Very Highly Toxic	40098001 Mayer, 86	Core
	50WP	0.029	Very Highly Toxic	40098001 Mayer, 86	Core
	50WP	0.092	Very Highly Toxic	FAOBEN06 McCann, 77	Core

* Exact formulation unknown.

** Formulated product used.

*** Yolk sac fry.

**** Raw data missing.

The above results (13 tests) indicate that benomyl technical, MBC technical, and Benomyl 50WP formulation are all very highly toxic to catfish and that benomyl technical, MBC technical, and Benlate 50WP formulation are more toxic on an acute basis to channel catfish than to tested scale fish. In 19 of 20 studies for the rainbow trout the tested materials benomyl technical, MBC technical, MBC50WP formulation, and Benlate 50DF formulation were rated "highly toxic" to rainbow trout. Of the 19 total bluegill sunfish studies using benomyl technical and Benlate 50WP, toxicity ranged from "moderately toxic" to "highly toxic" for each test material. All 4 fathead minnow studies were rated "moderately toxic" for benomyl technical and Benlate 50WP. The guideline requirement (72-1) is fulfilled [MRID # 40098001 (Rainbow trout), FAOBEN07 (Bluegill sunfish)]. An acute characteristic of exposed catfish is that they swim with their heads out of the water and swing the head to and fro. Their eyes appear to be sunken in their heads. They are characteristically referred to as "stargazers" in this incident report (1976).

(2) Freshwater Fish, Chronic

A fish early life stage test is required for benomyl due to high acute toxicity to fish and MBC metabolite half-life greater than 4 days in soil and water. Results of this test are tabulated below. The preferred test species is the channel catfish. The preferred test substance is TGAI/MBC.

Table : Freshwater Fish Early Life-Stage

Table : Freshwater Fish Early Life-Stage Toxicity

Species	% A.I.	NOEC/LOEC (ppm)	MATC (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Channel catfish (Icalurus punctatus)	99MBC	<0.00211 >0.00099	0.00144	Larval Survival	43872801 Rhodes, 95	Core
Rainbow trout (Oncorhynchus mykiss)	99	<0.0340 >0.0110	0.01900	Larval survival	43011801 Baer, 93	Core
	99MBC	<0.0540 >0.0260	0.0375	Larval survival	42882301 Baer, 93	Suppl.*

* Study design, solvent control.

The guideline requirement (72-4) is fulfilled (MRID # 43872801).

In the channel catfish study above (MRID 43872801), eggs hatched, survival 34 days post-hatch, and growth data (length and weight 34 days post-hatch) were analyzed. In the growth data, mean-length and wet weights showed no clear dose-response and were less sensitive endpoints than larval survival (survival 35 days post-hatch). During the channel catfish study, one water control fish, 2 fish at 0.270 ug/L, and one fish at 1.500 ug/L exhibited spinal curvature.

In the rainbow trout study above (MRID 42882301), the only usable data from this study was fingerling survival. This study was terminated at day 21 and should have been continued to day 32 post-hatch. Only 2 replicates per treatment were used and the concentration of solvent used was not reported. Fingerling size at the start of the study was not reported. Embryos should have been used. Because they were not used, it was not possible to assess the important reproductive endpoints: hatching success, time to hatch, embryo mortality, and time to swim up.

In the rainbow trout study above (MRID 43011801), embryos, alevins, and fingerlings were exposed in a flow-through 79 day chronic study. Risk calculations have been based on embryo survival, which was the most sensitive endpoint measured. All embryos at 34 and 92 ug/L did not survive to hatch. No statistically significant differences occurred in the first and last day of hatching, percent hatch, larval survival, abnormal larvae, standard length, or wet weights in the remaining test concentrations.

A freshwater fish life-cycle test using the technical grade of the active ingredient is required when an end-use product is intended to be applied directly to water or is expected to be transported to water from the intended use site, and when either of the following conditions exist: (1) the EEC is equal to or greater than one-tenth of the NOEL in the fish early life-stage or invertebrate life-cycle test; or (2) studies of other organisms indicate the reproductive

physiology of fish may be affected. The preferred test species is fathead minnow. A fish life-cycle test is triggered (the EEC is equal or greater than $1/10^{th}$ the NOEL in the fish early life-stage study) but not required for benomyl at this time. The channel catfish is clearly more sensitive to benomyl and MBC than the fathead minnow in acute toxicity tests. Therefore, this study is not required at this time but held in reserve.

MBC has a low potential for bio-accumulation in fish. Reported BFC's for MBC are 23 and 27 for whole fish and 460 and 380 for viscera (EFGWB/EFED/OPP).

(3) Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the technical grade of the active ingredient is required to assess the toxicity of a pesticide to invertebrates. The preferred test species is *Daphnia magna*. The preferred test substance is TGAI/MBC.

Table :
Freshwater
Invertebrate
Toxicity

Species	% A.I.	LC_{50}/ EC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Daphnia magna (Water Flea)	99MBC	0.3900	Highly Toxic	42414201 Baer, 92	Core
	99MBC	0.1300	Highly Toxic	43205502 Fischer, 88	Core
	98MBC	0.3500	Highly Toxic	00260572 Stahl, 85	Core
	99MBC	0.3500	Highly Toxic	00154667 Hall, 85	Core
	98	0.2850	Highly Toxic	FAOBEN07 Palmateer, 79	Core
	99	0.0390	Very Highly Toxic	42414201 Baer, 92	Core
	99	2.8000	Moderately Toxic	40098001 Mayer, 86	Core
	50WP	0.0680	Very Highly Toxic	40802101 Hutton, 88	Core
	50DF	0.0800	Very Highly Toxic	40802102 Hutton, 88	Core
Scud (Gammarus pseudolimneus)	99	0.7500	Highly Toxic	40098001 Mayer, 86	Core
Crayfish (Ornectes nais)	99	>10.00	Slightly Toxic	40098001 Mayer, 86	Supplemental*
Crayfish (Procambarus sp.)	99	>100.00	Practically Non-toxic	40098001 Mayer, 86	Supplemental*

* Not a guideline species.

Test results for *Daphnia magna* (water flea) indicate that benomyl technical is "moderately

toxic" to "very highly toxic", MBC technical is "highly toxic", and that the Benlate 50WP and DF formulations are "very highly toxic" on an acute basis. Benomyl technical was "highly toxic" to *Gammarus pseudolimneus* (scud) and was "slightly toxic" to "practically non-toxic" to *Ornectes nais and Procambarus sp.* (crayfish). The guideline requirement (72-2) is fulfilled (MRID # 42414201).

(4) Freshwater Invertebrate, Chronic

An aquatic invertebrate life-cycle test is required for benomyl because of high acute toxicity to aquatic invertebrates and MBC half-life persistence greater than 4 days in soil and water. Results of this test are tabulated below. The preferred test species is *Daphnia magna.* The preferred test substance is MBC.

Table :
Freshwater
Aquatic
Invertebrate Life-
Cycle Toxicity

Species	% A.I.	NOEC/LOEC (ppm)	MATC (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Daphnid (Daphnia magna)	99MBC	<0.0066 >0.0031	0.0040	% Survival, % Reproduction, Adult length	42529401 Baer, 92	Core
Daphnid (Daphnia magna)	99	<0.0250 >0.0130	0.0180	% Survival, % Reproduction, Adult length	40987901 Hutton, 86	Supplemental*

* High control mortality.

The guideline requirement (72-4) is fulfilled (MRID #42529401).

In the Daphnid test MRID 42529401, the most sensitive endpoint analyzed was the average number of young produced per adult reproductive day. This was a static renewal test. In the Daphnid test MRID 40987901, the most sensitive endpoint analyzed was first day reproduction. Other endpoints assessed include 21 day adult survival and adult length. This study is not repairable due to high mortality in water (43%) and solvent (28%) controls.

(5) Estuarine and Marine Animals, Acute

Acute toxicity testing with estuarine/marine organisms (fish, shrimp and oyster) using the technical grade of the active ingredient is required when an end-use product is intended for direct application to the marine/estuarine environment or the active ingredient is expected to reach this environment because of its use in coastal counties. The preferred test species are sheepshead minnow, mysid and eastern oyster. Estuarine/marine acute toxicity tests are required, and have been conducted for benomyl because high acute toxicity to freshwater aquatic organisms, MBC half-life persistence greater than 4 days in soil and water, and benomyl use on crops grown in coastal counties. Results of these tests are tabulated below.

Table : Estuarine/Marine
Acute Toxicity

Species	% A.I.	LC_{50}/EC_{50} (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Eastern oyster (Crassostrea virginica)	95	1.3760	Moderately Toxic	40975104 Boeri, 88	Core
	50WP	2.1420	Moderately Toxic	40975105 Boeri, 88	Core
	50DF	0.0780	Very Highly Toxic	42626401 Graves, 93	Core
Mysid (Americamysis bahia)	98MBC	0.0980	Very Highly Toxic	40964701 Boeri, 88	Core
	95	0.1750	Highly Toxic	40964802 Boeri, 88	Core
	50WP	0.1400	Highly Toxic	42414202 Ward, 92	Core
	50DF	0.1730	Highly Toxic	40964801 Boeri, 88	Core
Grass shrimp (Palomonetes vulgaris)	50WP	45.800	Slightly Toxic	00078579 Sleight, 72	Core
Sheepshead minnow (Cyprinodon variegatus)	95	3.7920	Moderately Toxic	40975101 Boeri, 88	Core
	50WP	3.6700	Moderately Toxic	40975103 Boeri, 88	Supplemental*
	50DF	>3.980	Moderately Toxic	40975102 Boeri, 88	Supplemental*, **
	50DF	26.000	Slightly Toxic	42626402 Graves, 93	Core
Dungeness crab (Cancer magister)	50WP	15.200	Slightly Toxic	00127865 Armstrong, 76	Supplemental*, **, ***, ****

* Formulated product test.
** Test concentration inadequate.
*** Test concentrations not reported.
**** Not a guideline species.

The results from mysid shrimp studies indicate that TGAI/MBC, 50%WP, and 50%DF are "Very highly toxic" and that TGAI/benomyl is "highly toxic". Benlate 50%DF is "very highly toxic" to the Eastern oyster and TGAI/benomyl and 50%WP are moderately toxic. The 50%WP formulation is slightly toxic to the Dungeness crab.

The results of estuarine/marine fish studies using the sheepshead minnow indicate that TGAI/benomyl, 50%WP, and 50%DF are moderately toxic an acute basis. The guideline requirement (72-3) is fulfilled (MRID #s 40975104, 40964701, 40975101).

(6) Estuarine and Marine Animals, Chronic

(6) Estuarine and Marine Animals, Chronic

Estuarine/marine fish early life-stage and/or aquatic invertebrate life-cycle toxicity tests are required for benomyl because of high acute toxicity and MBC half-life persistence of greater than 4 days in soil and water. Results of this test are tabulated below. The preferred test species are sheepshead minnow and/or mysid shrimp.

Table :
Estuarine/Marine
Chronic Toxicity

Species	% A.I.	NOEC/LOEC (ppm)	MATC (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Mysid (Americamysis bahia)	99MBC	<0.0504 >0.0248	0.0354	% Survival, Reproductive success,	42723701 Ward, 92	Core

The guideline requirement (72-4) is fulfilled (MRID #42723701).
In Mysid study MRID 42723701 above, the number of young per female and number of young per reproductive day were the most sensitive endpoints analyzed. Other endpoints were male/female wet and dry weights and mysid length.

An estuarine/marine fish life-cycle test using the technical grade of the active ingredient is required when an end-use product is intended to be applied directly to water or is expected to transport to water from the intended use site, and when any of the following conditions exist: (1) the EEC is equal to or greater than one-tenth of the NOEC in the fish early life-stage or invertebrate life-cycle test or; (2) studies of other organisms indicate the reproductive physiology of fish may be affected. The preferred test species is sheepshead minnow. The channel catfish is clearly more sensitive to benomyl and MBC than scale fish in acute and chronic fish toxicity tests. Therefore, this study is not required at this time, but held in reserve.

(7) Aquatic Field Testing

A rice monitoring field study was conducted in 1990 (MRID's 42969101, 44296910). This study did not assess biological effects except for one fish kill that occurred in an area adjacent to the treatment area.

c. Toxicity to Plants

(1) Terrestrial

Currently, terrestrial plant phytotoxicity testing under Subdivision J of FIFRA is not required for pesticides other than herbicides except on a case-by-case basis (e.g. labeling bears phytotoxicity warnings; incident data or literature which demonstrate phytotoxicity). In the time period 1987-1991 following the introduction and widespread use of Benlate 50%DF formulation, approximately 1800 plant phytotoxicity incident reports relating to Benlate 50%DF formulation were received by the agency. The majority of the reported incidents were associated with benomyl 50%DF use as a fungicide for control of diseases in greenhouse and shade-cloth grown ornamentals, cucumbers, and tomato plants in Florida, Texas, and Puerto Rico. However, incidents involving the field grown crops strawberries, blackberries, blueberries, tomatoes, beans, peanuts, pumpkins, squash, yams, mushrooms, watermelons, peaches, apples, plums, vineyards and papayas, were also received. Injury to the nontarget plants hickory, scrub oak, and some weeds was also reported in a limited number of incidents. In response to these incidents, the agency requested the conduct of early life-stage terrestrial plant Tier I studies under Subdivision J of FIFRA (see summaries below). DuPont Chemical Co. self-initiated a battery of early life-stage greenhouse studies to evaluate Benlate 50DF phytotoxicity to plants. Field tests on ornamental plants were also initiated by DuPont in Florida. The results of these tests were extensive. These data were evaluated and summarized by EPA Region 4. In 1994, the registrant voluntarily canceled all labeled uses of the 50%DF formulation and all greenhouse and ornamental used on the 50%WP labels. In their efforts to identify toxic metabolites and/or contaminates in Benlate 50DF formulation, DuPont identified: atrazine herbicide in multiple samples, simazine herbicide in one sample, chlorthalonil fungicide in multiple samples, flusilazole fungicide in multiple samples, dibutylurea (DBU) metabolite, and butylisocyanite (BIC) metabolite. DuPont has reported zero levels of sulfonylurea herbicides (ALS inhibitors) in all samples tested.

The formulated product Benlate 50DF was used in all terrestrial plant phytotoxicity tests: seed germination, seedling emergence, and vegetative vigor. The following plant species and groups were tested in each of the 3 studies: (1) six species of at least four dicotyledonous families, one species of which is soybean (*Glycine max*), and the second of which is a root crop, and (2) four species of at least two monocotyledonous families, one of which is corn (*Zea mays*). Seed germination tests were conducted for Benlate 50DF as this study was conducted before the agency generally waived this test requirement in 1994. Tier I tests measure the response of plants, relative to a control, at a test level that is equal to the highest use rate (expressed as lbs ai/A). Results of Tier 1 toxicity testing using Benlate 50DF formulation are tabulated below:

Table : Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier I)

Species	% A.I.	Dose (lb ai/A)	% Response and Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot- Corn	50DF	1.50	NSD* - Shoot ht, Dry wt, Emergence.	42817002 Carski, 93	CORE
Monocot- Wheat			NSD* - Shoot ht, Dry wt, Emergence.		CORE
Monocot- Sorghum			NSD* - Shoot ht, Dry wt, Emergence.		CORE
Dicot- Pea			NSD* - Shoot ht, Dry wt, Emergence.		CORE
Dicot- Onion			9.3% Sign. Inhibition, Shoot Wt.		CORE
Dicot- Soybean			NSD* - Shoot ht, Dry wt, Emergence.		CORE
Dicot- Tomato			7.9% Sign. Inhibition, Shoot wt.		CORE
Dicot- Rape			NSD* - Shoot ht, Dry wt, Emergence.		INVALID
Dicot- Sugarbeet					INVALID
Dicot- Cucumber					

* NSD= No significant difference in response compared to untreated controls.

Seed germination studies are no longer required but were included in the plant phytotoxicity data submission. Of the 10 tested plant species (MRID42817002), data from three of the species were invalid (rape, sugarbeet, cucumber). No significant adverse effects on germination were noted in any of the valid tests.

The seedling emergence test is considered a more scientifically rigorous test. In the seedling emergence study (MRID 42817002), significant adverse effects were noted in 2 of the 8 valid species tests - onion (9.3%) and tomato (7.9%). Neither of these responses achieved the Tier II trigger of 25% or greater effect. The sugarbeet test was invalid due to the unapproved use of chloroneb and lindane to control insects. The cucumber test was invalid due to the unapproved use of Poinsett 76 to regulate root and shoot growth.

The guideline requirement (122-1) is not fulfilled (MRID # 42817002); however, no further testing using 50%DF is required.

Table : Nontarget
Terrestrial Vegetative
Vigor Toxicity (Tier I)

Species	% A.I.	Dose (lb ai/A)	% Response and Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn	50DF	1.5	———	42817002 Carski, 93	INVALID**,***
Monocot - Wheat	50DF	1.5	NSD* - Shoot height	43363401 McKelvey, 94	CORE
Monocot - Sorghum	50DF	1.5	NSD* - Shoot height, Dry weight	43363401 McKelvey, 94	CORE
Dicot - Pea	50DF	1.5	———	42817002 Carski, 93	INVALID**
Dicot - Onion	50DF	1.5	1.2% Inhibition, NDS* - Shoot height	43363401 McKelvey, 94	CORE
Dicot - Soybean	50DF	1.5	———	42817002 Carski, 93	INVALID**
Dicot - Sugarbeet	50DF	1.5	NSD* - Shoot dry weight	43363401 McKelvey, 94	CORE
Dicot - Tomato	50DF	1.5	———	42817002 Carski, 93	INVALID**
Dicot - Cucumber	50DF	1.5	———	42817002 Carski, 93	INVALID**,***
Dicot - Rape	50DF	1.5	———	42817002 Carski, 93	INVALID**

* NSD = No significant difference in response compared to untreated controls.
** = Unapproved pesticide applied twice to test plants.
*** = Unapproved pesticide caused visible phytotoxicity to test plants soon after application.

Two different vegetative vigor studies were submitted. In study MRID 42817002 all ten test results were invalid due to the use of two applications of Mavrik insecticide as a foliar spray during the study. Visible phytotoxicity from the insecticide treatments was documented. In the second study, only 4 of the 10 required plant species were tested (MRID 43363401). While the symptoms of slight chlorosis, necrosis, and leaf curl were observed on a few select plants the injury was considered nonsignificant compared to the untreated controls. The University of Florida reported the following benomyl induced phytotoxicity symptoms on treated plants: stunting, smaller than normal leaves, twisted leaves, leaf margins turned down or cupped, chlorosis and yellowing similar to iron or manganese deficiency symptoms, leaves look mottled, necrotic leaf tips or margins, new stems elongated with long narrow leaves, leaf drop, and root portions darker than normal a few inches behind an active growing root tip with the outer root tissue stripped from the darkened area. In incident reports, plants often turned yellow and died, and/or yields were adversely affected (Chase, 1991).
The vegetative vigor Tier I test requirement (122-1) is not fulfilled; however, no further testing is required using the 50%DF formulation.

