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

FEB 15 1996

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review Of Tebuconazole Mesocosm Study

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Leonard Cole, PM 21
Registration Division (7505C)

Attached is an abbreviated review of aquatic mesocosm data for the fungicide, tebuconazole (Lynx®). The mesocosm study was required due to chronic effects on fish. This study was designed to evaluate ecologically significant effects on fish, identify impacts on aquatic community structure and ecosystem function, determine effects of treatment on water quality parameters, and verify exposure in aquatic systems subsequent to treatment with tebuconazole.

Tebuconazole altered juvenile fish growth and survival at the highest mesocosm test concentrations (24.4 to 149 ppb in water), which exceeded laboratory test chronic NOECs for fish and invertebrates (12 to 120 ppb). Although EECs (see Risk Assessment by C. Rodriguez, 1994, D198525) in water are not expected to exceed 12 ppb, residues in sediment may range in concentration from 10 to 1020 ppb. Effects to non-target organisms associated with sediment have not been adequately investigated, therefore sediment-related risk is undetermined at this time. The effects of high residue loads in sediment on non-target organisms should be investigated.

Tebuconazole also affected invertebrates (i.e. periphyton, phytoplankton, and macroinvertebrates) indirectly. Although these effects were biologically significant at the highest treatment level, they were only moderate and transient. Diversity of macroinvertebrates were effected at dose 2 (sediment = 10 to 370 ppb; Water = 10.2 to 35.3) and dose 3 (sediment = 10 to 1020 ppb; Water = 24.4 to 149 ppb).

The aquatic mesocosm study (72-7) was categorized as acceptable and fulfills the guideline requirement.

2046062

DP BARCODE: D193983

CASE: 192687
SUBMISSION: S440656

DATA PACKAGE RECORD
BEAN SHEET

DATE: 08/11/93
Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 405 6(A)(2) ADVERSE DATA
CHEMICALS: 128997 Tebuconazole 0.0000

ID#: 003125-GOO LYNX 25
COMPANY: 003125 MILES INC
PRODUCT MANAGER: 21 SUSAN LEWIS 703-305-5200 ROOM: CM2 217
PM TEAM REVIEWER: BENJAMIN CHAMBLISS 703-305-7382 ROOM: CM2 233
RECEIVED DATE: 05/05/93 DUE OUT DATE: 07/14/93

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 193983 EXPEDITE: N DATE SENT: 08/11/93 DATE RET.: / /
CHEMICAL: 128997 Tebuconazole
DP TYPE: 001 Submission Related Data Package
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT	
DIV : EFED	08/13/93	/ /	ADMIN DUE DATE: 09/05/93
BRAN: EEB	08/13/93	/ /	NEGOT DATE: 09/05/93
SECT:	/ /	/ /	PROJ DATE: / /
REVR :	/ /	/ /	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

Please review this mesocosm study with tebuconazole. The priority pts. for this data package = 30 pts.(20 pts for B/2 plus 10 pts for O/16). MRID # 427601-00 & 01 (2 vol.).

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package.

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

DP Barcode : D194003
PC Code No : 128997
EEB Out :

To:
Product Manager
Registration Division (H7505C)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 192686
Chemical Name : Tebuconazole
Type Product : Fungicide
Product Name : LYNX 2
Company Name : Miles, Inc.
Purpose : Review of Mesocosm Data

Action Code : 405 6(A)(2) Date Due : 05/26/95
Reviewer : Tom A. Bailey Date In EEB: 08/13/93

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)	427601-00,01	Y
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			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

ABBREVIATED FIELD STUDY REVIEW

1. **Pesticide Name:** Tebuconazole

2. **Study Type:** Aquatic Mesocosm Study

3. **Pesticide Use:** Tebuconazole is a broad spectrum systemic foliar fungicide developed to control *Sclerotium* stem rot (white mold), *Rhizoctonia* limb rot, early and late leafspot, and leaf rust in peanuts.

4. **Study Purpose:** The study was required because the proposed label use of Lynx could result in chronic problems for fish. The purposes of this study were to 1) evaluate ecologically significant effects of Lynx on fish; 2) identify any impacts on aquatic community structure and ecosystem function; 3) discern ecologically and statistically significant alterations in physical/chemical water quality parameters as a result of treatment with Lynx; and 4) analytically verify exposure in aquatic systems (mesocosm) after treatment with Lynx.

5. **System Description:** The study was conducted at the University of North Texas Research Field Station in Denton County, Texas. Sixteen ponds were used in this study. The ponds were 30 meters in length by 16 meters wide (480 m^2). The sides sloped moderately (2:1) to a maximum depth of 2 meters. The volume was 652,180 liters. The treatment design was a 4 x 4 x 4 x 4 replicated system with one control group and three test levels.

6. **Exposure Regime:** The ponds received 4 applications of Lynx applied at two week intervals beginning June 10 and terminating July 22, 1991. The applications were subsurface injections. The nominal test doses ranged from 2.5 to 40 ppb. The measured concentrations presented in table 1 are averaged over the 15 week study period.

Table 1. **Test doses (grams ai per pond) and concentrations (ppb) used in the Tebuconazole Mesocosm Study.**

Treatment Levels	Doses per mesocosm (grams a.i.)	Nominal Concentration (ppb)	Maximum Measured Concentration (ppb)		Maximum Mean Measured Concentration (ppb)	
			Sed.	Water	Sed.	Water
Control	0	0	<0.01	2.55	<0.01	<0.01
Dose 1	1.6	2.5	290	15.8	170	11.7
Dose 2	6.5	9.9	580	47.1	370	35.3
Dose 3	26	39.8	1140	190	1020	149

7. **Study Methods**:

a. **Biological Structure**:

1) **Phytoplankton**:

Monthly samples were collected using a depth-integrated tube sampler. Six random samples were taken from the pelagic and littoral zones. The samples from each zone were composited. The samples were taken for phytoplankton identification and for measurement of chlorophyll a and pheophytin a pigments. The pigments were measured using standard spectrophotometric techniques (APHA 1985).

2) **Periphyton**:

Samples were collected at three specified times: after the third application, two weeks after the final application, and at study termination. Glass microscope slides were placed in floating peryphytometers below the water surface. Two peryphytometers were in the littoral region and three were in the pelagic region. Colonization period was 2 weeks. The samples were used to determine total biomass by ash-free dry weight and for determination of chlorophyll a and pheophytin a with standard spectrophotometric techniques (APHA 1985).

3) **Ecosystem Metabolism:**

Ecosystem metabolism was measured biweekly using the three point diel oxygen method suggested by Lind (1979). Measurements were taken at dusk, dawn and the following dusk. Total Community Respiration (mg/l) was calculated as the first dusk DO value minus the 24 hour respiration slope value. The Gross Community Photosynthesis was calculated as the second dusk DO minus the 24 hour respiration slope value. The production to respiration ratio (P/R) was calculated as the ratio of the gross community photosynthesis to the total community respiration.

4) **Zooplankton:**

Zooplankton were sampled bimonthly with an integrated tube sampler using the same procedure that was used for phytoplankton sampling. The samples were filtered through a 35μm mesh plankton bucket. Each sample was preserved in 1-2% Lugol's solution. Protozoans, rotifers and crustaceans were counted and identified. Zooplankton counts were reported as number of individuals per liter pond water.

5) **Macroinvertebrates:**

Macroinvertebrates were collected and identified from artificial substrate samplers, Ekman grab samples and emergence trap samples. Three artificial substrate samplers were placed in the pelagic regions and two in the littoral regions. Sampling started two weeks after the first application and continued biweekly and were analyzed on a monthly basis. Ekman grab samples were taken prior to the first application, following the final application and at study termination. One sample was taken in the littoral region and two in the pelagic region. One emergence trap was in the littoral region and two traps in the pelagic regions. Sampling was every two weeks and analyzed monthly. Organisms were identified and enumerated.

6) **Fish:**

Twenty four adult bluegill sunfish (*Lepomis macrochirus* Rafinesque) were stocked in each pond. Fish were individually weighed, measured and tagged with a transponder implanted intramusculary beneath the first dorsal fin. Each fish was scanned for identification. Growth and survival of fish were determined. Spawning boxes were placed and observations were made. Juvenile fish were numbered weighed and grouped into one-centimeter size classes.

b. **Statistical Analysis:**

ANOVA was used to test for differences in biological parameters between control and doses. Biological count data was normalized by log transformation or ranking. Dunnett's Test was used to identify differences among means. All analyses were conducted at an alpha level of 0.05.

For the community structure analysis the Percent Similarity (PS) index by Bray and Curtis (1957) was used. Cluster analysis was performed on the calculated PS values and Statistically significant clusters were identified with the "bootstrap" technique. This is a non-parametric statistical technique .

8. **Study Evaluation:**

The two highest nominal test doses, 10 ppb and 40 ppb, respectively approximated or exceeded the NOEL (12 ppb) for the most sensitive species tested, the rainbow trout (Table 2). The maximum measured concentrations ranged from 2.55 (control) to 190 ppb (dose 3) in water and from 290 (dose 1) to 1140 ppb (dose 3) in sediment. Maximum mean measured concentrations ranged from 11.7 (dose 1) to 149 (dose 3) ppb in water and from 170 to 1020 ppb in sediment. The k_d for tebuconazole predicts that roughly 10 times as much material will bind to sediment compared to the amount solubilized in water. Calculations showed that there were 18X, 12X, and 6X as much material in sediment as water for doses 1, 2, and 3 respectively. Proportionately more material is detected in the water column as dosage increases.

