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Petroleum Hydrocarbon Resistance in the Marine Worm *Neanthes arenaceodentata* (Polychaeta: Annelida), Induced by Chronic Exposure to No. 2 Fuel Oil

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INTRODUCTION

Several organochlorine and -phosphate compounds induce a variety of resistant adaptations in fish (20), mammals (6), and terrestrial arthropods (7). Petroleum hydrocarbons (PHCs) likewise induce changes in detoxification metabolic processes of exposed fish (11), invertebrates (4), and mammals (12). It is not known whether metabolic alterations induced by PHCs infer resistance modification, as is often the case with chlorinated pesticides (13). In conjunction with work concerning the effects of chronic PHC exposure on growth and reproduction, we have studied induction of PHC resistance in the marine annelid, Neanthes arenaceodentata, including examination of specific PHC (naphthalene) uptake, release, and metabolism kinetics in resistant and control worms.

MATERIAL AND METHODS

All work was performed with worms obtained from a laboratory population of Neanthes arenaceodentata which has been isolated from the natural environment for more than 12 years (50 generations). Equal numbers of males and females were used, and were separated according to the techniques of Reish and Alosi (14). Seawater parameters identical to those used in culturing experimental animals were employed throughout (15).

Groups of polychaetes were continuously exposed to selected (sublethal) concentrations of No. 2 Fuel Oil water-soluble-fractions (WSFs) for three successive generations (3 months/generation), as described by Rossi and Anderson (18). Larval juvenile, and immature adult worms were harvested at various times during the above exposure regime to examine their sensitivity to PHCs through standardized toxicity tests. Sensitivities were examined by replicate bioassays with WSFs from a crude and refined oil, using procedures given in Rossi et al. (19). To study the lability of PHC resistance, selected groups of immature adult worms were removed from exposure

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(either 7 or 14) days prior to testing. Oil WSFs were prepared as outlined by Anderson et al. (2). Levels of specific and total hydrocarbons were monitored by ultraviolet (UV) and infra-red (IR) spectrophotometry, respectively (10;1).

Metabolic conversion of ^{14}C-naphthalene in PHC resistant (chronically exposed) and susceptible (control) Neanthes was investigated in the following experiments. Using previously described (17), kinetics of ^{14}C-naphthalene accumulation and depuration, as well as conversion into polar derivatives, were derived for both resistant (F_3 exposed) and susceptible (control) strains. In addition, relative rates of ^{14}C-naphthalene (or metabolite) excretion were studied by analyses of depuration waters containing respective strains of Neanthes.

RESULTS

Results of bioassays with consecutive generations of No. 2 Fuel Oil-exposed Neanthes are given in Table I. The resistance of each generation of oil exposed worms to No. 2 Fuel Oil and south Louisiana crude oil WSF was tested for comparison with unexposed (=susceptible=control) animals. Larvae (= 3 segment worms or 'metatrocophores') from all exposure concentrations (2.5, 5, 10% WSF) in each generation (F_1, F_2, F_3) were quite similar in sensitivity to WSFs of both test oils. That is, their respective (96 hr) TLm values overlapped. Exposed larvae were identical in sensitivity to the two oils when compared with control larvae. Therefore, induction of PHC resistance was not evident at this early stage (9-10 days old) of development.

As for newly hatched larvae, sensitivities (=TLms) among adult worms did not differ from generation to generation, or between exposure concentrations. Unlike larvae, exposed adults were considerably more resistant to the two oils than were unexposed adults. Increased resistance in adults was neither related to exposure concentration, nor was there increase in resistance with successive generations. For all exposure concentrations F_1 adults were just as resistant as were F_3 adults.

Unexpectedly, exposed juveniles were less resistant than their unexposed counterparts. Juveniles within each generation exhibited similar sensitivites, in that TLm values were not significantly related to exposure concentration. There was a distinct trend towards increasing resistance in later generations. F_3 juveniles were more resistant than F_1 juveniles for bioassays with No. 2 Fuel Oil. Among susceptible (control) Neanthes the order of sensitivity for both test oils proceeds: adults > juveniles > larvae (16), whereas for exposed worms one observes the sensitivity order: juveniles > adults > larvae.