In response to concerns that the 50%WP formulation may be phytotoxic as well, a review of published literature was conducted. In general, laboratory tests were conducted in the 60's and 70's to determine if subtle growth effects of chemicals on treated plants occurred. Based on this information, Benomyl 50%WP has demonstrated phytotoxicity to selective crop cultivars such as cabbages, cauliflower, muskmelon, pea, squash, tomato, tobacco, onions, lettuce, sweet corn, mushrooms, potato tubers, barley, cucumbers, chrysanthemum, American elm, sycamore, and buckthorn. The phytotoxic effects occurred when these plants were emerging through treated soil or were very young and transplanted into treated soil. No phytotoxicity occurred from applications to plant foliage. The cultivar of the plant, stage of plant growth, method of application, concentration of Benlate used, presence or absence of surfactants, and type of rooting media as related to soil texture and organic matter content are all important variables in these experiments. When phytotoxicity occurred, it was described as chlorosis of the leaf tip and center, newly developed leaves showed phytotoxic symptoms, yellowing occurred at the borders of affected areas of the leaf along veins, loss of turgidity, leaves become whitish- brittle- and dried, and dead leaves hang from green stems. In some cases, the seedling plants were stunted but appeared normal. To date, the EPA has received no reports of adverse effects from use of benomyl 50%WP formulations. Using cabbage as an example, the registrant has labeled around potential phytotoxicity problems by only allowing foliar sprays at 14 day intervals at reduced rates. One cannot conclude from these data that the active ingredient is the sole toxicant. Fifty percent of the WP formulations contain inerts which may or may not be independently phytotoxic (Klingensmith ,1961; Reyes, 1975; Schreiber et.al., 1975; Roberts et. al., 1973; Wensley, 1972; and Yang, 1976).
More recently, researchers at the University of Florida and the University of Hawaii have identified the phytotoxicants dibutylurea (DBU) and butylisocyanate (BIC) in benomyl formulations (Shilling et. al., 1994; Moye et. al., 1994; Agragak et.al., 1994; Tang et.al., 1992; Uchida et.al., 1993; and Tang, Yee, Yanagihara , 1993).

Because the 50%DF formulation is now canceled, no further terrestrial plant tests are required. Terrestrial plant phytotoxicity tests for the other benomyl formulations are held in reserve.

(2) Aquatic

Currently, Tier I aquatic plant testing is only required for a fungicide that has outdoor non-residential terrestrial uses and that may move off-site by runoff (solubility >10 ppm in water), and/or by drift (aerial or irrigation) or that is applied directly to aquatic use sites (except residential). Because of numerous incident reports regarding benomyl 50%DF formulation, a full battery of 5 nontarget aquatic vascular and algal plant species were requested at the Tier I level (122-2). Results follow:

Table : Nontarget Aquatic Vascular and Algal Plant Toxicity (Tier I)

Species	% A.I.	Dose (ppm)	% Response	MRID No. Author/Year	Study Classification
Vascular Plant- Duckweed Lemna gibba	50DF	1.1 mg ai/L (1.5# ai/Acre)	NSD*	42854802 Thompson, 93	CORE
Freshwater Diatom - Navicula pelliculosa	50DF	1.1 mg ai/L	40% growth stimulation	42854801 Thompson, 93	CORE
Marine Diatom - Skeletonema costatum	50DF	1.1 mg ai/L	3% growth inhibition, NSD*	42854801 Thompson, 93	CORE
Non-Vascular Plant - Blue-green algae - Anabaena flos-aquae	50DF	1.1 mg ai/L	4% growth inhibition, NSD*	42854801 Thompson, 93	CORE
Non-Vascular Plant- Green algae Kirchneria subcapitata	50DF	1.1 mg ai/L	100% growth inhibition	42854801 Thompson, 93	CORE

*NSD = No significant difference in response compared to untreated controls.

The guideline requirement (122-2) is fulfilled for the 50%DF formulation. No significant growth effects occurred for the vascular aquatic plant *Lemna gibba*, the marine diatom *Skeletonema costatum*, or the blue-green algae *Anabaena flos-aquae*. A significant growth stimulation of the freshwater diatom *Navicula pelliculosa* occurred (40% stimulation), and a significant growth inhibition of the green algae *Selenastrum capricornutum (Kirchneria subcapitata)* occurred (100% inhibition). These tests show that oversprays of the maximum label dosage will significantly reduce levels of green algae and significantly increase levels of freshwater diatoms. Because the 50%DF formulation is no longer registered, no further aquatic plant growth tests are required. Aquatic plant growth tests using other benomyl formulations are held in reserve.

3. Exposure and Risk Characterization

Risk characterization integrates the results of the exposure and ecotoxicity data to evaluate the likelihood of adverse ecological effects. The means of integrating the results of exposure and ecotoxicity data is called the quotient method. For this method, risk quotients (RQs) are calculated by dividing exposure estimates by toxicity values, both acute and chronic.

RQ = EXPOSURE/TOXICITY

RQs are then compared to OPP's levels of concern (LOCs). These LOCs are criteria used by OPP to indicate potential risk to nontarget organisms and the need to consider regulatory action. The criteria indicate that a pesticide used as directed has the potential to cause adverse effects on nontarget organisms. LOCs currently address the following risk presumption categories: (1) **acute high** - potential for acute risk is high and regulatory action may be warranted in addition to restricted use classification (2) **acute restricted use** - the potential for acute risk is high, but this may be mitigated through restricted use classification (3) **acute endangered species** - the potential for acute risk to endangered species is high regulatory action may be warranted, and (4) **chronic risk** - the potential for chronic risk is high and regulatory action may be warranted. Currently, EFED does not perform assessments for chronic risk to plants, acute or chronic risks to nontarget insects, or chronic risk from granular/bait formulations to mammalian or avian species.

The ecotoxicity test values (i.e., measurement endpoints) used in the acute and chronic risk quotients are derived from the results of required studies. Examples of ecotoxicity values derived from the results of short-term laboratory studies that assess acute effects are: (1) LC50 (fish and birds) (2) LD50 (birds and mammals (3) EC50 (aquatic plants and aquatic invertebrates) and (4) EC25 (terrestrial plants). Examples of toxicity test effect levels derived from the results of long-term laboratory studies that assess chronic effects are: (1) LOEC (birds, fish, and aquatic invertebrates) (2) NOEC (birds, fish and aquatic invertebrates) and (3) MATC (fish and aquatic invertebrates). For birds and mammals, the NOEC value is used as the ecotoxicity test value in assessing chronic effects. Other values may be used when justified. Generally, the MATC (defined as the geometric mean of the NOEC and LOEC) is used as the ecotoxicity test value in assessing chronic effects to fish and aquatic invertebrates. However, the NOEC is used if the measurement end point is production of offspring or survival.

Risk presumptions, along with the corresponding RQs and LOCs are tabulated below.

Risk Presumptions for Terrestrial Animals

Risk Presumption	RQ	LOC
Birds		
Acute High Risk	EEC[1]/LC50 or LD50/sqft[2] or LD50/day[3]	0.5

Risk Presumptions for Terrestrial Animals

Risk Presumption	RQ	LOC
Acute Restricted Use	EEC/LC50 or LD50/sqft or LD50/day (or LD50 < 50 mg/kg)	0.2
Acute Endangered Species	EEC/LC50 or LD50/sqft or LD50/day	0.1
Chronic Risk	EEC/NOEC	1
Wild Mammals		
Acute High Risk	EEC/LC50 or LD50/sqft or LD50/day	0.5
Acute Restricted Use	EEC/LC50 or LD50/sqft or LD50/day (or LD50 < 50 mg/kg)	0.2
Acute Endangered Species	EEC/LC50 or LD50/sqft or LD50/day	0.1
Chronic Risk	EEC/NOEC	1

[1] abbreviation for Estimated Environmental Concentration (ppm) on avian/mammalian food items

[2] $\frac{mg/ft^2}{LD50 * wt. of bird}$ [3] $\frac{mg\ of\ toxicant\ consumed/day}{LD50 * wt. of bird}$

Risk Presumptions for Aquatic Animals

Risk Presumption	RQ	LOC
Acute High Risk	EEC[1]/LC50 or EC50	0.5
Acute Restricted Use	EEC/LC50 or EC50	0.1
Acute Endangered Species	EEC/LC50 or EC50	0.05
Chronic Risk	EEC/MATC or NOEC	1

[1] EEC = (ppm or ppb) in water

Risk Presumptions for Plants

Risk Presumption	RQ	LOC
	Terrestrial and Semi-Aquatic Plants	
Acute High Risk	EEC[1]/EC25	1
Acute Endangered Species	EEC/EC05 or NOEC	1
	Aquatic Plants	
Acute High Risk	EEC[2]/EC50	1
Acute Endangered Species	EEC/EC05 or NOEC	1

[1] EEC = lbs ai/A

[2] EEC = (ppb/ppm) in water

a. Exposure and Risk to Nontarget Terrestrial Animals

For pesticides applied as a nongranular product (e.g., liquid, dust), the estimated environmental concentrations (EECs) on food items following product application are compared to LC50 values to assess risk. The predicted 0-day maximum and mean residues of a pesticide that may be expected to occur on selected avian or mammalian food items immediately following a direct single application at 1 lb ai/A are tabulated below.

Estimated Environmental Concentrations on Avian and Mammalian Food Items (ppm) Following a Single Application at 1 lb ai/A)

Food Items	EEC (ppm) Predicted Maximum Residue[1]	EEC (ppm) Predicted Mean Residue[1]
Short grass	240	85
Tall grass	110	36
Broadleaf/forage plants, and small insects	135	45
Fruits, pods, seeds, and large insects	15	7

[1] Predicted maximum and mean residues are for a 1 lb ai/a application rate and are based on Hoerger and Kenaga (1972) as modified by Fletcher *et al.* (1994).

The acute risk quotients for broadcast applications of nongranular products are tabulated below:

GENERAL LABEL INFORMATION OF IMPORTANCE TO SITE SELECTION

Benomyl is currently registered for use on a broad range of agronomic crops, tree crops (fruits and nuts), vineyards and some tropical crops. The manufacturer of benomyl, DuPont, met with the benomyl RED team on 7/16/96 and provided the following top 10 crop ranking based on total pounds benomyl used per year (ranked from highest usage to lowest): rice, cucurbits, tomatoes, berries, beans, stone fruit, pome fruit, tree nuts, citrus, and soybeans. Maximum single treatment rates for the top 10 crops range from 0.125 (cucurbits) to 1.5 (citrus) lb ai/acre. The total number of applications allowed per season for these crops ranges from 2 (soybeans) to 13 (apples). The total lbs. ai/acre/year or crop season for the top 10 crops ranges from 0.5 (soybeans) to 3.0 (cherries, citrus). While we have no residue decline data for benomyl on plant foliage, the conversion of benomyl to carbendazim is expected to be slower than that which occurs in the aquatic environment (based on a published WHO report - EHC 148). Earthworm toxicity in apple orchards occurred following 13 applications of benomyl over a 2 year period. Therefore, the analysis below has used the worst case analysis regarding residue decline following multiple applications.

Non-ag. use sites turf, ornamentals (conifers, Christmas trees) are not significant use sites in terms of product usage (sales) as determined in the DuPont/EPA RED team meeting of

7/16/96. A small number of turf granular labels (benomyl impregnated on clay granules with fertilizer) exist. The amount of benomyl active ingredient ranges from 1.1 to 1.9%; therefore, benomyl treated granular fertilizer is not expected to be a significant route of toxicity to birds or mammals.

While this document also concerns carbendazim phosphate [Methyl (2-benzimidazole) carbamate phosphate], the sole use of this active ingredient is as a tree injection to control Dutch elm disease. While no eco-toxicity data are currently available for this AI, minimal environmental exposure is expected because the compound is confined within the tree and it will be minimally used in the U.S.

Top 10 Benomyl Use Crops - From Top to Bottom - From Benlate SP (EPA REG. NO. 352-564 and Benlate Fungicide (EPA REG. NO. 352-354).

CROP	LB. AI/TREATMENT	TOTAL APPS./YR.	APPL. INTERVAL - DAYS	TOTAL LB.s AI/ACRE/YR
RICE	1.000	2	10-14	2.0
CUCURBITS	0.125	8	7-14	1.0
TOMATOES	0.500	5	7-14	2.5
CANE BERRIES	0.375	5	14	1.9
BEANS	0.666	3	7-10	2.0
POME FRUITS	0.417 (Pear) 0.192 (Apple)	6 (Pear) 13 (Apple)	14	2.5
TREE NUTS	0.500	3		1.5
STONE FRUITS	0.500	6 (Cherries)	10	3.0
CITRUS	1.50	2	20	3.0
SOYBEANS	0.25	2	14-21	0.5

Other crops with high total use rates per acre per year:

3.00 lb. ai/acre/yr.= avocados, brassica crops (broccoli, brussels sprouts, cabbage, Chinese cabbage, cauliflower, collards, kohlrabi, mustard greens, rutabagas, and turnips), grapes, mangoes, and papayas.
2.63 lb. ai/acre/yr.= macadamia nuts.
2.50 lb. ai/acre/yr.= blueberries, conifers (fir), and strawberries.

i. Birds

Avian Acute values range from LC50's of >5000 to >10000 ppm. EEB branch policy for values > 5,000 ppm dictates that adverse acute effects are not expected from use of the maximum labeled benomyl rates (total of 3 lbs. ai/acre/year).

The chronic NOEC value of 322 ppm was established for the Mallard duck and 3600 ppm ai for the Bobwhite quail.

The chronic risk quotients for multiple applictions of nongranular products (broadcast aerial application) are based on the Mallard duck NOEC of 322 ppm ai.

Site/App. Method	App.Rate (lbs ai/A) No. of Apps.	Food Items	Maximum EEC* (ppm)	LC50 (ppm)	NOEC (ppm)	Acute RQ (EEC/ LC50)	Chronic RQ (EEC/ NOEC)
Citrus aerial	1.5 (2)	Short grass	697*		322		2.24
		Tall grass			322		
		Broadleaf plants/Insects			322		
		Seeds			322		

* Assumes degradation using FATE program (2 applications of 1.5 lb.ai/acre at 30 day interval; maximum short grass residue at 154 days, average short grass residue at 154 days = 561 ppm).

The highest benomyl application rate per acre per season or year is 3.0 lb. ai. This amount can currently be used on the following crops: avocados (FL, PR only), brassica crops, cherries, citrus, grapes, mangoes, papayas, and yams. Citrus is used as the example crop in the table above. Assuming a worst case scenario, the results indicate that multiple broadcast applications of nongranular products may result in residue buildup that exceeds the chronic risk level of concern. Adverse reproductive effects observed in an avian reproduction study for the mallard duck include significant reductions in the percentage of viable embryos, normal hatchlings, and 14 day old survivors of eggs set (MRID 44103002).

ii. Mammals

Birds and mammals have similar responses to xenobiotics, their differences being more quantitative than qualitative. Since benomyl and MBC do not present an acute risk to endangered birds, mammals are also presumed to be protected. Estimating the potential for adverse effects to wild mammals is based upon EEB's draft 1995 SOP of mammalian risk assessments and methods used by Hoerger and Kenaga (1972) as modified by Fletcher *et al.* (1994). The concentration of benomyl or MBC in the diet that is expected to be acutely lethal to 50% of the test population (LC50) is determined by dividing the LD50 value (usually rat LD50) by the % (decimal of) body weight consumed. A risk quotient is then determined by dividing the EEC by the derived LC50 value. Risk quotients are calculated for three separate

weight classes of mammals (15, 35, and 1000 g), each presumed to consume four different kinds of food (grass, forage, insects, and seeds).

Mammalian (Herbivore/Insectivore) Acute Risk Quotients Multiple Applications of Nongranular Products (Broadcast) Based on a (laboratory rat) LD50 of >5,000 mg/Kg. EEB branch policy regarding > values, especially of this magnitude, dictate that minimal acute risk is expected from use of up to 3 lbs. ai benomyl per acre per year. The calculation of risk quotients is not necessary.

iii. Insects

Currently, EFED does not assess risk to nontarget insects. Results of acceptable studies are used for recommending appropriate label precautions.

b. Exposure and Risk to Nontarget Freshwater Aquatic Animals

Because of high acute and chronic toxicity concerns for benomyl in the aquatic environment, a rice monitoring study was required by EPA and was conducted by DuPont Chemical Co. Due to the extensive nature of residue monitoring in this study (rice field, drainage ditches, and streams), actual field residue data will be used for EEC values for rice on which the largest amount of benomyl is used each year. Two different studies were submitted (MRID 42969101 and 44296910). Refined EEC's for the benomyl use sites citrus and tomatoes were provided by the Environmental Fate and Groundwater Branch/EFED (Mostaghami, 1997). The benomyl use sites, rice, citrus, and tomatoes were selected as representatives of the top 10 usage sites and high label dosages. There are crops potentially grown/occurring in areas where runoff may occur to aquatic/estuarine habitats, and represent different application methods and agronomic practices. The EECs are used for assessing acute and chronic risks to aquatic organisms. Acute risk assessments are performed using either 0-day EEC values for single application or peak EEC values for multiple application. Chronic risk assessments are performed using the 21-day EECs for invertebrates and 56-day EECs for fish.

The model calculates the surface runoff concentration (i.e. EEC) of a pesticide into a water body, taking into account the following: (1) adsorption to soil or sediment (2) soil incorporation (3) degradation in soil before washoff to a water body and (4) degradation within the water body. The model also accounts for direct deposition of spray drift into the water body (assumed to be 1% and 5% of the application rate for ground and aerial applications, respectively). The interval between applications is included in the calculations. The environmental fate parameters used in the model for this pesticide are: soil K_{OC} 2100,

photolysis stable, aquatic aerobic metabolism half-life of 61 days. EECs are tabulated below.

Estimated And Monitored Environmental Concentrations (EECs) For Aquatic Exposure

Site	Application Method	Application Rate (lbs ai/A)	# of Apps./ Interval Between Apps.	Initial (PEAK) EEC (ppm)	21-day EEC (ppm)	56-day EEC (ppm)
Rice* (MONITORED)	aerial, liquid	1.00	2 treatments, 10 day Intv.	0.203**	0.096	0.037***
Citrus (PRIZM/EXAMS)	aerial, liquid	1.50	2 treatments, 30 day Intv.	0.122	0.108	0.095
Tomatoes (PRIZM/EXAMS)	aerial, liquid	0.50	5 treatments, 7 day Intv.	0.084	0.074	0.069

* Field residue monitoring study (Actual stream residues used in this analysis). Maximum median value from 5 AR and 2 LA streams.

** This residue level remained steady for 7 day drainage period - Stream 2, S.Louisiana.

*** Last sample (55 days), Stream 2, S. Louisiana

RICE MONITORING STUDY, MRID's 42969101, 44296910 (1990-1993): Two aerial applications of benomyl 50%WP were made, the first at the "boot stage"* of rice development and the second application 10 days after the first - generally at the "heading stage"** of rice development. The application rate for each treatment was 1 lb ai/acre Benlate® 50WP. Treatments were made to rice paddies in Arkansas and Louisiana. Water and sediment samples in the paddies, adjacent drainage ditches and receiving streams were taken in Central Arkansas, Eastern Arkansas, and Southern Louisiana. Samples were collected 1 day prior to paddy drain, 1 day prior to pesticide application, days 1,2,3,5, and 7 after paddy drain, and day 55 following paddy drain. Additional samples were collected following significant manmade or natural runoff events. A total of 7 streams were sampled, two in Louisiana and 5 in Arkansas. The closer to the paddy, the higher the soil and water residues detected. The ELISA (enzyme-linked immunosorbent assay) procedure was used to analyze soil and water samples and was validated with HPLC (high pressure liquid chromatgrophy). The limits of detection were 38 ppb in soil and 1 ppb in water.

* boot stage: when the flag leaf sheath swells because of an increase in size of the panicle as it grows up the leaf sheath, after the flag leaf is completely extended (DD50 stage).

** heading stage: when the seedhead (panicle) begins to come out of the boot (14-21 days after early booting stage). Heading stage lasts 10-14 days.

ii. Freshwater Fish

Risk Quotients for Freshwater Fish Based on a catfish LC50 of 0.0074 ppm and a catfish NOEC/MATC of 0.0014 .

