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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Protocol review of aquatic testing of microbial pest control agents

TO: Lois Rossi, PM-17
Registration Division (TS-767C)

THRU: for Raymond W. Matheny, Head, Section 1 Dennis McLane 1/6/87
Ecological Effects Branch
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and

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FROM: Robert W. Pilsucki, Microbiologist Robert W Pilsucki 1/6/88
Ecological Effects Branch
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I have reviewed the protocols sent in by Springborn Life Sciences Incorporated for testing microbial pest control agents in bluegill sunfish and Daphnia magna. These protocols will be acceptable with the following changes and additions.

Changes and comments applicable to both tests

1. The aqueous concentration should be 1000 times the expected environmental concentration after application to a six-inch layer of water. Thus, if the application rate is 2×10^{12} CFU/acre, the maximum test concentration should be:

$$\frac{2 \times 10^{12} \text{ cfu/A}}{(43560 \text{ sq ft/A})(0.5 \text{ ft})(7.5 \text{ gal/cu ft})(3785 \text{ mL/gal})} \times 1000$$

$$= \frac{2 \times 10^{12} \text{ cfu}}{6.18 \times 10^{8} \text{ mL}} \times 1000 = 3.2 \times 10^{6} \text{ cfu/mL}$$

If the water is renewed 3 times a week, as stated, There should

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be little adverse effect on water quality because it not expected that the microorganisms will elaborate a great deal of metabolic byproducts during that period due to the lack of a readily available carbon source. And, in fact, the concentration of the microorganism will probably decline somewhat over that period.

2. The second general comment relates to measurement of the concentration of the microorganism in one replicate when the test solution is renewed. This only works if the test solution for all replicates is made up in a single batch and is thoroughly mixed. Unless this is done, concentrations of the microorganism should be measured in each test vessel.

3. If the microorganism is grown in culture medium and both cells and spent culture medium are added to the test vessels, then a "solvent" control containing spent culture medium without cells should be performed. Comparison of the treatment groups with this control should account for any effects of metabolic byproducts produced by the microorganisms during culture.

Changes and comments specific to the fish test.

1. The figure for dietary concentration (less than or equal to 10^5 cells per mg) is not clear; the minimum concentration should be stated. This concentration should be the highest possible concentration achievable.

2. The food should be stored at 4° C or frozen if it is not made up fresh daily. Food should be assayed microbiologically periodically during the study to determine the number of viable cells that the fish are actually exposed to through the diet.

3. With regard to the histopathological examinations, rather than doing water washes of the gill and stomach tissues, tissues should be washed, homogenized and the homogenate serially diluted and plated. The bacteria that are adhering to the tissues are probably more important from a pathogenic point of view than those loosely associated with the surface - which is what will be washed off.

All tissue excised for histopathological examination should be done so under sterile conditions and those tissues should be observed for bacteria associated with eucaryotic cells. It is also a prudent idea to look at a stained blood smear during histopathological exam to see if there is a systemic involvement. It is obvious too that any lesions observed on gross examination should undergo culture and identification for the test microorganism.

Changes and comments specific to the aquatic invertebrate test

1. Water quality measurement can be made in one replicate but one replicate of each test concentration should be performed.

If you have comments or questions, feel free to contact me.