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

11-28-94

NOV 28 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Mancozeb (014504) data review (FELS)

From: Anthony F. Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 11/28/94

To: Walter Waldrop, PM 71
Special Review and Reregistration Division (7508W)

EEB has completed the fish early life-stage study submitted by the Mancozeb task force. The following is a brief summary of the data reviewed:

CITATION: Rhodes, Downing and Bielefeld. 1994. Early life-stage toxicity of Mancozeb to the Fathead Minnow (*Pimephales promelas*) under flow-through conditions. Project Final Report #41148. Study conducted by ABC Laboratories, Inc., Columbia, MO. Submitted by Mancozeb Task Force, Washington, DC. EPA MRID No. 432307-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. Based on the most sensitive endpoint (survival) evaluated in this fathead minnow early life-stage toxicity study, the MATC for Mancozeb is 3.16 µg/L as measured by gas chromatography. Based on survival and the lack of growth effects, the NOEC is 2.19 µg/L and the LOEC is 4.56 µg/L as measured by gas chromatography.

If there are any questions contact Renee Lamb at 305-5294.

DATA EVALUATION RECORD

CHEMICAL: Mancozeb; Shaughnessey No. 014504

TEST MATERIAL: Mancozeb, 79.3% radiopurity, a yellow powder.

STUDY TYPE: 72-4. Freshwater Fish Early Life-Stage Test. Species Tested: Fathead Minnow (*Pimephales promelas*).

CITATION: Rhodes, Downing and Bielefeld. 1994. Early life-stage toxicity of Mancozeb to the Fathead Minnow (*Pimephales promelas*) under flow-through conditions. Project Final Report #41148. Study conducted by ABC Laboratories, Inc., Columbia, MO. Submitted by Mancozeb Task Force, Washington, DC. EPA MRID No. 432307-01.

REVIEWED BY:

Renee Costello
Biologist
EFED/EEB

Signature: Renée Costello
Date: 6/23/94

APPROVED BY:

for Ann Stavola
Supervisory biologist
EFED/EEB

Signature: Allen W. Vaughan
Date: 11.23.94

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. Based on the most sensitive endpoint (survival) evaluated in this fathead minnow early life-stage toxicity study, the MATC for Mancozeb is 3.16 µg/L as measured by gas chromatography. Based on survival and the lack of growth effects, the NOEC is 2.19 µg/L and the LOEC is 4.56 µg/L as measured by gas chromatography.

MATERIALS AND METHODS:

Test Animals: Newly fertilized eggs (*Pimephales promelas*), ≤ 24 hours old, were obtained from an in-house culture. The study was initiated when 30 impartially selected eggs were placed into incubation cups.

Test System: The test system consisted of a 2-L proportional diluter system which intermittently delivered a solution of mancozeb and control dilution water to replicate test chambers. Flow-splitting and mixing cells divided the dilution water control and each of the seven test concentrations into two aliquots before they; were delivered to duplicate test aquaria. Each of these was again divided in half before delivery to replicate test chambers. Flow of dilution water and proper

operation of the proportional diluter and all mechanical systems was verified at least twice each day.

The test vessels were glass aquaria each divided into two replicate chambers with a glass partition. Individual chambers measured 15.7 x 31 cm with a water depth of 25 cm, yielding a volume of ≈ 12.2 L. Test aquaria were randomly placed in a thermostatically heated waterbath.

Developing embryos were incubated in glass cups constructed from 9-cm diameter flint glass jars with 40-mesh Nytex screen replacing the bottom.These cups were suspended int he test chambers and oscillated vertically to facilitate test solution circulation. Developing embryos were kept in semi-darkness until hatch was nearly complete. At that time all aquaria were illuminated with wide-spectrum fluorescent lighting. Sixteen hours of light with a simulated dawn-to-dusk transition period was provided.

The dilution water was obtained from a deep well and screened for contaminants. Hardness ranged from 140 to 160 mg/L and pH ranged from 7.47 to 7.93 as measured in a single replicate of the dilution water control. Over the course of the study, control dilution water and test solutions were delivered to each chamber an average of 84.7 L/day, a rate sufficient to replace the 12-L chamber volume an average of 7.1 times in a 24 hour period. As the study progressed, flow rate was increased to maintain water quality and reduce biomass loading. The last 7 days of the study, a 90.9 L/rep/day flow rate provided 7.6 volume replacements per day. At this flow rate, a maximum biomass loading of 0.0635 g/L/day at study termination was calculated in a single replicate of level 2.

Water quality measurements, temperature, DO, conductivity, pH, hardness and alkalinity were measured on days 0, 1, 7, 14, 21, 28, and 34.