Site/ Application Method	Rate in lbs as/A (No. of Apps.)	LC50 (ppm)	NOEC/ MATC (ppm)	EEC Initial/ Peak (ppm)	EEC 56-Day Average	Acute RQ (EEC/LC50)	Chronic RQ (EEC/NOEC or MATC)
Rice/ aerial	1 (2)	0.0074	0.0014	0.203	0.037	27.43	26.43
Citrus/ aerial	1.5 (2)	0.0074	0.0014	0.122	0.095	16.49	67.86
Tomatoes/ aerial	0.5 (5)	0.0074	0.0014	0.084	0.069	11.35	49.29

The results indicate that aquatic acute high risk, restricted use, and endangered species levels of concern are exceeded for freshwater fish for all 3 sites (rice, citrus, and tomatoes). The chronic risk level of concern is exceeded for freshwater fish for all 3 sites. The maximum application rates for these sites range from 2.0 to 3.0 lbs. ai/acre/year.

ii. Freshwater Invertebrates

The acute and chronic risk quotients are tabulated below.

Risk Quotients for Freshwater Invertebrates Based on a Daphnia magna EC50/LC50 of 0.039 ppm and a Daphnia magna NOEC/MATC of 0.0040 ppm.

Site/ Application Method	Rate in lbs as/A (No. of Apps.)	LC50 (ppm)	NOEC/ MATC (ppm)	EEC Initial/ Peak (ppm)	EEC 21-Day Average	Acute RQ (EEC/LC50)	Chronic RQ (EEC/NOEC or MATC)
Rice/ aerial	1 (2)	0.039	0.004	0.203	0.096	5.21	24.00
Citrus/ aerial	1.5 (2)	0.039	0.004	0.122	0.108	3.13	27.00
Tomatoes/ aerial	0.5 (5)	0.039	0.004	0.084	0.074	2.15	18.50

The results indicate that aquatic acute high risk, restricted use, and endangered species levels of concern are exceeded for freshwater invertebrates for all 3 sites (rice, citrus, and tomatoes). The chronic level of concern is exceeded for all 3 sites as well. Maximum application rates for these sites range from 2.0 to 3.0 lbs. ai/acre/year.

c. Estuarine and Marine Animals

Risk Quotients for Estuarine/Marine Fish Based on a Sheepshead minnow LC50 of 3.79 ppm.

Site/ Application Method	Rate in lbs ai/A (No. of Apps.)	LC50 (ppm)	NOEC/ MATC (ppm)	EEC Initial/ Peak (ppm)	EEC 56-Day Average	Acute RQ (EEC/LC50)	Chronic RQ (EEC/NOEC or MATC)
Rice/ aerial	1 (2)	3.79	___	0.203		0.054	___
Citrus/ aerial	1.5 (2)	3.79	___	0.122		0.032	___
Tomatoes/ aerial	0.5 (5)	3.79	___	0.084		0.022	___

The results of this analysis indicate that the aquatic acute level of concern is exceeded for endangered estuarine/marine fish (rice site only). Chronic fish data were not available.

Risk Quotients for Estuarine/Marine Aquatic Invertebrates Based on a Mysid shrimp LC50/EC50 of 0.098 and a Mysid shrimp NOEC/MATC of 0.035.

Site/ Application Method	Rate in lbs ai/A (No. of Apps.)	LC50 (ppm)	NOEC/ MATC (ppm)	EEC Initial/ Peak (ppm)	EEC 21-Day Average	Acute RQ (EEC/LC50)	Chronic RQ (EEC/NOEC or MATC)
Rice/ aerial	1 (2)	0.098	0.035	0.203	0.096	2.07	2.74
Citrus/ aerial	1.5 (2)	0.098	0.035	0.122	0.108	1.25	3.09
Tomatoes/ aerial	0.5 (5)	0.098	0.035	0.084	0.074	0.86	2.11

The results indicate that aquatic acute high risk LOC's are exceeded for all 3 sites, acute restricted use LOC's are exceeded for all 3 sites, and the acute endangered species levels of concern are exceeded for estuarine invertebrates for all 3 sites. The aquatic chronic level of concern is exceeded for estuarine invertebrates for all 3 use sites. Maximum application rates for these sites range from 2.0 to 3.0 lbs. ai/acre/year.

d. Exposure and Risk to Nontarget Plants

A risk assessment for terrestrial, aquatic vascular, and algal species cannot be conducted without Tier II data. Movement of benomyl off target is expected to adversely affect green algae based on Tier I data. The benomyl DF (dry flowable) formulation, when registered, was implicated in >1800 phytotoxicity incidents. Some of these incidents were associated with atrazine contamination and others are still unexplained. The Tier I terrestrial plant phytotoxicity studies did not reveal significant adverse effects to our 10 test species (>25% adverse effect) from the benomyl DF formulation. Injury to seedling plants exposed to benomyl 50% WP(wettable powder) is documented in the literature, however, the agency has no record of significant adverse effects (incident reports) from use of the 50% WP formulation. Benomyl is typically applied after the plants have emerged and are well established in the field, well after the seedling stage of growth. This may partially explain why no incidents for the 50% WP formulation have been reported.

4. Endangered Species

Endangered species LOCs are exceeded for freshwater fish, freshwater invertebrates, marine/estuarine invertebrates, and marine/estuarine fish (rice use).

The Endangered Species Protection Program is expected to become final in the future. Limitations in the use of benomyl may be required to protect endangered and threatened species, but these limitations have not been defined and may be formulation specific. EPA anticipates that a consultation with the Fish and Wildlife Service may be conducted in accordance with the species-based priority approach described in the Program. After completion of consultation, registrants will be informed if any required label modifications are necessary. Such modifications would most likely consist of the generic label statement referring pesticide users to use limitations contained in county Bulletins.

4. Labeling Requirements

a. Manufacturing-Use Products - No specific comments.

b. End-use Products - End-use product labels for Benlate® Fungicide (EPA Reg. No. 352-354 and Benlate SP® Fungicide (EPA Reg. No. 352-564) were reviewed for pertinent environmental hazard and drift statements. The labels appear adequate with the following exceptions:

1.) add: This pesticide is toxic to aquatic invertebrates, (rice section also)

2.) add: This pesticide is toxic to earthworms, (Environ. Hazards panel)

3.) add: This pesticide is toxic to aquatic plants, (rice section also)

4.) add: Drift and runoff from treated areas may be hazardous to fish, aquatic invertebrates, and aquatic plants in adjacent areas, (rice section also)

5.) add: When using aerial application or chemigation, maintain a within-the-field buffer to adjacent non-target water bodies of one swath width for aerial application and XX feet for chemigation application. (rice section also)

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Guideline Study Number:141-1

05001991 Stevenson, J.H. 1978. The acute toxicity of unformulated pesticides to worker honey bees (*Apis mellifera L.*). Plant Pathol. 27(1): 38-40

NONTARGET PLANT PHYTOTOXICITY DATA

Guideline Study Number:122-1

42817002 Carski, T., and R. McKelvey. (1993) Influence of Benlate DF Fungicide on Seed Germination, Seedling Emergence, and Vegetative Vigor of Several Terrestrial Plants: Lab Project Number:AMR 2626-93. Unpublished study prepared by E.I. du Pont de Nemours and Co. 92p.

43363401 McKelvey, R. (1994) Influence of Benlate 50 DF Fungicide on Vegetative Vigor of Four Terrestrial Plants: Lab Project Number: MAR 2812-93. Unpublished study prepared by E. I. du Pont de Nemours and Co., Inc. 51 p.

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42854801 Thompson, S.; McKelvey, R. (1993) Benlate 50 DF Fungicide: Influence on Growth and Reproduction of Four Select Algal Species: Lab Project Number: AMR 2695-93: 112A-114. Unpublished study prepared by Wildlife International Ltd. and Du Pont Agricultural Products. 40 p

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WHO (1993) - Environmental Health Criteria 149: Carbendazim. Geneva, World Health Organization. Pgs. 1-132

WHO (1993) - Environmental Health Criteria 148: Benomyl. Geneva, World Health Organization. Pgs. 1-132

Date:
Case No:
Chemical No:

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2] A,B,C,D	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
6 Basic Studies in Bold					
71-1(a) Acute Avian Oral, Quail/Duck			YES	43129604 (Grimes, 93)	NO
71-1(b) Acute Avian Oral, Quail/Duck	(TEP)				
71-2(a) Acute Avian Diet, Quail			YES	00073598 (Fink, 76)	NO
71-2(b) Acute Avian Diet, Duck			YES	00073597 (Fink, 76)	NO
71-3 Wild Mammal Toxicity					
71-4(a) Avian Reproduction Quail			YES	44103001 (Frey, 96)	YES
71-4(b) Avian Reproduction Duck			YES	44103002 (Frey, 96)	NO
71-5(a) Simulated Terrestrial Field Study					
71-5(b) Actual Terrestrial Field Study					
72-1(a) Acute Fish Toxicity Bluegill			YES	FAOBEN05 (McCann, 76)	NO
72-1(b) Acute Fish Toxicity Bluegill	(TEP) (WP)		YES	FAOBEN05 (McCann, 77)	NO
72-1(c) Acute Fish Toxicity Rainbow Trout			YES	40098001 (Mayer, 86)	NO
72-1(d) Acute Fish Toxicity Rainbow Trout	(TEP) (WP)		YES	40098001 (Mayer, 86)	NO
72-2(a) Acute Aquatic Invertebrate Toxicity			YES	42414201 (Baer, 92)	NO
72-2(b) Acute Aquatic Invertebrate Toxicity	(TEP) (WP)		YES	40802101 (Hutton, 88)	NO
72-3(a) Acute Estu/Mari Tox Fish			YES	40975101 (Boeri, 88)	NO
72-3(b) Acute Estu/Mari Tox Mollusk			YES	40975104 (Boeri, 88)	NO
72-3(c) Acute Estu.Mari Tox Shrimp			YES	40964701 (Boeri, 88)	NO

* In Bibliographic Citation column indicates study may be up-gradeable

Date:
Case No:
Chemical No:

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2] A,B,C,D	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?

* In Bibliographic Citation column indicates study may be up-gradeable

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72-3(d) Acute Estu/Mari Tox Fish	(TEP) (WP)	YES	40975103 (Boeri, 88)	NO
72-3(e) Acute Estu/Mari Tox Mollusk	(TEP) (WP)	YES	40975105 (Boeri, 88)	NO
72-3(f) Acute Estu/Mari Tox Shrimp	(TEP) (WP)	YES	42414202 (Ward, 92)	NO
72-4(a) Early Life-Stage Fish		YES	43872801 (Rhodes, 95)	NO
72-4(b) Life-Cycle Aquatic Invertebrate		YES	42529401 (Baer, 92)	NO
72-4(b) Life-Cycle Aquatic Invertebrate		YES	42723701 (Ward, 92)	NO
72-6 Aquatic Org. Accumulation				
72-7(a) Simulated Aquatic Field Study				
72-7(b) Actual Aquatic Field Study				
122-1(a) Seed Germ./Seedling Emerg.	(TEP) (DF)	YES	42817002 (Carski, 93)	NO
122-1(b) Vegetative Vigor	(TEP) (DF)	YES	43363401 (McKelvey, 94)	NO
122-2 Aquatic Plant Growth	(TEP) (DF)	YES	42854801 (Thompson, 93)	NO
123-1(a) Seed Germ./Seedling Emerg.				
123-1(b) Vegetative Vigor				
123-2 Aquatic Plant Growth				
124-1 Terrestrial Field Study				
124-2 Aquatic Field Study		YES	05001991 (Stevenson, 78)	NO
141-1 Honey Bee Acute Contact				
141-2 Honey Bee Residue on Foliage				
141-5 Field Test for Pollinators				

* In Bibliographic Citation column indicates study may be up-gradeable

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[1]...Composition: TGAI = Technical grade of the active ingredient; PAIRA = Pure active ingredient, radiolabeled; TEP = Typical end-use product

[2]...Use Patterns: 1 = Terrestrial/Food; 2 = Terrestrial/Feed; 3 = Terrestrial Non-Food; 4 = Aquatic Food; 5 = Aquatic Non-Food (Outdoor); 6 = Aquatic Non-Food (Industrial); 7 = Aquatic Non-Food (Residential); 8 = Greenhouse Food; 9 = Greenhouse Non-Food; 10 = Forestry; 11 = Residential Outdoor; 12 = Indoor Food; 13 = Indoor Non-Food; 14 = Indoor Medical; 15 = Indoor Residential

* In Bibliographic Citation column indicates study may be up-gradeable

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF fungicide (IN-T1991-570); 2-benzimidazole carbamic acid, 1-(butylcarbamoyl)-methyl ester; 49.2% active ingredient.

3. **STUDY TYPE:** 122-1. Non-Target Plants: Seed Germination, Seedling Emergence & Vegetative Vigor Phytotoxicity Tests - Tier 1. Species Tested: Soybean, Sugarbeet, Pea, Tomato, Cucumber, Rape, Sorghum, Wheat, Corn, and Onion.

4. **CITATION:** Carski, T.H. and R.A. McKelvey. 1993. Influence of Benlate® 50 DF Fungicide on Seed Germination, Seedling Emergence, and Vegetative Vigor of Several Terrestrial Plants. Du Pont Project ID No. AMR 2626-93. Conducted and submitted by E.I. du Pont de Nemours and Company, Newark and Wilmington, DE. EPA MRID No. 428170-02.

5. **REVIEWED BY:**

Richard C. Petrie
Senior Agronomist
EEB, EFED, OPP

Signature: Richard C. Petrie
Date: 11/06/96

6. **APPROVED BY:**

Ann Stavola
Section Head
Section 5
EEB, EFED, OPP

Signature: Ann Stavola
Date: 12/17/96

7. **CONCLUSIONS:**
Seed Germination: This 5 day seed germination study is scientifically sound and fulfills the guideline requirement for 7 of the 10 test species using a formulated product - corn, pea, onion, wheat tomato, sorghum, and soybean. The rape seed study is invalid due to poor germination and must be repeated. The sugarbeet and cucumber test species were treated with unapproved pesticides (sugarbeet - Dexon, Chloroneb 65W, and lindane; cucumber - Poinsett 76). The only two pesticides currently approved for use as seed

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treatments are captan and thiram fungicides. Therefore, the rape, sugarbeet and cucumber tests are not scientifically sound and do not fulfill the guideline requirement. All test plants were exposed to a concentration equivalent to the maximum Benlate DF label application rate of 1.5# ai/Acre. Only 1 of the 7 valid tests, sorghum, was significantly affected although the magnitude of this reduction was not greater than 25% (7.4%).

Seedling Emergence: The test plants were exposed to a concentration equivalent to the maximum Benlate DF label application rate of 1.5# ai/Acre, with test termination after 2 weeks. The seeds of sugarbeet and cucumber were treated with unapproved pesticides (sugarbeet - Dexon, Chloroneb 65W, and lindane; cucumber - Poinsett 76). The only two pesticides currently approved for use as seed treatments are captan and thiram fungicides. The results from these two species are not scientifically sound and do not fulfill the guideline requirement. The results obtained with the remaining eight species (soybean, pea, tomato, rape, sorghum, wheat, corn, and onion) are scientifically sound and fulfill the requirements for a Tier 1 seedling emergence study using a formulated product. The most sensitive monocot and dicot species parameter (onion and tomato dry weight) was not reduced greater than 25% (9.3 and 7.9% reduction respectively).

Vegetative Vigor: The test plants were exposed to a concentration equivalent to the maximum Benlate DF label application rate of 1.5# ai/Acre, with test termination after 3 weeks. All test plants (all species) were treated with an unapproved pesticide (Mavrik insecticide) twice during the study. On pages 46 and 47 of the report, phytotoxicity was observed on some test plants and believed to have occurred from use of the unapproved pesticide. The results of the vegetative vigor test are not scientifically sound and do not meet the guideline requirement.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, sugarbeet, pea, tomato, cucumber, and rape). Monocotyledon plants were represented by four species

from two families (i.e., corn, sorghum, wheat, and onion). Seed source, cultivar, and lot number information were included in the report. Corn and pea seeds were treated with captan and cucumber and sugarbeet seeds were treated with a combination of fungicides and/or insecticide for all three studies.

B. **Test System:**

Seed Germination: One or more circles of glass micro-fiber filter (125 mm diameter) paper were placed in the bottom of a glass petri plate (150 mm in diameter and 20 mm in height). The test solution was prepared in buffer (pH 7.0) and applied to the petri plates.

Twenty seeds of each crop were added to each petri plate. The plates were covered and placed in a dark incubator maintained at 25 ±1°C for 5 days.

Seedling Emergence: Twenty seeds of each crop were planted in 8-inch trays filled with a sand-amended loam soil (pH = 6.2, 1% organic matter content). Onion, tomato, sugarbeet, and rape seeds were planted at a 1-cm depth, and the remaining species were planted at a depth of 2.5 cm.

The trays were sprayed with a solution of Benlate®50 DF prepared in buffer (pH 7). Application was accomplished with a rotating belt lab sprayer calibrated to deliver 40 gallons per acre at a pressure of 40 psi. The nozzle was positioned 14.5 inches above the tray.

Plants were allowed to emerge in a greenhouse under natural lighting which was supplemented with artificial lighting to produce a 16-hour photoperiod. The temperature was maintained at 20-45°C. Top-watering was conducted on an as needed basis with tap water.

Vegetative Vigor: Solutions of Benlate® 50 DF were prepared in buffer (pH 7) and sprayed onto established plants that were either 13 cm in height or had 3-5 leaves. The pots (15 cm diameter x 15 cm depth) that each species were planted in contained either 1 plant (corn, soybean, tomato, cucumber), 3 plants (sugarbeet, pea, rape), or 6 plants (onion, wheat, sorghum). Planting, application, and growth conditions were identical to those in the seedling emergence test with the caveat that foliage was avoided when watering the pots and the temperature range was 17-35°C.

C. **Dosage:** Benlate® 50 DF was applied at the rate of 3 lb/acre (A) or 1.5 lb active ingredient (ai)/A for all three tests.

D. **Design:**

Seed Germination: The treatment/crop combination was replicated six times (i.e., 20 seeds/plate, 6 plates/treatment). After 5 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured. Percent seed germination was determined by counting the number of seeds with radicle lengths of 5 mm or greater. The physical appearance of the seedlings was also monitored.

Seedling Emergence: Each crop/treatment combination was replicated four times (i.e., 20 seeds/tray, 4 trays/treatment level). At 7 and 14 days after treatment (DAT), each replicate tray was assessed for abnormalities, seedling emergence, and seedling height. Dry weights were obtained from the shoot portions of each replicate by drying the tissue at 70° for a minimum of 48 hours.

Vegetative Vigor: Each crop/treatment combination was replicated four times (i.e., 1 vessel with 6 plants/replicate, 2 vessels with 3 plants/replicate, or 6 vessels with 1 plant/replicate per treatment level). At 7 and 21 DAT, each replicate pot was assessed for abnormalities and seedling height was measured. Shoot, root, and whole plant dry weights were determined by drying the plants for a minimum of 48 hours at 70°C.

Temperature, relative humidity, water applied per pot, and light intensity measured during the period of growth were provided in the report for the emergence and vegetative vigor studies. Plants were treated with an N-P-K fertilizer and iron chelate 7 DAT for the vegetative vigor study. Additionally, the plants were treated with Mavrik® insecticide at 1 and 7 DAT.

Test solutions for all three studies were analyzed for carbendazim by liquid chromatography. Carbendazim was the analyte because benomyl quickly degrades to this compound in water, and the rate is accelerated by the presence of methanol. The test solutions were diluted with methanol and allowed to stand for 24 hours, at which time analysis was conducted.

E. **Statistics:** All studies were conducted using randomized complete block designs. Percent inhibition and the associated 95% confidence intervals (calculated using the method for ratios with unequal variances) were determined. Welch's t-test was used to determine if a statistically significant reduction of 25% or greater had occurred in comparison to the control data ($p \leq 0.1$).