For the purposes of this report, test dosages will be reported as either maximum mean measured concentrations (averaged over length of the study) or in some cases as the respective weekly mean measured concentrations.

Table 2. Laboratory Toxicity Data for aquatic organisms

SPECIES	LC50	NOEL/LOEL	CHRONIC ENDPOINTS
Rainbow Trout	4.4 ppm	12/25 ppb	Larval Survival
Bluegill Sunfish	5.7 ppm	-	-
Daphnia magna	4.0 ppm	120/230 ppb	Adult Length Reproduction[1]
Sheepshead minnow	5.9 ppm	22/48 ppb	Reproduction
Mysid Shrimp	0.49 ppm	35/61 ppb	Reproduction
Eastern Oyster	5.9 ppm	-	-

[1] Reduction in the number of young Daphnids produced.

Phytoplankton

Chlorophyll a: There were no statistically significant effects of tebuconazole on phytoplankton in any treated pond. However biological significance is conceivable particularly with the coefficients of variation ranging from 23 to 95 percent. Chlorophyll a increased in all ponds after week 3, but was surmised by the study author to have been due to increased temperature and nutrient availability. It is noteworthy to mention that according to charts provided in the report, temperature decreased slightly after week 3, nitrates increased slightly between week 3 and week 7 prior to leveling off, and nitrites increased slightly, but only after week 7. Phosphate remained rather stable throughout the study period with only a slight decrease after week 3. Measures of chlorophyll a were comparable among the two lowest dosed ponds and the control ponds throughout the study (week 1 through week 15). In the highest dosed pond, chlorophyll a was higher than in the control after week three. Although no causal relationship to tebuconazole exposure is explainable, these results appear to be more than random events.

Community structure: Tebuconazole did not appear to significantly impact total number or taxonomic richness of phytoplankton. However, four of seven Divisions of phytoplankton observed during the study exhibited effects due to tebuconazole. Chlorophyta and Pyrrhophyta numbers were significantly reduced, while numbers of Cryptophyta and Euglenophyta were significantly increased at various times during the study. Community structure was altered by tebuconazole.

Periphyton

Chlorophyll a: During weeks 11 and 15 of the study, mean periphyton chlorophyll a was higher in the treated ponds. However, when averaged over time, chlorophyll a concentrations were actually lower in treatments than in the control (Fig. 1). No treatment related reduction was statistically significant. However during week 9, chlorophyll a was significantly higher at the highest dose (114.8 ppb). It is surmised that the lack of statistical significance may be due to the sizable coefficients of variation. Therefore a qualitative assessment suggests that the greater chlorophyll a production in treated ponds is due to the reduction or eradication of certain predator species, which results in an increased amount of periphyton biomass and associated chlorophyll a production.

Total biomass: Biomass levels were somewhat comparable among the two lowest treatment levels and control ponds (Figs. 2a,b,c). During week 9, total biomass was significantly higher at the highest dose (114.8 ppb). If chlorophyll a is an indicator of biomass, there is no explanation for why chlorophyll production would be higher in treated ponds despite biomass being roughly equal between treatment and control ponds. It is surmised that sampling and measurement error may have contributed to this discrepancy. No clear inference of tebuconazole effects on periphyton biomass was possible.

Community metabolism: Respiration was higher in treated ponds than in controls through week 13. Respiration increased in both treated and control ponds between week 13 and week 15, but was higher in the control ponds. There were no statistically significant differences in community metabolism values between treatment and control groups. Based on measured concentrations during week 1 and week 15, gross community photosynthesis was significantly lower in dose 3 ponds (week 1 = 24.4 ppb and week 15 = 79.6 ppb) than in the controls. Tebuconazole effects on community metabolism were transient.

Zooplankton

The zooplankton isolated and identified were representative of three major groups: Protozoa, Rotifera, and Crustacea. Rotifers were the most abundant group followed by protozoa and crustacea. The total number of zooplankton were significantly higher at dose 3 during weeks -2 to 3 (63 ppb) and week 15 (79.6 ppb) and at dose 2 during week 3 (15.2 ppb) and week 15 (20.8 ppb), respectively, when compared to the control groups. Overall, taxa richness was similar between treatments and controls. Direct effects on zooplankton by tebuconazole were unlikely, however indirect effects due to fish foraging were probable.

Protozoa: The predominate protozoans present were *Cyclotrichium* and *Vorticella*. The density of protozoans in the control groups was similar to the density observed at the highest measured concentrations of 11.7 (dose 1, week 3) and 35.3 ppb (dose 2, week 7). Prior to the first application of tebuconazole numbers of protozoans were greater in the highest dosed ponds (dose 3) than in the control. By week 7, this trend was reversed whereby numbers of protozoans exposed to 149 ppb (dose 3, week 7) were lower than the number of protozoans in the control. Direct effects of tebuconazole on protozoans was likely at the highest test concentration.

Rotifera: The total number of rotifers was higher in the 149 ppb treated ponds through week 11 when the number of rotifers in the control groups were at their peak. The increased number of rotifers in ponds treated with 149 ppb tebuconazole may be related to a reduced bluegill population. At 11.7 and 35.3 ppb, the number of rotifers was silimar to the numbers seen in the control ponds. There was a significantly greater number of rotifera when exposed to 149 ppb than in the control group in the pelagic zone during week 7 and greater in the littoral zone during week 15 (78.9 ppb). *Keratella cochlearis* populations were generally larger than seen in the control group, but were completely gone by week 7. It is unclear whether this decimation is due to tebuconazole or bluegill predation. *Polyarthra remata* populations at 11.7 and 35.3 were generally similar to those in the control ponds. However at 149 ppb, the number of *P. remata* was markedly higher when compared to the controls. The trends observed for rotifera could be due to indirect effects of tebuconazole, specifically by reductions of young bluegill populations.

Crustacea: There was a decline in the population after week 3 in both control and treated ponds due to an increase in bluegill population. Copepods were the most common microcrustaceans observed in the ponds early in the study. Copepod populations were not significantly altered by tebuconazole. The crustacean population declined as the study progressed and by week 6 was markedly low in all ponds. Copepods are a reliable food source for young bluegill. Copepod populations were higher in the treated ponds when compared to control. The impact of tebuconazole on juvenile fish had an indirect affect on copepod populations. Reduction in fish number would result in decreased feeding pressure.

Community structure and similarity: At week 7, there were no significant differences in community clusters for any ponds (*Brachionus havanensis* was the dominant species with subdominant taxa being Polyarthra remata, *Filinia longiseta* and *Anuraeopsis fissa*).

There were some significant differences among zooplankton clusters during week 11: the control and 5.9 ppb treatment level and the 26.4 and 94.5 ppb treatment levels clustered together respectively. *Brachionus havanensis* was the dominant species at both the 11.7 ppb treatment and control ponds, while a mixture of three species (*Filinia longisets*, *Branchionus havanensis* and *Polyarthra remata)* were observed to be dominant at maximum measured concentrations of 35.3 (dose 2) and 149 ppb (dose 3).

Zooplankton community composition at the end of the study was similar in both the control and treated ponds. No clear effect of tebuconazole on the zooplankton community was evident.

Macroinvertebrates

Oligochaeta (worms): Oligochaete numbers were generally higher in treatment ponds during week 3 and were lower during week 7 when compared to control ponds. Taxon were significantly reduced at the highest treatment level (Sediment Concentration = 10 to 1020 ppb and Water concentration = 24.4 to 149 ppb).

Ephemeroptera (mayflies): The Caenidae were the most dominant. Caenis was significantly reduced during week 7 and week 15 at the highest treatment level, but was highest during week 15 at dose level two.

Baetidae nymph populations were similar or higher in number (*Callibaetis nr. floridanus*) from treatment ponds than from control ponds. The highest population densities were observed at 15.2 and 63 ppb during week 3.

Trichoptera: The leptocerid, *Oecetis inconspicua*, was the largest population collected from artificial substrate emergence traps. No assessment was performed due to too few samples taken.

Leptocerid (larvae) populations were similar in both treatment and control ponds except higher numbers of leptoceridae were observed in the littoral zone of ponds treated with 35.3 ppb during week 7.

Hydroptilidae larvae (primarily *Orthotrichia*) were similar in treatment and control ponds throughout the study period.

Chironomidae: Chironomidae were the most common species collected from artificial substrates in the treated ponds. Tanypodinae (*Procladius*, *Labrudinia* and *Ablabesmyia*) and Chironominae (viz. *Endochironomus*, *Xenochironomus* and *Polypediulum*) were the most dominant species collected throughout the study .

The numbers of Chironominae collected from artificial substrates were similar in treatment and control ponds.

Chironominae larvae collected from ekman grab samples (two lowest test levels) were higher between week 1 and week 7 (sediment/water conc. = 10-20 ppb/2.5-10 ppb for dose 1 and 10-90 ppb/10.2-35.3 ppb for dose 2) , but were similar or slightly lower at sediment concentrations ranging from 10-150 ppb (dose 3) and pelagic water concentrations of 24.4 to 149 ppb (dose 3) when compared to control populations.

