UPTAKE, ASSIMILATION, AND LOSS OF DDT RESIDUES BY *Euphausia pacifica*, A EUPHAUSIID SHRIMP

ABSTRACT

JAMES L. COX[1]

Euphausia pacifica Hensen, an abundant euphausiid shrimp from the California Current, can acquire sufficient DDT residue from its food to account for amounts found in its tissues. Assimilation efficiencies for DDT in ingested food are similar to published figures for assimilation of carbon from food. The concentration vs. size function suggested by gas-liquid chromatographic analyses of DDT residues in *E. pacifica*, however, was quite different from the function predicted by a theoretical food assimilation model. Direct uptake of ^{14}C-DDT from water was rapid and partially reversible by returning animals to unlabelled flowing seawater. Uptake equilibrium was reached within 72 hr for smaller animals (<3 mg dry weight); larger animals apparently equiliberated after a longer period. ^{14}C-DDT present in animals after 2 weeks exposure to unlabelled flowing water was retained in higher amounts in larger animals (>3 mg dry weight). The possible effects of dietary changes, moulting, and surface to volume ratios on observed natural levels are discussed.

DDT and its congeners are manmade substances which have achieved global distribution. This fact has produced widespread concern over their long-term impact in ecosystems and has stimulated efforts to study DDT transport from a systems analysis viewpoint (Harrison et al., 1970). Indirect evidence (Cox, 1970) suggests an accretion of DDT residues in oceanic food chains and underscores the need to produce information about mechanisms and rates of DDT acquisition and loss by plankton organisms. This paper reports the results of an experimental study of the euphausiid crustacean *Euphausia pacifica* dealing with quantitative aspects of DDT acquisition from food and water, rates of loss of acquisition from food and water, rates of loss of acquired DDT, and factors affecting equilibration with the surrounding water.

Euphausiid crustaceans are among the most abundant zooplankters in many oceanic regions. They are the food of commercially important fishes and in general represent an important link of oceanic food chains. *E. pacifica* is the most abundant euphausiid of the California Current. Ponomareva (1954, 1955, 1959, 1963) has summarized behavioral and population data on this species, and Lasker (1966) has made extensive laboratory studies of its feeding, growth, respiration, and carbon utilization.

METHODS AND MATERIALS

Laboratory maintenance of *E. pacifica* has been described by Lasker and Theilacker (1965). Animals were maintained in a 40-liter capacity tub with flowing seawater at 10 to 12° C and fed daily rations of freshly hatched *Artemia* nauplii. Individuals were kept long enough during the course of the experimental work for noticeable growth. Mortality was extremely low after the first day that the animals were kept in the tub.

In direct uptake experiments, ^{14}C-DDT was added in small carrier volumes of ethanol (ca. 100 μliter) to GFC glass fiber filtered seawater (volumes from 1 to 10 liter) under constant stirring from a magnetic stirrer. Animals were introduced in groups from a small net or turkey baster. At the completion of an uptake run, animals were removed, rinsed briefly with fresh water, and placed in a desiccator for 6 days at

[1] Department of Biology, Southeastern Massachusetts University, North Dartmouth, Mass. 02747.

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room temperature. Losses of ^{14}C-DDT during desiccation were insignificant. Dried animals were removed, weighed on a Cahn electrobalance to ± 0.01 mg, placed in scintillation vials with equal volumes of NCS solubilizer (Nuclear-Chicago), and digested 1 hr at 70° C before introduction of scintillation fluid and subsequent counting on a Nuclear Chicago Unilux II scintillation counter.[2]

Loss experiments were done by taking labelled animals, subsampling them for initial ^{14}C-DDT levels, and placing them back in a flowing seawater tank. Water in the tank had a turnover time of less than 10 min, so lost ^{14}C-DDT was rapidly removed from the system. Groups of animals were removed from the tank at intervals and analyzed as described above.

In addition to work with ^{14}C-DDT, freshly caught *E. pacifica* were processed and analyzed for naturally occurring levels of DDT residues according to published methods (Cox, 1970), except that whole euphausiids were ground in the homogenizer, rather than algae on filters.