12. **REPORTED RESULTS:** The measured concentrations of the test solutions for all studies ranged from 125 to 138% of nominal. The results for all studies are based on nominal concentrations.

Seed Germination: Conditions inside the incubator for the test were reported as a temperature of 24-25°C and a relative humidity of 50-57%. Responses on germination of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows (negative inhibition indicates growth stimulation):

sugarbeet (-17) < corn (-3.5) < tomato (-3.4) < pea (-2.6) < soybean = cucumber (-0.9) < wheat (4.3) < onion (4.4) < rape (4.7) < sorghum (7.4).

Observations of abnormalities are presented in Table II (attached). None of the test species were advanced to Tier 2 testing based on less than 10% inhibition in germination for all the species.

Seedling Emergence: Conditions inside the greenhouse were reported as a temperature of 20-45°C and a relative humidity of 14-62%. Water applied to each tray ranged between 0 and 320 ml/tray/day. Responses of the 2-week emergence of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sugarbeet (-11) < rape (-2.9) < sorghum (-2.8) < pea (-2.6) < corn (0) < soybean (2.6) < onion (3.3) < cucumber (3.9) < wheat (5.3) < tomato (7.8).

Responses of 2-week height of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

soybean (-9.4) < cucumber (-5.4) < tomato (-3.0) < rape (-2.5) < sorghum (-2.3) < onion (-1.7) < pea (-0.7) < wheat (-0.6) < sugarbeet (1.5) < corn (1.6).

Response of 2-week dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sugarbeet (-8.0) < sorghum (-5.3) < wheat (-3.6) < cucumber (-1.8) < soybean (-1.1) < rape (-0.2) < pea (0.3) < corn (5.9) < tomato (7.9) < onion (9.3).

None of the ten species were advanced to Tier 2 testing based on the amount of inhibition witnessed in the emergence test (<10% for all measured parameters). Observations of abnormality are presented in Table X (attached).

Vegetative Vigor: Conditions inside the greenhouse were reported as a temperature of 17-35°C and a relative humidity of 14-62%. Water applied to each tray ranged between 90 and 470 ml/pot/day. Responses of the 3-week height of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-12) < cucumber (-9.3) < pea (-3.0) < tomato (-2.8) < soybean (-1.5) < rape (0.6) < corn (0.7) < onion (0.9) < wheat (1.3) < sugarbeet (2.5).

Responses of 3-week shoot dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-44) < pea (-26) < cucumber (-12) < tomato (-7.3) < rape (-4.3) < soybean (-4.2) < onion (1.7) < wheat (3.0) < corn (3.7) < sugarbeet (7.8).

Response of 3-week root dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

tomato (-41) < sorghum (-39) < rape (-33) < cucumber (-27) < pea (-22) < corn (-3.9) < soybean (0.4) < wheat (7.0) < onion = sugarbeet (14).

Response of 3-week total dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-43) < pea (-25) < cucumber (-14) < tomato (-12) < rape (-9.0) < soybean (-3.4) < corn (2.2) < wheat (4.5) < onion (4.6) < sugarbeet (9.0).

None of the ten species were advanced to Tier 2 testing based on the amount of inhibition witnessed in the vigor

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test (<15% for all measured parameters). Observations of abnormality are presented in Table XIX (attached). It was noted that the root dry weight data were highly variable for the onion, wheat, sugarbeet, and pea.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Germination, early seedling growth, and vegetative vigor of all ten test plants were not reduced by ≥25% at the rate of 3.0 lb/A (1.5 lb ai/A) for all ten test species. Therefore, Benlate® 50 DF fungicide when applied at the maximum labeled rate does not pose a threat to terrestrial plants.

Good Laboratory Practice (GLP) compliance and Quality Assurance statements were included in the report indicating that the study was conducted under the EPA GLP standards set forth in 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:**
For the GERMINATION STUDY, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

No germination ratings for the seeds used were reported.

Seeds of sugarbeet and cucumber were treated with unapproved pesticides (sugarbeet - Dexon, Chloroneb 65W, and lindane; cucumber - Poinsett 76).

Rape seeds were of poor quality (53% germination in the controls, 51% in the test plots). A minimum germination of 75% plus or minus 10% is required.

For the SEEDLING EMERGENCE study, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

Seeds of sugarbeet and cucumber were treated with unapproved pesticides (sugarbeet - Dexon, Chloroneb 65W, and lindane; cucumber - Poinsett 76).

No spray solution or application calculations were reported.

The study was terminated at the end of 2 weeks. A 3- or 4-week evaluation was not conducted.

For the VEGETATIVE VIGOR study, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

Seeds of some species were treated with unapproved pesticides.

Plants were treated twice with an unapproved pesticide (Mavrik insecticide).

No spray solution or application calculations were reported.

B. **Statistical Analysis:** SEED GERMINATION TEST: The reviewer used a t-test to determine if significant reductions in percentage germination occurred for sorghum (the most sensitive valid monocot species). Germination for valid dicot species was not negatively affected, and therefore, analyses were not conducted for those species. Sorghum germination was significantly reduced by Benlate® 50 DF treatment (α= 0.05).

SEEDLING EMERGENCE TEST: A t-test was also used to determine if significant reductions in dry weight occurred for onion and tomato (the most sensitive valid monocot and dicot species, respectively, for the emergence study). Significant weight reductions were not observed for these two species (see attached printouts).

VEGETATIVE VIGOR TEST: Statistical analyses were not conducted.

C. **Discussion/Results:** The reviewer believes that the nominal rates reported in these studies reflected the actual amount of test material applied. The discussion presented is therefore reported using nominal rates of the formulated product.

Seed Germination: The seeds of sugarbeet and cucumber were treated with unapproved pesticides. The results from these two plant species are not scientifically sound and do not fulfill the requirements. The control seeds of rape demonstrated less than 75% germination (plus or minus 10%), and the results from this species are also invalid. The results obtained with onion, wheat, tomato, sorghum, pea, corn, and soybean are scientifically sound and fulfill the requirements for a Tier 1 germination study. Germination of sorghum was

significantly affected by Benlate® 50 DF at a concentration equivalent to 3.0 lb/A, although the magnitude of this reduction was minimal (7.4%).

The author did not report the amount of control or treatment solution that each petri plate received. In previous studies conducted by the same laboratory, plates of corn, pea, and soybean received 30 ml of solution and plates of the remaining species received 15 ml of solution. The reviewer assumes that these were the volumes applied in this study.

Seedling Emergence: The seeds of corn, pea, sugarbeet, and cucumber were treated with fungicide and/or insecticide. The results from these four species are not scientifically sound and do not fulfill the requirements. The results obtained with the remaining six species are scientifically sound and fulfill the requirements for a Tier 1 seedling emergence study. The most sensitive monocot and dicot species parameter (onion and tomato dry weight) was not significantly reduced (9.3 and 7.9% reduction respectively).

Vegetative Vigor: The seeds of cucumber, corn, sugarbeet, and pea were treated with fungicide and/or insecticide. All plant species were treated with Mavrik® (fluvalinate) insecticide twice during the study. Therefore, the results from all species are not scientifically sound and do not meet the guidelines.

D. **Adequacy of the Study:**

(1) **Classification:** Seed Germination - Invalid for sugarbeet, corn, pea, rape, and cucumber. Core for a formulated product for onion, soybean, sorghum, tomato, and wheat.

Seedling Emergence - Invalid for corn, pea, sugarbeet, and cucumber. Core for a formulated product for wheat, sorghum, soybean, onion, tomato, and rape.

Vegetative Vigor - Invalid for all species.

(2) **Rationale:** Invalid results were due to fungicidal and/or insecticidal treatments.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 8-15-92.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF fungicide (IN-T1991-570); 2-benzimidazole carbamic acid, 1-(butylcarbamoyl)-methyl ester; 49.2% active ingredient.

3. **STUDY TYPE:** 122-1. Non-Target Plants: Seed Germination, Seedling Emergence & Vegetative Vigor Phytotoxicity Tests - Tier 1. Species Tested: Soybean, Sugarbeet, Pea, Tomato, Cucumber, Rape, Sorghum, Wheat, Corn, and Onion.

4. **CITATION:** Carski, T.H. and R.A. McKelvey. 1993. Influence of Benlate® 50 DF Fungicide on Seed Germination, Seedling Emergence, and Vegetative Vigor of Several Terrestrial Plants. Du Pont Project ID No. AMR 2626-93. Conducted and submitted by E.I. du Pont de Nemours and Company, Newark and Wilmington, DE. EPA MRID No. 428170-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 8/15/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 8/18/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry T. Craven
Date: 11/4/96

7. **CONCLUSIONS:**
Seed Germination: The seeds of corn, pea, sugarbeet, and cucumber were treated with fungicide and/or insecticide. The results from these four plant species are not scientifically sound and do not fulfill the requirements. The control seeds of rape demonstrated less than 70% germination, and the results from this species are also invalid. The results obtained with onion, wheat, tomato, sorghum, and soybean are scientifically sound and fulfill the requirements for a Tier 1 germination study using a

formulated product. Germination of sorghum was significantly affected by Benlate® 50 DF at a concentration equivalent to 3.0 lb/A (1.5 lb ai/A), although the magnitude of this reduction was minimal (7.4%).

Seedling Emergence: The seeds of corn, pea, sugarbeet, and cucumber were treated with fungicide and/or insecticide. The results from these four species are not scientifically sound and do not fulfill the requirements. The results obtained with the remaining six species are scientifically sound and fulfill the requirements for a Tier 1 seedling emergence study using a formulated product. The most sensitive monocot and dicot species parameter (onion and tomato dry weight) was not significantly reduced (9.3 and 7.9% reduction respectively).

Vegetative Vigor: The seeds of cucumber, corn, sugarbeet, and pea were treated with fungicide and/or insecticide. All plant species were treated with an insecticide twice during the study. Therefore, the results from all species are not scientifically sound and do not meet the guidelines.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, sugarbeet, pea, tomato, cucumber, and rape). Monocotyledon plants were represented by four species from two families (i.e., corn, sorghum, wheat, and onion). Seed source, cultivar, and lot number information were included in the report. Corn and pea seeds were treated with captan and cucumber and sugarbeet seeds were treated with a combination of fungicides and/or insecticide for all three studies.

B. **Test System:**
Seed Germination: One or more circles of glass micro-fiber filter (125 mm diameter) paper were placed in the bottom of a glass petri plate (150 mm in diameter and 20 mm in height). The test solution was prepared in buffer (pH 7.0) and applied to the petri plates.

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Twenty seeds of each crop were added to each petri plate. The plates were covered and placed in a dark incubator maintained at 25 ±1°C for 5 days.

Seedling Emergence: Twenty seeds of each crop were planted in 8-inch trays filled with a sand-amended loam soil (pH = 6.2, 1% organic matter content). Onion, tomato, sugarbeet, and rape seeds were planted at a 1-cm depth, and the remaining species were planted at a depth of 2.5 cm.

The trays were sprayed with a solution of Benlate®50 DF prepared in buffer (pH 7). Application was accomplished with a rotating belt lab sprayer calibrated to deliver 40 gallons per acre at a pressure of 40 psi. The nozzle was positioned 14.5 inches above the tray.

Plants were allowed to emerge in a greenhouse under natural lighting which was supplemented with artificial lighting to produce a 16-hour photoperiod. The temperature was maintained at 20-45°C. Top-watering was conducted on an as needed basis with tap water.

Vegetative Vigor: Solutions of Benlate® 50 DF were prepared in buffer (pH 7) and sprayed onto established plants that were either 13 cm in height or had 3-5 leaves. The pots (15 cm diameter x 15 cm depth) that each species were planted in contained either 1 plant (corn, soybean, tomato, cucumber), 3 plants (sugarbeet, pea, rape), or 6 plants (onion, wheat, sorghum). Planting, application, and growth conditions were identical to those in the seedling emergence test with the caveat that foliage was avoided when watering the pots and the temperature range was 17-35°C.

C. **Dosage:** Benlate® 50 DF was applied at the rate of 3 lb/acre (A) or 1.5 lb active ingredient (ai)/A for all three tests.

D. **Design:**
Seed Germination: The treatment/crop combination was replicated six times (i.e., 20 seeds/plate, 6 plates/treatment). After 5 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured. Percent seed germination was determined by counting the number of seeds with radicle lengths of 5 mm or greater. The physical appearance of the seedlings was also monitored.

Seedling Emergence: Each crop/treatment combination was replicated four times (i.e., 20 seeds/tray, 4 trays/treatment level). At 7 and 14 days after treatment (DAT), each replicate tray was assessed for abnormalities, seedling emergence, and seedling height. Dry weights were obtained from the shoot portions of each replicate by drying the tissue at 70° for a minimum of 48 hours.

Vegetative Vigor: Each crop/treatment combination was replicated four times (i.e., 1 vessel with 6 plants/replicate, 2 vessels with 3 plants/replicate, or 6 vessels with 1 plant/replicate per treatment level). At 7 and 21 DAT, each replicate pot was assessed for abnormalities and seedling height was measured. Shoot, root, and whole plant dry weights were determined by drying the plants for a minimum of 48 hours at 70°C.

Temperature, relative humidity, water applied per pot, and light intensity measured during the period of growth were provided in the report for the emergence and vegetative vigor studies. Plants were treated with an N-P-K fertilizer and iron chelate 7 DAT for the vegetative vigor study. Additionally, the plants were treated with Mavrik® insecticide at 1 and 7 DAT.

Test solutions for all three studies were analyzed for carbendazim by liquid chromatography. Carbendazim was the analyte because benomyl quickly degrades to this compound in water, and the rate is accelerated by the presence of methanol. The test solutions were diluted with methanol and allowed to stand for 24 hours, at which time analysis was conducted.

E. **Statistics:** All studies were conducted using randomized complete block designs. Percent inhibition and the associated 95% confidence intervals (calculated using the method for ratios with unequal variances) were determined. Welch's t-test was used to determine if a statistically significant reduction of 25% or greater had occurred in comparison to the control data ($p \leq 0.1$).

12. **REPORTED RESULTS:** The measured concentrations of the test solutions for all studies ranged from 125 to 138% of nominal. The results for all studies are based on nominal concentrations.

Seed Germination: Conditions inside the incubator for the test were reported as a temperature of 24-25°C and a

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relative humidity of 50-57%. Responses on germination of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows (negative inhibition indicates growth stimulation):

sugarbeet (-17) < corn (-3.5) < tomato (-3.4) < pea (-2.6) < soybean = cucumber (-0.9) < wheat (4.3) < onion (4.4) < rape (4.7) < sorghum (7.4).

Observations of abnormalities are presented in Table II (attached). None of the test species were advanced to Tier 2 testing based on less than 10% inhibition in germination for all the species.

Seedling Emergence: Conditions inside the greenhouse were reported as a temperature of 20-45°C and a relative humidity of 14-62%. Water applied to each tray ranged between 0 and 320 ml/tray/day. Responses of the 2-week emergence of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sugarbeet (-11) < rape (-2.9) < sorghum (-2.8) < pea (-2.6) < corn (0) < soybean (2.6) < onion (3.3) < cucumber (3.9) < wheat (5.3) < tomato (7.8).

Responses of 2-week height of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

soybean (-9.4) < cucumber (-5.4) < tomato (-3.0) < rape (-2.5) < sorghum (-2.3) < onion (-1.7) < pea (-0.7) < wheat (-0.6) < sugarbeet (1.5) < corn (1.6).

Response of 2-week dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sugarbeet (-8.0) < sorghum (-5.3) < wheat (-3.6) < cucumber (-1.8) < soybean (-1.1) < rape (-0.2) < pea (0.3) < corn (5.9) < tomato (7.9) < onion (9.3).

None of the ten species were advanced to Tier 2 testing based on the amount of inhibition witnessed in the emergence test (<10% for all measured parameters). Observations of abnormality are presented in Table X (attached).

Vegetative Vigor: Conditions inside the greenhouse were reported as a temperature of 17-35°C and a relative humidity of 14-62%. Water applied to each tray ranged between 90 and 470 ml/pot/day. Responses of the 3-week height of the ten

test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-12) < cucumber (-9.3) < pea (-3.0) < tomato (-2.8) < soybean (-1.5) < rape (0.6) < corn (0.7) < onion (0.9) < wheat (1.3) < sugarbeet (2.5).

Responses of 3-week shoot dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-44) < pea (-26) < cucumber (-12) < tomato (-7.3) < rape (-4.3) < soybean (-4.2) < onion (1.7) < wheat (3.0) < corn (3.7) < sugarbeet (7.8).

Response of 3-week root dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

tomato (-41) < sorghum (-39) < rape (-33) < cucumber (-27) < pea (-22) < corn (-3.9) < soybean (0.4) < wheat (7.0) < onion = sugarbeet (14).

Response of 3-week total dry weight of the ten test species, in increasing sensitivity to Benlate® 50 DF (in percent inhibition), are as follows:

sorghum (-43) < pea (-25) < cucumber (-14) < tomato (-12) < rape (-9.0) < soybean (-3.4) < corn (2.2) < wheat (4.5) < onion (4.6) < sugarbeet (9.0).

None of the ten species were advanced to Tier 2 testing based on the amount of inhibition witnessed in the vigor test (<15% for all measured parameters). Observations of abnormality are presented in Table XIX (attached). It was noted that the root dry weight data were highly variable for the onion, wheat, sugarbeet, and pea.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Germination, early seedling growth, and vegetative vigor of all ten test plants were not reduced by ≥25% at the rate of 3.0 lb/A (1.5 lb ai/A) for all ten test species. Therefore, Benlate® 50 DF fungicide when applied at the maximum labeled rate does not pose a threat to terrestrial plants.

Good Laboratory Practice (GLP) compliance and Quality Assurance statements were included in the report indicating that the study was conducted under the EPA GLP standards set forth in 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** For the germination study, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

No germination ratings for the seeds used were reported.

Seeds of some species were treated with fungicide and/or insecticide.

Rape seeds were of poor quality (<70% germination in the controls).

For the seedling emergence study, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

Seeds of some species were treated with fungicide and/or insecticide.

No spray solution or application calculations were reported.

The study was terminated at the end of 2 weeks. A 3- or 4-week evaluation was not conducted.

For the vegetative vigor study, the test design was generally in accordance with Subdivision J and SEP guidelines except for the following:

Seeds of some species were treated with fungicide and/or insecticide.

Plants were treated twice with an insecticide.

No spray solution or application calculations were reported.

B. **Statistical Analysis:** The reviewer used a t-test to determine if significant reductions in percentage germination occurred for sorghum (the most sensitive valid monocot species). Germination for valid dicot species was not negatively affected, and therefore, analyses were not conducted for those species. Sorghum germination was significantly reduced by Benlate® 50 DF treatment (α= 0.05). A t-test was also used to determine if significant reductions in dry weight occurred for onion and tomato (the most sensitive valid

monocot and dicot species, respectively, for the emergence study). Significant weight reductions were not observed for these two species (see attached printouts). Statistical analyses were not conducted for the vegetative vigor section due to the factors mentioned in the following section.

c. **Discussion/Results:** The reviewer believes that the nominal rates reported in these studies reflected the actual amount of test material applied. The discussion presented is therefore reported using nominal rates of the formulated product.

Seed Germination: The seeds of corn, pea, sugarbeet, and cucumber were treated with fungicide and/or insecticide. The results from these four plant species are not scientifically sound and do not fulfill the requirements. The control seeds of rape demonstrated less than 70% germination, and the results from this species are also invalid. The results obtained with onion, wheat, tomato, sorghum, and soybean are scientifically sound and fulfill the requirements for a Tier 1 germination study. Germination of sorghum was significantly affected by Benlate® 50 DF at a concentration equivalent to 3.0 lb/A, although the magnitude of this reduction was minimal (7.4%).