TABLE 1

Results of sensitivity bioassays with No. 2 Fuel Oil and South Louisiana crude oil (So. La.) on three life stages from each of three successive generations of polychaetes (Neanthes arenaceodentata) chronically exposed to sublethal concentrations of No. 2 Fuel Oil WSF. 96 hr TLm values + 95% confidence intervals and corresponding slope functions were calculated according to Litchfield and Wilcoxon (1949). Values represent mean results of at least two bioassays, with 10 animals used per each of 6 WSF concentrations (including a control group) in each. * = no mortality in a 100% WSF after 96 hr. 100% WSF No. 2 Fuel Oil and So. La. crude oil = 8.7 ppm and 19.8 ppm total dissolved hydrocarbons, respectively. For 0% chronic exposure concentration (control) results, F_2 and F_3 sensitivities were identical to those stated (F_1).

Generation	Life Stage	Chronic Exposure Concentration (% WSF No. 2 Fuel Oil) 0		2.5		5		10	
		No. 2	So. La.	No. 2	So. La.	No. 2	So. La.	No. 2	So. La.
F_1	Larvae	$\underset{1.8}{8.4 \pm 0.4}$	*	$\underset{1.2}{8.3 \pm 5}$	*	$\underset{1.7}{8.6 \pm 0.3}$	*	$\underset{1.65}{8.1 \pm 2}$	*
	Juveniles	$\underset{1.2}{5.7 \pm 0.4}$	*	$\underset{1.2}{4.9 \pm 0.6}$	$\underset{1.4}{16.1 \pm 0.3}$	$\underset{1.3}{4.5 \pm 0.8}$	$\underset{1.3}{12.5 \pm 1.4}$	$\underset{1}{5.8 \pm 0.2}$	$\underset{1.6}{15.6 \pm 0.5}$
	Adults	$\underset{2.0}{2.7 \pm 1.1}$	$\underset{1.2}{12.5 \pm 1}$	$\underset{1.4}{7.1 \pm 0.2}$		$\underset{1.7}{7.2 \pm 0.3}$		$\underset{1.4}{6.9 \pm 0.5}$	*
F_2	Larvae	See above		$\underset{1.2}{8.0 \pm 0.6}$	*	$\underset{1.4}{8.6 \pm 0.2}$	*	$\underset{1.7}{7.8 \pm 0.5}$	*
	Juveniles			$\underset{1.4}{5.6 \pm 0.3}$	$\underset{1.85}{18.2 \pm 0.7}$	$\underset{1.7}{6.0 \pm 0.2}$	$\underset{1.4}{17.6 \pm 1.1}$	$\underset{1.65}{6.0 \pm 0.2}$	$\underset{1.2}{16.5 \pm 2.0}$
	Adults			$\underset{1.4}{7.2 \pm 0.3}$	*	$\underset{1.7}{6.7 \pm 0.6}$	*	$\underset{1.7}{7.6 \pm 0.5}$	*
F_3	Larvae	See above		$\underset{1.7}{6.5 \pm 1.0}$	*	$\underset{1.2}{8.7 \pm 0.8}$	*	$\underset{1.2}{8.7 \pm 0.3}$	*
	Juveniles			$\underset{1.4}{6.7 \pm 0.3}$	$\underset{1.4}{16.8 \pm 1.7}$	$\underset{1.4}{5.8 \pm 0.4}$	$\underset{1.1}{17.8 \pm 1.0}$	$\underset{1.4}{6.3 \pm 0.4}$	$\underset{1.2}{16.9 \pm 0.4}$
	Adults			$\underset{1.3}{6.7 \pm 0.5}$	*	$\underset{1.2}{7.0 \pm 0.5}$	*	$\underset{1.7}{5.8 \pm 1.2}$	*

Data from sensitivity tests performed with groups of adult Neanthes removed from chronic exposure prior to bioassays are presented in Table II. Worms removed from exposure 7 days before

TABLE II

Results of bioassays on groups of adult Neanthes removed from chronic exposure to 5% No. 2 Fuel Oil WSF either 7 or 14 days prior to resistance testing. 96 hr TLm values and their respective ± 95% confidence intervals (C.I.) and slope functions (S) are given. TLm values expressed in ppm total dissolved hydrocarbons initially present in a 100% WSF of the challenge oil (No. 2 Fuel Oil). Mean values from 2 bioassays are given, with 10 animals for each of six concentrations (including a control group) used per bioassay.

Generation	Prior Removal Time (Days)	TLm	C.I.	S
Control	-	2.7	3.8-1.9	2.0
	0	7.2	7.5-6.9	1.7
F_1	7	7.1	7.5-6.7	1.2
	14	4.9	5.4-4.4	1.5
	0	6.7	7.3-6.1	1.7
F_2	7	7.1	7.7-6.5	1.3
	14	6.4	6.9-5.9	1.3
	0	7.0	7.5-6.5	1.2
F_3	7	7.0	7.5-6.5	1.2
	14	7.2	7.6-6.8	1.4

testing were just as resistant as constantly exposed animals, for all three generations. Only first generation adults removed 14 days prior to testing were significantly less resistant than constantly exposed adults. Note that F_1 worms removed 14 days before testing were still more resistant than unexposed animals.

