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FATE AND DISTRIBUTION OF ^{14}C-DDT IN THE WINTER FLOUNDER, Pseudopleuronectes americanus

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Coastal and estuarian waters are ultimately the recipient of large quantities of the persistent organochlorine pesticides, posing a serious potential hazard to these vastly productive and important ecosystems. The extent of this danger is not yet understood for little is known of the sub-lethal effects of such pesticides. As an initial step in examining these effects, the distribution of DDT (1,1,1-trichloro-2,2-bis [p-chlorophenyl] ethane) was studied in a marine teleost, P. americanus, using ring labeled ^{14}C-DDT obtained from Amersham/Searle Corporation. The flounder (150-400 g) were injected intravenously with doses of 0.1 mg ^{14}C-DDT/kg dissolved in 50% ethanol. After injection all fish used in short term study were maintained in enameled metal containers in 1 liter of aerated seawater at 13° C. Fish studied for one week were placed in free-flowing aquaria. Urine was collected via a catheter inserted into the external urinary papilla and tied in place. Blood samples of 0.3 ml were drawn by venipuncture of the caudal vein. Bile and tissue samples were obtained after decapitation of the fish. Tissue and fluid samples were dissolved in 1 ml of Soluene (Packard Instrument Co.), 10 ml of toluene scintillation fluid (5 g/liter PPO and 250 mg/liter POPOP) were added, and the samples were counted in a Nuclear Chicago scintillation counter. Initially, total recovery was determined by homogenizing the entire carcass (i.e., the body exclusive of organs sampled directly) and counting as above. However, for the majority of the animals, recovery from the carcass was estimated on the basis of the radioactivity in the skin and muscle samples. The radioactivity recovered in these experiments represents DDT and any of its metabolites produced by the fish. The nature of such metabolites is currently under investigation with the aid of Dr. A. Gaurino.

The data describing the disappearance of the radioactivity from flounder plasma is summarized in Table 1. When these data are plotted logarithmically and the resulting curve is stripped three components are seen. Initially, plasma pesticide levels decayed rapidly with a $t_{1/2}$ of on 5 min, presumably representing exit from the vascular compartment. The second half time of 2 hr probably arises from the redistribution of the drug from tissues of high blood flow and/or low affinity to tissues of higher lipid content and affinity. Finally, there is a very slow decrease in plasma levels ($t_{1/2}$ = 24 days) probably reflecting excretion of the pesticide.

Table 1

PLASMA LEVELS OF ^{14}C-DDT FOLLOWING INTRAVENOUS INJECTION OF 0.1 mg/kg

	5 min	15 min	1 hr	4 hr	8 hr	24 hr	48 hr	1 wk
Plasma (μg/ml)	2.48	0.93	0.28	0.16	0.10	0.08	0.07	0.07
SE	0.38	0.32	0.04	0.02	0.02	0.02	0.02	0.03
n	4	10	11	8	7	6	4	4

Total recoveries were generally between 60 and 100 (average 77) percent of the injected dose. However, included in the average are five animals which showed recoveries as low as 30% of the initial dose. (The most likely explanations of these low recoveries would be partially missed injections or spuriously low muscle counts due to unequal distribution.) Because of the variability in recovery of the injected dose, the pesticide content in each organ or tissue (Table 2) has been expressed as a percentage of the total recovered ^{14}C-DDT. Each value in Table 2

Table 2

TISSUE DISTRIBUTION OF ^{14}C-DDT FOLLOWING INTRAVENOUS INJECTION

Organ content as percent of total pesticide recovered ± SE and, in parentheses, tissue to plasma concentration ratio based on wet weight content