All direct uptake work was done at concentrations less than 33 ppt (parts per 10^{12}) ^{14}C-DDT in seawater, ranging down to 5 ppt. In uptake and loss experiments, individual samples were taken by removing about 10 to 15 animals from the experimental system, processing them, and plotting the results on log-log (full logarithmic) paper and fitting a least-squares regression line to the logarithmically transformed data. Depending upon the extent of the dry weights of the animals taken in each of the described groups, points corresponding to 1.0, 2.0, 3.0, or 10.0 mg dry weight were taken from the regression line for comparisons.

RESULTS

UPTAKE

Since the lipid constituents of planktonic organisms are not in direct contact with seawater, it is necessary to postulate a two-step process of uptake of DDT residues—first, adsorption on-

[2] Reference to trade name in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

to surfaces in contact with seawater and second, diffusion or transport of the adsorbed residues into the lipid constituents of the organism. Initial uptake by *E. pacifica* was rapid; Figure 1 shows the results of a 2-hr uptake experiment. Approximately equal numbers of animals were added to two 7-liter jars containing ^{14}C-DDT at a low ppt concentration. Two hours later, animals were removed and analyzed. The concentration vs. dry weight functions were found to be exponentials, yielding a straight line on the

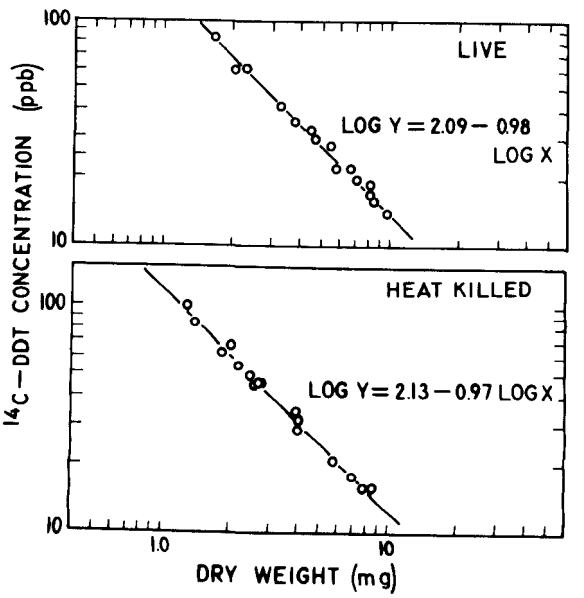

FIGURE 1.—Uptake of ^{14}C-DDT by *Euphausia pacifica* of different weights after 2 hr of exposure to labelled medium.

log-log plot. Initial uptake appeared to be unrelated to the animals' activity or respiration since heat-killed animals had the same total uptake as live animals. The amounts of ^{14}C-DDT taken up per animal were almost identical in these experiments (exactly equal amounts would yield a slope of —1 in the regression function).

In a different series of experiments, the slopes of the log-log concentration vs. dry weight functions changed from —1.05 at 2 hr and —0.99 at 8 hr to —0.67 at 24 hr. This change resulted from increased uptake by larger animals after longer exposure. Figure 2 summarizes the over-

all patterns of uptake for the 72-hr period. Three arbitrary dry weights of animals (2.0, 3.0, and 10.0 mg) were chosen to illustrate different weight effects during uptake. The points corresponding to these dry weights were taken from regression lines like those shown in Figure 1. The values on the ordinate were converted from concentration to total picograms ($g \times 10^{-12}$) of ^{14}C-DDT. After 72 hr of exposure, the 10-mg animal did not reach equilibrium; the 2 and 3 mg animals did reach equilibrium after 72 hr.