Due to the low water and organic solvent solubility of mancozeb, as well as its rapid rate of hydrolysis, an electronically controlled dosing system was used to prepare and deliver test solutions. Mancozeb was weighed into 90° polypropylene elbow that was attached to a solenoid valve immediately below a 1-L reservoir of dilution water. At six hour intervals, the valve would open and flush the chemical into the mixing jar containing 3 L of dilution water being continuously stirred. After an additional hour of stirring, a second valve would open for six hours and feed the stock solution into a manifold system which in turn was fed the diluter mixing cell.

Dosage: Nominal mancozeb concentrations tested were 0.30, 0.60, 1.3, 2.5, 5.0, 10, and 20 µg/L along with a dilution water control.

Design: Developing embryos were observed daily for mortality. Dead eggs were removed each day. Positive counts of the number of embryos present were made on test initiation and on days 2 and 3. The number of larvae were estimated from the cumulative egg mortality and observed larval mortality.

Observations of abnormal behavior, abnormal physical change, and mortality were recorded daily by visual inspection, Dead fry wee removed. Cumulative mortality estimates were based on fry mortality.

Fry were fed live rotifers and live brine shrimp soon after hatch began. Growing fry were fed *ad libitum* three times a day at 4 hour intervals during the week and twice a day on the weekends at the same intervals. Food was withheld 24 hours before study termination.

Fry growth, standard length and blotted wet weight, was determined on day 28 post-hatch.

Statistics: A nested experimental design was used. Data recorded on a discrete scale (egg hatchability and survival data) were analyzed using contingency table methods. Results were pooled prior to analysis from the chambers within the nested aquaria and aquaria nested within concentrations. Pairwise comparisons between the control and each test concentration were performed using a frequency analysis based on a chi-square estimate from the two-way contingency tables.

Data recorded on a continuous scale (standard length and blotted wet weight) were analyzed using ANOVA. Dunnett's was used to assess differences between the control and treatment levels.

REPORTED RESULTS: Mean measured concentrations (LSC) during the biological exposure period were 0.236, 0.535, 1.08, 2.37, 4.65, 9.57, and 19.0 µg/L. These values were 79 to 96% of the nominal test concentrations of 0.30, 0.60, 1.3, 2.5, 5.0, 10, and 20 µg/L. Mancozeb concentrations were measured by GC in the top 5 concentrations. Mean measured concentrations were 0.592, 1.07, 2.19, 4.56, and 7.97 µg/L. These values ranged from 40 to 46% of nominal. Recoveries of mancozeb fortification samples ranged from 78 to 91%.

DO was measured in each replicate of each treatment of days 0, 7 and weekly thereafter, including study termination day. DO ranged from 6.7 to 8.3 mg/L, representing 85 - 105% of saturation. Mean percent saturation was > 94% in all treatments.

Water temperature was measured in each replicate of each treatment of days 0, 7 and weekly thereafter, including study termination day. Temperature ranged from 24.2 to 25.8°C. The average across all treatments ranged from 24.9 to 25.0 °C.

Continuous temperature measurement in a centrally located chamber, monitored by an electronic datalogger, indicated no deviations from the 25 ± 2°C range.

pH, conductivity, hardness, and alkalinity were measured in a single replicate of the control, low, and high concentrations at study initiation and weekly thereafter. pH ranged from 7.47 to 7.94, with test solution measurements generally consistent with those of the dilution water control. Conductivity ranged from 331 to 376 µS, and hardness and alkalinity ranged form 140 to 172 mg/L and 140 to 172 mg/L, respectively.

Egg hatch began on day 3 and was complete by day 8. Percent hatch is based on cumulative egg mortality and the estimated number of larvae at the end of hatch. A single embryo in the A replicate of level 7, the B replicate of levels 2 and 7, and the C replicate of the control developed but had not hatched by day 8. These were removed when the larvae were released into the chamber. The initial number of eggs placed was adjusted to account for these unhatched eggs. Overall percent hatch represents the pooled results from chambers nested within aquaria and aquaria nested within concentrations.

Overall hatching success in the control and test levels was 84.9, 87.5, 95.8, 87.5, 90.0, 93.4, 89.2, and 92.4%, respectively. Within individual test chambers, egg hatchability ranged from 73.3 to 100%. No significant reduction between the control and treatments were noted.

Overall percent survival was 87.1, 97.1, 91.2, 94.3, 93.5, 91.1, 62.6, and 22.9%, respectively in the control and test levels. A significant reduction in survival in the 9.57 and 19.0 test concentrations when compared to the control. One fish from the C replicate of level 1, escaped into the A replicate of level 6 during termination. This fish was included in the total number present in level 1, replicate C at termination.

