Bull. Environm. Contam. Toxicol. 24,575-581 (1980)

Uptake and Excretion of Aminocarb, Nonylphenol, and Pesticide Diluent 585 by Mussels (*Mytilus edulis*)

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Aminocarb (4-(dimethylamino)-3-methylphenyl methylcarbamate), nonylphenol and pesticide diluent 585 (585 oil) comprise the aminocarb formulation used recently in forest spray operations for the control of spruce budworm in New Brunswick, Canada.

In studies of the lethality of aminocarb and nonylphenol, ZITKO et al. (1979) found that nonylphenol was more toxic than aminocarb to juvenile Atlantic salmon and that the two compounds were of equal toxicity to the shrimp Crangon septemspinosa.

The few studies on the accumulation and excretion of carbamate pesticides by aquatic species (KORN 1973, SANGHA 1971, ROBINSON & FISHER 1978) indicate that fish and aquatic invertebrates do not concentrate these compounds to a great degree. No information was found on the bioaccumulation of alkylphenols or 585 oil by aquatic organisms. However, the fate of petroleum hydrocarbons in aquatic species (NEFF et al. 1976, LEE et al. 1977, STEGEMAN & TEAL 1973) may be related to accumulation of 585 oil.

Bioaccumulation studies with bivalves are of particular importance because of the high potential for accumulation afforded by suspension-feeding organisms and because some bivalves are eaten by man. This paper describes the uptake and excretion of aminocarb, nonylphenol, and 585 oil by mussels (Mytilus edulis).

METHODS

Mussels with valve lengths of about 5.0 cm were exposed in static tests at 15°C to aminocarb at average concentrations of 0.352 and 0.403 mg/L of sea water for 4 d. At the start of each test, four mussels/L of sea water were placed in test vessels. The animals were transferred to clean flowing water to measure excretion. Similarily, mussels were exposed to aminocarb formulation at nominal concentrations of 0.2 and 2.5 mg of formulation/L. Mussels were sampled at 1, 2 and 4 d during the exposure period and at 1 and 2 or 8 d during the excretion period. Some samples were taken in replicate. Each sample, consisting of tissue from four mussels, was analyzed immediately after sampling.

Mussels were exposed to 0.4 mg/L of 585 oil for 2.5 d in a flow-through system. The 585 oil (2 g) dissolved in spectrograde hexane (20 mL) was mixed with glass beads (400 g, 4 mm diameter).

0007-4861/80/0024-0575 $01.40

The hexane was allowed to evaporate and the beads were placed in a 2.5-cm O.D. glass column. Sea water was siphoned through the column at 5 mL/min into a 10-L vessel containing the test mussels. Unadulterated sea water was dripped at a similar rate into a vessel containing control mussels. Mussels were sampled at 0.5, 1, 2 and 2.5 d during the exposure period. After they were transferred to clean flowing water, the mussels were sampled at 0.5, 1 and 2 d.

Aminocarb from 100-mL samples was extracted into 30 mL of pesticide-grade dichloromethane. Spectrograde hexane (30 mL) was used to extract nonylphenol from a 100-mL water sample and 10 mL of spectrograde hexane was used to extract 585 oil from a 25-mL sample. Extracts were evaporated to 5 mL for aminocarb, 1 mL for nonylphenol and 3 mL for 585 oil. Aminocarb was determined by gas chromatography using a N-P detector. The 0.6 m x 2 mm I.D. column was packed with 3% OV-17 on Chromsorb W-HP, and flows for nitrogen carrier gas, hydrogen and air were 25, 3 and 100 mL/min, respectively. The injector and column temperatures were 200 and 160°C, respectively. Nonylphenol was analyzed using a flame-ionization detector. The 2 m x 2 mm I.D. column was packed with 3% OV-101 on Chromosorb W-HP and flows for nitrogen carrier, hydrogen and air were 40, 50 and 100 mL/min, respectively. The injector, column and detector manifold temperatures were 200, 180 and 250°C, respectively. The 585 oil extracts were analyzed by fluorometry. Fluorescence was excited at 300 nm and the emission spectrum was recorded between 310 and 410 nm. Sample spectra were quantified by comparison with the fluorescence emissions of 585 oil standards at 330 nm.

Extraction efficiencies were determined by extracting fortified seawater samples. Analysis results were adjusted to account for the unextracted material. Average concentrations of aminocarb and nonylphenol in water during each test were calculated by the method of ZITKO et al. (1977).