The numbers of adult chironominae observed in littoral zone emergence traps (treatment) were significantly higher than controls with regards to number at sediment/water concentrations of 190 ppb/24.2 ppb and 580 ppb/95.0 ppb respectively during week 11. The population fluctuated in both control and treated ponds throughout the study.

Tanypodinae larval densities in the lower two doses were higher comparative to controls. For samples from ponds with maximum mean measured concentrations in sediment of 1020 ppb and pelagic waters of 149 ppb, numbers were somewhat lower than in controls. There were no significant differences between control and treated ponds for Tanypodinae larvae collected from artificial substrates. There was a significant increase in Tanypodinae imagoes collected from emergence traps in the pelagic zone of high dose ponds during week 11. The variability of these data obfuscates the conclusions regarding tebuconzaole effects.

Coleoptera: The collection of coleopterans was infrequent. Bluegill predation on both larvae and adult beetles may have contributed to the reductions.

Adult elmids had significantly higher numbers than controls in ponds receiving 35.3 ppb tebuconazole prior to pesticide application. Gyrinidae larvae were significantly greater at 6.9 ppb during week 3.

Hydrophilidae larvae were the most numerous beetle collected on artificial substrates. Hydrophilidae (primarily genus *Berosus*) populations were generally higher in 11.7 and 35.3 ppb ponds when compared to control ponds. High densities were maintained in these ponds throughout the study. These differences were never statistically significant, however high coefficients of variation may have contributed to the lack of statistical significance. The densities observed in the high rate (149 ppb) ponds were similar to those observed in control ponds and suggests that the trends exhibited are not treatment related.

There does not appear to be any direct effects of tebuconazole on macroinvertebrates, however in some instances indirect effects may be inferred.

Fish

Survival: The survival of stocked fish was low in all treatments as well as in control ponds (See Fig. 3). Survival ranged from 52 to 67%. The low survival rates evidenced by low numbers of fish did not permit sound conclusions, and could have obfuscated the results from the reproduction portions of the study. However, since the control groups also had low survival it was assumed that reasonable inferences were achievable.

Numbers and Growth: At harvest, the greatest numbers of juvenile fish collected were of the 2-2.9 and 3-3.9 size classes. At the highest dose, the total number of juveniles (i.e. 1 cm to 9.9 cm size classes) was reduced to less than half that of the control group (Fig. 4). The total average number of juveniles at doses 1 and 2 were comparable to numbers collected from the controls.

The number of smaller size-classed juveniles (i.e. 1 cm to 4.9 cm) collected from the highest dosed ponds was lower than the number of juveniles of this size class harvested from either the control ponds or the two lower dosed ponds. Contrastly, in the size classes greater than 5 cm (i.e. 5.0 to 9.9 cm) the number of juveniles collected from treated ponds were greater than the number of juveniles collected from control ponds. A similar trend was also observed for juvenile weight (Fig. 5). With the possible exception of the 14 - 14.9 cm size-class, dose 3 associated fish outweighed control juvenile fish. The highest dose consistently reduced the length and weight of juvenile fish. However the weight of fish in the 7-7.9 cm size-class was significantly (alpha = 0.05) greater than that of controls. Effects at the highest dose (24 - 149 ppb) were expected, because it exceeds the NOEL for the rainbow trout (12 ppb) by a factor of 2X to 12X.

At the middle dose (10 - 35.3 ppb) the number of juveniles was comparable to the control up to the 6 cm to 6.9 cm size class. In the remaining size classes the number of juvenile fish was higher in treated goups than in the control groups. The maximum mean measured concentration of 35.3 exceeded the NOEL (12 ppb) for fish reproduction. Effects at this dose are unclear. It is possible that some bluegill reproduction had already commenced prior to the first reported nest activity observations (week 1) which coincided with the initial application. Any treatment effects on fecundity, hatching success, or embryo survival beyond week 1 would be obfuscated.

At dose 2 juvenile weight was comparable with that of the controls with the exception of size-classes 7-7.9 and 8-8.9 where fish weight was higher among the treated fish.

At the lowest tested dosage (2.5 - 11.7 ppb) the total number of juvenile fish was comparable to the control group as well as the median dosed group. Within juvenile fish size-classes less than 5 cm, numbers of fish at dose 1 were less than in the control, but greater than in either dose 2 or dose 3. This trend was reversed for fish greater than 5 cm.

Observations of the nests

The overall frequency of active nest sightings did not differ significantly among control and treatment groups (Fig. 6). However when the frequency of zero activity occurrences (i.e. no activity on the nest) exhibted in fig. 6 were summed and compared, a clear dose related trend was evident (fig 7). The frequency of zero nesting activity at treatment level 3 was approximately half that of the control. While there was no statistical significance, there could be biological significance.

Residues

The loading procedures used in this study followed the protocol and were acceptable. The procedures used in water and sediment sampling along with chemical analysis were appropriate. However, LYNX concentrations found in tank mixtures were erroneous with Coefficient of Variation values of up to 24%.

Residues of tebuconazole in fish increased with increasing dose (Fig. 8). Average residues ranged from 0.095 ppm in dose 1 (11.7 ppb dose) to 2.45 ppm in dose 3 (149 ppb dose). Using these maximum mean measured concentrations, the body residues represent bioconcentraion factors of 6X to 13X, respectively, which approximates the sediment to water partitioning ratios (6X to 18X).

9. **Conclusions:**

Overall tebuconazole was shown to have mostly indirect effects on invertebrates, and in most cases these affects were transient and moderate. There was little statistical significance found among the study endpoints of fish survival or juvenile growth, however this may be due to immoderately high coefficients of variation.

For phytoplankton, the highest tested dose resulted in higher chlorophyll a measurements than in the control. Although no causal relationship to tebuconazole exposure is explainable, the impacts on chlorophyll a production appear to be more than random events. The community structure of phytoplankton was also altered by tebuconazole.

Four weeks following the final application, periphyton chlorophyll a was higher at 149 ppb than in the control. Although not statistically significantly, a qualitative assessment suggests that the greater chlorophyll a production in treated ponds is due to the reduction or eradication of certain predator species, which would result in an increased amount of periphyton biomass and associated chlorophyll production. Because chlorophyll a and total biomass results did not correlate, no clear inferences of tebuconazole effects on periphyton biomass were possible. Tebuconazole effects on periphyton community metabolism were transient.

The major groups of zooplankton isolated were protozoans, rotifers, and crustaceans. Direct effects on zooplankton by tebuconazole were unlikely, however indirect effects were probable. Specifically, Indirect effects of tebuconazole on protozoans was likely at the highest test concentration. The trend observed for increased numbers of rotifera could have been due to indirect effects of tebuconazole, primarily by reductions of young bluegill populations. The affect of tebuconazole on juvenile fish numbers likely contributed to the increase observed in the copepod population. No clear impact of tebuconazole on the zooplankton community was evident.

Macroinvertebrates do not appear to have been directly effected by exposure to tebuconazole. There were instances however where indirect effects may have occurred. The numbers of Chaoboridae and chironomidae larvae were lower in treated ponds than in controls during week 15, and there were increased numbers of specific invertebrate taxa at 149 ppb.

Adult and juvenile fish survival was affected at the highest dose of tebuconazole. Effects were observed at the middle dose (10.2 to 35.3 ppb), which is comparable to the rainbow trout NOEC of 12 ppb. The effects observed at this dose are difficult to interpret, because the highest survival and the highest number of juvenile fish were observed at this dose. However bluegill reproduction may have commenced prior to the initial test application. Inestimable treatment effects on fecundity, hatching success, or embryo survival beyond week 1 coupled with low survival of stocked adults make interpretation of the reproduction results difficult.

Fish nesting per se was not dramatically altered by tebuconazole. However, the frequency of observations in which there was no spawning or nesting activity was dose related. The highest treatment level (1020 ppb in sediment and 149 ppb in water) had twice the number of zero nesting observances as the control group.

Residues of tebuconazole were still evident in bluegill at study termination. This phenomenon along with the persistence of tebuconazole in sediment causes some concern for the aquatic ecosystem if it is consistently tainted with residues of this fungicide.

Tebuconazole exhibited effects on specific invertebrates and fish at a nominal dose of 40 ppb which resulted in maximum mean measured concentrations of 149 ppb in water following 4 applications at 2 week intervals. Tebuconazole did accumulate in sediment as well as fish, and potentially impacts fish growth, reproduction, and survival.

10. **Executive Summation:**

- Tebuconazole altered juevnile fish growth and survival at the highest mesocosm test concentrations which ranged from 24.4 to 149 ppb in water. The two highest mesocosm test levels equaled or exceeded laboratory test chronic NOECs for fish and invertebrates (12 to 120 ppb). Although EECs (see Risk Assessment by C. Rodriguez, 1994, D198525) in water are not expected to exceed 12 ppb, residues in sediment may range in concentration from 10 to 1020 ppb. Effects to non-target organisms associated with sediment have not been adequately investigated, therefore sediment-related risk is undetermined at this time. The effects of high residue loads in sediment on non-target organisms should be investigated.

- Tebuconazole effects on invertebrates (i.e. periphyton, phytoplankton, and macroinvertebrates) were indirect and biologically significant at the highest treatment level, but were transient and moderate. Diversity of macroinvertebrates were effected at doses 2 (sediment = 10 to 370 ppb; Water = 10.2 to 35.3) and 3 (sediment = 10 to 1020 ppb; Water = 24.4 to 149 ppb).