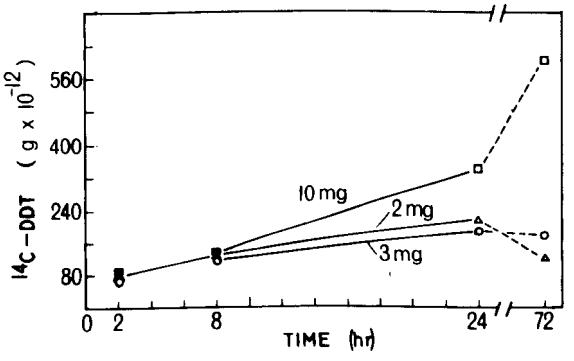

FIGURE 2.—Uptake of ^{14}C-DDT by *Euphausia pacifica* in a closed system. Equilibrium concentration of ^{14}C-DDT in the water was 20 parts per trillion. The three dry weight values were taken from log-log regression lines for subsamples of 10 animals or more. See text for details.

EFFECT OF TEMPERATURE

Temperature appeared to have little effect on initial uptake rates. The Q_{10} for short-term (2 hr of exposure) uptake between 5° and 15° C for an animal of a given dry weight was computed by comparing log-log regression functions for two groups of animals exposed to the same nominal concentrations of ^{14}C-DDT in the medium—one group at 5° C and the other at 15° C. This procedure yielded a Q_{10} of 1.11 for an animal of 2.0 mg dry weight and a Q_{10} of 1.29 for an animal of 10.0 mg dry weight. Both figures suggest a physical process as the limiting step for direct uptake of DDT; the higher figure for larger animals may reflect a higher Q_{10} for transfer into the lipid reservoir of the larger animal.

Del Nimmo (personal communication, 1970) has evidence that DDT residues are transported to internal sites of accumulation by a protein fraction in the haemolymph of penaeid shrimps. If *E. pacifica* is comparable in this regard to the penaeid shrimp, then transport of DDT in the circulatory system must not be the rate-limiting step in uptake, since circulatory rates may be expected to have a higher Q_{10} than those found. Respiratory rates, which are directly dependent upon circulatory rates, exhibit Q_{10} values in excess of 2.2 in *E. pacifica* (Paranjape, 1967).

CONCENTRATION FACTORS

The short-term uptake concentration factors (the ratio of the concentration of DDT in the animals to the concentration in the water after brief exposure) for ^{14}C-DDT changed little over the range of concentrations employed. Table 1 summarizes data that were taken from log-log plots for animals of 1.0 and 3.0 mg dry weight. It is evident that short-term uptake of DDT for an animal of a given size is proportional to the concentration of the DDT in water.

TABLE 1.—Concentration factors after 2 hr exposure.

Equilibrium concentration of ^{14}C-DDT in seawater	Concentration factor × 10^3 Concentration in animal (dry): concentration in water (w/v)	
Parts per trillion	*1.0 mg*	*3.0 mg*
5	4.4	1.1
[1]20	3.2	1.1
26	4.1	1.2
33	4.1	1.2

[1] This includes data from one-half hour run.

LOSS

If short-term exposure to DDT in the seawater medium of *E. pacifica* results in surface adsorption, one expects that these adsorbed residues will be lost to the medium if the ambient concentration of the DDT is lowered. If all the labelled DDT in a short-term experimental exposure is adsorbed, the animals would be expected to lose eventually all of their label when returned to unlabelled flowing seawater.

Figure 3 shows the results of 2 weeks of "rinsing" on animals originally exposed to ^{14}C-DDT for 2 hr. The lower data points show that a fraction of the ^{14}C activity was retained, although the size vs. estimated ^{14}C-DDT concentration function was altered considerably by the treatment. Figure 4 shows a loss curve constructed from a series of log-log plots such as those in Figures 1 and 3. The 10.0 mg animal apparently neared equilibrium at the end of the 2-week period, but the 2.0 and 3.0 mg animals were still declining. Presumably, the ^{14}C-activity loss occurred by diffusion of the parent compound (^{14}C-DDT) or metabolites into the flowing seawater medium. Some loss may have occurred through moulting. Unfortunately, the conditions of the experiment did not allow any record of moult production.