The mussels were analyzed for aminocarb and nonylphenol by combining the tissues of four mussels, homogenizing with sodium sulfate and Ottawa sand, and extracting in a Soxhlet apparatus for 1 h with ethyl acetate as solvent. Each extract was reduced in volume and cleaned up by gel permeation column chromatography (GPC). "Bio-beads SX-2" were used in the cleanup column (2.5-cm diameter with a resin height of 28-30 cm). The column was eluted with 50:50 dichloromethane-cyclohexane and the portion collected for analysis was 80-140 mL of the eluent. Quantitation of aminocarb and nonylphenol was performed by gas chromatography using systems and conditions described above for the water sample analyses.

The mussels were analyzed for 585 oil by homogenizing the soft tissues of two mussels with sodium sulfate and extracting with spectrograde hexane for 1 h in a Soxhlet apparatus. Extracts were cleaned up by column chromatography as described by ZITKO (1975) except that only 10 mL of the second fraction of eluent was collected and analyzed directly. The samples were analyzed by fluorometry using the same instrument and conditions described for analysis of water extracts.

The composition of 585 oil was determined by gas chromatographic analysis of 585 oil standards dissolved in carbon disulphide. Chromatograms were characterized by comparison with a standard of alkanes (C_8 to C_{20}), naphthalene, 2-ethyl-naphthalene and 2-methylnaphthalene. Instrument conditions were the same as previously described for the analysis of nonylphenol, except that the column temperature was programmed from 90-200°C at 8°/min.

Concentration factors were calculated as the concentration of the compound in the mussel on a wet weight basis divided by the average concentration of the compound in sea water. Aminocarb and nonylphenol concentrations decreased with exposure time. The accumulation and excretion rate constants for these compounds were estimated according to the methods described by ZITKO (in press). For 585 oil data, in which there was no decrease in exposure concentration with time, rate constants were calculated using the computer program of BLAU & AGIN (1978) for one-compartment uptake models.

RESULTS AND DISCUSSION

The efficiencies of the extractions for aminocarb, nonylphenol and 585 oil from sea water averaged 93, 79 and 72%, respectively. The concentration of aminocarb in water declined by an average of 40% during 4 d, with most of the decline occurring during the first 2 d. Nonylphenol concentrations decreased slightly (25%) over the 4 d and 585 oil concentrations remained relatively constant over 2.5 d.

Aminocarb in the mussels reached a maximum concentration at 2 d, declined slightly by 4 d (Table 1), and was not detected after 1 d post-exposure. Maximum concentration factors from the four tests ranged from 1.0 to 1.9. The pattern of aminocarb uptake by mussels from exposure to the formulation closely resembled that from exposure to aminocarb alone.

Data reported by KORN (1973) indicates that ^{14}C labelled carbaryl reaches an equilibrium concentration factor of 0.04 in channel catfish (Ictalurus punctata) after about 8 d exposure. Little excretion of carbaryl residues was noted during 28 d post-exposure.

SANGHA (1971) reported concentration factors of 4 to 210 for mosquito fish (Gambusia), snail (Physa) and Daphnia exposed to each of six carbamate insecticides. Snails had the lowest concentration factors. The three species metabolized the residues to conjugated compounds and hydroxylation products. A freshwater bivalve (Elliptio) metabolized about half of an injected dose of carbofuran within a 48-h period (ROBINSON & FISHER 1978).

Nonylphenol in the mussels reached a maximum concentration at 2 d and decreased by 4 d (Table 2). Not all the nonylphenol had disappeared by 1 d excretion. Maximum concentration factors were 12 and 13.

TABLE 1

Aminocarb concentration in mussels (Mytilus edulis) exposed to aminocarb in sea water at 15°C for 4 d and transferred to clean water. Concentration factors in brackets.

Average aminocarb concentration (mg/L)	Aminocarb concentration in mussels (µg/g wet weight) 1-d uptake	2-d uptake	4-d uptake	1-d excretion
0.025[a]	0.022(0.9)	0.035(1.4)	0.022(0.9) 0.026(1.0)	ND[c]
0.275[a]	0.38(1.4)	0.43(1.6)	0.17(0.6)	ND
0.352[b]	0.42(1.2)	0.65(1.9)	0.50(1.4) 0.24(0.8) 0.22(0.6)	ND
0.402[b]	0.25(0.6)	0.38(1.0)	0.39(1.0) 0.28(0.7)	ND

[a]Aminocarb formulation added to test vessels.
[b]Aminocarb added to test vessels.
[c]Not detected, <0.007 µg/g.

TABLE 2

Nonylphenol concentration in mussels (Mytilus edulis) exposed to aminocarb formulation in sea water at 15°C for 4 d and transferred to clean water. Concentration factors in brackets.

Average nonylphenol concentration (mg/L)	Nonylphenol concentration in mussels (µg/g wet weight) 1-d uptake	2-d uptake	4-d uptake	1-d excretion	8-d excretion
0.1	0.90(9.0)	1.3(13)	0.79(7.9) 0.79(7.9)	NA[a]	NA
1.13	9.9(11) 10.4(12)	6.9(7.9) 7.4(8.5)	1.2(1.4)	1.4	ND[b]

[a]Not analyzed.
[b]Not detected, <0.3 µg/g.