Kinetics of ^{14}C-naphthalene uptake and release by susceptible (control) and resistant (F_3 exposed to 5% WSF) Neanthes are seen in Figure 1. Both groups accumulated and released ^{14}C-naphthalene in nearly identical fashion. There was apparently little difference in permeability to ^{14}C-naphthalene between resistant and control animals, noting that F_3 worms were acutely exposed to slightly higher concentrations of ^{14}C-naphthalene.

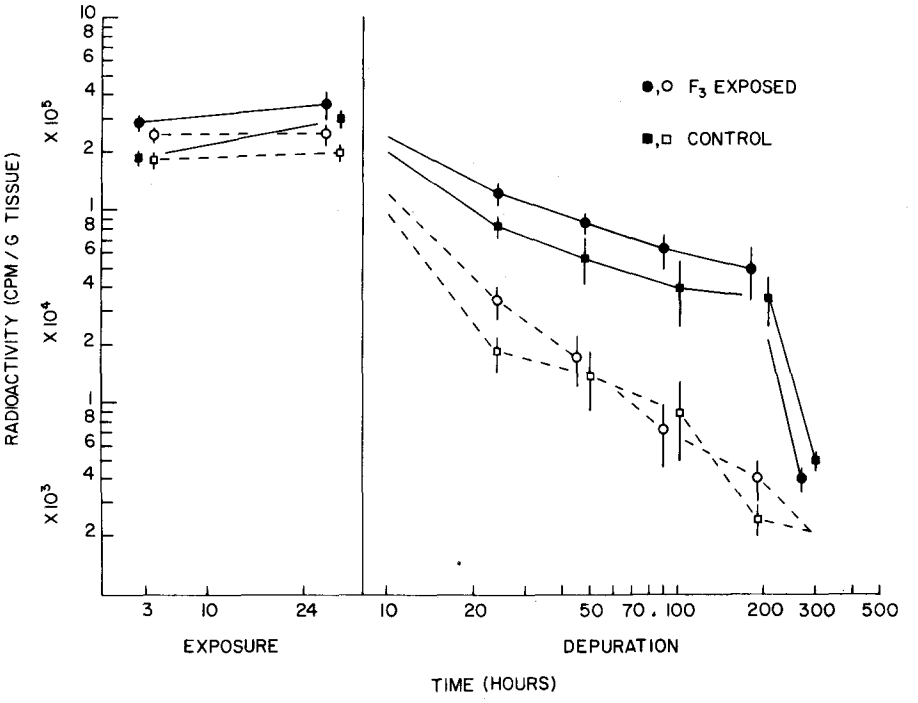

Fig. 1. Radioactivity in adult Neanthes tissues, during and after placement in clean sea water following acute exposure to ^{14}C-naphthalene. Open symbols depict unmetabolized (hexane extractable) radioactivity, dark symbols indicate total extractable radioactivity (unmetabolized + metabolized ^{14}C-naphthalene). Symbols represent mean values of 8 samples ± S.D. (vertical bars).

The quantitative role of ^{14}C-naphthalene metabolism appeared similar for both worm groups. After placement in clean sea water, both groups released radioactivity in the unmetabolized (native) as well as converted (more polar) form. Analyses of depuration water indicated possible metabolic differences between F_3 and control Neanthes, as is seen in Figure 2. However, these differences