If you have any questions with regards to this review, please contact Henry Craven (703-305-5320) or Tom A. Bailey (703-305-6666).

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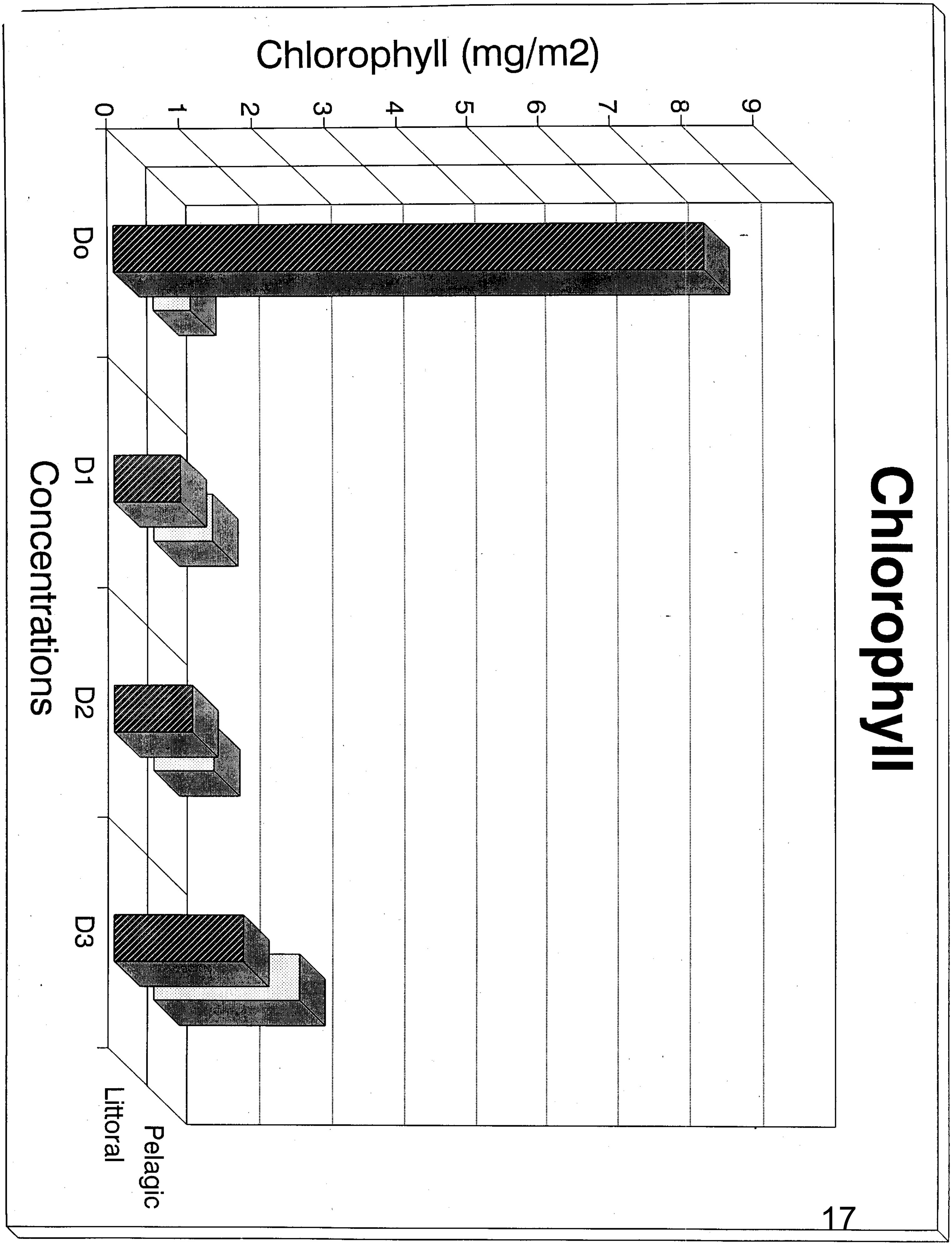
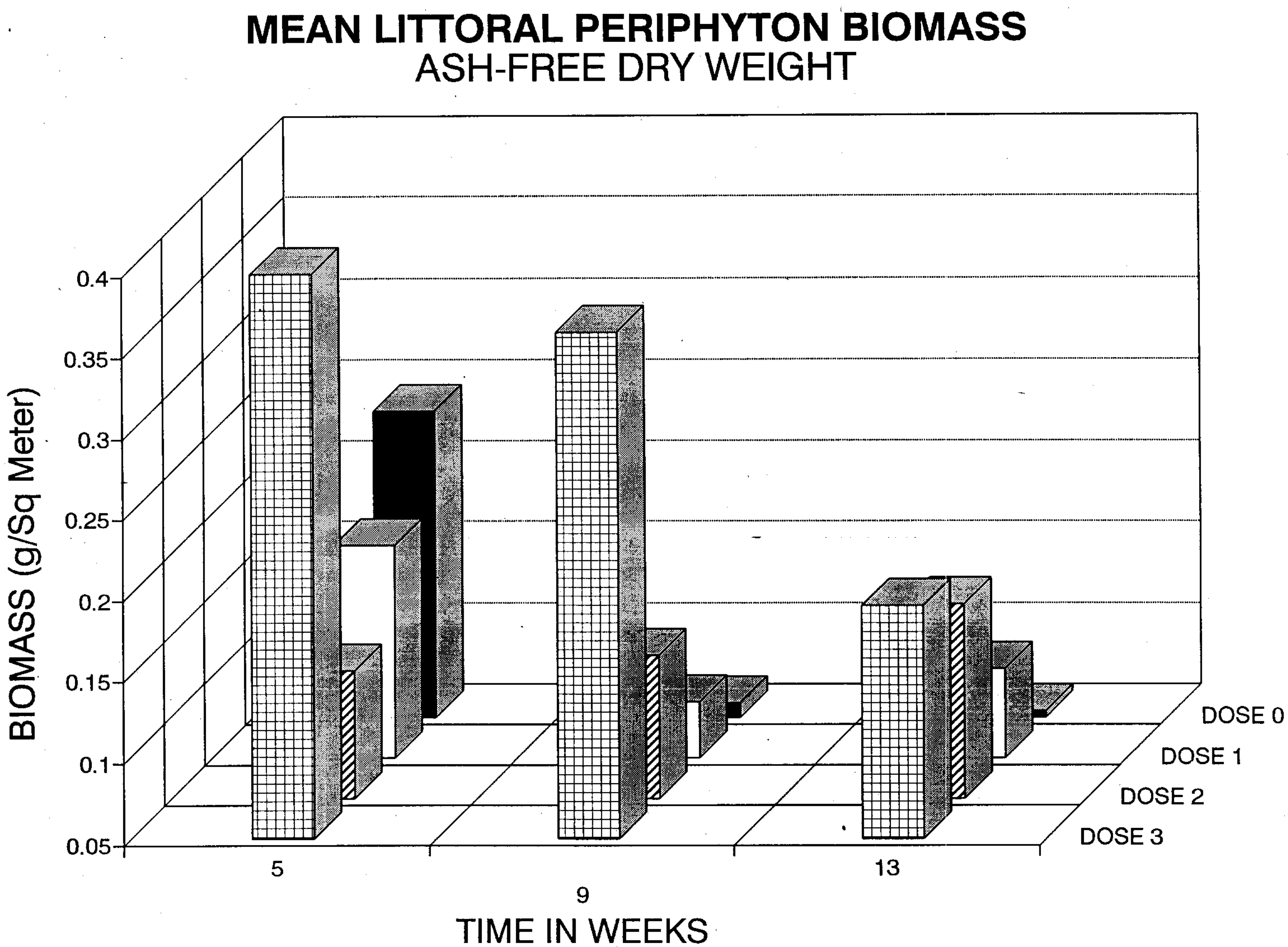
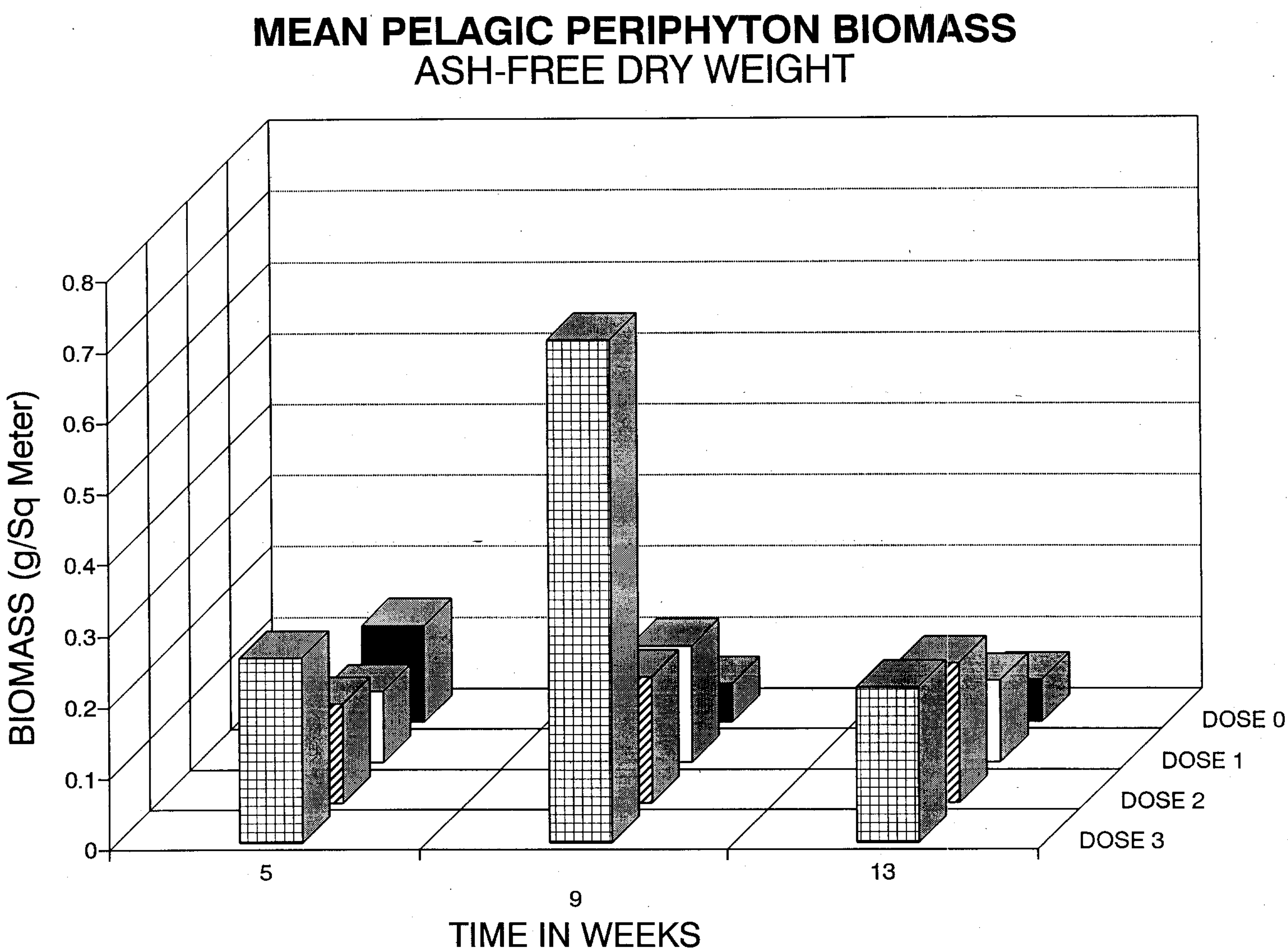
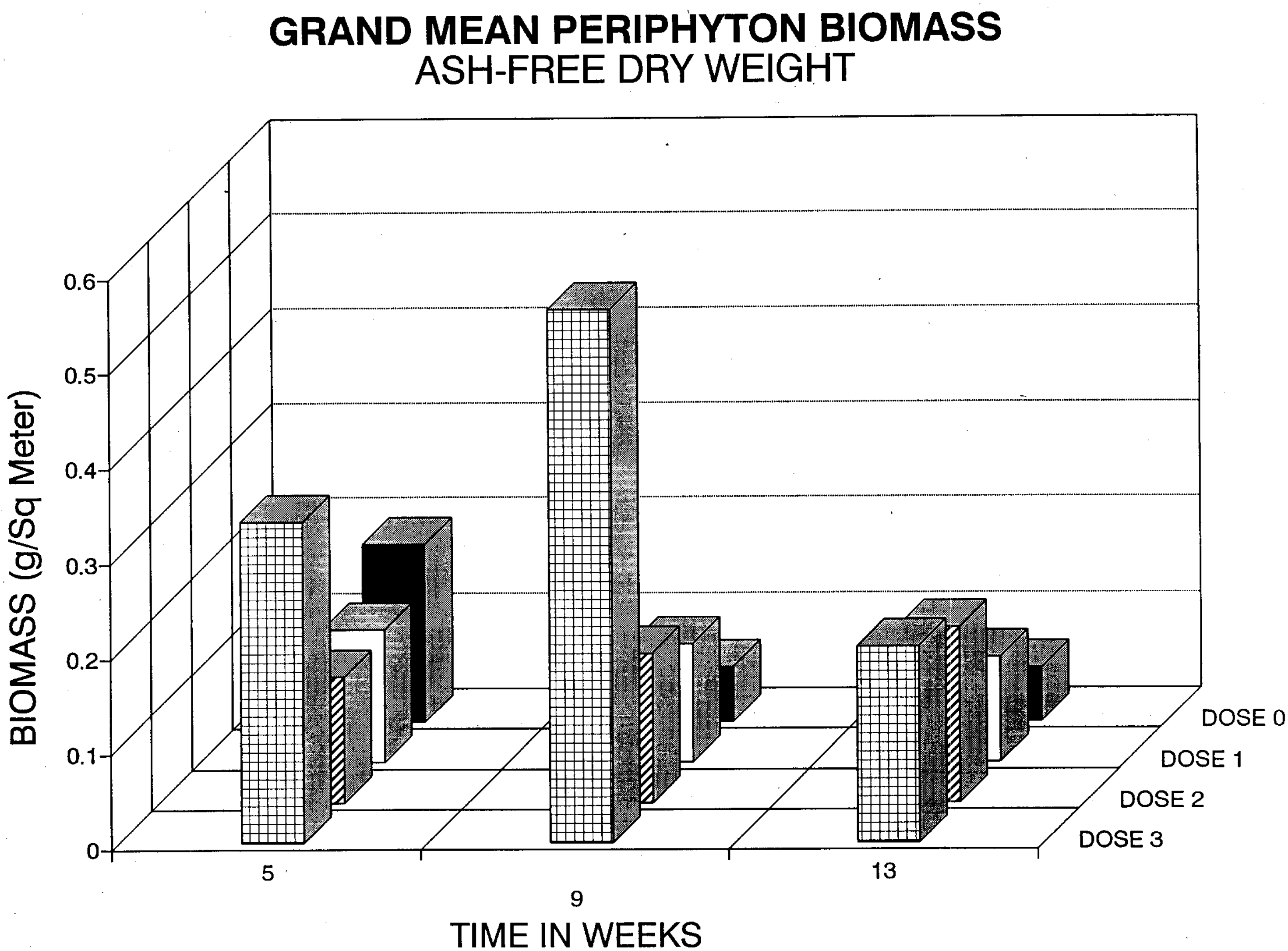
FIGURE 1

