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Uptake and Excretion of Organochlorine Pesticides by *Nereis virens* under Normoxic and Hypoxic Conditions

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The marine polychaete worm, Nereis virens, is resistant to organochlorine pesticides. When exposed to each of five pesticides (endosulfan, chlordane, endrin, dieldrin, and DDT) in concentrations ranging from 0.03 mg/L (DDT) to 22.0 mg/L (chlordane), only endosulfan and chlordane killed Nereis (228 h LC50 = 0.10 mg/L and 0.22 mg/L, respectively) (McLeese et al. 1982). In comparison, the same compounds were much more toxic to another marine invertebrate, Crangon septemspinosa (for example, 96-h LC50 = 0.0002 mg/L for endosulfan to 0.002 mg/l for chlordane; McLeese and Metcalfe 1980). We wondered if the resistance of N. virens to organochlorines was related to their response to hypoxia.

N. virens is a sediment dweller often found in intertidal regions and consequently may experience periods of severe oxygen deprivation (Schottler and Weinhausen); varying degrees of hypoxia can initiate a switch to anaerobic energy metabolism (Jorgensen and Kristensen 1980; Magnum 1973; Schottler 1979; Schottler and Weinhausen 1981; Theede et al. 1973). Exposure to the organophosphate pesticide, phosphamidon, causes freshwater mussels to switch to anaerobic metabolic pathways (Moorthy et al. 1983). When N. virens encounter hypoxic conditions, they can also exhibit a compensatory ventilation response (Kristensen 1983). A shift to anaerobic metabolism might also be accompanied by a change in epidermal rates of passive diffusion. Thus, uptake and excretion kinetics of xenobiotics may be altered. In the present study, we measured the bioaccumulation of endosulfan, dieldrin and DDT by N. virens under normoxic and hypoxic conditions.

MATERIALS AND METHODS

Worms were collected from Passamaquoddy Bay, N.B., Canada and maintained in clean sediment and flowing seawater (ambient temperature; 30 o/oo salinity). For 48 h before testing, worms were held in normoxic or hypoxic seawater in glass aquaria (20 L) without sediment. Hypoxic conditions were produced and

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maintained by bubbling nitrogen through the water. Glass covers kept head space to a minimum (about 1 cm). Water temperature was maintained at 7°C.

Hexane (pesticide grade) solutions of the 14C-DDT (p,p'-DDT (ring-UL-14C)) and 14C-dieldrin (dieldrin (1,2,3,4,10-14C)) (California Bionucelar Corporation, California, U.S.A) were pipetted onto the bottom of a second set of aquaria. After evaporation of the hexane, hypoxic and normoxic seawater and worms (mean wt ± S.D. = 4.5 ± 2.1 g) were transferred to the aquaria. Worms placed in unspiked hypoxic and normoxic seawater in similar aquaria served as controls. Worms were transferred after 48 h to a similarly treated set of aquaria. Six worms and 2 x 1 mL of water were sampled at 0, 6, 12, 24, 48, 72 and 96 h. At 96 h the remaining worms were transferred to control hypoxic or normoxic seawater for a further 96 h. During this excretion, phase 5-6 worms were sampled at 6, 12, 24, 48, 72 and 96 h.

Water samples were mixed with 15 ml of Aquasol scintillation cocktail (New England Nuclear, Lachine, Que., Canada). Individual worms were homogenized in an equal (w/v) amount of water using a Polytron tissue homogenizer. An aliquot of the homogenate was added to 15 mL of Aquasol and 2.5 mL Protosol (New England Nuclear). The radioactivity was measured 1 wk later (allows for disappearance of photoluminescence) using a Beckman LS-100 scintillation counter. The concentration (µmol/L or µmol/g lipid) of each compound was calculated from a quench curve. The quench curve was established using aliquots of homogenate spiked with 14C-$NaHCO_3$ and Protosol (2.5 ml maximum).

Lipid content in worms exposed to 14C-labelled pesticides was determined according to the techniques of Bligh and Dyer (1959) except that only 0.5 g of tissue was used for each determination.

