Toxicity of Water-Soluble Fractions of No. 2 Fuel Oil and South Louisiana Crude Oil to Selected Stages in the Life History of the Polychaete, *Neanthes arenaceodentata*

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INTRODUCTION

Few generalizations can be made concerning the toxicity of petroleum hydrocarbons (PHCs) for larval or juvenile forms of marine invertebrates, as compared to the sensitivity of their adult counterparts. Contrary to the widely held belief that younger stages of a given species are more susceptible to environmental stress (THORSON, 1950), some studies have shown marine larvae and juveniles to be quite similar to adults in thier susceptibility to PHCs (TATEM, 1975). In a few cases, sensitivity to PHCs has been observed to increase with age and development (COX, 1974; KUHNHOLD, 1972). Attempts to characterize the toxicity of a pollutant (or mixture thereof) for a given species should ideally include an examination of its toxicity for early or critical stages in the life history of the organism studied.

Previous studies concerned with the toxicity of PHCs for marine larvae and juveniles have been confined to species with pelagic or epibenthic young life stages. More work is needed concerning the effect of PHCs on truly infaunal, burrowing organisms. Many polychaetes are found in PHC-containing sediments contaminated by chronic (refinery effluents) or acute (spills) inputs, and as a consequence, may be exposed to PHCs throughout their entire life cycle (REISH, 1973). The Nereid polychaete, *Neanthes arenaceodentata*, is known to reside in sediments moderately influenced by petroleum refinery effluents (REISH, 1957a). Unlike most polychaetes, *N. arenaceodentata* lacks a pelagic stage in its life history. Consequently, this species completes its entire life cycle within quiet-water sediments (REISH, 1957b). We report here results of bioassays performed on four juvenile and 2 adult stages in the life history of *N. arenaceodentata*.

METHODS AND MATERIALS

Bioassays were performed on the following life history stages: 4, 18, 32 and 40 segment juveniles, as well as 60 segment mature adult worms. Juvenile stages were obtained from mature worm couples (one gravid female and one male), previously isolated from a laboratory population of this species. At the appropriate time following fertilization, developing worms (4

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 1 © 1976 by Springer-Verlag New York Inc.

and 18 segment juveniles) were removed from the parent tube for testing. Older juvenile and adult stages were also harvested from the offspring of isolated worm couples. In most cases, one couple produced enough offspring for one bioassay with the two oils studied: No. 2 Fuel Oil and South Louisiana crude oil. In each bioassay 5 concentrations of water-soluble fraction (WSF) and a control were employed. For all testing, ten animals were used at each concentration. For juveniles, 10 animals were placed in uncovered 100 ml culture dishes, containing 50 mls WSF of a given concentration (100, 80, 60, 40, 20, or 0%). Adults were held individually in unstoppered 125 ml erlenmeyer flasks containing 50 mls test solution. Each bioassay was repeated four times so that $n \geq 40$ for each concentration tested. In all bioassays, no control mortality was observed. All experiments were performed at room temperature (22 ± 1°C), using 32 o/oo salinity artificial sea water (Instant Ocean).

Oil water-soluble fractions (WSFs) employed here were prepared as described by ANDERSON *et al.* (1974a). Ultraviolet spectrophotometric analysis by the technique of NEFF and ANDERSON (1975) showed that all equivalent test solutions in this study were statistically ($p < 0.05$) identical to each other as well as those used in earlier bioassays with immature adult polychaetes (ROSSI *et al.*, 1975). The hydrocarbon composition of WSFs from No. 2 Fuel Oil and South Louisiana crude oil is given by ANDERSON *et al.* (1974a).

RESULTS

Complete results of bioassays on juvenile and adult stages are presented in Tables 1 and 2, respectively. Results reported for immature adults, produced in an earlier study, are included in Table 2 for comparative purposes. Data presented in these two tables show the broad differences in sensitivity to oil WSFs among selected stages in the life cycle of *Neanthes arenaceodentata*. As was shown for adults, WSFs prepared from No. 2 Fuel Oil were more toxic to juvenile worms than were those from South Louisiana crude oil. A great deal of the toxicity of No. 2 Fuel Oil WSF is attributed to its comparatively high concentration of diaromatic compounds (naphthalenes) (ANDERSON *et al.*, 1974b). It would appear that the toxic action of naphthalenes is similar for juvenile and adult polychaetes. To dramatize differences in sensitivity, 96 Hr TLm values for all stages are plotted as a function of age in Figure 1. TLm values are considered significantly different if their respective 95% confidence intervals fail to overlap one another. Although some differences in sensitivity were apparent after 48 hours of testing, 96 Hr TLm values provide more accurate comparisons, since the WSFs employed failed to elicit significant mortality in some juvenile stages after only 48 hours of testing. As can be seen from Figure 1, the toxicity of WSFs from both oils for *Neanthes* increases as worms grow older and increase in size. Differences in sensitivity to the two oils are greatest between the earliest juvenile stage tested (9 day old animals) and adults,

TABLE 1.