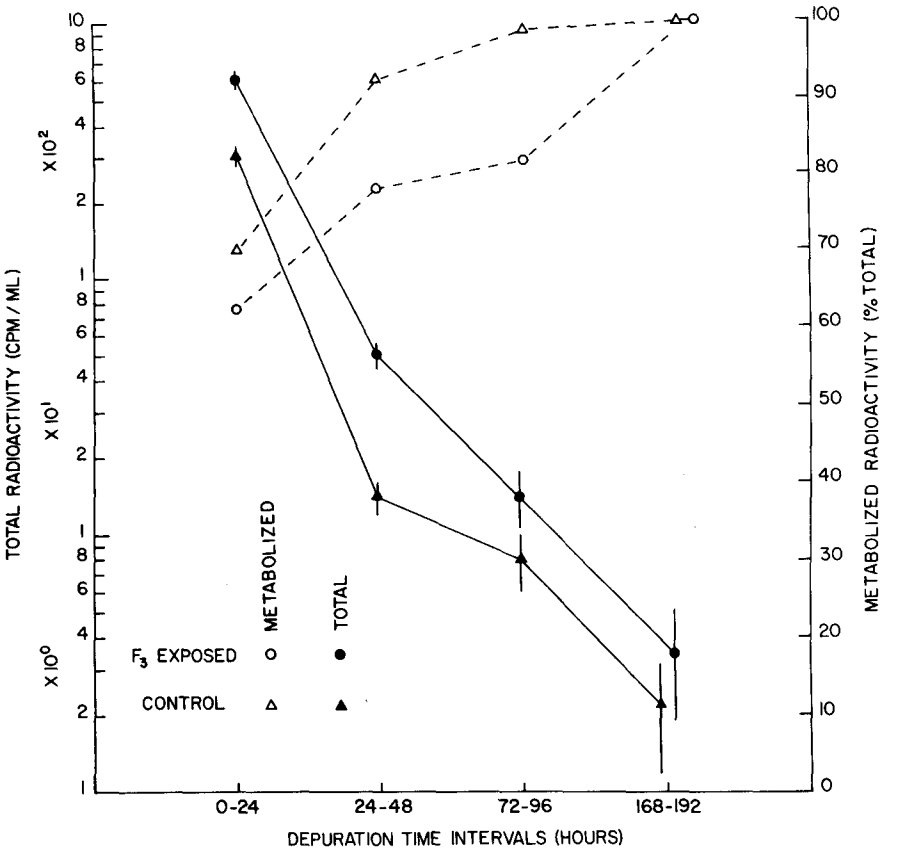

Fig. 2. Radioactivity in sea water containing Neanthes for selected 24 hr intervals after acute exposure to ^{14}C-naphthalene. Symbols depict mean values for 8 samples ± S.D. (vertical bars). Metabolized radioactivity = non-hexane extractable; total = metabolized + unmetabolized (hexane extractable) radioactivity.

proved inconsequential since loss of the two forms of radioactivity from tissues was nearly identical for both groups. From Figs. 1 and 2, relative percentages of radioactivity released during depuration closely paralleled those which simultaneously occurred within tissues. Specifically, concentrations of unmetabolized ^{14}C-naphthalene approached undetectable levels in tissues of both groups at the same time detectable amounts were no longer found in external media.

DISCUSSION

Results presented here offer the first report of toxicological resistance adaptation in a marine invertebrate. Induction of resistance to chlorinated hydrocarbons has not been studied among marine invertebrates, and chronic sublethal exposure to glycol esters failed to produce resistance in a closely related polychaete worm, Capitella capitata (3). However, aspects of findings presented here conflict with those of related studies (with chlorinated hydrocarbons), while many of our results substantiate those of previous work on xenobiotic resistance development. In this study little evidence of increased resistance with successive generations (or exposure level) was observed, as has been noted in sheepshead minnows (Cyprinodon variegatus) during exposure to DDT (8). That is, F_3 adult worms were no more tolerant than F_1 animals, and polychaetes exposed to 2.5% WSF were just as tolerant as were those exposed to 10% WSF (Table I). With the exception of F_1 worms removed 14 days prior to challenge, we observed little termination or reduction of resistance shortly after cessation of exposure (especially among F_2 and F_3 worms). Significant reductions in brood survival (notably between larval and juvenile stages) below control values were observed among exposed worms (18). It appears that resistance in Neanthes was attributable to a selection process in that direct pressure of PHC exposure selected, through mortality, animals with genetic characteristics conferring resistance.

The failure of exposed larvae and juveniles to exhibit PHC resistance above control levels may be due to the incomplete development of biochemical machinery (active in resistance mechanisms) in these early stages. As has been suggested for developing mammals (6), younger worms (larvae and juveniles) may only gradually acquire resistance mechanisms as they mature. Lack of sensitivity differences between exposed and control larvae suggests that the extremely high lipid content (up to 85% dry weight) in larvae may effectively sequester cytotoxic hydrocarbons away from developing tissues, so as to overshadow potential differences in sensitivity between resistant and control larvae (5).