Additional experiments were carried out with non-radiolabelled endosulfan, dieldrin and DDT. The excretion phase was extended to 336 h. Water (20 ml) was sampled from each aquaria at 0, 6, 12, 24, 48, 49, 54, 60, 72 and 96 h. Worms (three) were sampled from each aquaria at 0, 24, 48 and 96 h exposure and at 24, 48, 96, 168 and 336 h during excretion. Dissolved oxygen in hypoxic seawater was measured daily by the azide modification of the Winkler titration (Rand et al. 1976). Whole worms were homogenized by grinding with anhydrous sodium sulfate, then extracted in a Soxhlet apparatus with hexane for 1 h. The cooled extract was brought to 100 mL and a 10-mL aliquot was taken for lipid determination.

Water samples were extracted with 2 mL of pesticide grade hexane, dried with anhydrous sodium sulfate, evaporated with a roto-evaporator and brought to volume (1 mL) with hexane. The tissues were cleaned by column chromatography (2 g of a 5% deactivated alumina and eluted with 15 mL of hexane). The eluate was concentrated to 1 mL. The hexane extracts were analyzed on a Varian 3700 gas chromatograph equipped with a 63Ni electron capture

detector and 2 m x 3 mm i.d. glass column packed with 3% OV-101 on Chromosorb-W (80-100) mesh. Temperatures were 210, 200 and 300°C for injector, column and detector, respectively. Extraction efficiencies were determined for water and tissue in all test by spiking water and worms with the test compounds and extracting by the appropriate technique. Average concentrations of the pesticides in water were calculated by using the method of Zitko et al. (1977).

Uptake and excretion rate constants (K1 and K2, respectively) for a one-compartment model were calculated according to Zitko (1980). Briefly, K2 is defined as the slope of the line:

ln (conc. in tissue) vs. depuration time

$$K1 = CF*K2/(CW*(1-EXP(-K2*T))) \quad \text{(Equation 1)}$$

where: CF = conc. in tissue at T, CW = average concentration in water, and
T = time.

When K2 is small, K1 can be estimated from:

$$K1 = CF/(CW*T) \quad \text{(Equation 2)}$$

In the present study, K2 is small relative to K1, uptake was essentially linear and the data were evaluated using linear regression. Uptake constants (slopes from linear regression) during hypoxic conditions were compared to those under normoxic conditions using analysis of co-variance (Wine 1964).

RESULTS AND DISCUSSION

The average concentrations of pesticides in water (Table 1) were similar in tests using labelled and unlabelled DDT (0.012 and 0.013 µmol/L normoxic; 0.009 and 0.010 µmol/L hypoxic, respectively), but varied by a factor of 3 to 5 between the labelled and non-labelled dieldrin (0.005 and 0.023 µmol/L normoxic 0.006 and 0.19 µmol/L hypoxic, respectively). Endosulfan concentration in water was not different ($p > 0.05$) in the hypoxic and normoxic exposures (0.14 and 0.16 µmol/L).

Dissolved oxygen values were 11-12% saturation (17 mm Hg) in hypoxic seawater at 7°C. At this oxygen concentration, N. virens exhibited a reduced oxygen uptake and an increased ventilation (Kristensen 1983; Thede 1973). N. virens is capable of surviving in hypoxic water up to 72 h at 15°C or longer at lower temperatures (Theede 1973). Worms exposed to hypoxia at 7°C in our study survived for 400 h. However, worms sampled after 400 h in hypoxic conditions appeared to be near death (flaccid, inactive and showing very little reaction to prodding).

There was very little excretion of the organochlorine pesticides during the depuration phase except for endosulfan (Figures 1-2).

Table 1. Uptake and excretion rate constants of organochlorine pesticides for Nereis virens exposed to the pesticides in normoxic and hypoxic seawater. (*p < 0.05 between treatments.)

