ACUTE TOXICITY OF BIODIESEL TO FRESHWATER AND MARINE ORGANISMS

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Abstract

Biodiesel fuels are reported to be nontoxic resulting in less potential hazard to fish and other aquatic life in case of accidental spills. This paper reports on static tests with rapeseed methyl ester (RME) and rapeseed ethyl ester (REE) performed according to EPA/600/4-90/027. The acute aquatic toxicity tests were conducted with both rainbow trout and daphnia magna by CH2M Hill in Corvallis, Oregon under contract to the University of Idaho.

The LC50 (the point at which 50% have died and 50% are still alive determined by interpolation) values for each of the substances tested with daphnia magna in parts per million were as follows: control (table salt (NaCl)) = 3.7, D2 = 1.43, RME = 23, REE = 99, and Methyl Soyate = 332. Duplicate tests with rainbow trout were run with 10 organisms per replicate. LC50 numbers were not reported because of the failure to kill a sufficient number of fish at the concentrations tested, even with the diesel control fuel. The 20 percent and 50 percent blends had scattered losses of fish but none of the tests had less than 85 percent survival at any concentration after 96 hours.

Introduction

Current test guidelines for acute aquatic toxicity produced by the United States Environmental Protection Agency (EPA), American Standard Testing of Materials (ASTM), and other federal and state governing agencies are not generally applicable to oil products.

As part of the ongoing research of Biodiesel at the University of Idaho's Department of Agricultural Engineering a test of acute aquatic toxicity to freshwater organisms was conducted by CH2M Hill in Corvallis, Oregon. Biodiesel fuels are reported to be nontoxic resulting in less potential hazard to fish and other aquatic life in case of an accidental spill. Biodiesel has been shown to be readily biodegradable (Peterson et al., 1994). To satisfy a growing interest in renewable, biodegradable, and non-toxic fuel sources, ecotoxicity testing was conducted on methyl and ethyl esters of rapeseed oil and blends of these esters with number two diesel fuel.

Literature Review

Cheng et al. (1991) compared hydraulic oils of mineral oil, vegetable oil, polyglycol, and a synthetic ester for biodegradability, toxicity, and fluid performance parameters. They used an oil-water dispersion procedure by the Ministry of Agriculture, Fisheries and Food, England, and provided mechanical agitation to continually disperse the test materials as small droplets in the water column. This procedure simulates physical dispersion by wave and current action. Rainbow trout were exposed to five concentrations of each test material and a control. Toxicity was expressed as the concentration of material in ppm to kill 50% of the fish after 96 hours of exposure (LC50). They reported that the base stocks for the vegetable oil formulations were nontoxic (> 1000 ppm) but the formulated products did not meet this criteria because of one or more of the additives. The LC50 with rainbow trout for mineral oil was 389 ppm; vegetable oil, 633 ppm; polyglycol, 80 ppm; and synthetic ester, > 5000 ppm.

Lockhart et al. (1984) studied rainbow trout in forty-eight hour-LC50 (acute lethal toxicity) tests exposed to water-soluble fractions (WSF) for a range of crude and refined oils. The results were compared to the concentrations of volatile very low-boiling (<115°C) and low-boiling (115-270°C) hydrocarbons determined by a two-stage analytical method. The results suggest that toxicity of the water-soluble fractions is associated largely with the substituted benzenes and naphthalenes, with boiling points between 115 and 270°C.

Lockhart et al. (1984) found that the LC50 values were highly dependent on the particular exposure conditions. Thus, survival of rainbow trout and their LC50 values were consistently higher in test containers that were open to the atmosphere than in sealed closed containers. Aeration of test containers, by bubbling air through the WSF, virtually eliminated toxicity to the trout in even the most toxic test preparations. The results were discussed in terms of the design of bioassays relevant to ice-covered environments and to other situations where volatilization may be low or reduced.