Statistical analysis of results of bioassays on juvenile *Neanthes arenaceodentata*. Ninety five percent confidence intervals (C.I.) and slope functions (S) are given for each TLm value, as calculated by the method of Litchfield and Wilcoxon (1949). TLm values represent ppm of total hydrocarbons calculated from the amount present in a 100% water-soluble fraction (WSF), while those in parentheses represent the equivalent percentages.

Stage and Age (Days)	Statistical Value	No. 2 Fuel Oil WSF			South Louisiana Crude Oil WSF		
		24h	48h	96h	24h	48h	96h
4 Segment (9)	TLm	>8.7(100)	>8.7	8.4(96)	>19.8(100)	>19.8	>19.8
	C.I.	-	-	8.6-8.2	-	-	-
	S	-	-	1.0	-	-	-
18 Segment (21)	TLm	>8.7(100)	>8.7	5.7(66)	>19.8(100)	>19.8	>19.8
	C.I.	-	-	6.1-5.3	-	-	-
	S	-	-	1.2	-	-	-
32 Segment (30)	TLm	>8.7(100)	7.8(90)	5.4(62)	>19.8(100)	>19.8	17.8(90)
	C.I.	-	8.0-7.6	6.3-4.5	-	-	18.2-17.4
	S	-	1.05	1.7	-	-	1.0
40 Segment (40)	TLm	>8.7(100)	6.2(71)	4.0(46)	>19.8(100)	17.0(86)	15.2(77)
	C.I.	-	6.5-5.9	4.4-3.6	-	1.3	1.1
	S	-	1.4	1.8	-	17.7-16.3	15.8-14.6

TABLE 2.

Statistical analysis of results of bioassays on adult *Neanthes arenaceodentata*. Ninety five percent confidence intervals (C.I.) and slope functions (S) are given or each TLm value, as calculated by the method of Litchfield and Wilcoxon (1949). TLm values represent ppm of total hydrocarbons calculated from the amount present in a 100% water-soluble fraction (WSF), while those in parentheses represent the equivalent percentages.

Stage and Age (Days)	Statistical Value	No. 2 Fuel Oil WSF			South Louisiana Crude Oil WSF		
		24h	48h	96h	24h	48h	96h
Immature* Adults	TLm	>8.7(100)	3.2(37)	2.7(31)	18.0(91)	13.9(70)	12.5(63)
48 Segment	C.I.	-	4.1-2.3	3.8-1.6	19.2-16.8	14.0-13.8	13.5-11.5
(46)	S	-	1.8	2.0	1.2	1.2	1.2
Mature Males	TLm	>8.7(100)	3.0(35)	2.6(30)	18.1(92)	13.6(69)	12.0(61)
60 Segment	C.I.	-	3.5-2.5	3.3-1.9	18.9-17.3	14.4-12.8	12.5-11.5
(54)	S	-	1.9	2.0	1.0	1.2	1.4
Gravid Females	TLm	>8.7(100)	5.6(69)	4.2(48)	>19.8(100)	18.0(91)	17.6(89)
60 Segment	C.I.	-	6.0-5.2	4.5-3.9	-	17.0-16.0	18.1-17.1
(54)	S	-	1.5	1.6	-	1.0	1.0

*From ROSSI *et al*. (1975)