Compound	Treatment[1]	Conc. in[2] water (µmol/L)	Diss. O_2[3]	K1[4] (1/h)	K2[5] (1/h)	Linear regression Slope[6]	Linear regression Corr. coef.
14C-DDT	N	0.012	100	0.2	0.002	0.002*	0.847
14C-DDT	H	0.009	ND[7]	0.9	0.011	0.004*	0.838
DDT	N	0.013	100	0.1	0.001	0.001	0.079
DDT	H	0.010	11	0.3	0.006	0.002	0.065
14C-Dieldrin	N	0.005	100	1.4	0.0004	0.007	0.763
14C-Dieldrin	H	0.006	ND	0.9	0.001	0.006	0.843
Dieldrin	N	0.023	100	0.6	0.001	0.013	0.906
Dieldrin	H	0.019	12	0.9	0.0006	0.018	0.944
Endosulfan	N	0.16	100	0.3	0.018	0.042*	0.906
Endosulfan	H	0.14	12	0.8	0.009	0.111*	0.784

[1]N = normoxic; H = hypoxic
[2]Average measured concentration during uptake phase
[3]Dissolved oxygen concentration in % saturation
[4]Estimated using Equation 2 in Methods
[5]Estimated using Equation 1 in Methods
[6]µmol/(g lipid x h)
[7]ND = not determined

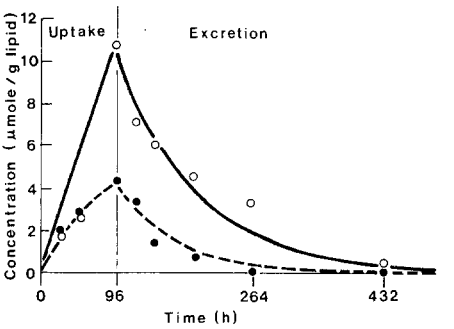

Figure 1. Uptake and excretion of endosulfan by Nereis virens during normoxic and hypoxic exposure. Curves were predicted by one-compartment model. Each symbol represents the mean of three worms. --o-- normoxic conditions; --o-- hypoxic conditions.

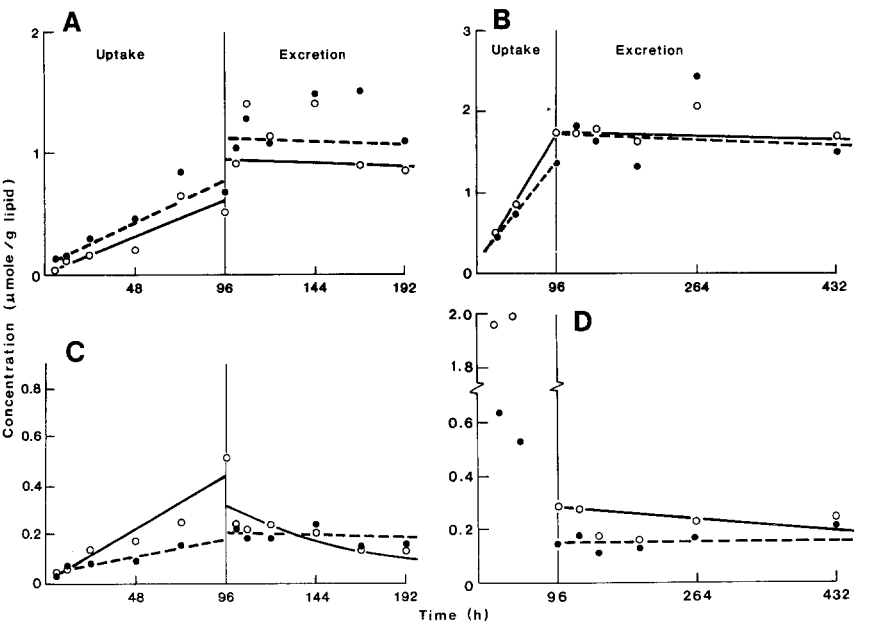

Figure 2. Uptake and excretion of (A) 14C-dieldrin, (B) dieldrin, (C) 14C-DDT and (D) DDT during normoxic and hypoxic exposure. Uptake curves are predicted by linear regression, and excretion curves are those prediced by the one compartment model (Zitko 1979). Each symbol represents the mean of 5-6 worms (A and C); 3 worms (B and D); --o-- normoxic conditions; --o-- hypoxic conditions.