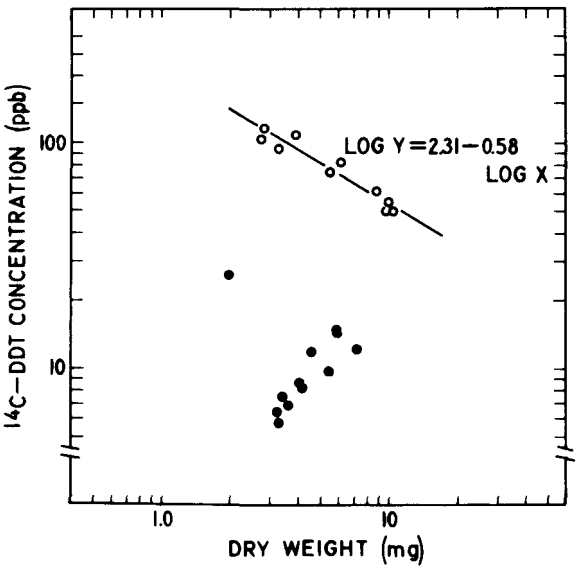

FIGURE 3.—Loss of ^{14}C-DDT from *Euphausia pacifica* kept in a flowing water system. Values for the different dry weights were obtained as indicated in the methods section of the text. The solid dots indicate ^{14}C-DDT concentrations after 2 weeks of exposure to unlabelled flowing seawater. The open dots are for animals exposed to ^{14}C-DDT for 2 hr, then "rinsed" in the flowing seawater system for 2 hr before sampling.

ASSIMILATION FROM FOOD

Animals were isolated in Carolina dishes and kept at 10° C in the dark in 200 ml of GFC filtered seawater and fed known numbers of freshly hatched *Artemia* nauplii previously labelled with ^{14}C-DDT ($2.7 \pm 0.02 \times 10^{-12}$ g ^{14}C-DDT/nauplius, on the average for groups of 10 to 50). After 24 hr, animals were removed to new dishes and fed daily rations of unlabelled nauplii to ensure flushing of the undigested remains of the labelled nauplii from the guts of the experimental animals. After 2 days, the animals were removed, rinsed, dried in a desiccator, and weighed. Amounts of ^{14}C-DDT activity retained were computed by measuring the activity of the dried animals as described in the section on methods. Amounts of labelled nauplii eaten were calculated by counting the numbers left in the dishes after the 24-hr feeding period. Table 2 summarizes the results of the experiment.

Animal 5 may have had a higher assimilation efficiency because of delayed excretion of the gut contents, presumably attributable to the postmoult condition, i.e., passivity and lack of feeding or swimming movements (Paranjape, 1967). Consequently animal 5 was excluded from further calculations. Animal 1 may have had a lower assimilation efficiency because some loss of labelled material with the moult. The average ^{14}C-DDT assimilation efficiencies for animals 2 to 4 is only slightly lower than Lasker's (1966) estimates of carbon incorporation efficiency for *E. pacifica*.

TABLE 2.—^{14}C-DDT assimilation from labelled *Artemia* nauplii by *Euphausia pacifica*.

Animal	Nauplii eaten nauplii offered (labelled)	Percent consumption	Amt. ^{14}C-DDT ingested picograms ($g \times 10^{-12}$)	Amt. ^{14}C-DDT assimilated picograms ($g \times 10^{-12}$)	Moult[1]		Percent assimilation efficiency
					Pre	Post	
1	30/30	100	81	28	−	+	34
2	44/53	83	119	70	−	−	58
3	38/38	100	103	81	−	−	78
4	49/75	65	132	106	−	−	80
5	21/62	34	62	58	+	−	93

[1] Premoult means moult was recovered after feeding on labelled nauplii. Postmoult means moult was recovered after feeding on unlabelled nauplii.

In another experiment, 12 animals were placed in a vessel and fed ^{14}C-DDT labelled nauplii for 1 hr. Six animals were taken and processed for ^{14}C activity, and the remaining six were allowed to feed on unlabelled nauplii for 2 days before they were processed. The assimilation efficiencies were computed as a ratio of ^{14}C activity in the animals processed after 2 days to the ^{14}C activity in the animals processed immediately after the 1-hr feeding period. This method yielded an assimilation efficiency of 76%.