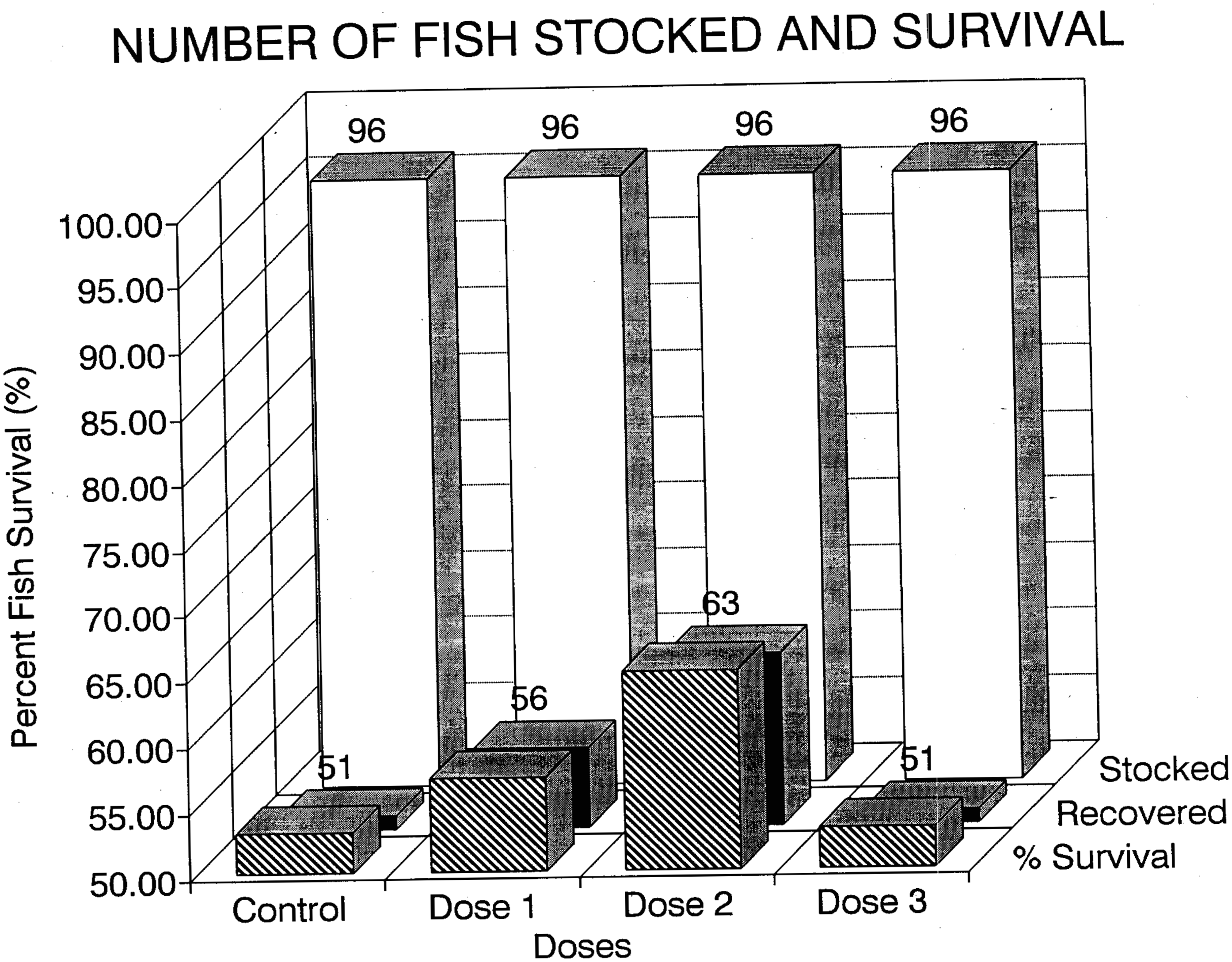


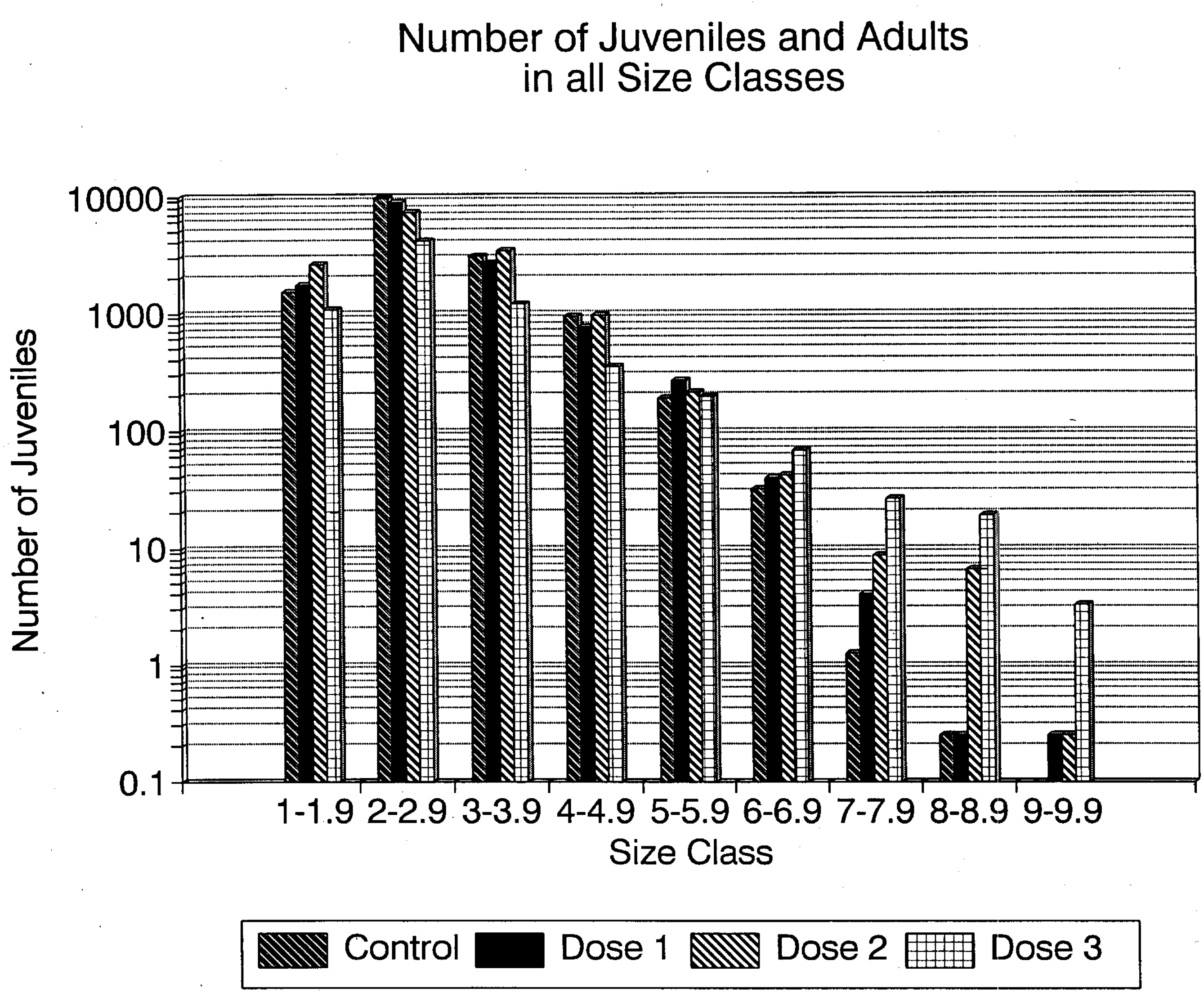
Chlorophyll
Chlorophyll (mg/m2)
0
1
2
3
4
5
6
7
8
9
Do
D1
D2
D3
Concentrations
Pelagic
Littoral


MEAN LITTORAL PERIPHYTON BIOMASS
ASH-FREE DRY WEIGHT
BIOMASS (g/Sq Meter)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
5
9
13
TIME IN WEEKS
DOSE 0
DOSE 1
DOSE 2
DOSE 3

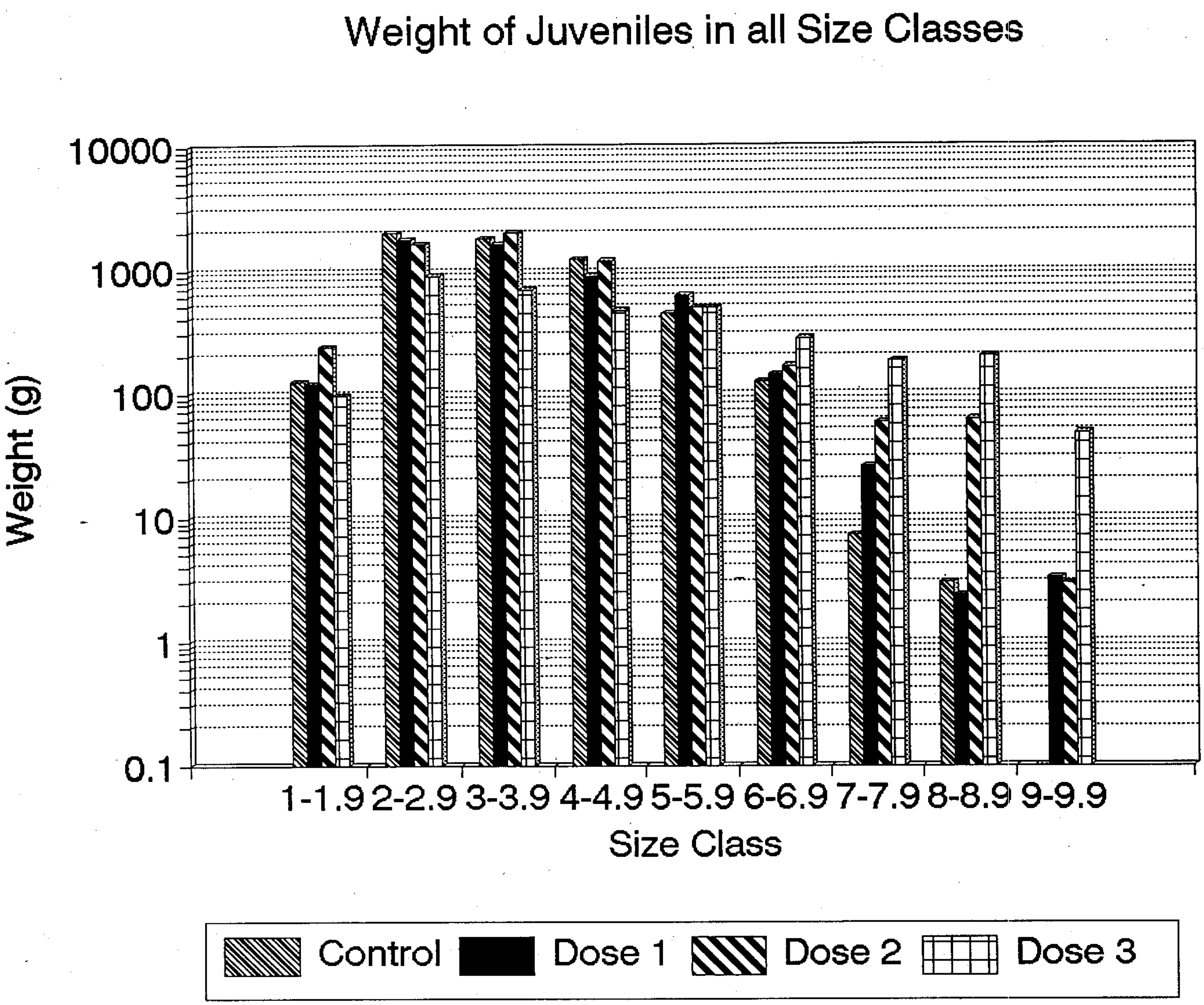

MEAN PELAGIC PERIPHYTON BIOMASS
ASH-FREE DRY WEIGHT
BIOMASS (g/Sq Meter)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
9
13
TIME IN WEEKS
DOSE 0
DOSE 1
DOSE 2
DOSE 3


GRAND MEAN PERIPHYTON BIOMASS
ASH-FREE DRY WEIGHT
BIOMASS (g/Sq Meter)
0.6
0.5
0.4
0.3
0.2
0.1
0
DOSE 0
DOSE 1
DOSE 2
DOSE 3
5
9
13
TIME IN WEEKS

