

Uptake, absorption efficiency and elimination of DDT in marine phytoplankton, copepods and fish

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Biomagnification and exposure of DDT in a marine food chain is demonstrated by measurements of uptake and elimination rates and kinetic modeling.

Abstract

Uptake, absorption efficiency and elimination of DDT were measured in marine phytoplankton, copepods (*Acartia erythraea*) and fish (mangrove snappers *Lutjanus argentimaculatus*). The uptake rate constant of DDT from water decreased with increasing trophic level. The dietary absorption efficiency (AE) of DDT was 10–29% in copepods and 72–99% in fish. Food concentration did not significantly affect the AEs of DDT, but the AEs varied considerably among the different food diets. The elimination rate constants of DDT by the copepods were comparable following uptake from the diet and from the water. Elimination of DDT from the fish was exceedingly low. Both aqueous and dietary uptake are equally important for DDT accumulation in the copepods. In fish, dissolved exposure is a more significant route than intake from the diet. The predicted trophic transfer factors in the copepods and the fish are consistent with the field measurements in marine zooplankton and fish.

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1. Introduction

DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] is a well known, persistent and highly lipid soluble organochlorine pesticide with a long half-life in the environment. Previously, it was widely used to fight insect-borne diseases (especially malaria) until legislative restrictions were imposed following the manifestation of ecological impairment. Xenobiotic effects (e.g. steroid biosynthesis, biotransformation, oogenesis and sper-

matogenesis) have been observed at all trophic levels (Reijnders and Brasseur, 1997). With the bans or controls on the use of DDT in the 1970s, the DDT concentrations in aquatic animals have decreased significantly, but it can still be found in various compartments of the environment such as air, sediments, fish and mammals, and may continue to present a threat to human health (Hites et al., 2004). The marine environment receives considerable input of DDT from various anthropogenic sources such as direct discharges, or indirectly from river flows and runoff, as well as from long-distance transport through the atmosphere. DDT concentrations increase with increasing trophic level in the aquatic food chain, suggesting that this compound is biomagnified (Butler and Schutzmann, 1979; Cullen and Connell, 1992; Kidd et al., 2001).

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Many previous studies have focused on the bioaccumulation and biomagnification of organochlorine compounds in the aquatic food chains of various ecosystems (Strandberg et al., 1998a,b; Ruus et al., 1999; Dietz et al., 2000; Borga et al., 2001; Kidd et al., 2001; Voutsas et al., 2002). However, few laboratory studies have evaluated the mechanisms of biomagnification of these compounds in the marine food chain, especially at the lower trophic levels. Most field studies have focused on the higher trophic levels in marine environments, such as from Arctic cod to ringed seals in Canadian arctic archipelago (Muir et al., 1988), from fish *Diaphus suborbitalis* to the striped dolphin (*Stenella coeruleoalba*) (Tanabe et al., 1982, 1984), and from the lesser sand eel (*Ammodytes marinus*) and cod (*Gadus morhua*) to the harbour seal (*Phocavitulina*) and grey seal (*Halichoerus*) in Jarfjord, northern Norway (Ruus et al., 1999).

Phytoplankton are the primary producers in aquatic ecosystems and play a key role in the transport of organic contaminants through the food chain to higher trophic levels. Zooplankton (especially the copepods) are typically the primary consumers and play an important ecological role in aquatic ecosystems by controlling the phytoplankton communities as well as acting as direct or indirect food sources for higher trophic animals (Huntly et al., 1986). Uptake by zooplankton represents another redistribution pathway for organic contaminants. Wallberg et al. (2001) have suggested that trophic transfer is a significant transport route for the more hydrophobic organic contaminants in plankton communities. The bioaccumulation at lower trophic levels forms the first step in the transfer of chemicals through the food chain. Conversely, marine fish are able to accumulate lipophilic contaminants such as DDT from both the aqueous phase and dietary sources (e.g. trophic transfer). Previous studies reported that trophic transfer resulted in a high concentration of residual DDT in fish (Cullen and Connell, 1992; Monirith et al., 1999). Leblanc (1995) found a direct correlation between the trophic level and lipid concentration, thus animals at a higher trophic level tended to have a higher lipid concentration and DDT concentration. These conclusions were primarily based on the measured bioaccumulation at higher trophic levels, e.g. in fish, gulls, seals, and whales.

Few studies have quantified the biomagnification of DDT at lower trophic levels in marine food chain. In the present study, the bioaccumulation and biomagnification of this compound at different trophic levels (phytoplankton, zooplankton, and fish) of a planktonic food chain was measured. We measured the uptake rate constant, absorption efficiency, and elimination rate constant of DDT, and then quantified the relative contribution of aqueous and dietary uptake to the total DDT body burden in each animal. There are very few data available on the biokinetic parameters of DDT in

marine planktonic food chain. The influences of food concentration and prey species on the trophic transfer of DDT were specifically measured. Finally, the potential biomagnification of DDT in the simulated food chain was modelled using a simple kinetic equation in an attempt to understand the mechanisms controlling the biomagnification of this organochlorine.

2. Materials and methods

2.1. Chemicals and organisms

[¹⁴C]4',4-DDT (with a specific activity 12.8 mCi mmol⁻¹) was purchased from Sigma, and dissolved in toluene solution and stored at 2–8 °C. The solution was subsequently diluted with acetone to facilitate its solubility in seawater before the radioactive experiments described below.

