UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
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February 3, 1988

SUBJECT: Trifluralin: Effect on and accumulation by sheepshead minnows, Cyprinodon variegatus.

FROM: David J. Hansen
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TO: John A. Couch, Ph.D.
Pathobiologist

Henry Craven
Acting Branch Chief, Ecological Effects Branch (TS-769C)

The effects of trifluralin and its breakdown products on sheepshead minnows, Cyprinodon variegatus, were investigated by the Environmental Research Laboratory at Gulf Breeze, Florida. Tests conducted include: an entire life-cycle toxicity test (Parrish et al., 1978); an early life-stage toxicity test with trifluralin and trifluralin III (2,6-dinitro- α,α,α - trifluoro-p-toluidine) (Goodman et al., Manuscript); and acute lethality tests with trifluralin, trifluralin III, and two other breakdown products (2,6-dinitro-N,n-propyl- α,α,α-trifluoro-p-toluidine and 2-amino-6-nitro- α,α,α-trifluoro-p-toluidine) (Goodman et al., Manuscript). In addition, special tests were conducted to examine the vertebral lesion response by sheepshead minnows to trifluralin exposure (Couch et al., 1979 and 1981).

This memorandum and that by Dr. Couch to Henry Craven (attachment) constitute a report of the results of an early life-stage toxicity test with the sheepshead minnow exposed to trifluralin (Experiment TRF-20) conducted between December 16, 1976 and January 13, 1977. Methods used were similar to those in ASTM (1987), Draft 12 of the "Proposed new standard guide for conducting toxicity tests with early life-stages of fishes" and the sheepshead minnow specific methods in Hansen et al. (1983). Trifluralin used in the experiment was labeled "Treflan Tech. 99% #5GB70 X-14788 Lilly". Trifluralin was dissolved in triethylene glycol (TEG) and delivered to test aquaria at nominal concentrations of 3.1, 6.2, 12.5, 25 and 50 µg/l. Hereafter, only measured concentrations will be noted. A seawater and solvent (TEG) control were also treatments. The TEG concentration was 22 µg/l in all treatments except the seawater control. The test was begun with 20 embryos from newly fertilized eggs in each of two egg cups in each of two aquaria per treatment; total 560 embryos. The test lasted 28 days and embryonic development, hatching (days 4 to 6), survival of hatched fish and standard lengths of surviving fish at test termination were monitored. Ten fish from each treatment were preserved in Davidsons analyses at test termination for future histopathological examination.

Concentrations of trifluralin were measured in water samples from each treatment at least weekly and in fish at the end of the test. Analyses of variance and Duncan's multiple range tests were used to determine treatment differences in embryo hatching, juvenile survival, overall survival and length at test termination(α =0.05).

Survival and standard lengths of juvenile sheepshead minnows were reduced in trifluralin (Table 1). Survival was reduced in 20 and 31 µg/l but not in $\leq$ 5.5 µg/l. Fish exposed to 5.5 to 31 µg/l were lethargic, edematous and visibly shortened in the trunk. Lengths of fish surviving all concentrations of trifluralin tested 1.2 to 31 µg/l, were significantly less than the average length of fish in the TEG control. The magnitude of decrease was concentration dependant; 9.4 to 25 percent of the average length of fish from the TEG control. Differences in lengths of seawater and TEG control fish suggest a solvent mediated increase in fish size; possibly from enhancement of growth of microorganisms that were then used by fish as food or from microorganisms that restricted flushing of Brine shrimp from the egg cups. This response should also occur in trifluralin treatments receiving identical TEG concentrations. Therefore, the TEG control is the proper control for statistical analyses and growth reductions in trifuralin exposed fish are likely related to this herbicide.

Trifluralin and one breakdown product (2,6-dinitro-N-n-propyl- α,α,α-trifluoro-p-toluidine) were bioaccumulated by sheepshead minnows surviving the 28-day early life-stage toxicity test (Table 1). Concentrations of trifluralin in fish increased from 1.7 to 43 µg/g wet weight with increase in concentration of exposure. Concentrations of the metabolite for all trifluralin treatments averaged 2.1 percent of the concentration of trifluralin; range 1.2 to 2.4 percent. The concentration of trifluralin and its product measured in sheepshead minnows divided by the average measured concentration of trifluralin in exposure water, bioconcentration factor, averaged 1330.

References

ASTM (American Society for Testing and Materials). 1987. Proposed new standard guide for conducting toxicity tests with early life-stages of fishes. ASTM. Draft 12. 85p.