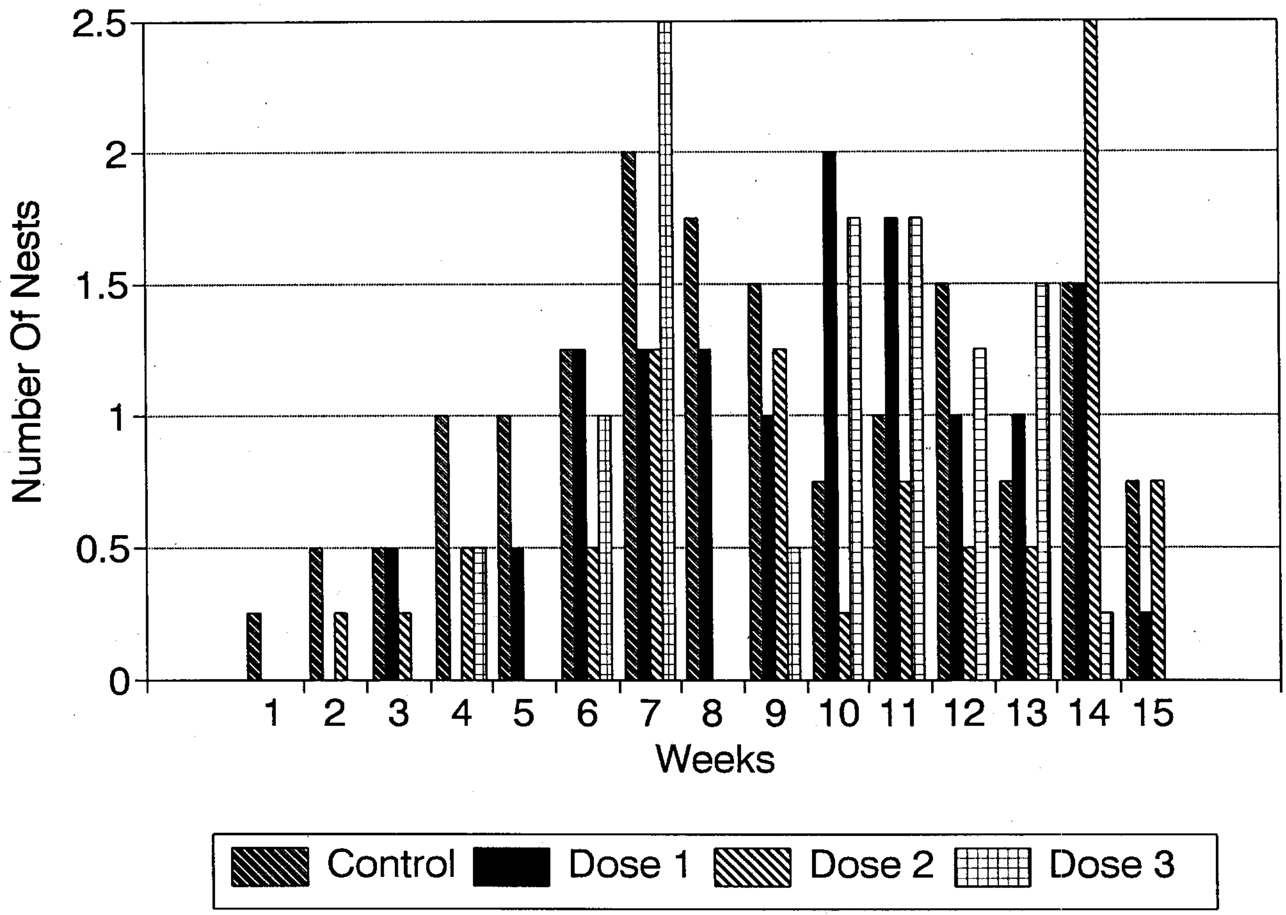

NUMBER OF FISH STOCKED AND SURVIVAL
96
96
96
96
63
56
51
51
100.00
95.00
90.00
85.00
80.00
75.00
70.00
65.00
60.00
55.00
50.00
Percent Fish Survival (%)
Control
Dose 1
Dose 2
Dose 3
Doses
Stocked
Recovered
% Survival