The author did not report the amount of control or treatment solution that each petri plate received. In previous studies conducted by the same laboratory, plates of corn, pea, and soybean received 30 ml of solution and plates of the remaining species received 15 ml of solution. The reviewer assumes that these were the volumes applied in this study.

Seedling Emergence: The seeds of corn, pea, sugarbeet, and cucumber were treated with fungicide and/or insecticide. The results from these four species are not scientifically sound and do not fulfill the requirements. The results obtained with the remaining six species are scientifically sound and fulfill the requirements for a Tier 1 seedling emergence study. The most sensitive monocot and dicot species parameter (onion and tomato dry weight) was not significantly reduced (9.3 and 7.9% reduction respectively).

Vegetative Vigor: The seeds of cucumber, corn, sugarbeet, and pea were treated with fungicide and/or insecticide. All plant species were treated with Mavrik® (fluvalinate) insecticide twice during the

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study. Therefore, the results from all species are not scientifically sound and do not meet the guidelines.

D. **Adequacy of the Study:**

(1) **Classification:** Seed Germination - Invalid for sugarbeet, corn, pea, rape, and cucumber. Core for a formulated product for onion, soybean, sorghum, tomato, and wheat.

Seedling Emergence - Invalid for corn, pea, sugarbeet, and cucumber. Core for a formulated product for wheat, sorghum, soybean, onion, tomato, and rape.

Vegetative Vigor - Invalid for all species.

(2) **Rationale:** Invalid results were due to fungicidal and/or insecticidal treatments.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 8-15-92.

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EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 68 through 72 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

orghum germination
ile: sor Transform: ARC SINE(SQUARE ROOT(Y))

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 ~~(SOLVENT CRTL)~~* MEAN =	1.2524	CALCULATED t VALUE =	2.7469
GRP2 ~~(BLANK CRTL)~~* MEAN =	1.1538	DEGREES OF FREEDOM =	10
DIFFERENCE IN MEANS =	0.0986		

'ABLE t VALUE (0.05 (2),10) = 2.228** SIGNIFICANT DIFFERENCE at alpha=0.05
'ABLE t VALUE (0.01 (2),10) = 3.169 NO significant difference at alpha=0.01

* GRP1 = control
GRP2 = treatment (3.0lb/A)

Emergence -
Towards dry wt.

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¤ STUDENT'S T-TEST (two-tailed) ¤
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Enter the name of the DATAFILE you wish to analyze: tom
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 'control'	2 'trt'
Means =	.57	.525
'ariances =	4.466668E-03	1.433334E-03

Are these INDEPENDENT or PAIRED samples? (I or P) i

T = 1.171698 df = 6

p = .2857349

The MEANS of these 2 samples are NOT significantly different.

:he confidence limits on the DIFFERENCE between the means of these samples
:an be calculated as:

4.500002E-02 +/- T(6) * 3.840581E-02

Do you want another T-TEST using this datafile?

Emergence -
Onion dry wt.

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¤ STUDENT'S T-TEST (two-tailed) ¤
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Enter the name of the DATAFILE you wish to analyze: oni
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 'control'	2 'trt'
Means =	.135	.1225
Variances =	1.666668E-04	1.583334E-04

Are these INDEPENDENT or PAIRED samples? (I or P) i

T = 1.386747 df = 6

p = .2148442

The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

.0125 +/- T(6) * 9.013902E-03

Do you want another T-TEST using this datafile?

Ecological Effects Branch One-Liner Data Entry Form

Chemical Benomyl Shaughnessy No. 099101 Pesticide Use Fungicide

MOST SENSITIVE TERRESTRIAL VEG. GERM/EMERGE	% AI	EC_{25} (95%CL)	DAYS	NOEL lbs/A or ppm	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
MONOCOT								
Germ — 1. Sorghum	49.2	signif. reduct in germ (7%)	5	n/A	1993/1993	426170-02 *	DVP	MM
Emerg — 2. onion	"	no sig reduct. in dry wt	14	"	"	426170-02 **	"	"
DICOT								
Germ — 1. soybean	"	1% stimulation of germination	5	"	"	426170-02 *	"	"
Emerg — 2. tomato	"	no sig reduct. in dry wt.	14	"	"	426170-02 **	"	"
VEGETATIVE VIGOR	**% AI**	**EC_{25} (95%CL)**	**DAYS**	**NOEL lbs/A**	**STUDY/REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
MONOCOT								
1. All species	49.2	n/A	21	n/A	1993/1993	426170-02 Invalid	DVP	MM
2.								
DICOT								
1.								
2.								

COMMENTS: * Core for a formulated product for onion, wheat, tomato, sorghum, soybean. Invalid for corn, pea, cucumber, rape, sugarbeet

** " " " " " " onion, wheat, tomato, sorghum, soybean, ~~rape~~ rape: Invalid for corn, pea, cucumber, sugarbeet.

9C

DATA TO KBN
DATE: 7-27-93
REVIEWER: DAN RIEDER
CHEMICAL: BENOMYL
SHA#: 099101

(A5)

TYPE ACTION: REREG 627
LIST: A
DPBARCODE: D193153
REREG CASE#: 0119
DUE DATE: 10-17-93

STUDY TITLE	MRID#
122-1 TERRESTRIAL PLANT TEST INCLUDES SEED GERMINATION, SEEDLING EMERGENCE AND VEGETATIVE VIGOR	42817002

24

Tier I

Petrie

MRID No. 428548-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF (IN T1991); Lot No. 570; 49.2% active ingredient.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Navicula pelliculosa*, and *Skeletonema costatum*.

4. **CITATION:** Thompson, S.G. and R.A. McKelvey. 1993. Benlate® 50 DF Fungicide: Influence on Growth and Reproduction of Four Select Algal Species. Laboratory Project No. 112A-114. Conducted by Wildlife International Ltd., Easton, MD. Submitted by E.I. du Pont de Nemours and Company, Wilmington, DE. EPA MRID No. 428548-01.

5. **REVIEWED BY:**

Richard C. Petrie
Senior Agronomist
EEB, EFED, OPP

Signature: Richard C Petrie
Date: 11/06/96

6. **APPROVED BY:**

Ann Stavola
Section Head
Section 5
EEB, EFED, OPP

Signature: Ann Stavola
Date: 12/12/96

7. **CONCLUSIONS:** These studies are scientifically sound and meet the guideline requirements for Tier 1 non-target aquatic plant studies. Based on nominal concentrations, the 5-day growth of *A. flos-aquae*, *N. pelliculosa*, and *S. costatum* was not significantly reduced by exposure to 1.1 mg ai/l (2.2 mg/l of Benlate® 50 DF - equivalent to the maximum label rate of 1.5# ai/acre Benalte DF formulation applied directly to a 6 inch deep water body). The growth of *S. capricornutum* was totally inhibited (100% inhibition) over the 5-day exposure period.

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respectively (negative inhibition indicates growth stimulation in the treatment solutions). Since *S. capricornutum* was the only algae that demonstrated greater than 50% inhibition, a 9-day recovery test was initiated with cells from the terminal treatment and control solutions. Resuspension of the cells in growth medium alone indicated that the effects of Benlate® 50 DF were algistatic rather than algicidal as cell growth increased from 4,000 cells on day one of the recovery period to 750,000 cells on day 9 (Appendix E, attached). Added A.C. Patra 8/20/96

The pH, temperature, and light intensity for the four tests were generally within the recommended range (Tables I and II, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study authors.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviation:

The test was conducted with a formulated product rather than a technical grade material.

Generally, the light intensities were slightly higher and the temperatures were slightly lower than recommended.

B. **Statistical Analysis:** The reviewer used a t-test to determine if a significant reduction in cell density had occurred for *S. capricornutum* and *A. flos-aquae*. The other two algae demonstrated growth stimulation in the treatment solutions, and consequently, analysis of data for these two species was not conducted. The results confirm that growth of only *S. capricornutum* was significantly reduced in comparison to the control (see attached printouts).

C. **Discussion/Results:** The report did not indicate that the test vessels were covered with stoppers. Previous reports by the same laboratory indicated that this indeed was the case. Therefore, the reviewer assumes that the flasks were covered with sterile stoppers.

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The reviewer believes that the nominal concentrations are conservative representatives of the measured concentrations. Therefore, the results will be expressed in terms of nominal concentrations.

These studies are scientifically sound and meet the guideline requirements for Tier 1 non-target aquatic plant studies. Based on nominal concentrations, the 5-day growth of *A. flos-aquae*, *N. pelliculosa*, and *S. costatum* was not significantly reduced by exposure to 1.1 mg ai/l (2.2 mg/l of Benlate® 50 DF). The growth of *S. capricornutum* was totally inhibited (100% inhibition) over the 5-day exposure period.

D. **Adequacy of the Study:**

(1) **Classification:** Core for a formulated product (Benlate® 50 DF).

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 8-17-93.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF (IN T1991); Lot No. 570; 49.2% active ingredient.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Navicula pelliculosa*, and *Skeletonema costatum*.

4. **CITATION:** Thompson, S.G. and R.A. McKelvey. 1993. Benlate® 50 DF Fungicide: Influence on Growth and Reproduction of Four Select Algal Species. Laboratory Project No. 112A-114. Conducted by Wildlife International Ltd., Easton, MD. Submitted by E.I. du Pont de Nemours and Company, Wilmington, DE. EPA MRID No. 428548-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]

Date: 8/23/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat

Date: 8/23/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:

Date:

7. **CONCLUSIONS:** These studies are scientifically sound and meet the guideline requirements for Tier 1 non-target aquatic plant studies. Based on nominal concentrations, the 5-day growth of *A. flos-aquae*, *N. pelliculosa*, and *S. costatum* was not significantly reduced by exposure to 1.1 mg ai/l (2.2 mg/l of Benlate® 50 DF). The growth of *S. capricornutum* was totally inhibited (100% inhibition) over the 5-day exposure period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** The four algal species (*Selenastrum capricornutum*, *Anabaena flos-aquae*, *Navicula pelliculosa*, and *Skeletonema costatum*) were obtained from the University of Texas Culture Collection. The algae were kept in culture at the laboratory and were transferred to fresh medium every three to five days.

B. **Test System:** Test vessels used were 250-ml flasks. The test medium used for *S. capricornutum* and *A. flos-aquae* was algal assay (AAP) medium. This same medium was used for *N. pelliculosa*, but silica was added to the solution. The pH of these two media was adjusted to 7.5 ±0.1. The medium used for the *S. costatum* test was marine algal assay (MAA) medium. This medium contained 30 parts per thousand salt and the pH was adjusted to 8.0 ±0.1. All media were filter sterilized (0.2 µm) prior to inoculation.

Fifty milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept at 24 ±2°C (20 ±2°C for *S. costatum*) in an environmental chamber under 24 hours (16 hours for *S. costatum*) of cool-white fluorescent illumination per day. The light intensity was set to provide 4310 ±650 lux (2150 ±320 lux for *A. flos-aquae*). The test vessels were continuously shaken at 100 rpm (except *S. costatum* flasks, which were hand-shaken once or twice daily).

C. **Dosage:** Five-day growth and reproduction test. One nominal concentration of 1.1 mg active ingredient (ai)/l (2.2 mg of formulation/l) was selected for the test. A medium control and an uninoculated control (medium plus test material only) were also prepared. The maximum single application rate was reported to be 1.5 lb ai/acre.

Primary stock solutions (20 mg/ml) were prepared by dissolving the test material in the appropriate test medium. The test solutions were prepared by diluting the stock solutions with medium. The amount of test material added to the stock was not adjusted for percent purity.

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D. **Test Design:** The study was arranged in a randomized design with three replicates (flasks). An inoculum of cells designed to provide 3,000 cells/ml (*S. capricornutum*, *A. flos-aquae*, and *N. pelliculosa*) or 10,000 cells/ml (*S. costatum*) was added to each flask. Inoculum volume ranged between 500 and 811 µl/flask. Cell density was determined daily using a hemacytometer. Samples of *A. flos-aquae* were sonicated for approximately two minutes before counting to reduce the length of the algal strands.

The pH was measured at the beginning and end of the study. Temperature within the growth chamber was also monitored.

Samples of the treatment and control solutions were collected from freshly prepared medium on day 0 and from old solutions at test termination. The samples were analyzed for the test material using liquid chromatography. Carbendazim (MBC) and 1,3,5-triazinol[1,2-a]benzimidazole-2,4(1H,3H)dione,3-butyl- (STB) were the analytes due to the rapid hydrolytic conversion of benomyl to these compounds. The samples were allowed to stand for 24 hours before analysis to ensure that all benomyl would be converted to MBC and STB).

E. **Statistics:** Percentage growth inhibition was computed from mean cell density data. The 90% confidence interval for percent inhibition was calculated and the null hypothesis of 50% or greater inhibition was tested at the 90% confidence level using Welch's t-test for ratios.

12. **REPORTED RESULTS:** The day-0 measured concentrations of Benlate® 50 DF (determined from back-calculation of MBC and STB concentrations) ranged between 110 and 125% (for the inoculated treatment solution) and 111 and 119% of nominal (for the uninoculated solution). Day-5 samples ranged between 107 and 119% (for the inoculated treatment solution) and 113 and 129% of nominal (for the uninoculated solution). Full details of the results are presented in Table IV (attached).

Cell density data (Table III, attached) indicated *S. capricornutum*, *A. flos-aquae*, *N. pelliculosa*, and *S. costatum* were inhibited by 100%, 4%, -40%, and -3%, respectively (negative inhibition indicates growth stimulation in the treatment solutions). Since *S. capricornutum* was the only algae that demonstrated greater

than 50% inhibition, a 9-day recovery test was initiated with cells from the terminal treatment and control solutions. Resuspension of the cells in growth medium alone indicated that the effects of Benlate® 50 DF were algistatic rather than algicidal (Appendix E, attached).

The pH, temperature, and light intensity for the four tests were generally within the recommended range (Tables I and II, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study authors.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviation:

The test was conducted with a formulated product rather than a technical grade material.

Generally, the light intensities were slightly higher and the temperatures were slightly lower than recommended.

B. **Statistical Analysis:** The reviewer used a t-test to determine if a significant reduction in cell density had occurred for *S. capricornutum* and *A. flos-aquae*. The other two algae demonstrated growth stimulation in the treatment solutions, and consequently, analysis of data for these two species was not conducted. The results confirm that growth of only *S. capricornutum* was significantly reduced in comparison to the control (see attached printouts).

C. **Discussion/Results:** The report did not indicate that the test vessels were covered with stoppers. Previous reports by the same laboratory indicated that this indeed was the case. Therefore, the reviewer assumes that the flasks were covered with sterile stoppers.

The reviewer believes that the nominal concentrations are conservative representatives of the measured concentrations. Therefore, the results will be expressed in terms of nominal concentrations.

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These studies are scientifically sound and meet the guideline requirements for Tier 1 non-target aquatic plant studies. Based on nominal concentrations, the 5-day growth of *A. flos-aquae*, *N. pelliculosa*, and *S. costatum* was not significantly reduced by exposure to 1.1 mg ai/l (2.2 mg/l of Benlate® 50 DF). The growth of *S. capricornutum* was totally inhibited (100% inhibition) over the 5-day exposure period.

D. **Adequacy of the Study:**

(1) **Classification:** Core for a formulated product (Benlate® 50 DF).

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 8-17-93.

85

EEB Review dated 1/5/1999 Benomyl

Page _____ is not included in this copy.

Pages 86 through 92 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

A. flos-aquae

```
ÉÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ»
º STUDENT'S T-TEST (two-tailed) º
ÈÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¼
```

Enter the name of the DATAFILE you wish to analyze: ana
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 `control'	2 `trt'
Means =	326666.7	313333.4
Variances =	5.833333E+09	4.263333E+10

Are these INDEPENDENT or PAIRED samples? (I or P) i

T = .1049004 df = 4

p = .9215046

The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

13333.31 +/- T(4) * 127104.5

Do you want another T-TEST using this datafile?

S. capricornutum

° STUDENT'S T-TEST (two-tailed) °
ÈÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍÍ¼

Enter the name of the DATAFILE you wish to analyze: sel
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 `control'	2 `trt'
Means =	2466667	4000
Variances =	2.533334E+11	4000000

Are these INDEPENDENT or PAIRED samples? (I or P) i

The T-TEST may not be appropriate because these variances
are so different (F = 63333.34 p = 1.579523E-05).

T = 8.474542 df = 4

p = 1.062751E-03

The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

2462667 +/- T(4) * 290595.9

Do you want another T-TEST using this datafile?

Ecological Effects Branch One-Liner Data Entry Form

Chemical Benomyl Shaughnessy No. 099101 Pesticide Use Fungicide

PHYTOTOXICITY AQUATIC SPECIES	% AI	~~EC50~~ (~~95%CL~~)	HRS/ DAYS	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Selenastrum capricornutum	49.2	100% S*	5 days	N/A	1993/1993	428548-01 Core for	WIL	Mal
2. Anabaena flos-aquae	"	4% NS**	"	"	"	A formulated product "	"	"
3. Navicula pelliculosa	"	-40% ~~3%~~ NS**	"	"	"	"	"	"
4. Skeletonema costatum	"	-3% NS**	"	"	"	"	"	"
5.								

negative % inhibition = stimulation

COMMENTS: * - significant reduction ** non-significant reduction or stimulation.

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF (IN/DPX No. T1991-570); Batch No. 1210870300; 49.2% active ingredient; a white granule.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1993. Benlate® 50 DF Fungicide: Acute Toxicity to the Vascular Plant, *Lemna gibba* G3. Laboratory Project No. 112A-119. Conducted by Wildlife International Ltd., Easton, MD. Submitted by E.I. du Pont de Nemours and Company, Wilmington, DE. EPA MRID No. 428548-02.

5. **REVIEWED BY:**

Richard C. Petrie
Senior Agronomist
EEB, EFED, OPP

Signature: Richard C. Petrie
Date: 11/06/96

6. **APPROVED BY:**

Ann Stavola
Section Head
Section 5
EEB, EFED, OPP

Signature: Ann Stavola
Date: 12/17/96

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for a Tier 1 toxicity study using a non-target aquatic macrophyte when exposed to the single concentration of 1.1 mg ai/L - equivalent to the maximum label appliction rate of 1.5# ai/Acre Benlate DF applied directly to the surface of a 6 inch water body. No detrimental effect on frond growth was observed over the 14-day treatment period.

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Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The test was conducted with a formulated product rather than a technical grade material.

The light intensity during the test (4.3-5.4 klux) was occasionally higher or lower than recommended (5 klux).

B. **Statistical Analysis:** Since the mean frond number of the treatment group was greater than the mean of replicates A and B of the medium control, it is apparent that the test material had no significant detrimental effect on the growth of *L. gibba*.

C. **Discussion/Results:** The mean measured concentration of 1.62 mg Benlate® 50 DF/l (0.8 mg ai/l) is only 74% of the theoretical concentration in water of a 3 lb/A application applied to a 15-cm water column. Therefore, this study should have been conducted as a static renewal study in order to keep the measured concentration at the desired level or greater; however static renewal is not currently a requirement for this test under FIFRA.

This study is scientifically sound and fulfills the guideline requirements for a Tier 1 toxicity study using a non-target aquatic macrophyte when exposed to the single concentration of 1.1 mg ai/L - equivalent to the maximum recommended label rate of 1.5# ai/Acre Benlate 50DF when applied directly to the surface of a 6 inch water body. No detrimental effect on frond growth was observed over the 14-day treatment period.

8/23/96

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D. Adequacy of the Study:

(1) Classification: Core for a formulated product.