The excretion of the organochlorines could be described reasonably well by the one-compartment model (depuration phase, Figures 1-2). In all cases, hypoxia had no effect on the excretion rates.

A reasonable fit between observed and predicted (one compartment model) uptake data was obtained only for endosulfan (Figure 1). However, since K2 is small relative to K1 in all cases, uptake of all these pesticides is linear. The uptake rate constants (Table 1) were determined as the slope from linear regression (Equation 2 in Methods). The uptake of endosulfan, dieldrin, 14C-dieldrin and 14C-DDT was linear (Figures 2A-2C) but was not linear for DDT on exposure to non-radiolabelled DDT (Figure 2D).

Several anomalies were observed in the DDT experiments. The concentration in worms was maximum after 96 h of exposure to 14C-DDT (Figure 2C). However, maximum concentration of DDT in worms was reached after 24 and 48 h of exposure to non-radiolabelled DDT in normoxic and hypoxic seawater, respectively (Figure 2D). By 96 h the concentrations had dropped significantly, for example from 2.0 to 0.3 µmol/g lipid during hypoxic exposure to non-radiolabelled DDT. Uptake of radioactivity during exposure to 14C-DDT was linear, but uptake of DDT during exposure to non-radiolabelled DDT was not. At similar exposure concentrations the maximum uptake of DDT was higher for the non-radiolabelled exposure than for the 14C-DDT exposure (2.04 vs 0.51 µmol/g lipid, respectively under hypoxic conditions). The non-radiolabelled DDT experiment indicates that differences in biotransformation of the pesticides in hypoxic compared to normoxic conditions may contribute to the observed differences in bioaccumulation. Peaks with retention times corresponding to the metabolites of DDT, namely DDD, DDE, and possibly DDMU, were found in the chromatograms for worms sampled after 96 h of exposure. There were trace concentrations of DDD and the concentrations of DDE were 5% of DDT on a weight basis. Similar concentrations of these metabolites were found in all samples of worms held under normoxic conditions during the depuration phase. Significant concentrations of DDT metabolites were found in worms held under hypoxic conditions only in worms sampled after 96 h of depuration.

The maximum uptake of dieldrin was also greater during exposure to non-radiolabelled dieldrin than to radiolabelled dieldrin. For example, the uptake of dieldrin and 14C-dieldrin under hypoxic conditions after 96 h of exposure was 1.36 and 0.61 µmol/g lipid, respectively (Figures 2A, 2B). The greater uptake of dieldrin in the study using non-radiolabelled dieldrin than that in the study using radiolabelled dieldrin is probably the result of the differences in exposure concentrations (Table 1). Uptake and excretion rates of chemicals from water by aquatic animals are dependent on the water concentration of the chemical (Zitko 1980).

There was no difference in uptake rate constants of dieldrin for worms held in hypoxic or normoxic conditions (slopes, Table 1). There was a significant increase ($p < 0.05$) in the uptake rate constants of endosulfan and 14C-DDT for worms under hypoxic conditions compared to those held under normoxic conditions (slopes, Table 1). Although the uptake of DDT was not linear during exposure to non-radiolabelled DDT, the results support greater uptake in hypoxic conditions compared to that under normoxic conditions. The tissue concentration of DDT was greater under hypoxic conditions compared to normoxic conditions within each sampling period (Figure 2D). If the uptake line is forced through zero, the uptake rate constant (slope, Table 1) is three times larger for uptake under hypoxic conditions compared to that under normoxic conditions (0.005 and 0.014 μmol/g lipid x hour, respectively).

The results suggest that the bioaccumulation rates of organochlorines are greater under hypoxic than normoxic conditions for N. virens. This is probably due to increased water flow over the parapodia under hypoxic conditions (i.e., increased ventilation; Kristensen 1983). This response is more effective in burrows than in the free swimming form used in our experiment. Thus the differences in uptake of organochlorines by worms may be quite different at high tide compared to that at low tide. The results of the present study did not indicate a relationship between the response of Nereis to hypoxia and resistance to the toxic effects of organochlorines.

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