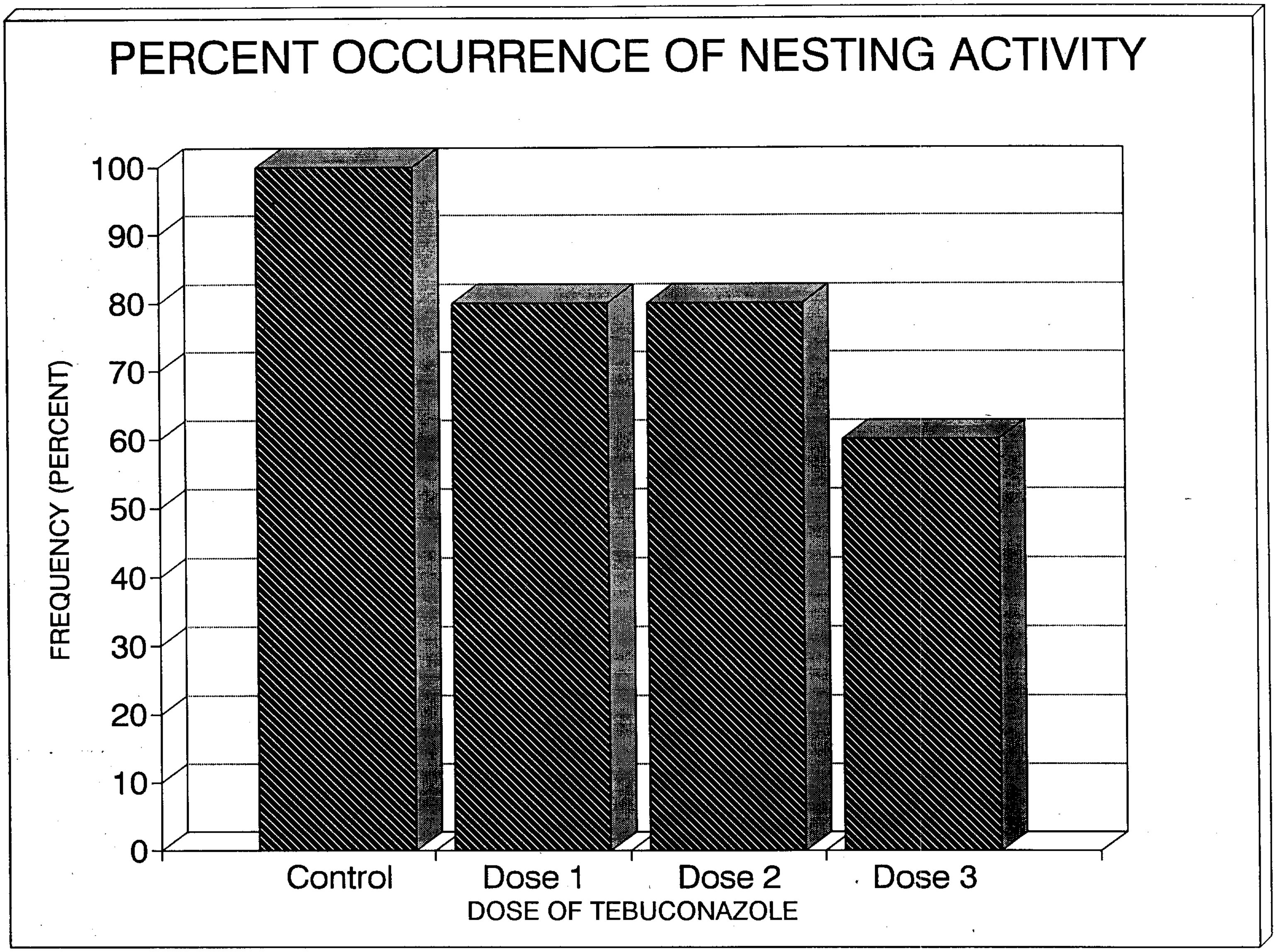


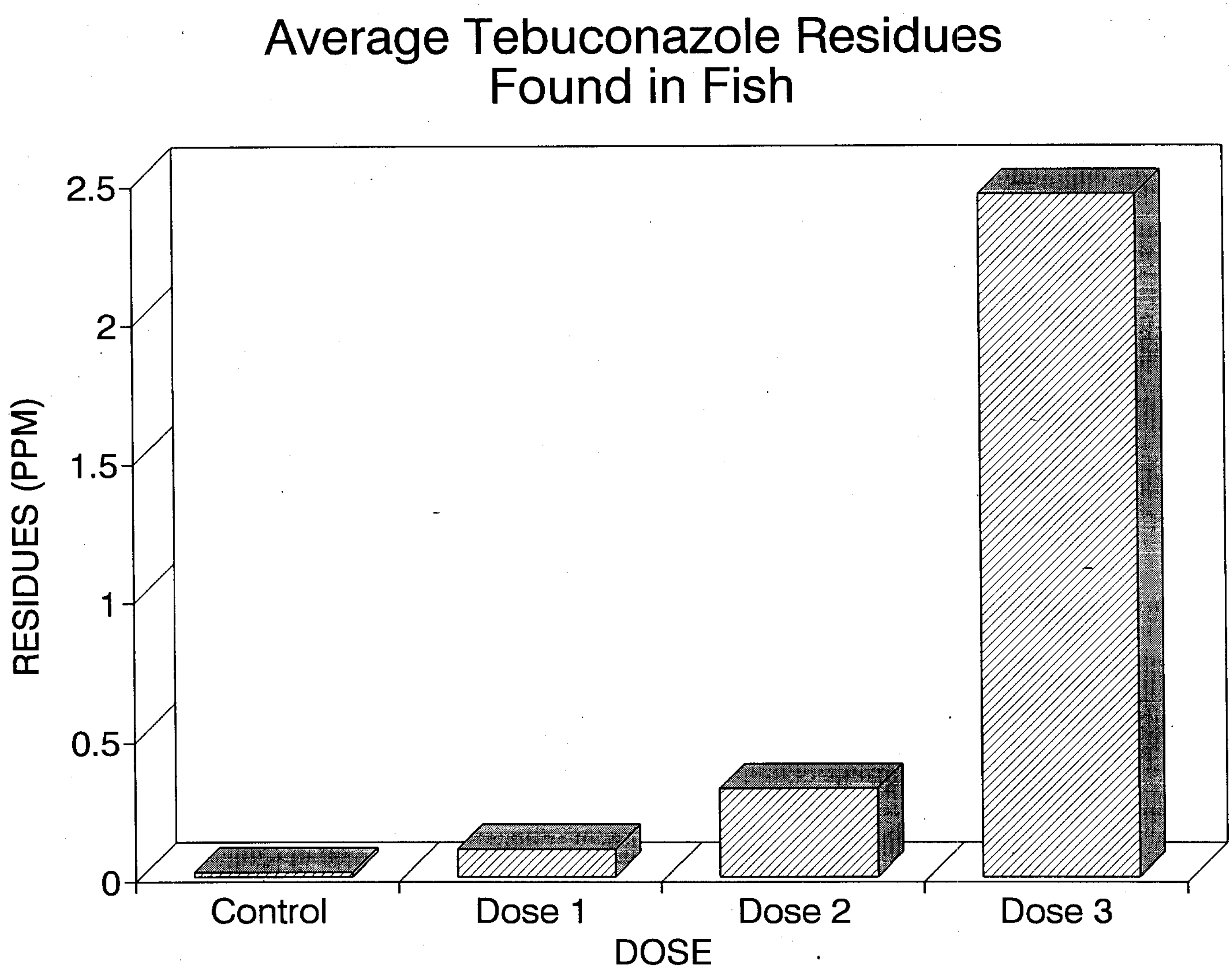
Number of Juveniles and Adults
in all Size Classes
10000
1000
100
10
1
0.1
Number of Juveniles
1-1.9
2-2.9
3-3.9
4-4.9
5-5.9
6-6.9
7-7.9
8-8.9
9-9.9
Size Class
Control
Dose 1
Dose 2
Dose 3

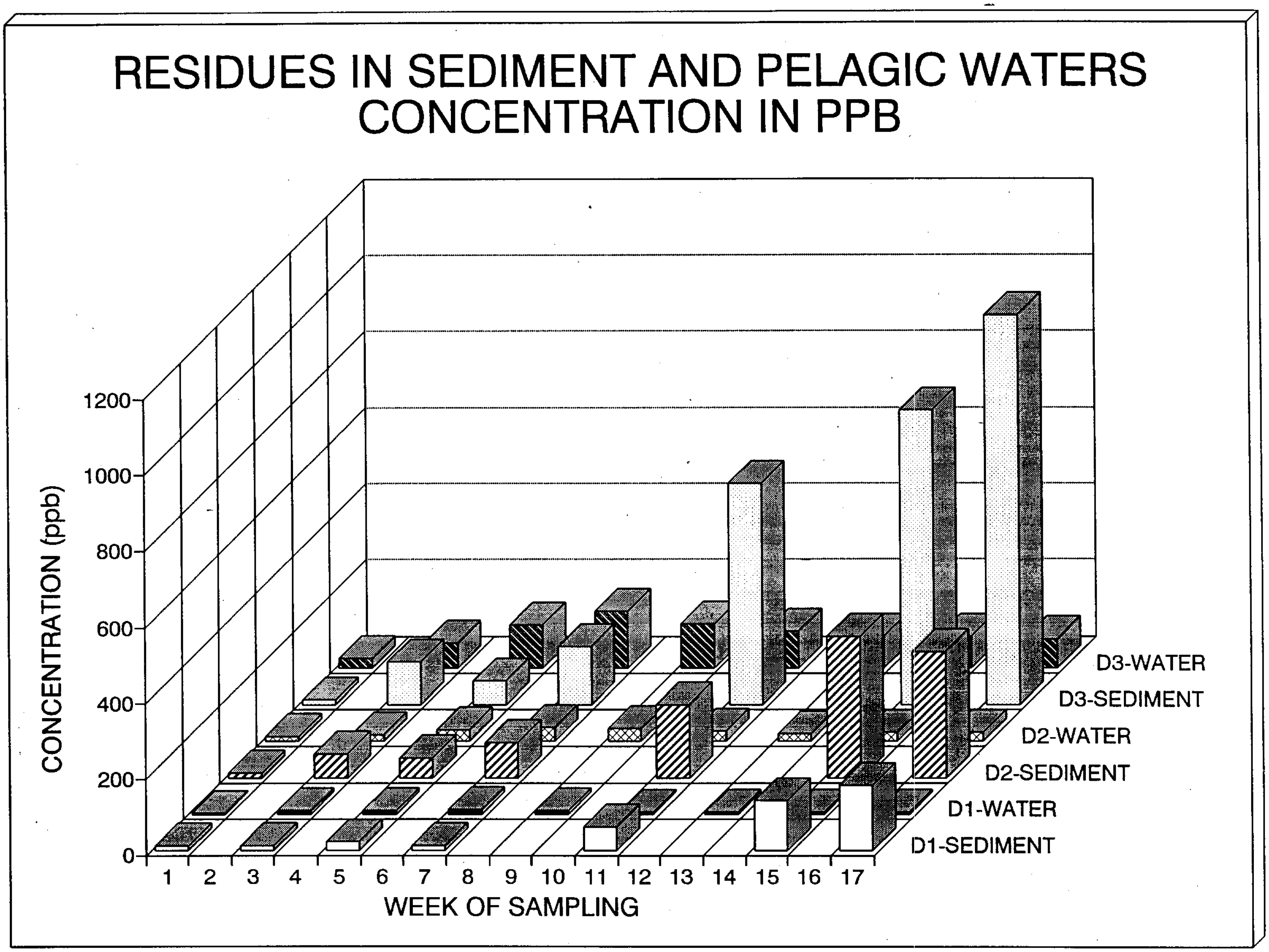

Weight of Juveniles in all Size Classes
Weight (g)
10000
1000
100
10
1
0.1
1-1.9
2-2.9
3-3.9
4-4.9
5-5.9
6-6.9
7-7.9
8-8.9
9-9.9
Size Class
Control
Dose 1
Dose 2
Dose 3

NUMBER OF NESTS PER REPLICATE




PERCENT OCCURRENCE OF NESTING ACTIVITY
FREQUENCY (PERCENT)
100
90
80
70
60
50
40
30
20
10
0
Control
Dose 1
Dose 2
Dose 3
DOSE OF TEBUCONAZOLE


Average Tebuconazole Residues
Found in Fish
RESIDUES (PPM)
2.5
2
1.5
1
0.5
0
Control
Dose 1
Dose 2
Dose 3
DOSE


RESIDUES IN SEDIMENT AND PELAGIC WATERS
CONCENTRATION IN PPB
CONCENTRATION (ppb)
0
200
400
600
800
1000
1200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
WEEK OF SAMPLING
D3-WATER
D3-SEDIMENT
D2-WATER
D2-SEDIMENT
D1-WATER
D1-SEDIMENT

Appendix 1. Residue data for tebuconazole measured in aquatic mesocosm ponds

WK NO.	RESIDUES IN SEDIMENT				BACKGROUND RESIDUES				RESIDUES (LITTORAL)				RESIDUES (PELAGIC)			
	D0	D1	D2	D3	D0	D1	D2	D3	D0	D1	D2	D3	D0	D1	D2	D3
1	10	10	10	10	0.5	0.5	0.5	0.5					0.5	2.5	10.2	24.4
2																
3	10	10	60	110	0.5	3.9	13.9	66.7	0.5	11.7	24.9	93.1	0.5	6.9	15.2	63
4																
5	10	20	50	60	1.01	5.5	17.1	131.3	0.5	11.1	31	131.3	0.5	9.3	27.6	111.2
6																
7	10	10	90	150	0.5	7.1	20.8	81.2	0.5	9.2	30.6	120	0.5	10	35.3	149
8									0.5			105	0.5			109.8
9									0.5	6.5	31.5	110.9	0.5	7.3	30.8	114.8
10																
11	10	60	190	580					0.5	5.8	24.2	95	0.5	5.9	26.4	94.5
12																
13									0.5	4.6	18.8	74	0.5	5.1	19.4	79.7
14																
15	10	130	370	770					0.5	4.7	20.5	78.9	0.6	4.9	20.8	79.6
16																
17	10	170	330	1020					0.5	5.2	20.2	73.1	0.5	4.8	19.9	75.6