(2) Rationale: N/A

R.C. 8/22/96

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, 8-15-93.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 DF (IN/DPX No. T1991-570); Batch No. 1210870300; 49.2% active ingredient; a white granule.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1993. Benlate® 50 DF Fungicide: Acute Toxicity to the Vascular Plant, *Lemna gibba* G3. Laboratory Project No. 112A-119. Conducted by Wildlife International Ltd., Easton, MD. Submitted by E.I. du Pont de Nemours and Company, Wilmington, DE. EPA MRID No. 428548-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: M.L. Mossler
Date: 8/25/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 8/25/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound ~~but does not fulfill the guideline requirements for a Tier 1 toxicity study using non target aquatic plants. The mean measured concentration (0.8 mg ai/l) was only 74% of the concentration which would result from the maximum use rate (1.5 lb ai/acre) being applied to a 15-cm water column. Based on a mean measured concentration of 1.62 mg Benlate® 50 DF/l (0.8 mg ai/l),~~ no detrimental effect on frond growth was observed over the 14-day treatment period.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Lemna gibba* G3 used in the test came from laboratory stock cultures.

B. **Test System:** Test vessels used were 250-ml glass beakers covered with petri dishes. The test medium was M-Hoagland's medium (without EDTA or sucrose) with the pH adjusted to 5.0 ±0.1. The medium was autoclaved before use.

One-hundred milliliters of the appropriate test or control solution were placed into each beaker. The test vessels were kept at 25 ±2°C in an environmental chamber located in a constant temperature room. The vessels were continuously illuminated with warm-white lighting at an intensity of 4.3-5.4 klux.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on the maximum application rate of 3 lb/acre [1.5 lb active ingredient (ai)/acre], one nominal concentration of 2.2 mg/l (1.1 mg ai/l) was selected for the test. A medium control and an uninoculated control (medium plus test material only) were also prepared.

A primary stock solution (20 mg/ml) was prepared by dissolving the test material in reverse-osmosis water. The test solution was prepared by diluting an appropriate volume of the stock solution (0.11 ml) with medium to the final volume of 1 l. The amount of test material added to the stock was not adjusted for percent purity.

D. **Test Design:** An inoculum of *Lemna gibba* consisting of 15-17 fronds, representing at least five plants, was added to each beaker (3 beakers per treatment). The beakers were randomly positioned in the chamber on each working day. Frond counts were made on test days 3, 6, 9, 12, and 14. Observations of frond death, colony formation, tissue chlorosis and necrosis, root destruction, and changes in color were also made at these times.

The pH values of the initial and terminal treatment and control solutions were determined. The temperature was

measured in a flask of water near the test vessels twice a day.

Samples of the treatment and control solutions were collected from freshly prepared medium on day 0 and from old solutions at test termination. The samples were analyzed for the test material using liquid chromatography. Carbendazim was the analyte due to the rapid hydrolytic conversion of benomyl to this compound. The samples were allowed to stand for 24 hours before analysis to ensure that all benomyl would be converted to carbendazim. Additionally, the stock solution was analyzed.

E. **Statistics:** Percentage growth inhibition was computed from frond number data. The 90% confidence interval for percent inhibition was calculated and the null hypothesis of 50% or greater inhibition was tested at the 90% confidence level using Welch's t-test for ratios.

12. **REPORTED RESULTS:** The day-0 measured concentrations of Benlate® 50 DF (determined from back-calculation of carbendazim concentration) ranged between 112% (for the inoculated treatment solution) and 113% of nominal (for the uninoculated solution). Day-14 samples ranged between 38% (for the inoculated treatment solution) and 56% of nominal (for the uninoculated solution). Analysis of the stock solution indicated that the concentration was 105% of nominal (Table 1, attached).

By test termination, replicate C of the medium control contained only 3% of the fronds present in replicates A and B. This was determined to be anomalous and the data from this replicate were excluded from analysis. The 2.5 mg Benlate® 50 DF/l treatment group exhibited frond and plant growth equivalent to the negative control. There were no significant differences in dead, necrotic, or chlorotic fronds in comparison to the medium control (Tables 4, 5, and 6, attached).

The pH was 4.9-5.0 in the treatment solution and the controls at test initiation and 5.0-6.8 at test termination. The temperature ranged from 22.2 to 26.0°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those stated were made by the authors.

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Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The test was conducted with a formulated product rather than a technical grade material.

The light intensity during the test (4.3-5.4 klux) was occasionally higher or lower than recommended (5 klux).

B. Statistical Analysis: Since the mean frond number of the treatment group was greater than the mean of replicates A and B of the medium control, it is apparent that the test material had no significant detrimental effect on the growth of *L. gibba*.

C. Discussion/Results: The mean measured concentration of 1.62 mg Benlate® 50 DF/l (0.8 mg ai/l) is only 74% of the theoretical concentration in water of a 3 lb/A application applied to a 15-cm water column. Therefore, this study should have been conducted as a static renewal study in order to keep the measured concentration at the desired level or greater.

This study is scientifically sound but does not fulfill the guideline requirements for a Tier 1 toxicity study using non-target aquatic plants. ~~Based on a mean measured concentration of 1.62 mg Benlate® 50 DF/l (0.8 mg ai/l), no detrimental effect on frond growth was~~ observed over the 14-day treatment period.

D. Adequacy of the Study:

CORE

(1) Classification: ~~Supplemental~~ for a formulated product.

(2) Rationale: ~~The mean measured concentration (0.8 mg ai/l) was only 74% of the concentration which would result from the maximum use rate (1.5 lb ai/acre) being applied to a 15-cm water column.~~

(3) Repairability: ~~No.~~ N/A

15. COMPLETION OF ONE-LINER: Yes, 8-15-93.

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EEB Review dated 1/5/1999 Benomyl

Page _____ is not included in this copy.

Pages 103 through 107 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Benomyl **Shaughnessy No.** 099101 **Pesticide Use** Fungicide

PHYTOTOXICITY AQUATIC SPECIES	% AI	EC_{50} (95%CL)	HRS/ DAYS	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Lemna gibba Tier I	49.2	N/A	14 days	No signif reduct of fronds	1993/1993	428548-02 ~~Supplemental~~ for a formulated prod.	WIL	MM
2.				At 1.62 mg Benlate ~~50~~ DF/l (1.1 mg ai/l)			CORE	
3.							QC Date 8/22/96	
4.								
5.								

COMMENTS: [redacted]

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DATA EVALUATION RECORD
VEGETATIVE VIGOR TEST
§ 122-1 (TIER I)

1. **CHEMICAL:** Benomyl PC Code No.: 099101

2. **TEST MATERIAL:** Benlate® 50 DF Fungicide Purity: 49.2%

3. **CITATION:**

Author: Robert A. McKelvey
Title: Influence of Benlate® 50 DF Fungicide on Vegetative Vigor of Four Terrestrial Plants
Study Completion Date: August 8, 1994
Laboratory: Stine-Haskell Research Center, Newark, DE
Sponsor: E.I. du Pont de Nemours and Company, Wilmington, DE
Laboratory Report ID: AMR 2812-93
MRID No.: 433634-01
DP Barcode: D208069

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature: M. L. Mossler **Date:** 3/13/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 3/13/96

5. **APPROVED BY:**

Signature: Anu Stavola **Date:** 10/30/96

6. **STUDY PARAMETERS:**

Definitive Study Duration: 21 days

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier I vegetative vigor test with terrestrial plants. Based on the nominal application rate, no significant reductions were observed for any measured parameter when treatment data were compared to solvent control data for all four test species.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental.

B. **Rationale:** Only four species, rather than ten, were tested.

C. **Repairability:** Yes, if the original report contains "core" data concerning the vegetative vigor of the other six required species, then the entire study may be upgraded to "core" status.

9. **GUIDELINE DEVIATIONS:**

1. The width of the spray swath was not reported. This information is required to confirm proper test substance application.

2. Only four species were tested. Ten species, which fulfill the guideline specifications, are required for testing.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: sugar beet Monocots: onion, sorghum, wheat
Number of plants per rep 5	10
Source of Seed	Commercial suppliers

B. Test System

Guideline Criteria	Reported Information
Solvent	pH 7 phosphate-buffered water
Site of test	Greenhouse
Planting method / type of pot	Individual plants grown in 11-cm square pots

Guideline Criteria	Reported Information
Method of application	Belt sprayer delivering 40 gallons per acre
Method of watering	Watering as needed avoiding foliage
Growth stage at application Past first true leaf stage	Onion: 13 cm in height, Wheat, sorghum, and sugar beet: 3-5 true leaves

C. **Test Design**

Guideline Criteria	Reported Information
Dose range 2x or 3x	N/A
Doses maximum label rate	1
Controls Negative and solvent	Negative (deionized water) and solvent (phosphate-buffered water) control
Replicates per dose At least 3	10 replicates
Duration of test 14 days	21 days
Were observations made at least weekly?	Observations made one and three weeks after application
Maximum labeled rate	1.5 lb ai/A

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was an NOEL observed for each species?	Yes
Phytotoxic observations	Yes

Guideline Criteria	Reported Information
Were initial chemical concentrations measured? (Optional)	Yes, measured concentration of 105% of nominal
Were adequate raw data included?	Yes

Results for the most sensitive parameter[a] of each species

Species	Parameter	% inhibition
Onion	shoot height	1.2
Sorghum	shoot height and dry weight	0
Wheat	shoot height	0.1
Sugar beet	shoot dry weight	0.1

[a]Determination of the most sensitive parameter is based on the amount of inhibition in comparison to the solvent control.

Observations: Symptoms of Benlate® toxicity included some chlorosis, necrosis, and leaf curl in a few select plants. Overall, minor phytotoxicity was observed.

Statistical Results: Based on the results of Welch's t-test for ratios, no significant reductions were observed for any measured parameter when treatment data were compared to solvent control data for all four test species.

13. **VERIFICATION OF STATISTICAL RESULTS:** All comparisons were made against the phosphate buffer control. Based on the results from t-tests, no significant reductions were observed.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound but does not fulfill the guideline requirements since only four species rather than ten were tested. The study is classified as **Supplemental.**

onion shoot height
File: oni Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	20.9900	CALCULATED t VALUE =	0.2915
GRP2 (BLANK CRTL) MEAN =	20.7700	DEGREES OF FREEDOM =	18
DIFFERENCE IN MEANS =	0.2200		

TABLE t VALUE (0.05 (2),18) = 2.101 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),18) = 2.878 NO significant difference at alpha=0.01

wheat shoot height
File: whe Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	40.1200	CALCULATED t VALUE =	0.1137
GRP2 (BLANK CRTL) MEAN =	40.0700	DEGREES OF FREEDOM =	18
DIFFERENCE IN MEANS =	0.0500		

TABLE t VALUE (0.05 (2),18) = 2.101 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),18) = 2.878 NO significant difference at alpha=0.01

Note: BLANK CRTL = Treatment

sugar beet shoot weight
File: sug Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	3.5740	CALCULATED t VALUE =	0.0488
GRP2 (BLANK CRTL) MEAN =	3.5690	DEGREES OF FREEDOM =	18
DIFFERENCE IN MEANS =	0.0050		

TABLE t VALUE (0.05 (2),18) = 2.101 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),18) = 2.878 NO significant difference at alpha=0.01

Note: BLANK CRTL = TREATMENT

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4

1. **CHEMICAL:** Benomyl — PC Code No.: 099101

2. **TEST MATERIALS:** Carbendazim (technical) — Purity: 99.3%
   ^{14}C-Carbendazim — Radiopurity: 100%

3. **CITATION:**

Author:	J.E. Rhodes, B. Hurshman, and T. Leak
Title:	Early Life-Stage Toxicity of Benomyl (as Carbendazim, DPX-E965-299) to the Channel Catfish (*Ictalurus punctatus*) Under Flow-Through Conditions
Study Completion Date:	December 1, 1995
Laboratory:	ABC Laboratories, Inc., Columbia, MO
Laboratory Report ID:	Haskell Laboratory Outside Report 236-95
Sponsor:	E.I. du Pont de Nemours and Company, Wilmington, DE
MRID No.:	438728-01
DP Barcode:	D221857

4. **REVIEWED BY:**

Richard C. Petrie, Senior Agronomist
EEB, EFED, OPP

Signature: Richard C. Petrie **Date:** 11/06/96

APPROVED BY:

Ann Stavola, Section Head, Section 5
EEB, EFED, OPP

Signature: Ann Stavola **Date:** 12/17/96

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater fish early life-stage test. Based on the most sensitive parameter (larval survival), the MATC for channel catfish exposed to carbendazim was between 0.99 and 2.11 µg/L (as mean measured, carbendazim concentrations). The geometric mean MATC was 1.44 µg/L.

Statistical Results:

Statistical Method: Cochran-Armitage trend test

NOEC: 1.5 µg/L benomyl **LOEC:** 3.2 µg/L benomyl **MATC:** 2.2 µg ai/L benomyl
Most sensitive endpoint: Larval survival.

Comments: Survival data for the exposure groups were compared to data for the pooled control.

11. **REVIEWER'S STATISTICAL RESULTS:**

Statistical Methods: Bonferroni's t-test
NOEC: 3.2 µg/L benomyl **LOEC:** 7.3 µg/L benomyl **MATC:** 4.8 µg/L benomyl
Most sensitive endpoint: Hatchability

Comments: Hatch, survival, and growth data from the exposure groups were compared to the data for the pooled control. The replicate means were used to analyze the growth, hatchability, and survival data.

12. **REVIEWER'S COMMENTS:** Although the individual growth data were not presented in the report, the reviewer accepts the conclusions of the authors. Upon visual examination of the means, length and wet weight showed no clear dose-response and appeared to be less sensitive endpoints than larval survival. This study is scientifically sound, meets the guideline requirements for a fish early life-stage study, and is classified as **Core**. Based on mean measured, calculated carbendazim concentrations, the MATC for exposed channel catfish was between 0.99 and 2.11 µg/L. The geometric mean MATC was 1.44 µg/L.

BASED ON THE RATIO OF BENOMYL MOLECULAR WEIGHT TO CARBENDAZIM MOLECULAR WEIGHT, nominal carbendazim concentrations of 0.0, 0.0 ,0.10. 0.22, 0.48, 1.10, 2.3, and 5.2 ug/L were converted by the study author to nominal benomyl equivalent concentrations of 0.0, 0.0, 0.15, 0.33, 0.73, 1.70, 3.50, and 7.90 ug/L using a conversion factor of 1.52. This conversion step is not necessary given that the test chemical is carbendazim, not benomyl. Agreement between the EPA and the registrant to use carbendazim instead of benomyl (due to rapid conversion of benomyl to carbendazim in water) was reached before study initiation. Therefore, study conclusions are reported as nominal CARBENDAZIM concentrations as opposed to nominal benomyl concentrations.
(R. Petrie, EEB/EFED/OPP)

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4

1. **CHEMICAL:** Benomyl PC Code No.: 099101

2. **TEST MATERIALS:** Carbendazim (technical) Purity: 99.3%
^{14}C-Carbendazim Radiopurity: 100%

3. **CITATION:**

Author: J.E. Rhodes, B. Hurshman, and T. Leak
Title: Early Life-Stage Toxicity of Benomyl (as Carbendazim, DPX-E965-299) to the Channel Catfish (*Ictalurus punctatus*) Under Flow-Through Conditions
Study Completion Date: December 1, 1995
Laboratory: ABC Laboratories, Inc., Columbia, MO
Laboratory Report ID: Haskell Laboratory Outside Report 236-95
Sponsor: E.I. du Pont de Nemours and Company, Wilmington, DE
MRID No.: 438728-01
DP Barcode: D221857

4. **REVIEWED BY:** Rosemary Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: for RM **Date:** 3/22/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 3/22/96

5. **APPROVED BY:**

Signature: **Date:**

6. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater fish early life-stage test. Based on the most sensitive parameter (larval survival), the MATC for channel catfish exposed to carbendazim was between 1.5 and 3.2 µg/L (as mean measured, calculated Benomyl concentrations). The geometric mean MATC was 2.2 µg/L.

7. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core.

B. **Rationale:** Although individual growth data were not included in the report, length and wet weight showed no clear dose-response based upon visual examination of the means. Larval survival appeared to be a more sensitive endpoint than growth.

C. **Repairability:** N/A.

8. **MAJOR GUIDELINE DEVIATIONS:**

1. Individual growth data were not presented in the report. There must be sufficient information presented in the report for the reviewer to verify the authors' statistical conclusions.

2. The embryo incubation chambers were aerated. Embryo and larval chambers should not be aerated.

3. The dissolved oxygen concentrations during the test were ≥58% saturation. With the exception of Days 35, 38, and 39, most D.O. level were ≥75%. The test system should maintain D.O. concentrations above 75% of saturation.

9. **MATERIALS AND METHODS:**

A. **Biological System**

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	*Ictalurus punctatus*
Source: Commercial fishery, wild, or brood stock.	Newly fertilized embryos were obtained from Osage Catfisheries, Inc., Osage Beach, MO.
Age at beginning of test: Embryos 2 to 24 hours old.	<24 hours post-fertilization at test initiation

Guideline Criteria	Reported Information
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	**Embryo exposure:** 20 eggs/incubation cup, 2 cups/replicate aquarium, 4 replicate aquaria/treatment **Larval exposure:** 20 fish per replicate, 4 replicates per treatment
Posthatch: % of embryos that produce live fry must be ≥50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	Percentage hatch was 87.7% in pooled controls (91% and 84.5% in water control 1 and water control 2, respectively).
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish were fed at least three times daily. Feeding was terminated 24 hours prior to test termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Surviving and dead fry were recorded daily up to Day 21. Thereafter, cumulative mortality was recorded based on observed dead fry. Surviving fry were counted again at test termination.
Controls: Avg. survival at end of test must be ≥80%. Survival in any control chamber must not be <70%.	Average survival of the pooled controls was 89.2%. Survival in each control replicate ranged from 72.2 to 100%.
Controls: Negative control and carrier control (when applicable) are required.	Two sets of dilution water controls were used.

Comments:

B. Physical System

Guideline Criteria	Reported Information
Test Water: 1) May be natural (well or spring) or reconstituted water. 2) Water should be sterilized with UV radiation and screened for contaminants. 3) Hardness of 40-48 mg/L as $CaCO_3$, pH of 7.2-7.6	1) A blend of treated (reverse-osmosis) well water and raw well water. 2) Dilution water was heated, filtered (5-µm), UV-sterilized and screened for contaminants prior to use. 3) Hardness of 132-150 mg/L as $CaCO_3$, pH range of 7.85-8.51.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For rainbow trout, 10°C is recommended.	Range of 24.2-25.9°C
Photoperiod: Recommend 16L/8D.	Continuous semi-darkness for embryos and 16L/8D after hatching.
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	Intermittent-flow proportional diluter. Nominal concentrations: 0.10, 0.22, 0.48, 1.1, 2.3, and 5.2 µg/L as Carbendazim (0.15, 0.33, 0.73, 1.7, 3.5, and 7.9 µg/L as Benomyl equivalent).

Guideline Criteria	Reported Information
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) Mixing chambers were used. 2) From test initiation until Day 8, incubation cups were aerated to keep embryos in constant movement and well oxygenated. 3) Appropriate mixing was confirmed by chemical analysis. 4) Flow splitting accuracy was verified prior to test initiation and on Day 38. The percentage accuracy was not reported.
Test Vessels: All glass or glass with stainless steel frame.	Glass aquaria (15.5 x 30.5 x 29.2 cm).
Embryo Cups: 120 Ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	1-liter, narrow-mouth, polyethylene bottles with the bottoms cut out. Two bottles were suspended upside down in each aquarium. The mouth and the hole on the side of the bottle were covered with 15-18 mesh, stainless-steel screen. An airline with an air stone was inserted through the top of the cap to increase circulation.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain D.O. at above 75% of saturation and maintain the toxicant level.	11.9 volume replacements per day until Day 34, then increased to 22.1 volume replacements/day thereafter. D.O. and chemical concentrations were monitored.

Guideline Criteria	Reported Information
Aeration: Dilution water should be aerated to insure D.O. concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	D.O. ≥61% of saturation at all times. Incubation chambers were aerated as discussed above.

