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Toxicity and Uptake Potential of an Acrylic Polymer Comonomer and its Biological Degradation Products

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Sodium p-sulfophenyl methallyl ether (SPME) is a comonomer used for acrylic polymer manufacture. Acrylics are used in a variety of industrial applications which may lead to contact with the environment. Thus, limited environmental release of SPME could be expected. Additionally, two SPME degradation products, sodium methallyl sulfonate (SMS) and sodium p-phenol sulfonate (SPS) would be expected in low quantities following environmental release.

The objective of this series of studies was to determine the acute and 34-day chronic toxicity of SPME to representative aquatic species and the bioconcentration potential and tissue concentrations of radiolabelled SPME, SPS and SMS in standard aquatic tests.

MATERIALS AND METHODS

Nonradioactive samples of sodium p-sulfophenyl methallyl ether (SPME), sodium p-phenol sulfonate (SPS) and sodium methallyl sulfonate (SMS) were obtained from the Monsanto Co., St. Louis, Missouri.

The following radioactive materials, with respective activities and radiochemical purity were synthesized by Dr. J.C. Masson of Monsanto Co.:

a) ^{14}C-SPME, 0.54 mCi/g., > 99%

b) ^{14}C-SPS, 0.18 mCi/g., > 99%

c) ^{35}S-SMS, 0.91 mCi/g., 83.5%

Bluegill sunfish (Leponis Macrochirus) with a mean wet weight of 4.9±1.7 g and a mean length of 67±12 mm were obtained from a Nebraska fish farm, as were fingerling rainbow trout (Salmo gairdeneri). All fish were acclimatized in holding tanks at the laboratory for at least 1 week prior to acute exposures and 30 days prior to radiotracer exposure. Fish were fed a dry pelleted ration ad libitum each day.

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Daphnia magna were obtained from cultures maintained in well water at the Monsanto environmental laboratory. Organisms less than 24h old were selected for testing from healthy cultures with a known history.

A modified continuous-flow proportional dilution apparatus (Mount and Brungs 1967) was used to establish and maintain the desired chemical concentrations in the test chambers throughout the studies. Thirty-liter aquaria with 30 to 50 fish each were used to expose the test organisms. Aerated well water (pH 7.1, hardness 35 mg/L $CaCo_3$, dissolved oxygen >5 mg/L, temperature 18-20°C) was provided to each unit at a flow rate of 5 L/hr. Water temperatures were recorded daily while dissolved oxygen was measured at least weekly throughout the study. The bioconcentration test procedures as described by Macek et al. (1978) were followed.

Radioactive SPME, SPS or SMS stock solutions were prepared once at the beginning of the studies by the addition of a sufficient amount of radiotracer and unlabeled chemical in deionized water to achieve desired concentrations with an acceptable specific activity for each study.

Subsequent solutions were generally prepared weekly through dilution of the stock. The stock solutions were refrigerated in the dark to minimize thermal and photodegradation during storage. The following nominal test concentrations were used in this series of studies: SPME - 0.5 and 50 mg/L; SPS - 0, 50 mg/L; SMS - 0, 50 mg/L. Water and fish were sampled from each aquarium after 1, 7, 10, 21 and 28 days of exposure. Fish remaining after 28 days were transferred to clean flowing water and samples taken on the following days postexposure: SPME - 1, 3, 7; SPS - day 7 only; SMS - 1, 4, 7, 11 and 14. Control fish and water were sampled on days 1 and 28 of exposure. On each sample day triplicate 5-mL water samples and 5 fish were collected for analysis. The fish were eviscerated and filleted to provide samples of edible tissues (muscle) and a composite of the remaining portions of the fish. Duplicate portions of muscle tissue (fillets) and individual visceral tissue (viscera and internal organs) of fish from each test group were analyzed by standard radiometric methods. Radioactivity present in aqueous samples were determined by addition of the 5-mL samples to scintillation vials containing cocktail and counted by a liquid scintillation counter. Portions of fish carcass were either combusted (^{14}C samples) or digested (^{35}S samples) prior to radioanalysis of the trapped $^{14}CO_2$ or dissolved tissue, respectively. All measurements of radioactivity were made using a Model 2112 Packard Tri-Carb Liquid Scintillation Spectrometer, respectively. All data were corrected for background, dilution, quenching and counting efficiency. Minimum detection limits were established for each sample based on the size and radiotracer counting error associated with that sample. Higher detection limits generally were applicable to the visceral tissue data, which was much smaller in sample size than the muscle tissue. Acute toxicity rangefinding tests were conducted with rainbow trout and bluegill sunfish with SPME and SPS. Ten fish per concentration were tested (except for SPS at 5000 mg/L which

only had five fish) in 25-L pickle jars using 20L of water. Test concentrations were 0, 0.1, 1.0, 10, 100 and 1000 mg/L for SPME and 0, 10, 100, 1000, and 5000 mg/L for SPS. The test chemicals were introduced as aqueous solutions without solvents prior to introducing the fish. Mortality, behavior, dissolved oxygen, pH and temperature were recorded daily for 96h.