Mean blotted wet weight in the control and each test level was 0.211, 0.205, 0.204, 0.195, 0.206, 0.199, 0.184, and 0.131 g, respectively. ANOVA and Dunnett's indicated no significant reduction in blotted wet weight at any concentration up to 4.65 when compared to the control. The 9.57 and 19.0 concentrations were eliminated from the analysis due to significant survival effects.

Physical abnormalities included spinal curvature. This was observed in 4 individuals in the 9.57 treatment level and 2 individuals in the 19.0 treatment level. The two in this level were observed resting on the bottom of the test chamber.

STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Egg hatchability was not significantly reduced by Mancozeb at any

test concentration. Survival was significantly reduced by Mancozeb concentrations of 9.57 and 19.0 µg/L. Growth, as measured by standard length and blotted wet weight, exhibited no significant reduction after exposure to Mancozeb.

Based on the most sensitive endpoint (ie survival) evaluated in this fathead minnow early life-stage toxicity study, the MATC for Mancozeb is 3.16 µg/L as measured by gas chromatography. Based on survival and the lack of growth effects, the NOEC is 2.19 µg/L and the LOEC is 4.56 µg/L as measured by gas chromatography."

A GLP compliance statement was included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 160). The report also included a quality assurance statement which was signed by a representative of the laboratory's quality assurance unit.

REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Test Procedure: The test procedure is generally in accordance with the SEP and ASTM guidelines.

Statistical Analysis: Individual length and weight data were analyzed using a 2-way ANOVA coupled with Dunnett's test for treatment comparisons (printouts, attached). It should be noted that growth data were individual measurements; however, the author statistically analyzed these data using a one-way ANOVA and the replicate means. Also, the two highest treatment levels were excluded from length and weight analysis due to low survival at 28 days post-hatch. Based on GC measured concentrations (except for the two lowest treatment levels where the nominal was used) the NOEC was calculated by the reviewer to be 2.19 µg/L; the LOEC = 4.56 µg/L, based on the most sensitive parameter, survival. The MATC is 3.16 µg/L.

Discussion/Results: This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. Based on the most sensitive endpoint (survival) evaluated in this fathead minnow early life-stage toxicity study, the MATC for Mancozeb is 3.16 µg/L as measured by gas chromatography. Based on survival and the lack of growth effects, the NOEC is 2.19 µg/L and the LOEC is 4.56 µg/L as measured by gas chromatography.

Adequacy of the Study:

(1) **Classification:** Core

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

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

mancozeb percent egg hatch
File: b:HATCH. Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	73.300	90.000	84.925
2	0.30	4	80.000	96.700	87.500
3	0.60	4	86.200	100.000	95.725
4	0.592	4	83.300	93.300	87.475
5	1.07	4	80.000	96.700	90.000
6	2.19	4	86.700	96.700	93.350
7	4.56	4	83.300	93.300	89.150
8	7.97	4	86.200	96.700	92.325

mancozeb percent egg hatch
File: b:HATCH. Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	62.282	7.892	3.946
2	0.30	54.927	7.411	3.706
3	0.60	42.743	6.538	3.269
4	0.592	25.056	5.006	2.503
5	1.07	51.927	7.206	3.603
6	2.19	22.223	4.714	2.357
7	4.56	17.630	4.199	2.099
8	7.97	27.563	5.250	2.625

mancozeb percent egg hatch
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	353.929	50.561	1.329
Within (Error)	24	913.050	38.044	
Total	31	1266.979		

Critical F value = 2.42 (0.05,7,24)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

mancozeb percent egg hatch
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	84.925	84.925		
2	0.30	87.500	87.500	-0.590	
3	0.60	95.725	95.725	-2.476	
4	0.592	87.475	87.475	-0.585	
5	1.07	90.000	90.000	-1.164	
6	2.19	93.350	93.350	-1.932	
7	4.56	89.150	89.150	-0.969	
8	7.97	92.325	92.325	-1.697	