Comments: The authors reported an oxygen saturation limit of 7.9 mg/L at 25°C, suggesting a minimum D.O. of 61% of saturation. The reviewer calculated a minimum D.O. of 58% of saturation based on an oxygen saturation limit of 8.25 mg/L at 25°C.

C. **Chemical System**

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	- Two sets of water controls and six exposure concentrations. - Test concentrations were measured in each replicate on Days 0, 6, 14, 21, 28, 35, and 41. - LOEC and NOEC were obtained.
Other Variables: D.O. must be measured at each conc. at least once a week.	D.O. and pH in each replicate were measured weekly until the last 7 days of the study when they were measured daily.
Solvents: Should not exceed 0.1 Ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	No solvent was used.

Comments: None.

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10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if appropriate); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Data include: - Number of eggs hatched; - 34-day post-hatch survival; - 34-day post-hatch length; - 34-day post-hatch wet weight.
Raw data included? (Y/N)	Yes, for survival and hatchability data. Only replicate means were reported for growth data.

Effects Data

Mean calculated Benomyl Concentration (µg/L)		Mean Percent Hatch	Survival (34 days Post-Hatch)	Standard Length (mm)	Wet Weight (g)
Nominal	Measured				
Control 1	-	91.0	84.4	43.5	1.179
Control 2	-	84.5	93.7	42.8	1.095
0.15	0.15	92.2	86.9	44.1	1.209
0.33	0.27	90.8	87.3	41.6	1.024
0.73	0.64	88.3	90.5	43.6	1.145
1.7	1.5	89.9	87.0	41.9	1.024
3.5	3.2	94.1	77.5	43.2	1.087
7.9	7.3	0	-	-	-

Toxicity Observations: During the test, one fish in water control 1, two fish at 0.27 µg/L, and one fish at 1.5 µg/L exhibited spinal curvature.

Statistical Results:

Statistical Method: Cochran-Armitage trend test

NOEC: 1.5 µg/L **LOEC:** 3.2 µg/L **MATC:** 2.2 µg ai/L

Most sensitive endpoint: Larval survival.

Comments: Survival data for the exposure groups were compared to data for the pooled control.

11. **REVIEWER'S STATISTICAL RESULTS:**

Statistical Methods: Bonferroni's t-test
NOEC: 3.2 µg/L **LOEC:** 7.3 µg/L **MATC:** 4.8 µg/L

Most sensitive endpoint: Hatchability

Comments: Hatch, survival, and growth data from the exposure groups were compared to the data for the pooled control. The replicate means were used to analyze the growth, hatchability, and survival data.

12. **REVIEWER'S COMMENTS:** Although the individual growth data were not presented in the report, the reviewer accepts the conclusions of the authors. Upon visual examination of the means, length and wet weight showed no clear dose-response and appeared to be less sensitive endpoints than larval survival. This study is scientifically sound, meets the guideline requirements for a fish early life-stage study, and is classified as **Core.** Based on mean measured, calculated benomyl concentrations, the MATC for exposed channel catfish was between 1.5 and 3.2 µg/L. The geometric mean MATC was 2.2 µg/L.

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.144	7.744	12.224	7.744	2.144
OBSERVED	0	11	9	12	0

Calculated Chi-Square goodness of fit test statistic = 8.8464
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.064

W = 0.982

Critical W (P = 0.05) (n = 32) = 0.930
Critical W (P = 0.01) (n = 32) = 0.904

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Benomyl: Percentage Hatch of Channel Catfish
FILE: 43872801.hat
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9570	0.9570
1	Control	2	0.9020	0.9020
1	Control	3	0.8670	0.8670
1	Control	4	0.9110	0.9110
1	Control	5	0.8260	0.8260
1	Control	6	0.7950	0.7950
1	Control	7	0.8330	0.8330
1	Control	8	0.9300	0.9300
2	0.15 ug/l	1	0.8840	0.8840
2	0.15 ug/l	2	0.9530	0.9530
2	0.15 ug/l	3	0.9500	0.9500
2	0.15 ug/l	4	0.9000	0.9000
3	0.27 ug/l	1	0.8220	0.8220
3	0.27 ug/l	2	0.8860	0.8860
3	0.27 ug/l	3	0.9300	0.9300
3	0.27 ug/l	4	1.0000	1.0000
4	0.64 ug/l	1	0.8160	0.8160
4	0.64 ug/l	2	0.9350	0.9350
4	0.64 ug/l	3	0.9510	0.9510
4	0.64 ug/l	4	0.8370	0.8370
5	1.5 ug/l	1	0.8440	0.8440
5	1.5 ug/l	2	0.9520	0.9520
5	1.5 ug/l	3	0.9270	0.9270
5	1.5 ug/l	4	0.8780	0.8780
6	3.2 ug/l	1	0.9530	0.9530
6	3.2 ug/l	2	0.9300	0.9300
6	3.2 ug/l	3	0.9270	0.9270
6	3.2 ug/l	4	0.9520	0.9520

7	7.3 ug/l	1	0.0000	0.0000
7	7.3 ug/l	2	0.0000	0.0000
7	7.3 ug/l	3	0.0000	0.0000
7	7.3 ug/l	4	0.0000	0.0000

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	2.859	0.477	185.025
Within (Error)	25	0.064	0.003	
Total	31	2.924		

Critical F value = 2.49 (0.05,6,25)
Since F > Critical F REJECT Ho: All equal

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.878	0.878		
2	0.15 ug/l	0.922	0.922	-1.420	
3	0.27 ug/l	0.910	0.910	-1.026	
4	0.64 ug/l	0.885	0.885	-0.229	
5	1.5 ug/l	0.900	0.900	-0.728	
6	3.2 ug/l	0.941	0.941	-2.023	
7	7.3 ug/l	0.000	0.000	28.238	*

Bonferroni t table value = 2.57 (1 Tailed Value, P=0.05, df=25,6)

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	0.15 ug/l	4	0.080	9.1	-0.044
3	0.27 ug/l	4	0.080	9.1	-0.032
4	0.64 ug/l	4	0.080	9.1	-0.007
5	1.5 ug/l	4	0.080	9.1	-0.023
6	3.2 ug/l	4	0.080	9.1	-0.063
7	7.3 ug/l	4	0.080	9.1	0.878

Benomyl: Percentage Survival of Channel Catfish
File: 43872801.sur Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	9	8	11	0

Calculated Chi-Square goodness of fit test statistic = 7.7946
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Benomyl: Percentage Survival of Channel Catfish
File: 43872801.sur Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.309

W = 0.959

Critical W (P = 0.05) (n = 28) = 0.924
Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

Benomyl: Percentage Survival of Channel Catfish
File: 43872801.sur Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.045	0.009	0.637
Within (Error)	22	0.309	0.014	
Total	27	0.354		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F FAIL TO REJECT Ho: All equal

Benomyl: Percentage Survival of Channel Catfish
File: 43872801.sur Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.890	0.890		
2	0.15 ug/l	0.866	0.866	0.327	
3	0.27 ug/l	0.875	0.875	0.200	
4	0.64 ug/l	0.908	0.908	-0.248	
5	1.5 ug/l	0.866	0.866	0.320	
6	3.2 ug/l	0.775	0.775	1.578	

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Benomyl: Percentage Survival of Channel Catfish
File: 43872801.sur Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	0.15 ug/l	4	0.182	20.5	0.024
3	0.27 ug/l	4	0.182	20.5	0.015
4	0.64 ug/l	4	0.182	20.5	-0.018
5	1.5 ug/l	4	0.182	20.5	0.023
6	3.2 ug/l	4	0.182	20.5	0.115

TITLE: Benomyl: Percentage Survival of Channel Catfish
FILE: 43872801.sur
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.7370	0.7370
1	Control	2	0.7220	0.7220
1	Control	3	0.9000	0.9000
1	Control	4	1.0000	1.0000
1	Control	5	1.0000	1.0000
1	Control	6	0.8570	0.8570
1	Control	7	1.0000	1.0000
1	Control	8	0.9000	0.9000
2	0.15 ug/l	1	0.8130	0.8130
2	0.15 ug/l	2	0.7500	0.7500
2	0.15 ug/l	3	0.9500	0.9500
2	0.15 ug/l	4	0.9500	0.9500
3	0.27 ug/l	1	1.0000	1.0000
3	0.27 ug/l	2	0.9000	0.9000
3	0.27 ug/l	3	0.9000	0.9000
3	0.27 ug/l	4	0.7000	0.7000
4	0.64 ug/l	1	0.9000	0.9000
4	0.64 ug/l	2	0.8500	0.8500
4	0.64 ug/l	3	0.9470	0.9470
4	0.64 ug/l	4	0.9330	0.9330
5	1.5 ug/l	1	0.7650	0.7650
5	1.5 ug/l	2	0.8000	0.8000
5	1.5 ug/l	3	0.9000	0.9000
5	1.5 ug/l	4	1.0000	1.0000
6	3.2 ug/l	1	0.9000	0.9000
6	3.2 ug/l	2	0.5500	0.5500
6	3.2 ug/l	3	0.7000	0.7000
6	3.2 ug/l	4	0.9500	0.9500

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	8	10	9	1

Calculated Chi-Square goodness of fit test statistic = 3.2814
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 43.373

W = 0.947

Critical W (P = 0.05) (n = 28) = 0.924
Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Benomyl: Length of Channel Catfish
FILE: 43872801.len
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	43.7000	43.7000
1	Control	2	44.8000	44.8000
1	Control	3	42.3000	42.3000
1	Control	4	43.7000	43.7000
1	Control	5	42.9000	42.9000
1	Control	6	42.2000	42.2000
1	Control	7	42.4000	42.4000
1	Control	8	43.6000	43.6000
2	0.15 ug/l	1	43.8000	43.8000
2	0.15 ug/l	2	45.4000	45.4000
2	0.15 ug/l	3	43.3000	43.3000
2	0.15 ug/l	4	44.2000	44.2000
3	0.27 ug/l	1	42.3000	42.3000
3	0.27 ug/l	2	41.8000	41.8000
3	0.27 ug/l	3	41.5000	41.5000
3	0.27 ug/l	4	40.7000	40.7000
4	0.64 ug/l	1	46.1000	46.1000
4	0.64 ug/l	2	43.5000	43.5000
4	0.64 ug/l	3	41.8000	41.8000
4	0.64 ug/l	4	43.0000	43.0000
5	1.5 ug/l	1	43.9000	43.9000
5	1.5 ug/l	2	42.3000	42.3000
5	1.5 ug/l	3	38.1000	38.1000
5	1.5 ug/l	4	43.8000	43.8000
6	3.2 ug/l	1	43.6000	43.6000
6	3.2 ug/l	2	43.3000	43.3000
6	3.2 ug/l	3	42.0000	42.0000
6	3.2 ug/l	4	43.7000	43.7000

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	19.294	3.859	1.957
Within (Error)	22	43.372	1.971	
Total	27	62.667		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F FAIL TO REJECT Ho: All equal

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	43.200	43.200		
2	0.15 ug/l	44.175	44.175	-1.134	
3	0.27 ug/l	41.575	41.575	1.890	
4	0.64 ug/l	43.600	43.600	-0.465	
5	1.5 ug/l	42.025	42.025	1.367	
6	3.2 ug/l	43.150	43.150	0.058	

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	0.15 ug/l	4	2.157	5.0	-0.975
3	0.27 ug/l	4	2.157	5.0	1.625
4	0.64 ug/l	4	2.157	5.0	-0.400
5	1.5 ug/l	4	2.157	5.0	1.175
6	3.2 ug/l	4	2.157	5.0	0.050

Benomyl: Length of Channel Catfish
File: 43872801.len Transform: NO TRANSFORMATION

WILCOXON'S RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	Control	43.200				
2	0.15 ug/l	44.175	36.00	12.00	4	
3	0.27 ug/l	41.575	11.50	12.00	4	*
4	0.64 ug/l	43.600	26.00	12.00	4	
5	1.5 ug/l	42.025	25.50	12.00	4	
6	3.2 ug/l	43.150	24.50	12.00	4	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	8	12	7	1

Calculated Chi-Square goodness of fit test statistic = 2.6725
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.274

W = 0.973

Critical W (P = 0.05) (n = 28) = 0.924
Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Benomyl: Weight of Channel Catfish
FILE: 43872801.wt
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.2040	1.2040
1	Control	2	1.2570	1.2570
1	Control	3	1.1180	1.1180
1	Control	4	1.1670	1.1670
1	Control	5	1.1120	1.1120
1	Control	6	1.0630	1.0630
1	Control	7	1.0450	1.0450
1	Control	8	1.1640	1.1640
2	0.15 ug/l	1	1.1880	1.1880
2	0.15 ug/l	2	1.3050	1.3050
2	0.15 ug/l	3	1.1540	1.1540
2	0.15 ug/l	4	1.2030	1.2030
3	0.27 ug/l	1	1.0850	1.0850
3	0.27 ug/l	2	1.0090	1.0090
3	0.27 ug/l	3	1.0480	1.0480
3	0.27 ug/l	4	0.9320	0.9320
4	0.64 ug/l	1	1.3700	1.3700
4	0.64 ug/l	2	1.1390	1.1390
4	0.64 ug/l	3	0.9990	0.9990
4	0.64 ug/l	4	1.0510	1.0510
5	1.5 ug/l	1	1.1720	1.1720
5	1.5 ug/l	2	1.0490	1.0490
5	1.5 ug/l	3	0.7470	0.7470
5	1.5 ug/l	4	1.1580	1.1580
6	3.2 ug/l	1	1.1320	1.1320
6	3.2 ug/l	2	1.1110	1.1110
6	3.2 ug/l	3	0.9770	0.9770
6	3.2 ug/l	4	1.1110	1.1110

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.114	0.023	1.840
Within (Error)	22	0.274	0.012	
Total	27	0.388		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F FAIL TO REJECT Ho: Alt equal

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1.141	1.141		
2	0.15 ug/l	1.213	1.213	-1.043	
3	0.27 ug/l	1.019	1.019	1.797	
4	0.64 ug/l	1.140	1.140	0.022	
5	1.5 ug/l	1.032	1.032	1.607	
6	3.2 ug/l	1.083	1.083	0.856	

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	0.15 ug/l	4	0.171	15.0	-0.071
3	0.27 ug/l	4	0.171	15.0	0.123
4	0.64 ug/l	4	0.171	15.0	0.002
5	1.5 ug/l	4	0.171	15.0	0.110
6	3.2 ug/l	4	0.171	15.0	0.058

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	8	1.141	1.141	1.165
2	0.15 ug/l	4	1.213	1.213	1.165
3	0.27 ug/l	4	1.019	1.019	1.079
4	0.64 ug/l	4	1.140	1.140	1.079
5	1.5 ug/l	4	1.032	1.032	1.057
6	3.2 ug/l	4	1.083	1.083	1.057

Benomyl: Weight of Channel Catfish
File: 43872801.wt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	1.165				
0.15 ug/l	1.165	0.348		1.72	k= 1, v=22
0.27 ug/l	1.079	0.910		1.80	k= 2, v=22
0.64 ug/l	1.079	0.910		1.83	k= 3, v=22
1.5 ug/l	1.057	1.232		1.84	k= 4, v=22
3.2 ug/l	1.057	1.232		1.85	k= 5, v=22

s = 0.112
Note: df used for table values are approximate when v > 20.

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.144	7.744	12.224	7.744	2.144
OBSERVED	0	11	9	12	0

Calculated Chi-Square goodness of fit test statistic = 8.8464
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Benomyl: Percentage Hatch of Channel Catfish
File: 43872801.hat Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.064

W = 0.982

Critical W (P = 0.05) (n = 32) = 0.930
Critical W (P = 0.01) (n = 32) = 0.904

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Benomyl: Percentage Hatch of Channel Catfish
FILE: 43872801.hat
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9570	0.9570
1	Control	2	0.9020	0.9020
1	Control	3	0.8670	0.8670
1	Control	4	0.9110	0.9110
1	Control	5	0.8260	0.8260
1	Control	6	0.7950	0.7950
1	Control	7	0.8330	0.8330
1	Control	8	0.9300	0.9300
2	0.15 ug/l	1	0.8840	0.8840
2	0.15 ug/l	2	0.9530	0.9530
2	0.15 ug/l	3	0.9500	0.9500
2	0.15 ug/l	4	0.9000	0.9000
3	0.27 ug/l	1	0.8220	0.8220
3	0.27 ug/l	2	0.8860	0.8860
3	0.27 ug/l	3	0.9300	0.9300
3	0.27 ug/l	4	1.0000	1.0000
4	0.64 ug/l	1	0.8160	0.8160
4	0.64 ug/l	2	0.9350	0.9350
4	0.64 ug/l	3	0.9510	0.9510
4	0.64 ug/l	4	0.8370	0.8370
5	1.5 ug/l	1	0.8440	0.8440
5	1.5 ug/l	2	0.9520	0.9520
5	1.5 ug/l	3	0.9270	0.9270
5	1.5 ug/l	4	0.8780	0.8780
6	3.2 ug/l	1	0.9530	0.9530
6	3.2 ug/l	2	0.9300	0.9300
6	3.2 ug/l	3	0.9270	0.9270
6	3.2 ug/l	4	0.9520	0.9520

DATA EVALUATION RECORD
§ 71-4 -- AVIAN REPRODUCTION TEST

1. **CHEMICAL:** Benomyl — PC Code No.: 099101

2. **TEST MATERIAL:** DPX-T1991-615 — Purity: 96.8%
   DPX-T1991-529 — 96.9%

3. **CITATION:**
   Authors: L.T. Frey, J. Grimes, M. Stence, J.B. Beavers, and M. Jaber
   Title: DPX-T1991-615,-529 (Benomyl): A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*)
   Study Completion Date: August 15, 1996
   Laboratory: Wildlife International Ltd., Easton, MD
   Sponsor: E.I. du Pont de Nemours & Company, Newark, DE
   Laboratory Report ID: 112-391
   MRID No.: 441030-01
   DP Barcode: D230010

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.

   Signature: [signature] **Date:** 11/6/96

   APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

   Signature: P. Kosalwat **Date:** 11/6/96

5. **APPROVED BY:**

   Signature: Anne Stavola **Date:** 12/17/96

6. **STUDY PARAMETERS:**

   Scientific Name of Test Organism: *Colinus virginianus*
   Age of Test Organisms at Test Initiation: 20 weeks
   Definitive Study Duration: 22 weeks

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an avian reproduction study using bobwhite quail. When compared to the control, there were no significant treatment related effects on any of the parameters measured at any concentrations tested (i.e., 322, 720, 1610, and 3600 ppm ai). It is not stated whether the test was conducted with the highest dosage level at or above the maximum field residue level (i.e., the expected concentration on avian food items when treated at recommended label rates).

Results Synopsis

Most sensitive endpoints: No significant adverse effects

NOEC: 3600 ppm ai LOEC: >3600 ppm ai

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Supplemental

B. **Rationale:** None of the parameters were affected at any test concentration; however, it is not stated whether the test was conducted with the highest dosage level at or above the maximum field residue level (i.e., the expected concentration on avian food items when treated at recommended label rates).

C. **Repairability:** Yes; if the expected maximum field residue level was 3600 ppm ai or lower.

9. **GUIDELINE DEVIATIONS:**

1. Neither the highest test concentration showed any significant effect nor the maximum field residue level was reported.

2. The temperature of the study room (19°C) was lower than recommended (21°C).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species A wild waterfowl species, preferably the mallard (*Anas platyrhynchos*), or an upland game species, preferably the northern bobwhite (*Colinus virginianus*)	Northern bobwhite (*Colinus virginianus*)
Age at beginning of test Birds should be approaching their first breeding season.	20 weeks old; birds were approaching their first breeding season.