	15 min	1 hr	4 hr	8 hr	24 hr	48 hr	1 wk
Carcass	36.1 ± 4.3 (0.04)	51.0 ± 7.7 (0.2)	74.0 ± 2.4 (0.4)	76.9 ± 4.8 (0.8)	81.3 ± 2.1 (0.9)	81.0 ± 4.1 (0.9)	72.9 ± 1.3 (0.6)
Liver	22.5 ± 2.0 (3.0)	26.0 ± 7.8 (7.30	9.3 ± 1.1 (6.3)	9.0 ± 2.8 (9.2)	8.3 ± 1.4 (8.6)	6.9 ± 3.2 (7.0)	5.7 ± 0.3 (4.9)
Plasma	16.3 ± 1.6 (1.0)	6.1 ± 0.6 (1.0)	3.0 ± 0.5 (1.0)	2.6 ± 0.7 (1.0)	1.9 ± 0.1 (1.0)	1.8 ± 0.3 (1.0)	1.9 ± 0.2 (1.0)
Kidney	14.0 ± 6.5 (6.2)	4.2 ± 1.9 (3.1)	2.4 ± 0.6 (4.6)	1.1 ± 0.4 (4.3)	1.3 ± 0.4 (3.9)	1.3 ± 0.6 (4.4)	1.1 ± 0.2 (1.7)
Gut	5.2 ± 0.6 (0.3)	7.2 ± 1.4 (1.0)	5.8 ± 0.8 (2.1)	6.2 ± 2.0 (2.6)	2.7 ± 0.5 (1.9)	2.6 ± 0.6 (1.7)	2.2 ± 0.3 (1.4)
Gill	2.7 ± 0.4 (0.5)	1.8 ± 0.3 (0.7)	1.2 ± 0.3 (1.1)	1.0 ± 0.1 (1.3)	1.0 ± 0.1 (1.4)	1.0 ± 0.2 (1.4)	1.7 ± 0.4 (1.4)
Stomach	1.4 ± 0.4 (0.2)	2.1 ± 0.2 (0.9)	2.3 ± 0.4 (1.8)	1.3 ± 0.2 (1.8)	1.3 ± 0.2 (2.3)	1.1 ± 0.2 (1.9)	1.4 ± 0.3 (1.6)
Heart	0.9 ± 0.05 (1.5)	0.4 ± 0.05 (1.9)	0.2 ± 0.03 (2.1)	0.2 ± 0.02 (3.2)	0.1 ± 0.02 (2.3)	0.1 ± 0.02 (2.0)	0.4 ± 0.27 (1.6)
Spleen	0.7 ± 0.10 (1.6)	0.3 ± 0.07 (2.4)	0.2 ± 0.06 (2.7)	0.1 ± 0.03 (2.9)	0.2 ± 0.10 (2.8)	0.1 ± 0.03 (2.1)	0.04 ± 0.02 (1.1)
Gonad	0.4 ± 0.09 (0.2)	0.6 ± 0.20 (0.8)	0.5 ± 0.09 (0.9)	0.5 ± 0.16 (1.1)	0.2 ± 0.09 (1.3)	0.4 ± 0.14 (1.0)	0.1 ± 0.02 (1.0)
Brain	0.2 ± 0.01 (0.3)	0.2 ± 0.05 (0.8)	0.3 ± 0.06 (2.0)	0.3 ± 0.03 (3.4)	0.2 ± 0.06 (2.4)	0.2 ± 0.01 (2.6)	0.3 ± 0.06 (2.3)
Bile	- -	0.1 ± 0.04 (0.1)	0.6 ± 0.2 (1.9)	0.7 ± 0.1 (3.8)	1.3 ± 0.4 (4.6)	3.0 ± 0.3 (10.3)	10.0 ± 0.4 (22.1)
Urine	- -	- -	0.1 ± 0.04 (0.1)	0.1 ± 0.02 (0.3)	0.3 ± 0.2 (0.3)	0.5 ± 0.3 (0.9)	2.2 ± 0.3 (0.2)
Muscle*	- (0.03)	- (0.1)	- (0.3)	- (0.4)	- (0.8)	- (0.7)	- (0.4)
Skin*	- (0.1)	- (0.4)	- (0.8)	- (2.3)	- (1.3)	- (1.1)	- (0.9)

*Muscle and skin content included in carcass.

is the average of four fish. At all times the carcass, primarily muscle, contained the bulk of the radioactivity recovered. Earlier (15 min and 1 hr) the carcass contained 35 to 50% of the recovered label, while at times from four hours to one week it contained 70 to 80%. Liver contained the next most significant fraction of the drug recovered (22% at 15 min to 6% at one week Plasma (16 to 2%), kidney (14 to 1%), bile (0.01 to 10%), and gut (5 to 2%) contained the greatest portion of the remainder.