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

mancozeb percent egg hatch
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DUNNETTS 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	4			
2	0.30	4	10.816	12.7	-2.575
3	0.60	4	10.816	12.7	-10.800
4	0.592	4	10.816	12.7	-2.550
5	1.07	4	10.816	12.7	-5.075
6	2.19	4	10.816	12.7	-8.425
7	4.56	4	10.816	12.7	-4.225
8	7.97	4	10.816	12.7	-7.400

mancozeb standard length
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	22.800	23.800	23.275
2	0.30	4	22.500	24.000	23.400
3	0.60	4	22.500	23.400	23.125
4	0.592	4	22.600	23.300	22.975
5	1.07	4	22.900	23.900	23.325
6	2.19	4	22.500	24.100	23.175

mancozeb standard length
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.182	0.427	0.214
2	0.30	0.407	0.638	0.319
3	0.60	0.182	0.427	0.214
4	0.592	0.089	0.299	0.149
5	1.07	0.216	0.465	0.232
6	2.19	0.489	0.699	0.350

mancozeb standard length
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.469	0.094	0.360
Within (Error)	18	4.697	0.261	
Total	23	5.166		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

mancozeb standard length
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	23.275	23.275		
2	0.30	23.400	23.400	-0.346	
3	0.60	23.125	23.125	0.415	
4	0.592	22.975	22.975	0.830	
5	1.07	23.325	23.325	-0.138	
6	2.19	23.175	23.175	0.277	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

mancozeb standard length
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DUNNETTS 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	4			
2	0.30	4	0.871	3.7	-0.125
3	0.60	4	0.871	3.7	0.150
4	0.592	4	0.871	3.7	0.300
5	1.07	4	0.871	3.7	-0.050
6	2.19	4	0.871	3.7	0.100

mancozeb 28 day post hatch survival
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	80.800	96.300	87.025
2	0.30	4	93.100	100.000	97.275
3	0.60	4	83.300	96.600	91.300
4	0.592	4	85.200	100.000	94.525
5	1.07	4	87.500	100.000	93.200
6	2.19	4	88.500	93.100	91.050
7	4.56	4	51.900	78.600	62.525
8	7.97	4	10.300	40.000	23.600

mancozeb 28 day post hatch survival
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	43.683	6.609	3.305
2	0.30	11.303	3.362	1.681
3	0.60	32.193	5.674	2.837
4	0.592	49.849	7.060	3.530
5	1.07	35.820	5.985	2.992
6	2.19	5.317	2.306	1.153
7	4.56	140.476	11.852	5.926
8	7.97	165.900	12.880	6.440

mancozeb 28 day post hatch survival
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	17876.355	2553.765	42.164
Within (Error)	24	1453.620	60.567	
Total	31	19329.975		

Critical F value = 2.42 (0.05,7,24)
Since F > Critical F REJECT Ho:All groups equal

mancozeb 28 day post hatch survival
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	87.025	87.025		
2	0.30	97.275	97.275	-1.863	
3	0.60	91.300	91.300	-0.777	
4	0.592	94.525	94.525	-1.363	
5	1.07	93.200	93.200	-1.122	
6	2.19	91.050	91.050	-0.731	
7	4.56	62.525	62.525	4.452	*
8	7.97	23.600	23.600	11.525	*

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

mancozeb 28 day post hatch survival
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DUNNETTS 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	4			
2	0.30	4	13.648	15.7	-10.250
3	0.60	4	13.648	15.7	-4.275
4	0.592	4	13.648	15.7	-7.500
5	1.07	4	13.648	15.7	-6.175
6	2.19	4	13.648	15.7	-4.025
7	4.56	4	13.648	15.7	24.500
8	7.97	4	13.648	15.7	63.425

mancozeb wet weight
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.201	0.223	0.212
2	0.30	4	0.188	0.225	0.206
3	0.60	4	0.191	0.220	0.205
4	0.592	4	0.186	0.203	0.196
5	1.07	4	0.196	0.220	0.208
6	2.19	4	0.186	0.225	0.199

mancozeb wet weight
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.000	0.010	0.005
2	0.30	0.000	0.015	0.008
3	0.60	0.000	0.012	0.006
4	0.592	0.000	0.008	0.004
5	1.07	0.000	0.013	0.006
6	2.19	0.000	0.018	0.009

mancozeb wet weight
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0007	0.0001	0.500
Within (Error)	18	0.0031	0.0002	
Total	23	0.0037		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

mancozeb wet weight
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.212	0.212		
2	0.30	0.206	0.206	0.625	
3	0.60	0.205	0.205	0.700	
4	0.592	0.196	0.196	1.625	
5	1.07	0.208	0.208	0.425	
6	2.19	0.199	0.199	1.225	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

mancozeb wet weight
File: B:\MANCOZEB\WEIGHT2. Transform: NO TRANSFORM

DUNNETTS 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	4			
2	0.30	4	0.024	11.4	0.006
3	0.60	4	0.024	11.4	0.007
4	0.592	4	0.024	11.4	0.016
5	1.07	4	0.024	11.4	0.004
6	2.19	4	0.024	11.4	0.012