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Guideline Criteria	Reported Information
Supplier All birds should be from the same source.	Trace Pheasantry, Douglassville, PA
Were birds pen-reared?	Yes
Were birds phenotypically indistinguishable from wild birds?	Yes
Health observation period 2 to 6 weeks.	3 weeks
Were birds healthy and without excessive mortality prior to the test?	Yes

B. **Test System**

Guideline Criteria	Reported Information
Were pens for adult birds of adequate size and designed to conform to good husbandry practices?	Yes
Were pens for chicks of adequate size and designed to conform to good husbandry practices?	Yes
Were pens constructed of a nonbinding material such as galvanized or stainless steel?	Yes
Was adequate ventilation provided?	Yes
Temperature Approx. 21°C (70°F)	Mean: 19.0°C SD: 1.5°C
Relative humidity Approx. 55%	Mean: 31% SD: 12%
Lighting First 8 weeks: 7 h per day. Thereafter: 16-17 h per day. At least 6 footcandles at bird level.	First 7 weeks: 8 h per day. Thereafter: 17 h per day. Mean illumination: 273 lux.

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Guideline Criteria	Reported Information
Diet A commercial breeder feed (or its equivalent) that is appropriate for the test species.	27% protein minimum 2.5% fat minimum 5% fiber maximum 5% limestone (adult diet only) Note: Offspring received a water soluble vitamin and electrolyte mix in their water from the day of hatch until the birds were 14 days old.
Preparation of test diet A premixed containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.	Test diets were prepared by mixing the test compound into a premix which was used for weekly preparation of the final diet.
Was the premix stored under conditions which maintain stability?	Yes, the premix was placed into labeled freezer bags and frozen.
Was the diet analyzed to verify homogeneity and stability of the test substance?	Since benomyl cannot be analyzed directly due to conversion to carbendazim upon exposure to moisture (see p.167 of report), homogeneity and stability was confirmed based on measured concentrations of carbendazim and STB.
Replenishment of feed	Adult diets were prepared weekly and presented to the birds on Wednesday of each week. Additional diets were prepared when necessary. Feed and water were provided *ad libitum* for the adults and offspring.

C. Test Design

Guideline Criteria	Reported Information
Nominal concentrations At least two concentrations other than the control are required; three or more are strongly recommended. The highest test concentrations should show a significant effect or be at or above the maximum field residue level.	Nominal concentrations: Control, 322, 720, 1610, and 3600 ppm ai. Max. residue level: Not reported.
Control Vehicle control.	No vehicle was used. The test substance was added directly to the basal diet.
Vehicle Corn oil or other appropriate vehicle.	N/A
Vehicle amount (% of diet by weight) Not more than 2%.	N/A
Number of birds per pen One male and 1 female per pen is strongly recommended. For quail, 1 male and 2 females may be acceptable. For ducks, 2 males and 5 females may be acceptable.	1 male and 1 female per pen
Number of pens per group At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs.	16 pens per group
Pre-laying exposure duration At least 10 weeks prior to the onset of egg-laying.	11 weeks
Exposure duration with egg-laying At least 10 weeks.	10 weeks

Guideline Criteria	Reported Information
Withdrawal period If reduced reproduction is evident, a withdrawal period of up to 3 weeks may be added to the test phase.	N/A

D. Egg Collection and Incubation

Guideline Criteria	Reported Information
Were eggs collected daily?	Yes
Egg storage temperature Approximately 16°C (61°F)	13.2 ±0.9°C
Egg storage humidity Approximately 65%	49 ±11%
Were eggs set weekly?	Yes
Were eggs candled for cracks prior to being set for incubation on Day 0?	Yes
Candling for fertility Quail: approx. Day 11 Ducks: approx. Day 14	Eggs were candled on day 11 or 12 for embryo viability and on day 21 for embryo survival.
Transfer of eggs to hatcher Bobwhite: Day 21 Mallard: Day 23	Eggs were transferred on Day 21.
Hatching temperature 39°C (102°F) is recommended	37.2°C
Hatching humidity 70% is recommended	76%
Day after egg set that chicks were removed and counted Bobwhite: Day 24 Mallard: Day 27	Chicks that had hatched were removed and counted on Day 25. All remaining hatchlings and unhatched eggs were removed on Day 26.

E. Eggshell Thickness Measurement

Guideline Criteria	Reported Information
Collection Schedule At least once every two weeks (Week 1, 3, 5, 7 and 9).	One Egg was collected weekly, when available, for eggshell thickness from odd numbered pens during odd numbered weeks and from even numbered pen during even numbered weeks.
Were shells opened, washed, and air dry for at least 48 hours before measuring?	Yes; shells air dried for 1 week.
Measurement 3-4 measurements per eggs to the nearest 0.01 mm.	5 measurements to the nearest 0.005 mm.

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Did diet analysis verify the concentrations of test material?	Yes
Did diet analysis show that the test substance was stable and homogeneous?	Yes
Were body weights of adults reported for test initiation and biweekly up to week 8 or the onset of egg laying?	Yes
Was average food consumption of adults reported at least biweekly?	Yes

Guideline Criteria	Reported Information
Reproductive Endpoints The following endpoints should be reported: • Eggs laid • Eggs cracked • Eggs set • Viable embryos • Live 3-week embryos • Normal hatchlings • 14-day-old survivors • Weights of 14-day-old survivors • Egg shell thickness • Total food consumption • Initial and final body weights, by sex	All endpoints listed at left plus hatchling weight, maximum number of eggs laid, and maximum number of eggs set.
Were data reported by pen for all endpoints?	Yes

Significant Results: There were no overt signs of toxicity or treatment related reductions in food consumption or body weights at any test concentration (322, 720, 1610, and 3600 ppm ai) when compared to the control. There were no statistically significant effects on any reproductive parameter measured at any test concentration when compared to the control. There was an apparent, but not statistically significant, reduction in the number of eggs laid at the 1610 ppm ai concentration. This effect was not concentration responsive and was not considered to be treatment related. The number of eggs laid at the highest concentration level (3600 ppm ai) was similar to the control.

13. **VERIFIED STATISTICAL RESULTS:**

Means of Endpoints

Endpoint	Control	322 ppm	720 ppm	1610 ppm	3600 ppm
Eggs laid (EL)	40 (18)	52 (16)	45 (16)	27 (18)	39 (19)
Eggs cracked (EC)	0.6 (0.6)	0.9 (2.3)	1.8 (3.1)	0.8 (1.4)	0.8 (0.9)
Eggs set (ES)	32 (19)	45 (16)	38 (17)	20 (16)	33 (17)
Viable embryos (VE)	30 (19)	41 (16)	31 (17)	18 (15)	27 (18)
Live 3-wk embryos (LE)	30 (19)	40 (16)	31 (17)	18 (15)	26 (18)
Normal hatchlings (NH)	28 (19)	36 (14)	29 (16)	16 (14)	24 (17)
14-day-old survivors (HS)	26 (17)	33 (14)	26 (14)	15 (13)	22 (16)
Egg shell thickness (THICK)	0.220 (0.023)	0.235 (0.011)	0.221 (0.017)	0.230 (0.016)	0.227 (0.020)
Hatchling weight (HATWT)	6.1 (0.5)	6.2 (0.6)	6.1 (0.5)	5.8 (0.6)	5.9 (0.3)
14-day survivor weight (SURVWT)	25.2 (2.9)	26.0 (1.5)	25.3 (3.7)	25.4 (2.3)	24.5 (2.8)
Mean food consumption (FOOD)	19.3 (1.7)	20.5 (1.2)	19.7 (1.3)	19.3 (1.4)	20.7 (1.9)
Final weight of males (POSTM)	218 (16)	216 (18)	217 (15)	212 (12)	214 (13)
Final weight of females (POSTF)	236 (35)	255 (26)	241 (16)	244 (18)	238 (20)

Statistically Significant Endpoints: No significant effects.

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14. **REVIEWER'S COMMENTS:** There were no significant treatment related effects on any of the parameters measured at any concentration tested (i.e., 322, 720, 1610, and 3600 ppm ai) when compared to the control. The authors noted that nominal concentrations were selected after consultation with the Sponsor and were based upon "Expected Environmental Concentrations" (page 14 of the report). However, it is unclear if the highest dosage level (3600 ppm ai) was at or above the maximum field residue level. This study will be classified as **Supplemental** pending the registrant's response.

DATA EVALUATION RECORD
§ 71-4 -- AVIAN REPRODUCTION TEST

1. **CHEMICAL:** Benomyl — PC Code No.: 099101

2. **TEST MATERIAL:** DPX-T1991-615 — Purity: 96.8%
DPX-T1991-529 — 96.9%

3. **CITATION:**

Authors: L.T. Frey, M. Stence, J.B. Beavers, and M. Jaber

Title: DPX-T1991-615,-529 (Benomyl): A Reproduction Study with the Mallard (*Anas platyrhynchos*)

Study Completion Date: August 15, 1996

Laboratory: Wildlife International Ltd., Easton, MD

Sponsor: E.I. du Pont de Nemours & Company, Newark, DE

Laboratory Report ID: 112-392

MRID No.: 441030-02

DP Barcode: D230010

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature: [signature] **Date:** 11/7/96

APPROVED BY: Pim Kosalwat, Ph.D, Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 11/7/96

5. **APPROVED BY:**

Signature: [signature] **Date:** 12/17/96

6. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Anas platyrhynchos*
Age of Test Organisms at Test Initiation: 21 weeks
Definitive Study Duration: 22 weeks

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an avian reproduction study using the mallard. The NOEC was determined to be 322 ppm ai based on significant reductions in the percentage of viable embryos, normal hatchlings, and 14-day old survivors of eggs set at 720 ppm ai.

Results Synopsis

Most sensitive endpoints: Viable embryos of eggs set, normal hatchlings of eggs set, and 14-day old survivors of eggs set.

NOEC: 322 ppm ai LOEC: 720 ppm ai

8. **ADEQUACY OF THE STUDY:**

   A. **Classification:** Core

   B. **Rationale:** Fulfills the guideline requirements.

   C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:**

   1. The maximum field residue level was not reported.

   2. The temperature of the study room (18.5°C) was lower than recommended (21°C).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

   A. **Test Organisms**

Guideline Criteria	Reported Information
Species A wild waterfowl species, preferably the mallard (*Anas platyrhynchos*), or an upland game species, preferably the northern bobwhite (*Colinus virginianus*)	Mallard (*Anas platyrhynchos*)
Age at beginning of test Birds should be approaching their first breeding season.	21 weeks old; birds were approaching their first breeding season.
Supplier All birds should be from the same source.	Whistling Wings, Inc. Hanover, Illinois
Were birds pen-reared?	Yes

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Guideline Criteria	Reported Information
Were birds phenotypically indistinguishable from wild birds?	Yes
Health observation period 2 to 6 weeks.	6 weeks
Were birds healthy and without excessive mortality prior to the test?	Yes

B. Test System

Guideline Criteria	Reported Information
Were pens for adult birds of adequate size and designed to conform to good husbandry practices?	Yes
Were pens for chicks of adequate size and designed to conform to good husbandry practices?	Yes
Were pens constructed of a nonbinding material such as galvanized or stainless steel?	Yes
Was adequate ventilation provided?	Yes
Temperature Approx. 21°C (70°F)	Mean: 18.5°C SD: 1.5°C
Relative humidity Approx. 55%	Mean: 39% SD: 16%
Lighting First 8 weeks: 7 h per day. Thereafter: 16-17 h per day. At least 6 footcandles at bird level.	First 8 weeks: 8 h per day. Thereafter: 17 h per day. Mean illumination: 365 lux.
Diet A commercial breeder feed (or its equivalent) that is appropriate for the test species.	27% protein minimum 2.5% fat minimum 5% fiber maximum 5% limestone (adult diet only)

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Guideline Criteria	Reported Information
Preparation of test diet A premixed containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.	Test diets were prepared by mixing the test substance into a premix which was used for weekly preparation of the final diet.
Was the premix stored under conditions which maintain stability?	Yes, the premix was placed into labeled freezer bags and frozen.
Was the diet analyzed to verify homogeneity and stability of the test substance?	Since benomyl cannot be analyzed directly due to conversion to carbendazim upon exposure to moisture (see p.164 of report), homogeneity and stability was confirmed based on measured concentrations of carbendazim and STB.
Replenishment of feed	Adult diets were prepared weekly and presented to the birds on Wednesday of each week. Feed and water were provided *ad libitum* for the adults and offspring.

C. **Test Design**

Guideline Criteria	Reported Information
Nominal concentrations At least two concentrations other than the control are required; three or more are strongly recommended. The highest test concentrations should show a significant effect or be at or above the maximum field residue level.	Nominal concentrations: Control, 64, 144, 322 and 720 ppm ai Max. residue level: Not reported

Guideline Criteria	Reported Information
Control Vehicle control.	No vehicle was used. The test substance was added directly to the basal diet.
Vehicle Corn oil or other appropriate vehicle.	N/A
Vehicle amount (% of diet by weight) Not more than 2%.	N/A
Number of birds per pen One male and 1 female per pen is strongly recommended. For quail, 1 male and 2 females may be acceptable. For ducks, 2 males and 5 females may be acceptable.	1 male and 1 female per pen
Number of pens per group At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs.	16 pens per group
Pre-laying exposure duration At least 10 weeks prior to the onset of egg-laying.	10 weeks
Exposure duration with egg-laying At least 10 weeks.	11 weeks
Withdrawal period If reduced reproduction is evident, a withdrawal period of up to 3 weeks may be added to the test phase.	N/A

D. Egg Collection and Incubation

Guideline Criteria	Reported Information
Were eggs collected daily?	Yes

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Guideline Criteria	Reported Information
Egg storage temperature Approximately 16°C (61°F)	13.4 ±0.8°C
Egg storage humidity Approximately 65%	45 ±11%
Were eggs set weekly?	Yes
Were eggs candled for cracks prior to being set for incubation on Day 0?	Yes
Candling for fertility Quail: approx. Day 11 Ducks: approx. Day 14	Eggs were candled on Day 14 for embryo viability and on day 20 or 21 for embryo survival.
Transfer of eggs to hatcher Bobwhite: Day 21 Mallard: Day 23	Eggs were transferred on Day 24.
Hatching temperature 39°C (102°F) is recommended	37.2°C
Hatching humidity 70% is recommended	76%
Day after egg set that chicks were removed and counted Bobwhite: Day 24 Mallard: Day 27	Chicks were removed and counted on Day 27 or 28.

E. Eggshell Thickness Measurement

Guideline Criteria	Reported Information
Collection Schedule At least once every two weeks (Week 1, 3, 5, 7 and 9).	One egg was collected for eggshell thickness measurement from each of the odd numbered pens during odd numbered weeks and from each of the even numbered pens during even numbered weeks.
Were shells opened, washed, and air dry for at least 48 hours before measuring?	Yes; shells air dried for 1 week.

Guideline Criteria	Reported Information
Measurement 3-4 measurements per eggs to the nearest 0.01 mm.	5 measurements to the nearest 0.005 mm

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Did diet analysis verify the concentrations of test material?	Yes
Did diet analysis show that the test substance was stable and homogeneous?	Yes
Were body weights of adults reported for test initiation and biweekly up to week 8 or the onset of egg laying?	Yes
Was average food consumption of adults reported at least biweekly?	Yes
Reproductive Endpoints The following endpoints should be reported: • Eggs laid • Eggs cracked • Eggs set • Viable embryos • Live 3-week embryos • Normal hatchlings • 14-day-old survivors • Weights of 14-day-old survivors • Egg shell thickness • Total food consumption • Initial and final body weights, by sex	All endpoints listed at left plus hatchling weight and maximum number of eggs set and eggs laid.

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Guideline Criteria	Reported Information
Were data reported by pen for all endpoints?	Yes

Significant Results: There were no overt signs of toxicity or treatment related mortalities at any test concentration (64, 144, 322, and 720 ppm ai). When compared to the control, there was a significant treatment related reduction in the percentage of viable embryos of eggs set at the highest concentration (720 ppm ai). There was also a significant difference in egg shell thickness at the 64, 144, and 720 ppm ai treatment levels when compared to the control. The authors did not consider this difference to be treatment related since: 1) the effect was not treatment responsive; 2) the control value was unusually high compared to historical data on this parameter; and 3) the values for each concentration where comparable to the historical (1978-1995) control mean value of 0.383 ±0.015mm. No other significant effects were observed.

13. VERIFIED STATISTICAL RESULTS:

Means of Endpoints

Endpoint	Control	64 ppm	144 ppm	322 ppm	720 ppm
Eggs laid (EL)	41 (17)	39 (15)	37 (21)	41 (18)	47 (15)
Eggs cracked (EC)	0.9 (1.2)	1.3 (2.0)	0.8 (0.8)	0.7 (1.1)	0.6 (0.7)
Eggs set (ES)	35 (15)	33 (13)	32 (20)	36 (16)	41 (15)
Viable embryos (VE)	28 (17)	29 (15)	30 (19)	32 (15)	24 (14)
Live 3-wk embryos (LE)	27 (17)	29 (14)	30 (19)	32 (15)	23 (14)
Normal hatchlings (NH)	20 (15)	23 (12)	23 (14)	23 (13)	17 (11)
14-day-old survivors (HS)	20 (15)	22 (12)	22 (14)	22 (13)	17 (11)
Egg shell thickness (THICK)	0.414 (0.019)	0.398 (0.019)	0.395 (0.021)	0.411 (0.016)	0.394 (0.020)

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Endpoint	Control	64 ppm	144 ppm	322 ppm	720 ppm
Hatchling weight (HATWT)	35.2 (3.0)	35.8 (2.3)	34.9 (3.8)	35.9 (3.4)	35.9 (3.6)
14-day-old survivor weight (SURVWT)	310 (23)	291 (24)	297 (24)	293 (17)	293 (17)
Mean food consumption (FOOD)	150 (27)	150 (36)	134 (18)	141 (26)	149 (26)
Final weight of males (POSTM)	1226 (74)	1216 (146)	1228 (127)	1226 (98)	1206 (113)
Final weight of females (POSTF)	1171 (145)	1242 (123)	1206 (137)	1180 (115)	1199 (117)

Statistically Significant Endpoints

Endpoint	Statistical Method	Levels at which Effect Was Observed
Reduction in the percentage of: - Viable Embryos of Eggs Set	Dunnett's	720 ppm ai
Reduction in the percentage of: - Viable Embryos of Eggs Set - Normal Hatchlings of Eggs Set - 14-Day Old Survivors of Eggs Set	Least Squares Means	720 ppm ai
Reduction in: - Eggshell Thickness	Dunnett's	64, 144, and 720 ppm ai*
Reduction in: - 14-Day Survivor Weight	Dunnett's	64 ppm ai*

* Not considered treatment related.

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14. **REVIEWER'S COMMENTS:** The reviewer agrees with the authors that the significant difference in eggshell thickness at 64, 144, and 720 ppm ai when compared to the control was not treatment related. The mean value for the control (0.414 mm) was higher than the highest value obtained from 14 different control groups from previously reviewed studies (range of 0.332 to 0.402 mm). Also, the eggshell thickness values for the four concentrations (0.398, 0.395, 0.411, and 0.394 mm for 64, 144, 322, and 720 ppm ai, respectively) were higher than the mean value for those 14 studies (0.379 mm). And finally, the effect was not concentration responsive. Therefore, the reviewer believes that the decrease in eggshell thickness observed when compared to the control was not treatment related.

Based on statistically significant reductions in the percentage of viable embryos, normal hatchlings, and 14-day old survivors of eggs set, the NOEL and LOEL are determined to be 322 and 720 ppm ai, respectively. This study is scientifically sound and fulfills the guideline requirements for an avian reproduction test using mallards. This study is classified as **Core**.

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EEB Review dated 1/5/1999 Benomyl

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