DEPURATION OF KEPONE BY ATLANTIC CROAKER IN A LABORATORY STUDY[a]

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Abstract: Contamination of the James River in Virginia by the organochlorine pesticide Kepone[R] prompted depuration studies of commercially important species. Approximately 400 croaker (*Micropogonias undulatus*) were taken from the James River and placed in Kepone-free York River water. Groups of 20 fish, maintained at ambient temperature, were sampled over time to determine depuration rate. Results suggest that there is no substantial depuration of Kepone by croaker until water temperature exceeds 15 C.

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Fishes taken in the James River, Virginia, have been shown to carry residues of the pesticide Kepone[R] of sufficient concentration to constitute a potential health hazard (Bender et al. 1977). As a result, the James River has been closed to commercial finfishing (except channel *Ictalurus punctatus*, and white catfish *I. catus*, and American shad, *Alosa sapidissima*, for a brief period) since early 1976. The river has been exposed to Kepone for considerable time and the observed Kepone residues in fish have been attributed to biomagnification through the food chain and direct uptake from water (Schimmel and Wilson 1977). Fish may depurate Kepone when placed in uncontaminated water (Bahner et al. 1977).

Predictions concerning the spread of Kepone itself along the East Coast and the body burden of contaminated migratory fish must be based on defined depuration rates. To this end we placed Atlantic croakers taken from the James River in Kepone-free York River water. Croaker was chosen as the test species because it is a commercially and recreationally important species in the middle Atlantic states. They migrate out of the James River and are known to be contaminated with Kepone.

METHODS AND MATERIALS

Approximately 400 croakers were collected from the lower James River in October of 1976 and transported to Virginia Institute of Marine Science. The fish were held in 1.2-m diameter tanks supplied with Kepone-free York River water in a flow-through system. Kepone-free squid were fed to the fish in amounts adequate to maintain health.

Temperature was close to that of ambient York River water, except during part of February and March when small heaters were necessary to maintain water temperature at approximately 10 C. Water temperature of the test tanks was recorded from December through the end of the experiment (Fig. 1).

Groups of 20 fish were sacrificed at weeks 2, 4, 8, 16, 24 and 28 and analyzed for Kepone. Whole fish were ground in a meat grinder into hamburger consistency, frozen at -5 C for 24 hours to rupture the cells and then thawed. After thawing, a mixture of anhydrous sodium sulfate and Quso[R] G-30 (precipitated silica, Philadelphia Quartz Co.) was added for desiccation. The proportions of sample to the desiccants were: 30 g fish - 54 g Na_2SO_4 - 6 g Quso. The samples were taken mixed and refrozen. After thawing the desiccated samples were ground with a blender to a powdery consistency and transferred

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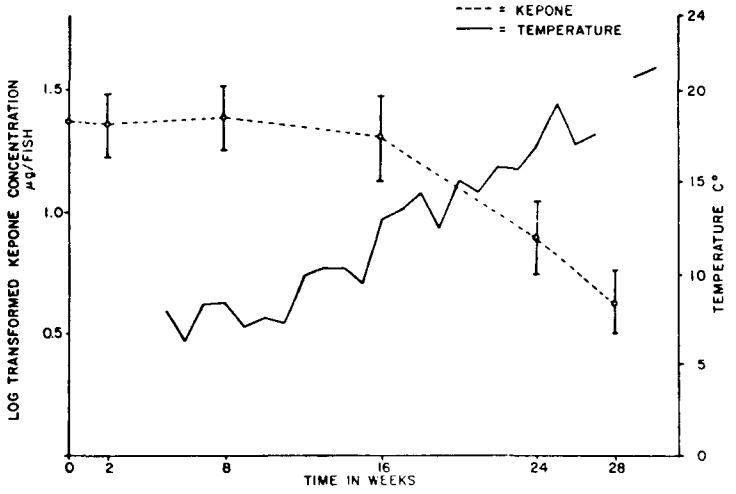

Fig. 1. Mean Kepone concentrations (log_{10} transformed) of groups of 20 croakers sampled over time (dotted line). Solid line denotes temperature of ambient York River water over same time period.

to pre-extracted paper thimbles for Soxhlet extration. Extraction was carried out using 1:1 ethyl ether-petroleum ether for 16 hours. Extracts were then concentrated by evaporation and cleaned by activated fluorisil column chromatography (Moseman et al. 1977). The Kepone containing elutriate was analyzed by electron capture gas chromatography utilizing packed columns with one or more of the following liquid phases: 4% SE-30 + 6% OV 210; 1.5% OV-17 + 1.95% QF-1 + 3% OV-1. On occasion, when concentrations were sufficiently high, Kepone presence was confirmed by mass spectrometry.

Analysis of variance was used to test treatment effect of time (1 factor AOV, treatment = time, 6 levels). Prior to analysis of variance Bartlett's test of homogeneity of variances (Sokal and Rohlf 1969) was performed to test the assumption of homogeneity (and normality since this test is also sensitive to departure from normality). Observations in the original scale of measurement (μg/fish) exhibited heterogeneity (and/or non-normality). These data were transformed by common logarithms (base 10) and retested. The transformed data showed no evidence of violation of the assumptions and were used for the analysis of variance. A posteriori multiple comparisons between treatment means were carried out using Student-Newman-Keuls procedure (Sokal and Rohlf 1969).

RESULTS

The mean levels of Kepone in the croakers showed little change through the first 16 weeks ranging from 27.4 to 30.7 μg/fish, whereas the 24- and 28-week means were greatly reduced. The analysis of variance displayed a highly significant ($\alpha = 0.001$) treatment effect of time ($F^{S} = 18.552$; 5,106 df;), therefore the H_0 of equal treatment means was rejected.

Student-Newman-Keuls procedure (Table 1) showed that T_0 to T_{16} means were not significantly different. T_{24} and T_{28} means also were not significantly different. However, these 2 sets of means were significantly different. An explanation for this pattern is that a threshold temperature is necessary before substantial depuration can occur. As can be seen in Fig. 1, no significant change in mean levels occurred until the temperature was above 15 C.

Table 1. Student-Newman-Keuls test of treatment means. Means underlined by the same line are not significantly different ($P > 0.05$). Treatment = time (6 levels).

	T_{28}	T_{24}	T_{16}	T_{2}	T_{0}	T_{8}
	1	2	3	4	5	6
y	0.631	0.989	1.302	1.356	1.367	1.385
n_i	14	20	20	19	19	20
s^2	0.052	0.103	0.138	0.072	0.065	0.082

DISCUSSION

It appears that there is a threshold of metabolic activity which must be attained before substantial depuration of Kepone is accomplished. Croakers collected just prior to migrating from the James River in the fall of 1976 were found to average 27.5 ± 6.5 μg Kepone/fish. This level of contamination was maintained through the winter and early spring of 1977 based on samples taken within that time period from our holding facilities (weeks 0-16). A significant drop in Kepone concentration was observed in the 24-week sample (10.7 ± 4.1 μg/fish). This drop in Kepone coincided with the rise in the ambient water temperature to above 15 C.

Therefore, since the adult portion of the natural population returning to the James River from the south (Haven 1959) would have likely encountered temperatures above 15 C, they may be able to depurate the majority of Kepone from their tissues before returning to Chesapeake Bay. Young of the year croaker overwinter in the estuaries (Chao 1977) and would not encounter temperatures which would allow depuration.

Future work needs to be done to substantiate the relationship between temperature and depuration of Kepone.

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