Results from experiments with ^{14}C-naphthalene indicate that the most important mechanisms of PHC resistance in Neanthes may not be related to external permeability and/or excretion rate differences. Factors potentially responsible for PHC resistance might include some form of membrane (external) barrier (20), and/or more effective metabolism and subsequent excretion (in resistant polychaetes) (13). However, resistant Neanthes were not less permeable, and did not more efficiently metabolize or excrete ^{14}C-naphthalene than (did) susceptible worms (Fig. 1). Thus, on a quantitative basis, susceptible and resistant Neanthes appear to handle challenge from the key PHC, naphthalene, in nearly identical fashion. It therefore seems likely that resistance differences are attributable to either or both: 1) Some form of an internal barrier associated with sensitive nervous tissue (20), or 2) qualitative changes in the metabolism of naphthalene.

SUMMARY

1. Three successive generations of the marine polychaetous annelid Neanthes arenaceodentata taken from a laboratory population, were continuously exposed to one of three sublethal concentrations of No. 2 Fuel Oil water-soluble-fraction (WSFs). During each generation larvae, juvenile, and immature adult polychaetes were challenged with acute (96 hr) doses of No. 2 Fuel Oil or south Louisiana crude oil WSF to test their sensitivity to petroleum hydrocarbons (PHCs).

2. Larvae from all 3 generations, at all exposure concentrations, were no different from control (susceptible) larvae in their sensitivity to the two test oils. F_1, F_2, and F_3 adults exhibited equally increased PHC resistance (X2) compared to control adults.

3. The only evidence of increased resistance beyond that observed in F_1 animals was seen in results of bioassays with juvenile worms, wherein PHC resistance increased from slightly below control levels in F_1 juveniles to slightly above control tolerance among F_3 juveniles.

4. With the exception of F_1 worms, removal from chronic exposure 7 or 14 days prior to challenge did not result in termination or reduction of resistance, implicating a genetic mechanism behind PHC resistance in N. arenaceodentata.

5. F_3 resistant and unexposed control polychaetes accumulated, metabolized, and excreted a key diaromatic PHC (naphthalene-^{14}C) in quantitatively identical fashion. Mechanisms responsible for resistance appeared unrelated to external permeability and/or excretion rates.

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REFERENCES

1. AMERICAN PETROLEUM INSTITUTE: Determination of volatile and non-volatile oily material. Infrared Spectrometric Method, No. 733 (1958).
2. ANDERSON, J.W., J.M. NEFF, B.A. COX, H.E. TATEM and G.M. HIGHTOWER: Mar. Biol. 27, 75 (1974).
3. BELLAN, G., D.J. REISH and J.P. FORET: C.R. Acad. Sci. Paris 272, 2476 (1971).
4. BURNS, K.A.: Mar. Biol. 36, 5 (1976).
5. FABACHER, D.L. and H. CHAMBERS: Bull. Environ. Contam. Tox. 6, 373 (1971).
6. HARTGROVE, R.W. and R.E. WEBB: Pest. Biochem. Physiol. 3, 61 (1973).
7. HODGSON, E., L.G. TATE, A.P. KULKARNI and F.W. PLAPP, JR.: J. Agr. Food Chem. 22, 360 (1974).
8. HOLLAND, H.T. and D.L. COPPAGE: Bull. Environ. Contam. Tox. 5, 362 (1970).
9. LITCHFIELD, J.T. and F. WILCOXON: J. Pharmac. exp. Ther. 96, 99 (1949).
10. NEFF, J.M. and J.W. ANDERSON: Bull. Environ. Contam. Tox. 14, 122 (1975).
11. PAYNE, J.F. nad W.R. PENROSE: Bull. Environ. Contam. Tox. 14, 112 (1975).
12. PELKONEN, O., E.H. KALTIALA, N.T. KARKI, K. JALOENE, and K. PYORALA: Xenobiotica 5, 501 (1975).
13. PETRELLA, V.J., J.P. FOX and R.E. WEBB: Tox. Appld. Pharmac. 34, 283 (1975).
14. REISH, D.J. and M.C. ALOSI: Bull. Sth. Calif. Acad. Sci. 67, 21 (1968).
15. REISH, D.J. and T.L. RICHARDS: Turtox News 44, 16 (1966).
16. ROSSI, S.S. and J.W. ANDERSON: Bull. Environ. Contam. Tox. 16, 18 (1976).
17. ROSSI, S.S.: Procs. J.C. Prev. Cont. Oil Spills, 621 (1977).
18. ROSSI, S.S.: Air, Wat., Soil Poll. 9, in press (1977).
19. ROSSI, S.S., J.W. ANDERSON and G.S. WARD: Environ. Poll. 10, 9 (1976).
20. WATKINS, J. and J.D. YARBROUGH: Bull. Environ. Contam. Tox. 14, 731 (1975).