Weber, C.I. (1993) documents the EPA methods for acute toxicity of effluents and receiving waters to freshwater and marine organisms for the use in the National Pollutant Discharge Elimination System Permits Program. Summaries of the test conditions for the daphnids, daphnia magna, fathead minnows, rainbow trout, brook trout, the mysid, sheepshead minnows, and silversides are given. Three test types are given: static non-renewal, static renewal, and flow-through. Test duration may vary from 24 to 96 hours depending on the objective of the test. The tests are designed to provide dose-response information, expressed as the percent effluent concentration that is lethal to 50 percent of the test organisms (LC50) within the prescribed period of time (24-96 hours), or the highest effluent concentration in which survival is not statistically different from the control.

Nearly identical procedures are outlined in 40 CFR part 797.1300 (Daphnid acute toxicity test) and part 797.1400 (fish acute toxicity test), and ASTM E 729-88. These procedures include, with the LC50 (median lethal concentration), an EC50 (median effective concentration), and an IC50 (inhibition concentration).

Materials and Methods

Toxicity Tests

The University of Idaho Department of Agricultural Engineering contracted with CH2M Hill in Corvallis, Oregon to conduct two static definitive bioassays. The first bioassay was a 48-hour bioassay using the water flea, daphnia magna. A 96-hour bioassay using rainbow trout was conducted for the second study. Two rounds of tests were conducted for this study. All tests were performed according to: Methods for Measuring the Acute Aquatic Toxicity of Effluents to Freshwater and Marine Organisms EPA600/4-90/027F.

Fuels

Fuel nomenclature is as follows: (1) Phillips D2 low-sulfur diesel control fuel (D2); (2) 100 percent rapeseed methyl ester (RME); (3) 100 percent rapeseed ethyl ester (REE); (4) 50 percent RME - 50 percent D2 (50RME); (5) 50 percent REE - 50 percent D2 (50REE); (6) 20 percent RME - 80 percent D2 (20RME); 20 percent REE - 80 percent D2 (20REE); and 100 percent soybean methyl ester (SME).

Test quantities of each Biodiesel fuel tested were supplied by the University of Idaho. The rapeseed oil was expelled at the University of Idaho's Agricultural Engineering farm scale process facility. The RME, REE, and SME fuels were produced using the process developed by University of Idaho researchers. Phillips 66 Company 0.05 low sulfur diesel fuel was used as the baseline for the Biodiesel fuels.

Test Methods

All static tests were performed according to: Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Weber C., et al. (1991); EPA/600/4-90/027.

Test Organisms

Daphnia Magna

The daphnia magna were obtained from CH2M Hill's in house cultures and were less than 24 hours old prior to initiation of the test. All organisms tested were fed and maintained

during culturing, acclimation, and testing as prescribed by EPA (1989). The test organisms appeared vigorous and in good condition prior to testing. The daphnia magna were placed below the test surface at test initiation due to the non-soluble nature of the sample.

Rainbow Trout

The rainbow trout used in the first round of tests were obtained from Thomas Fish Company, Anderson, California, and were 22 days old and 32±2 mm in length. The rainbow trout were acclimated to test conditions (dilution water and temperature) for 10 days prior to test initiation. The rainbow trout used in the second round of tests were obtained from Spring Creek Trout Hatchery, Lewiston, Montana, and were 24 days old and 28±1 mm in length. The rainbow trout were acclimated to test conditions (dilution water and temperature) for 12 days prior to test initiation. All the test organisms appeared vigorous and in good condition prior to testing.

Dilution Water

Daphnia Magna

The water used for acclimation and dilution during the static testing was reconstituted moderately hard water with a total hardness of 98 as $CaCO_3$, alkalinity of 64 mg/l as $CaCO_3$, and a pH of 8.0 to 8.2.

Rainbow Trout

The water used for acclimation and dilution during the rainbow trout static testing was reconstituted moderately hard water with a total hardness of 92-98 as $CaCO_3$, alkalinity of 70-74 mg/l as $CaCO_3$, and a pH of 7.9 to 8.0.