The 585 oil used in the spray formulation consists primarily of low molecular weight alkanes (C_9 to C_{14}) and naphthalenes.

Analysis of control mussels in the 585 oil test indicated that initial background fluorescence of mussels was equivalent to approximately 200 µg/g of 585 oil. The fluorescence of control mussels increased throughout the test, presumably as a result of contamination from the laboratory seawater supply. The concentrations of 585 oil in mussels, after subtraction of background fluorescence, reached a maximum after 2 d exposure and decreased to baseline levels after 1 d excretion (Table 3). The maximum concentration factor was 330.

TABLE 3

585 oil concentration in mussels (Mytilus edulis) exposed to 585 oil in sea water at 15°C for 2.5 d and transferred to clean water. Concentration factors in brackets.

Average 585 oil concentration (mg/L)	585 oil concentration in mussels (µg/g weight)					
	1/2-d uptake	1-d uptake	2-d uptake	2 1/2-d uptake	1/2-d excretion	1-d excretion
0.396	1.6 (4.0)	130 (330)	58 (150)	62 (160)	ND[a]	1.9 (7.3)
	ND	32 (80)	66 (170)	NA[b]	NA	ND

[a]Not detected, <1.0 µg/g.
[b]Not analyzed.

NEFF et al. (1976) exposed the marine clam Rangia cuneata to several naphthalenes for 24 h and reported concentration factors ranging from 2.3 for naphthalene to 27 for trimethylnaphthalene. In clean water, the clams excreted between 50 and 79% of the compounds within 24 h. Mytilus edulis accumulated ^{14}C labelled petroleum hydrocarbons in amounts equivalent to concentration factors of 970 for heptadecane (24 h exposure) and 220 for naphthalene (4 h exposure) (LEE et al. 1972). At 24 h post-exposure, these mussels excreted 80% of the heptadecane and 50% of the naphthalene. STEGEMAN & TEAL (1973) found that oysters, Crassostrea virginica, accumulated total petroleum hydrocarbons equivalent to a concentration factor of 420 during 2 d exposure.

The calculated rate constants for uptake (K1) and excretion (K2) for aminocarb, nonylphenol and 585 oil (Table 4) indicate relatively low uptake rates and high excretion rates. As a result,

TABLE 4

Uptake (K1) and excretion (K2) rate constants, accumulation coefficients (K1/K2) and times to maximum concentration (uptake) and ½ clearance (excretion) of aminocarb, nonylphenol and 585 oil in mussels (Mytilus edulis).

Compound	Average exposure conc. (mg/L)	K1 (day^{-1})	K2 (day^{-1})	K1/K2	Time max. conc. (d)	½ Time clearance (d)
Aminocarb	0.025	7.6	2.0	3.8	2.0	0.4
	0.275 0.352 0.402	6.8	1.4	4.9	1.8	0.6
Nonylphenol	0.100 1.130	23	2.3	10	0.4	0.3
585 oil	0.396	360	2.3	160	1.4	0.3

the accumulation coefficients (K1/K2) are low, being about 4 for aminocarb, 10 for nonylphenol and 150 for 585 oil. High rates of excretion of aminocarb are to be expected in light of the reported rapid metabolism of carbamate pesticides by other aquatic invertebrates (SANGHA 1971, ROBINSON & FISHER 1978). The rate constants and accumulation coefficient reported for 585 oil are representative of the fluorescent aromatic components of the oil. Other components of the 585 oil may be accumulated and excreted at faster or slower rates. The data reported here for 585 oil are similar to uptake and excretion data reported for Mytilus exposed to naphthalene (LEE et al. 1972).

The accumulation coefficients for aminocarb and for nonylphenol (Table 4) in mussels are considerably lower than the coefficients for fenitrothion of 19-35 in Mya arenaria and 78-130 in Mytilus edulis (McLEESE et al. 1979).

Because of the low accumulation coefficients it is concluded that aminocarb and nonylphenol concentrations in water of less than 0.01 mg/L are not likely to result in significant contamination of bivalves. The 585 oil disappears rapidly from sea water, with an estimated half-life of about 60 min (unpublished data). Therefore, even though the oil comprises about 30% by weight of the formulation and about 80% by weight of the spray solution, significant accumulation of the diluent by bivalves appears unlikely. The rapid loss of fluorescent compounds from mussels limits the usefulness of fluorescence analysis as an indicator of the presence of spray constituents.

ACKNOWLEDGMENTS

Drs. K. Haya and R. H. Peterson commented on the text. The manuscript was typed by the Word Processing Unit. Ms. R. Garnett edited the text.

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