Daphnia magna (<24h old) were exposed to purified (recrystallized) SPME for 48h using five concentrations and a control. Ten daphnids per beaker were used with three replicates per concentration. Each beaker contained 200 mL of well water. Test solutions were not aerated and no solvents were used. Dissolved oxygen, pH, alkalinity, hardness and temperature were measured at the beginning and end of the study. Procedures used followed those described by the EPA (U.S. EPA 1975).

Fathead minnow (Phimephales promelas) eggs were obtained from the Columbia National Fisheries Research Center, Columbia, Missouri and introduced into a test system at Monsanto. The eggs were less than 24h old at the start of the test. Five concentrations and a control, each in duplicate, were tested. The nominal exposure concentrations were 0, 3.8, 7.5, 15 and 30 mg/L. Test solutions were delivered to the all-glass aquaria (25 X 15 X 12 cm) by means of a solenoid diluter and syringe injector. No solvents were used. Each aquaria contained 5L of test solution. The dilution water was high quality (characterized for hardness, pH, conductivity, total organics, pesticides and metals) well water. The flow rate was 56 ml/min. The test temperature was maintained at 25 ± 1°C by means of a circulating water bath.

Fifty eggs were placed in each oscillating egg cap. When hatching was complete, 96h after test initiation, all dead eggs were counted and percent hatchability calculated. All surviving fry were placed in the test system for 30 days. Fathead minnow fry were fed 1 mL of concentrated brine shrimp nauplii three times daily during the week and twice daily on weekends. Observations on behavior and survival were made three times per week. At 30 days post hatch the study was ended and the fry were counted, weighed, and measured.

Dissolved oxygen was measured twice per week, temperature was measured daily, pH was measured once per week and alkalinity, hardness and conductivity were measured three times during the study. Water samples were collected for SPME analysis on days -1, 0, 2, 4, 7, 14, 20, 27 and 34. Twenty milliliters of water were collected from each aquaria and analyzed spectrophotometrically. The method of Sechist and Garmon (1975) was followed. Standards and two fortified samples were analyzed with each set of water samples to verify analytical results and recoverability.

RESULTS AND DISCUSSION

The mean measured aqueous concentrations of ^{14}C-SPME during the 28-day exposure period were 0, 6.3±0.3 mg/L and 60.3±2.5 mg/L. Survival (92%) of bluegill exposed to 6.3 mg/L ^{14}C-SPME was

considered comparable to that of the control group (98%). No abnormal behavioral signs suggestive of toxic effects were observed. Bluegill exposed to 60.3 mg/L ^{14}C-SPME appeared normal and fed readily until the last week of the study. During study days 25-28 feeding activity decreased, some fish became dark and lethargic. Death occurred in 28% of this test group. Necropsies conducted on moribund or dead fish failed to find internal or external parasites as a causative agent. Within 2 days of transfer of surviving bluegill to flowing, uncontaminated water these fish resumed a normal behavior and feeding activity increased. These observations suggest that the stress and mortality present in the bluegill population exposed to 60.3 mg/L ^{14}C-SPME may be related to treatment. However, these effects were not substantiated by the toxicity tests conducted.

The mean measured concentrations of ^{14}C-residues present in the muscle of bluegill exposed to 6.3 or 60.3 mg/L ^{14}C-SPME exhibited little, if any, significant accumulation above minimum detectable limits during the entire exposure period. Thus, the maximum bioconcentration factor in the muscle portion of bluegill exposed to either concentration of ^{14}C-SPME was <1X the concentration of ^{14}C-residues present in the water at any point during the entire exposure period. The mean measured concentration of ^{14}C-residues present in the muscle tissue remained below minimum detectable limits throughout the 7 day depuration period.

Radiometric analysis indicated a small but consistent accumulation of radioactivity in visceral portions of fish during the 28-day exposure period. This level never exceeded 4X the minimum detectable limits. No detectable residues were found during depuration.

The mean measured aqueous concentrations of ^{14}C-SPS were 0.0 and 60.7±4.1 mg/L ^{14}C-SPS during the 28-day exposure period. Cumulative mortality observed was 4.0% in the 60.7 mg/L treated group versus 3.2% for fish in the control population. There were no signs of abnormal behavior or chemical induced stress.