Test Concentrations

Daphnia Magna

The concentrations tested in definitive test on REE were 33, 167, 833, 4170, and 20800 ppm of sample and dilution water for the control. The concentrations tested in the definitive test on RME were 67, 333, 1330, 6670, and 26700 ppm of sample and dilution water for the control. The concentrations tested in the definitive test on D2 were 6.67, 13.3, 33.3, 66.7, and 1333 ppm of sample and dilution water for control. The concentrations tested in the definitive test on Methyl Soyate were 13.3, 33.3, 66.7, and 6667 ppm of sample and dilution water for control. The fuel mixture concentrations were run in quadruplicate with five organisms per replicate. Additional concentrations of 1.43 and 3.33 ppm were set up for D2 with 10 organisms in on chamber. The fuel was stirred into the water before the daphnia magna were introduced into the chamber. There was a sheen of fuel on the top of each chamber.

Rainbow Trout

The concentrations tested for round 1 in the definitive test on D2, 20RME, and REE were 100, 300, 600, 1200, and 2400 ppm with dilution water for control. The concentrations tested for round 2 in the definitive test on RME and 50REE were 100, 500, 750, 1000, and 7500 ppm and the 50RME sample was tested at 100, 500, 600, and 7500 ppm due to a shortage of the sample.

The rainbow trout bioassays were run in 5-gallon glass aquaria, with a volume of 5 liters. The samples were run in duplicate with 10 organisms per replicate. The photo period was 16 hours light 8 hours dark. The temperature range was 12±1°C. Loading of test organisms was 0.53g wet fish weight per liter in round one, and 0.26g wet fish per liter in round two. Mortality was measured by lack of response to tactile stimulation and lack of respiratory movement. The fuel was stirred into the water before the Daphnia Magna were introduced into the chamber. There was a sheen of fuel on the top of each chamber.

Monitoring of Bioassays

The static tests were monitored at test initiation for pH, hardness, alkalinity, conductivity, and dissolved oxygen, and every 24 hours thereafter for mortality and at test termination for pH, conductivity, and dissolved oxygen. Temperature was monitored continuously throughout the test periods. The response measured for the Daphnia Magna was mortality over the 48-hour exposure period. The median lethal response (LC50) was calculated using Toxis version 2.2.

Results

Daphnia Magna

The raw data is summarized in Table 1 below for 100 percent REE. Some of the mortality seen in the tests may have been caused by the physical nature of the test substances. The raw data sheets noted when the Daphnia Magna were trapped on the oil sheen at the surface of the test containers. The LC50 for the REE sample was 99 ppm.

Table 1
REE
Summary of Results
Percent Survival

Concentration (ppm)	0 hr	24 hr	48 hr
Control	100	100	100
33	100	80	65
167	100	70	40
833	100	65	30
4170	100	65	25
20800	100	50	20

Table 2 summarizes the results of the RME sample. The LC50 for RME was 23 ppm.

Table 2 RME Summary of Results Percent Survival			
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**
Control	100	100	100
67	100	90	40
333	100	95	40
1330	100	60	20
6670	100	35	5
26700	100	30	30

Table 3 summarizes the results of the D2 sample tested. The LC50 for the D2 sample was less than 1.43 ppm.

Table 3 D2 Summary of Results Percent Survival			
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**
Control	100	100	100
1.43	100	30	0
3.33	100	0	0
6.67	100	10	0
13.3	100	10	0
33.3	100	0	0
66.7	100	0	0
1333	100	0	0

Table 4 summarizes the results of the Methyl Soyate. The LC50 for the Methyl Soyate was 332 ppm.

Table 4 Methyl Soyate Summary of Results Percent Survival			
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**
Control	100	100	100
13.3	100	90	85
33.3	100	95	80
66.7	100	85	75
667	100	80	45
6667	100	45	10

The methyl and ethyl esters are not water soluble and form a sheen on the water surface. This sheen could be easily skimmed off, but the Daphnia Magna get captured in this sheen. Fifty percent of the Daphnia Magna in common table salt had died at a concentration of 3.7 parts per million (ppm). With diesel, 50 percent of them had died at less than 1.43 ppm and CH2M Hill reported all were dead at this concentration. When this test was first completed, CH2M Hill reported that the LC50 for diesel fuel was less than 6 ppm because all the Daphnia Magna had died. They were asked to test lower concentrations. They tested four more concentrations less than 6 ppm and the diesel fuel still killed all the Daphnia Magna. For the RME the LC50 was 23 ppm, and at 26,700 ppm 30 percent of them were still alive. With REE the LC50 was 99 ppm and 20 percent were still alive at 20,800 ppm. With methyl soyate the LC50 was 332 ppm; however, only 45 percent were alive at 667 ppm. This difference between rapeseed esters and SME may be due to the high Erucic acid content of the rapeseed. If one takes the worst case, the 23 ppm for REE, and compare it of the 1.4 ppm for diesel fuel, the acute aquatic toxicity is 15 times less. What is even more significant is the 20 percent and 30 percent that are still alive at very high concentrations of Biodiesel.