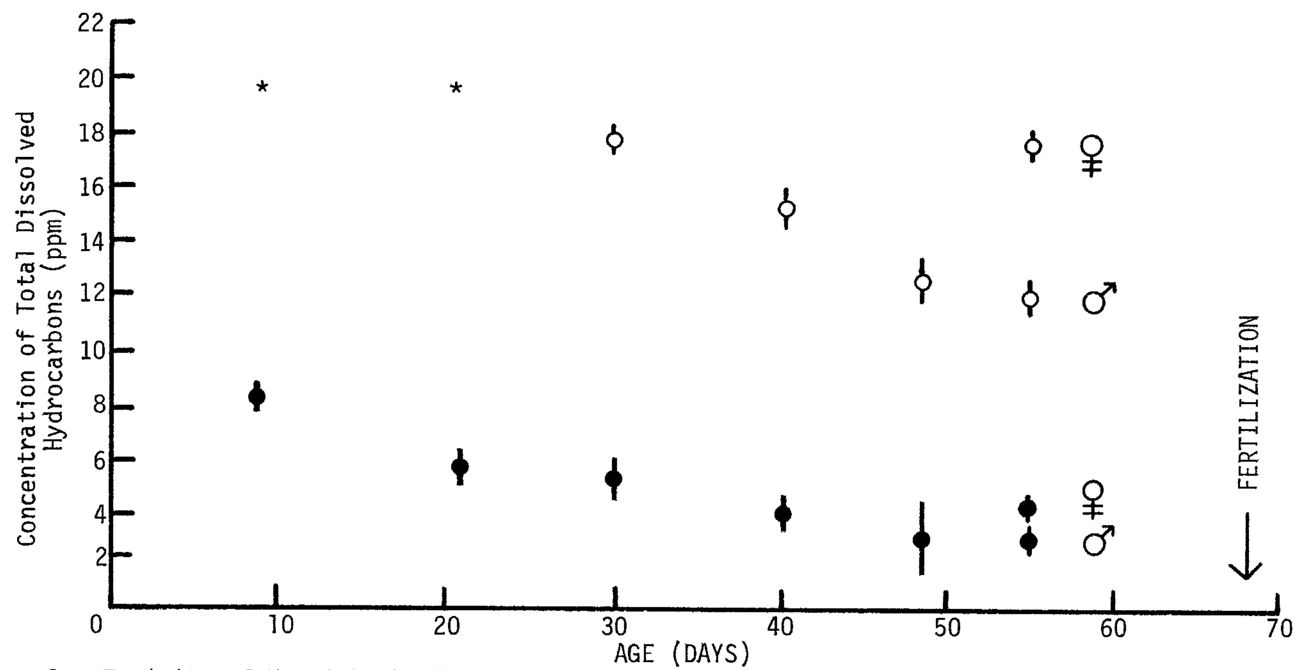

Figure 1. Toxicity of No. 2 Fuel oil and South Louisiana crude oil WSFs for six stages in the life history of *Neanthes arenaceodentata*. 96 H TLm values for South Louisiana crude oil (open circles) and No. 2 Fuel oil (dark circles) ± 95% confidence intervals (vertical bars) are plotted as a function of age. TLm values represent ppm of total dissolved hydrocarbons calculated from the amount present in a 100% WSF, as determined by infrared spectrometry (100% WSF No. 2 Fuel oil = 8.7 ppm; 100% WSF South Louisiana crude oil = 19.8 ppm), where * = TLm > 19.8 ppm.

except in the case of gravid females, whose sensitivity was similar to that of medium-sized (30 day old) juveniles. Bioassays performed on immature adult worms failed to show a significant difference in the sensitivity of male and female animals at this stage of development.

DISCUSSION

Comparisons involving larvae and juvenile sensitivities to PHCs are difficult to make since much of the earlier work was performed with oil-water test solutions which were not well characterized. ANDERSON *et al*. (1974) have documented the great discrepancies which can exist between calculated and actual PHC levels in sea water test solutions. Recently, several workers have begun to use chemically well-defined media. Using these recent studies for comparison, it would appear that juveniles and very young life stages of *Neanthes* are much more resistant to crude oil WSFs than are fish fry (KUHNHOLD, 1974 and RICE *et al*., 1975). Larvae and post-larvae of the grass shrimp (*Palaemonetes pugio*) are somewhat more sensitive to No. 2 Fuel Oil WSFs than are *Neanthes* juveniles. However, the toxicity of this WSF for adults of both species is quite similar (TATEM, 1975). Although the toxicity of No. 2 Fuel Oil WSFs for large juvenile stages of the commercial brown shrimp (*Penaeus aztecus*) is greater than that for large polychaete juveniles, the sensitivity of brown shrimp postlarvae to this WSF is very similar to that of *Neanthes* 18 segment (21 day old) juveniles (COX, 1974). These comparisons clearly indicate the dangers involved in inferring juvenile (larval) sensitivity to PHCs from adult toxicity data.

It is not known with certainty why the toxicity of refined and crude oil WSFs increases with increasing development in *Neanthes*. The results of bioassays with gravid adult female worms lend support to one possible explanation. We suggest that the greater concentration of yolk matter within (younger) worms is in some way responsible for the greater tolerance of these stages. This is indicated by the simultaneous increase in tolerance and yolk content of adult females as they become gravid. Likewise, the most dramatic decrease in tolerance to No. 2 Fuel Oil WSF is found between the 4 segment and 18 segment stages, which represents an eleven day time period during which juvenile worms use up a large percentage of their yolk supply in morphogenesis. Juveniles begin to rely on external food sources shortly after reaching the 18 segment stage (REISH, 1957b). It is possible that the more toxic PHCs (naphthalenes) are being sequestered into yolk material, known to contain significant amounts of neutrophilic lipids and glycolipids (COSTELLO, 1949 and ALLEN, 1962), to the extent that their effect on other tissues is reduced. Naphthalenes are known to be accumulated rapidly and retained by gravid female *Neanthes* (ROSSI and ANDERSON, unpublished manuscript). Similarly, sequestration of chlorinated hydrocarbons into lipid-containing tissues or organs has been proposed as an explanation for insecticide resistance in brown trout (HOLDEN, 1962) and mosquito fish (FABACHER and CHAMBERS, 1971).

These results indicate that significant differences in sensitivity to PHCs can exist among life stages of marine invertebrates. Of particular interest is the great tolerance to PHCs exhibited by the very young stages of the *Neanthes* life cycle. It would appear that young adult *Neanthes* are most susceptible to oil spillage or other sources of PHC introduction. More work is needed to assess the pattern of toxicity for life stages of other infaunal species.

Acknowledgements

This research was supported by contract #OS20C from the American Petroleum Institute to J.W. Anderson.

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