For calculations, I took a mean of the first four animals' assimilation efficiencies. It is uncertain whether this figure (62%) adequately reflects the influence of moulting on DDT assimilation efficiency. Moulting probably plays an important role in DDT loss from the organism; DDT incorporated into the moult is lost when the moult is shed.

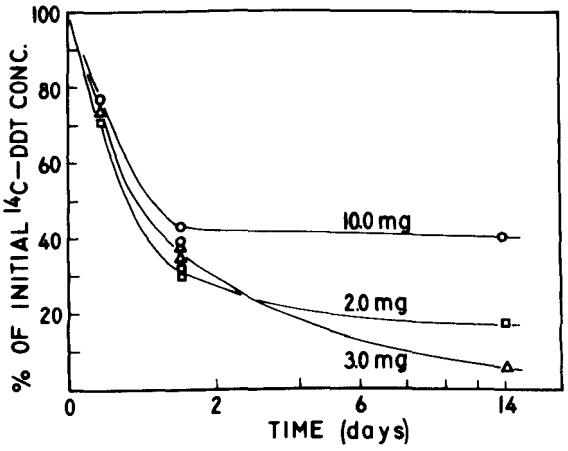

FIGURE 4.—Loss curve constructed from data such as that presented in Figure 3. See text for details.

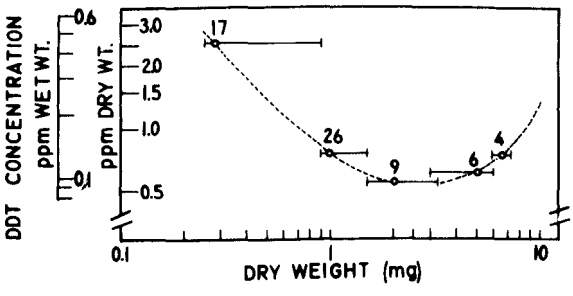

FIGURE 5.—DDT residue concentrations in different sizes of *Euphausia pacifica*. The numbers next to the data points indicate the numbers of animals in the pooled sample analyzed; horizontal brackets indicate the range of weights of individual animals within the groups.

NATURAL LEVELS OF DDT

Figure 5 shows the results of gas chromatographic analyses of *E. pacifica* samples collected in August 1970, the same time that most of the experimental animals were collected. On the basis of DDT acquisition from food, a rising trend in the DDT residue concentrations would be expected as animals grew and aged. In order to examine the discrepancy between the observed DDT values and that which might be expected from cumulative assimilation of DDT residues from food, a model was constructed.

Woodwell, Wurster, and Isaacson (1967) found 0.04 ppm in plankton hauls from a polluted estuary; I have found 0.25 ppm in large, pooled samples of copepods from Monterey Bay. The mean weight of these copepods, 0.95 mg, was only slightly higher than for those eaten by *E. pacifica*. *E. pacifica* also feeds on phytoplankton. The concentrations of phytoplankton, when the density of the standing crop of phytoplankton is high enough to stimulate feeding, are probably below 0.1 ppm, wet weight (Cox, 1970). An intermediate figure can be taken as representative of the DDT concentration of the food of *E. pacifica*. I chose 0.1 ppm as the mean concentration of DDT residues in food.

Employing the carbon budget parameters published by Lasker (1966) and the estimate of DDT residue concentration in food organisms, I calculated the cumulative DDT content of the ingested food of animals of three different dry weights (Table 3). The computed values are compared with values interpolated from the arbitrarily drawn dotted line in Figure 5.

Two conclusions may be drawn from a comparison of columns 7 and 8 in Table 3. First, the estimated values are close enough to the observed values to indicate that ingestion is a sufficient source of DDT residues in *E. pacifica*. Second, the concentration vs. size function of the observed values is quite different from that of the calculated values, indicating that processes other than simple accumulation of a fraction of

TABLE 3.—Calculation of expected DDT residues in different sizes of *Euphausia pacifica*.