Couch, John A., Lee A. Courtney and Steven S. Foss. 1981. Laboratory evaluation of marine fishes as carcinogen assay subjects. In: Phyletic Approaches to Cancer: Proceedings of the 11th International Symposium of the Princess Takanatsu Cancer Research Fund, Tokyo, 1980. Clyde J. Dawe, Ed. Japan. Scientific, Sciences Press, Tokyo, Japan, pp.125-139.

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Goodman, Larry R., David J. Hansen, Charles S. Manning, Richard G. Zepp and Jerrold Forester. Acute and chronic toxicity of trifluralin and selected degradation products and chronic toxicity of carbophenothion and phorate to sheepshead minnows (Cyprinodon variegatus). Manuscript in review.

Parrish, Patrick R., Elizabeth E. Dyar, Joanna M. Enos and William G. Wilson. 1978. Chronic toxicity of chlordane, trifluralin and pentachlorophenol to sheepshead minnows (Cyprinodon variegatus). U.S. EPA. Ecological Research Series. EPA-600/3-78-010. 53p.

Survival, average length, concentration of trifluralin and trifluralin II in whole fish, and bioconcentration factors for sheepshead minnows (Cyprinodon variegatus) continuously exposed to trifluralin for 28 days at 30°C in an early life-stage toxicity test.

Exposure concentation (ug/l)		Survival (%)			Average Length	Residue (ug/g)[a]			Bioconcentration
Nominal	Average Measured	Embryos	Juvenile Fish	Combined	(mm)	Trifluralin	Trifluralin II	Total	Factor[b]
SW-CTL	ND[c]	85	94	80	10.7[d]	0.021		0.021	-
C-CTL	ND[c]	80	95	76	12.8	0.058		0.058	-
3.1	1.2	80	98	79	11.6[d]	1.7	0.040	1.7	1,400
6.2	2.7	75	95	71	11.3[d]	2.9	0.069	3.0	1,100
12.	5.5	84	86	72	10.7[d]	7.9	0.18	8.1	1,500
25.	31.	82	68[e]	56[d]	10.2[d]	14.	0.29	14.	450
50.	20.	81	58[e]	46[d]	9.6[d]	44.	0.52	44.	2,200

a - Trifluralin, 2,6-dinitro-N-n,dipropyl- α,α,α-trifluoro-p-toluidine; trifluralin II, 2.6-dinitro-N-n-propyl α,α,α-trifluro-p-toluidine

b - Derived by dividing sum of Trifluralin I and II residues by the average concentration measured in water.

c - ND = Not detectable (<0.02ug/l in water): The carrier concentration was 22 µl of triethylene glycol/l of water in all treatments except the seawater control, SW-CTL.

d - Significantly different from both controls (α=0.05).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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January 12, 1988

SUBJECT: Statement of Effect, No-Effect Concentration of Trifluralin in Producing Vertebral Lesion in Cyprinodon

FROM: John A. Couch, Ph.D.
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TO: Dave Hansen
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In consideration of published and unpublished data on the effect of trifluralin on fish vertebrae, we would make the following guarded statements about effect vs. no-effect concentrations. In a long-term 19 month study in which fish were exposed continuously to from 1-5 µg/ℓ trifluralin, vertebral lesions, as described by Couch et al. (1979, 1981), were the rule rather than the exception in most exposed fish (>80% incidence). In a more recent unpublished study in which fish were exposed to five concentration ranges (1.2 to 31.0 µg/ℓ trifluralin inclusive) continuously for 28 days (early life cycle study), characteristic trifluralin-induced, vertebral lesions occurred only at concentrations of 4 µg/ℓ and above. The numbers of fish sampled from these five concentration ranges were from 2 to 10 per sample. Therefore, based on examination of relatively low numbers of specimens from the 28 day study, we must say we found no vertebral effects at concentrations below 4 µg/ℓ.

One should note that the induction of vertebral effects by trifluralin in fish is probably both duration-of-exposure dependent and concentration-of-exposure dependent. This statement is based on the results of the 19 month exposure study and the 28 day early life cycle exposure study.

Publications describing the nature of the trifluralin-induced lesions are available through the Gulf Breeze ERL library.

References

Couch et al. (1979) Vertebral dysplasia in young fish exposed to the herbicide trifluralin. J. Fish Diseases 2, 35-42.

Couch et al. (1981) Laboratory evaluation of marine fishes as carcinogen assay subjects. In Phyletic Approaches to Cancer (C.J. Dawe et al., eds) Japan Sci. Soc. Press, Tokyo. pp. 125-139.