The flounder tissues and organs may be divided into three classes with regard to their handling of the injected pesticide. (A) Liver, kidney, heart, and spleen fall into the first category. Each of these tissues shows a tissue to plasma ratio (T/P) greater than 1.0 as early as 15 minutes (Table 2). From this time on their content falls throughout the week. For the first 8 to 24 hours the rate of loss is less rapid than plasma decay and the T/P rises. At later times the plasma content stabilizes while the tissues continue to fall and T/P falls sharply. Initially, these tissues contain over 30% of the ^{14}C-DDT, while by one week their content is down to less than 10%. The high T/P when plasma carries large quantities of pesticide suggests that these tissues may be particularly subject to acute effects following pesticide exposure. (B) The second group is composed of those tissues which are considerably below plasma in initial content (T/P of 0.4 - 0.1 at 15 min) and decrease slowly thereafter. Therefore, their T/P ratios increase to 2- by 8 hours and then fall slowly toward unity by 1 week. These tissues include gut, stomach, gill skin, gonad, and brain. They (excluding skin) account for 10% of the pesticide present at 15 minutes, 9% at 8 hours, and 6% at one week. Since they contain little drug initially when plasma levels are high, they may be somewhat less prone to acute effects than group A. However, the slow release of the DDT which is taken up suggests that they may, nevertheless, be subject to chronic or cumulative effects of environmental pesticide. This suggestion may be particularly applicable to brain which appears to increase not only T/P, but also actual pesticide content (0.24 μg/g to 0.31 μg/g) between 15 minutes and 8 hours. Furthermore, its content is more stable between 8 hours and 1 week, decreasing its T/P only from 3.1 to 2.3, suggesting that its content is perhaps even more readily retained than that of the others in group B. (C) The third type includes only muscle. Its pesticide content is much less than plasma initially (T/P of 0.03), but it increases slowly both in T/P and actual content for the first 24 hours (T/P = 0.75). Over the next several days it decreases slowly until a T/P of 0.43 is reached at one week. The importance of this tissue is that it provides a large sink into which the pesticide leaving other tissues may flow. This is of particular importance during the first 24 hours when approximately 40% of the total pesticide present in the fish redistributes from the organs of groups A and B (25%) and the plasma (14%) to some other site. This site is clearly muscle, for during this same time the carcass, primarily muscle, increases in ^{14}C-DDT content by 45% (from 36 to 81%). It is this redistribution of pesticide, which is reflected in the second component ($t_{1/2}$ = 2 hr) of the plasma decay curve.

It perhaps is surprising that the liver, which in fish is considered to be high in lipid content, does not hold a greater fraction of this lipid soluble pesticide. However, the flounder has a very small liver (0.84 ± SE 0.05% of body weight); thus even though it may be high in lipid, its capacity is necessarily limited. The carcass (muscle, skin, and bone) on the other hand, comprises 89 ± 0.5% of body weight and even if rather limited in affinity it provides a significant sink for DDT in the flounder. The lipid content of flounder liver and muscle are being examined in Dr. Gaurino's laboratory. While the above discussion emphasizes that the liver is not the

main storage site for the organochlorine pesticides in the winter flounder, it must be noted that in the early post-injection period the liver, comprising less than 1% of body weight, contains over 20% of the pesticide. Even as late as 1 week it still accounts for almost 6% of the labeled DDT. Its capacity to store or bind DDT is therefore high, and it is potentially an important site for metabolism and excretion of this pesticide.

According to the previous discussion excretion plays little role in the early handling of injected pesticide. This may also be observed directly from the radioactivity in urine, bile, and the seawater in which the fish was maintained. During the first 24 hours after injection less than 0.5% of the recovered drug was present in the urine. Even at 1 week the total urinary excretion accounted for only 2% of the drug. Biliary excretion was more extensive (1.3% at 24 hr and 10% by 1 wk). However, these figures represent pesticide present in gall bladder bile, not in bile which has traversed the digestive tract. Since preliminary in vivo experiments show that DDT is well absorbed in the flounder gut, net biliary excretion may be much less. Significant excretion via the gills was not detected. The water of the experimental containers showed less than 1% of the recovered drug. However, several fish maintained in free-flowing aquaria showed marked decrease in total recovery, raising the possibility of increased gill excretion under these circumstances.

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DDA: AN INHIBITOR OF CHLORPHENOL RED TRANSPORT BY FLOUNDER KIDNEY TUBULES IN VITRO

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DDA (bis[p-chlorophenyl] acetic acid) is a major polar metabolite of DDT and constitutes the principle form found in the urine of mammals (Ann. Rev. Pharm. 5:27-52, 1965). Since, structurally, DDA is an organic acid, it might be predicted that it would be secreted via the well-known renal transport system for organic acids such as chlorphenol red. This system is particularly well-developed in flounder kidney tubules and the isolated renal tubule preparation was developed by Forster, who recognized its potential for screening possible inhibitors of organic acid transport (Science 108:65-67, 1948).

The kidneys of small (50-150 g) winter flounder, Pseudopleuronectes americanus, were teased into fragments (about 1 mg) and incubated for up to 2 hr at 17-20° C in depression slides containing the appropriate concentrations of chlorphenol red (CPR) and DDA in Forster's medium. In each depression there was 0.1 ml medium and about 5 mg tissue. The medium was changed at 10 min intervals to maintain constant concentrations of the organic acids.

The first series of experiments was conducted by incubating the teased tubules with unlabeled CPR (10^{-5} M) alone and with CPR plus 10^{-3} M (280 ppm) or 10^{-4} M (28 ppm) DDA. Results were determined by visually rating dye uptake into tubular lumens. With tubules from three fish, 10^{-3} M DDA completely inhibited dye uptake at all times examined (30, 60, 90 and 120 min). In the presence of 10^{-4} M DDA, dye uptake was not detectably different from control uptake at