Dry weight	Equivalent weight carbon	Carbon growth incorporation efficiency[1]	Cumulative amount nauplius carbon required	DDT equivalent[2] ($g \times 10^{-9}$)	Assumed DDT incorporation efficiency	Parts per 10^6 — dry	
						Expected DDT concentration	Observed DDT concentration
mg	*mg*		*mg*				
1.0	0.42	0.30	1.4	1.4	0.62	0.9	0.75
2.0	0.84	0.15	4.2	4.2	0.62	1.3	0.55
3.0	1.26	0.10	8.4	8.4	0.62	1.7	0.56

[1] Carbon growth incorporation efficiencies were taken from Table 2 of Lasker (1966); the figures shown are not means of the values presented by Lasker but are round-figure approximations which take account of the trends shown and of the different range of sizes of animals used in the laboratory experiments which yielded these figures.
[2] The DDT equivalent of the food was calculated from nauplius carbon assuming a wet weight DDT concentration in the food of 0.1 ppm, and a carbon weight to wet weight ratio of 0.1.

ingested DDT determine DDT residue concentrations in *E. pacifica*.

DISCUSSION

For *E. pacifica*, there are two important sources of DDT residues—direct uptake from water and assimilation from food. Short-term direct uptake is rapid and appears to be at least partially reversible, suggesting adsorption of DDT to exposed surfaces. Over longer periods, these initially acquired residues are transferred to internal deposition sites. The long-term uptake and loss experiments show that larger animals tend to retain more of the initially acquired DDT, possibly because of greater lipid content. Direct uptake from water is a possible mechanism for accumulation of residues if the initially adsorbed residues are continually transferred to internal deposition sites. The rate of initial uptake will depend upon the concentration in seawater (Table 1); retention of these initially acquired residues apparently depends on other factors, judging from the lower set of data points in Figure 3. One determinative factor may be lipid content; values given by Mauchline and Fisher (1969) indicate that lipids, expressed as percentage of body weight, can vary by as much as an order of magnitude in *Euphausia* spp., according to the body weight of the animal. The four lipid values listed by Mauchline and Fisher (1969) for *E. pacifica* correspond closely to the DDT concentration values after 2 weeks rinsing shown in Figure 3. However, in the absence of concurrent lipid values for the animals of the lower data points shown in Figure 3, no conclusion can be drawn about the relationship between retention of ^{14}C-DDT and the percentage lipid composition of the animals. It is reasonable to assume, nonetheless, that the changes in the lipid content of *E. pacifica* which accompany reproductive cycles and seasonal feeding changes will have some impact on the DDT residue content, regardless of the source of the DDT residues.

The second possible source of DDT residues, as previously discussed, is from food. In this case, DDT is almost certainly transported directly in the fat of the food organisms to the fat reservoir of the consumer. Numerous studies indicate that marine organisms do not alter lipids from ingested food (Lasker and Theilacker, 1962; Jezyk and Penicnak, 1966; Jeffries, 1970; and others). Comparison of published values of fatty acid composition for *E. pacifica* (Yamada, 1964) with values for its food, microzooplankton and phytoplankton (Jeffries, 1970), suggests that mass assimilation of fatty constituents along with DDT residues is taking place.

As has been suggested, food is probably a sufficient source of DDT residues in *E. pacifica* (Table 3). Direct uptake may contribute to DDT residues in *E. pacifica*, but its role cannot be assessed because of the lack of seasonal data on DDT concentrations in seawater as well as uncertainties about DDT's availability to organisms in the natural environment (Cox, 1971).

Some basis must be sought to explain the unexpected higher concentrations of DDT residues in the smaller animals. Three possibilities exist: (1) the food of immature *E. pacifica* may have higher DDT concentrations, (2) direct uptake from water is more important for the smaller animals because of their higher area:volume

ratios, or (3) smaller animals have not used any of their lipid reserves, which use may cause loss of some DDT residues. The data presented here do not allow conclusions on the relative importance of these possibilities.

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