Rainbow Trout

The raw data is summarized in Tables 5 to 10 below for 100 percent D2. The LC50 for D2 was not determined. This data compares cadmium chloride (CdCl), diesel fuel, and methyl and ethyl esters of rapeseed.

Table 5 D2 Summary of Results Percent Survival					
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**	**72 hr**	**96 hr**
Control	100	100	100	100	100
100	100	100	95	95	90
300	100	100	100	100	100
600	100	100	100	100	100
1200	100	100	100	95	85
2400	100	100	100	95	80

Table 6
20%RME - 80%D2
Summary of Results
Percent Survival

Concentration (ppm)	0 hr	24 hr	48 hr	72 hr	96 hr
Control	100	100	100	100	100
100	100	100	100	100	100
300	100	100	100	100	100
600	100	95	95	95	95
1200	100	100	100	100	85
2400	100	100	100	100	95

Table 7
20%REE - 80%D2
Summary of Results
Percent Survival

Concentration (ppm)	0 hr	24 hr	48 hr	72 hr	96 hr
Control	100	100	100	100	100
100	100	100	100	95	95
300	100	100	100	100	100
600	100	100	100	100	100
1200	100	100	95	95	95
2400	100	100	95	95	90

Table 8
100% REE
Summary of Results
Percent Survival

Concentration (ppm)	0 hr	24 hr	48 hr	72 hr	96 hr
Control	100	100	100	100	100
100	100	100	100	95	95
300	100	100	100	100	100
600	100	100	100	100	100
1200	100	100	100	100	100
2400	100	100	100	100	95

Table 9 100% RME Summary of Results Percent Survival					
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**	**72 hr**	**96 hr**
Control	100	100	100	100	100
100	100	100	100	100	100
500	100	100	100	100	100
600	100	100	100	100	100
1000	100	100	100	100	100
7500	100	100	100	100	100

The 50RME percent survival summary results were identical to the 100% RME results.

Table 10 50% REE - 50% D2 Summary of Results Percent Survival					
Concentration (ppm)	**0 hr**	**24 hr**	**48 hr**	**72 hr**	**96 hr**
Control	100	100	100	100	100
100	100	100	100	100	100
500	100	100	100	100	100
600	100	100	100	100	100
1000	100	100	100	100	100
7500	100	100	100	100	95

The 48-hour LC50 value and Control Chart limits for the reference toxicant (cadmium chloride) was at a concentration of 2.8μg/l for the round one rainbow trout and 4.6μg/l for the round two trout. The results indicate that the test organisms were within their expected sensitivity range. Comments included in round one test data at 24-hours was a general behavior of twitching and they were swimming on their sides and skittering; at 48-hours their condition was the same as at 24-hours. The trout in the 20REE containers at 100 and 300 ppm were swimming vertically, at 600 ppm the trout were on their sides at the bottom, and at 2400 ppm they were barely moving at the bottom of the tank. The trout in the REE containers were not as active as in the other three test substances. The end condition of survivors was reported as being poor. The only comment in round two was at 48-hours that the fish were dark and swimming vertical at concentrations as low as 500 ppm in the 50RME and 50REE with the end condition of survivors as being poor.

Conclusions

Biodiesel is not as toxic to Daphnia Magna as NaCl. When compared to the reference toxicant (sodium chloride) diesel fuel was 2.6 times more toxic, RME was 6.2 times less toxic, REE was 26 times less, and SME 89 times less toxic. When compared to number two diesel fuel RME is 16 times less toxic, REE is 69 times less toxic, and SME was 237 times less toxic. CH2M Hill has been asked to repeat the toxicity study with rainbow trout at the water accommodated fraction (WAF) and below to produce a LC50.

References

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