Mean measured ^{14}C-residues present in both muscle and visceral fish portions were below the minimum detectable limits during the entire exposure period. Thus, the maximum bioconcentration factor for bluegill exposed to ^{14}C-SPS was <1X of the concentration of ^{14}C-residues present in the water at any point during the entire study period. Residue measurements for the depuration period were not done since no significant residues were found during the exposure phase.

A mean measured aqueous concentration of ^{35}S-SMS of 56±10 mg/L was attained over the 28-day exposure period. Both the treated and control fish populations behaved normally and showed no signs of stress throughout the study period. Each test population had a cumulative mortality of 1%.

The mean measured ^{35}S-residue concentrations in the muscle and visceral tissues of bluegill during the 28-day exposure and a

14-day depuration period are presented in Table 1. All analyses of edible tissue throughout the study yielded values below respective detection limits. Thus, no bioconcentration in this fish portion was evident. Bluegills did, however, accumulate ^{35}-S-residues in the viscera. The concentration of ^{35}-S-residues peaked in the visceral sample after 4 days of exposure and remained near steady-state throughout the remainder of the 28 day exposure period. The mean concentration of ^{35}-S-residues between days 4 and 28 of exposure was calculated to be 65±12 mg/kg. This value yielded a bioconcentration factor of 1X. Twenty-four hours after depuration began, visceral samples were devoid of measurable ^{35}-S residues (Table 1).

Table 1. Measured ^{35}S-Sodium Methallyl Sulfonate (SMS) residues in bluegill during and after continuous exposure to 56 mg/L ^{35}S-SMS.

Study Period	Study Day	^{35}S Conc. (Mean±S.D.) in Fish Tissue (mg/kg) Muscle	Viscera
Exposure	0	---	---
	1	<4.9+0.4[a]	<12±2
	4	<6.2±2.1	75±49
	7	<6.1±1.6	44±16
	11	<7.4±1.5	69±30
	14	<6.7±1.1	60±18
	21	<7.1±1.5	65±24
	28	<7.0±1.7	76±35
Depuration	1	<6.8±1.3	<18±3
	4	<6.2±0.7	<19±5
	7	<6.5±0.7	<13±3
	11	<6.5±1.2	<15±5
	14	<6.9±1.0	<14±3

A less than (<) indicates limit of detection.

A 4-day static exposure to SPME and SPS resulted in no deaths in groups of fingerling trout or bluegill exposed up to 1000 (SPME) and 5000 (SPS) mg/L, the highest concentrations tested.

The results of a 48-h static acute toxicity test with Daphnia magna indicated that SPME is practically non toxic to this species. The 48-h LC50 was determined to be 23,000 (21,340-24,830) mg/L. No effects were observed below 14,800 mg/L.

Results of this study indicate SPME did not effect any of the observed parameters as compared with the control fish. The mean measured exposure concentrations during the 30-day study were 0, 2.5, 4.8, 9.6, 20.5, and 49.4 mg/L. The maximum acceptable toxicant concentration (MATC) for SPME and fathead minnow fry was >49.4 mg/L.

Bioconcentration is a process which results in higher concentrations in tissues of an aquatic specie than in the exposure water itself. Twenty eight-day exposure of bluegill to aqueous radioactive concentrations up to 60 mg/L of SPME resulted in little if any accumulation of radioactivity in fish muscle tissue. Residues which may have accumulated were rapidly eliminated (within 24h) after removal of fish from exposure conditions. Additionally, no accumulation was observed in muscle tissue of bluegills exposed to 56 mg/L SMS or 61 mg/L SPS, two SPME degradation products. Very low levels of radiotracer accumulation were observed in visceral fish tissue following 28-day exposures to SPME and SMS. No SPS was detected in the bluegill viscera. Detectable residues of SMS found in visceral tissue reached steady state after 4 days of exposure and were quickly eliminated.

In all cases, measured bioconcentration factors for all fish tissues never exceeded 4X at any time point. The lack of bioconcentration observed is consistent with the fact that the aqueous solubility of all three test chemicals is high, ie. greater than 10,000 mg/L (1%). Clearly, these chemicals would not be expected to bioconcentrate in aquatic organisms.

The results of the acute toxicity studies with bluegills, rainbow trout, and Daphnia magna indicate that SPME is of low toxicity to aquatic organisms. This is confirmed by the lack of toxic effects observed in a fathead minnow early life stage chronic toxicity test. Vieth et al. (1983) have shown that there is a significant direct relationship between water solubility and toxicity for chemicals with nonspecific modes of toxic action. The data obtained in the present study are consistent with these findings. These combined data lead to the conclusion that the metabolites of SPME (SMS and SPS) are also practically nontoxic to aquatic organisms.

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