Organisms examined in this study included phytoplankton, zooplankton (copepods) and fish (mangrove snappers). Four phytoplankton species, *Thalassiosira pseudonana* (diatom), *Thalassiosira weissflogii* (diatom), *Tetraselmis levis* (prasinophyte), and *Prorocentrum minimum* (dinoflagellate), were cultured under laboratory conditions and maintained in f/2 medium at 18 °C with a light illumination of 70 μmol photon m⁻²s⁻¹ with a 14:10 h light/dark cycle. The copepods (*Acartia erythraea*) were collected by net towing from Port Shelter, Clear Water Bay, Hong Kong. The mangrove snappers (*Lutjanus argentimaculatus*), with a size of about 1.5–2.5 cm body length, were purchased from a fish farm in Hong Kong. The fish were maintained in glass fibre filtered natural seawater and fed frozen shrimps twice daily. The clams (*Ruditapes philippinarum*) were collected from the Port Shelter, Clear Water Bay, Hong Kong. All experiments were conducted in glass beakers to minimize sorption of DDT onto the experimental glassware. All experimental seawater was collected from the Clear Water Bay, Hong Kong, with a salinity of 32 psu.

2.2. Uptake of DDT from the dissolved phase

Uptake of DDT by different marine organisms was quantified using a kinetic approach. In the first experiment, we measured the uptake of DDT by four species of phytoplankton (*T. pseudonana*, *T. weissflogii*, *T. levis*, and *P. minimum*). When the cells reached the exponential (for all algal species) or the stationary (for *T. weissflogii* only) growth phase, they were filtered and resuspended in 100 ml of 0.2 μm filtered seawater at a cell biomass of 1 mg L⁻¹. The algae were spiked with 0.5 μCi [¹⁴C]DDT (solvent-to-seawater ratio of 0.04%) and were exposed in dark. There were three replicates for each algal species. At 0.5, 1.0, and 1.5 h of exposure, a 10 ml sample was filtered onto two stacked 25 mm

glass-fibre filters (GF/F) and rinsed with 5 ml of 0.2 μm filtered seawater. The top filter retained the algae, and the bottom filter was used to calibrate the sorption of ^{14}C during the filtration step. The radioactivity was measured by a liquid scintillation counter after the addition of cocktail (Ultima Gold XR, Packard, CT, USA). A 1 ml water sample was taken for measurements of the radioactivity (including both the water and the algae). The cell dry weight was measured by filtering the cells onto a pre-weighed GF/F and rinsing with ammonium formate, followed by drying the filters at 90 °C overnight before weighing them again. Finally, the dry weight concentration factor (DCF) was calculated by the following equation:

$$\text{DCF} = \frac{{}^{14}\text{C}_{\text{organisms}} \text{ g}^{-1} \text{ dry weight}}{{}^{14}\text{C}_{\text{water}} \text{ L}^{-1} \text{ water}} \quad (1)$$

In the second experiment, the uptake of DDT by the copepods was measured. The copepods were fed with a mixture of algae (*T. pseudonana* + *T. weissflogii* + *T. levis* + *P. minimum*) for 1–2 days before the experiments. They were then transferred to a glass beaker containing 200 ml of 0.2 μm filtered seawater and 1.0 μCi of the radioactive DDT (solvent to seawater ratio of 0.2%). There were three replicated bottles. At 1, 2, 4, and 8 h of exposure, a 1 ml water sample was taken for measurements of the total radioactivity. Twenty individual copepods from each bottle were subsequently removed and rinsed with GF/F seawater by placing them in non-radioactive seawater for 4–5 min. The copepods were subsequently collected and solubilized, and the radioactivity was measured. The dry weights of the copepods were also measured using methods described above for phytoplankton. The DCF was calculated by Eq. 1.

In the third experiment, the uptake of DDT by the mangrove snappers was determined. To measure the dissolved uptake of DDT by the fish, individual mangrove snappers were placed in replicated 1 litre of 0.2 μm filtered seawater containing 10 μCi [^{14}C]DDT. At 1, 2, 3 and 4 h, three individual fishes were removed, rinsed, and placed in non-radioactive seawater for 5 min to remove any loosely bound DDT. A 1 ml water sample was also collected at each time point for radioactivity measurements. The whole fish was then dissected into three components: gill, viscera, and remaining tissue. After wet weight measurements, the tissues were added with Solvable and homogenized. After 1 day of tissue solubilization, 10 ml of the cocktail was added and the radioactivity was measured. The DCF was calculated by Eq. (1). The uptake was expressed based on the dry weight of the tissues, which was calculated from the dry:wet weight ratio of each tissue determined from 10 dissected individual fishes.

2.3. Absorption efficiency (AE) of DDT in copepods and fish

The absorption efficiency (AE) quantifies the efficiency of ingested DDT from the dietary source that is absorbed across the gut lining following food digestion. The AE of DDT by the copepods and fish was determined using both the mass balance and the ratio methods (^{14}C : ^{59}Fe) as described in Wang and Chow (2002) and Wang and Fisher (1999). ^{59}Fe was used as a tracer of food particles because it was little assimilated by the copepods and fish (typically with an AE <5% despite being an essential metal) (Wang and Chow, 2002).

The AE of DDT by the copepods was measured by pulse feeding on radiolabelled phytoplankton food. To radiolabel the phytoplankton cells, they were filtered from their f/2 medium and suspended into 20 ml of 0.2 μm seawater. The medium was then spiked with 1 μCi ^{59}Fe (in 0.1 N HCl) without nutrients under dark conditions. After 12 h of incubation, the culture was spiked with 5 μCi [^{14}C]DDT for 2 h. The cells were subsequently collected by centrifugation twice (20 min, 2500 $\times g$ at 10 °C) and resuspended in 10 ml of filtered seawater before being fed to the copepods.

A pulse-chase feeding technique was used to quantify the AEs of [^{14}C]DDT in the copepods. The radiolabelled phytoplankton were added to 100 ml of 0.2 μm seawater containing 100 individual copepods. A 1 ml water sample was first taken for food concentration measurement. After 20–30 min of radioactive feeding, the copepods (50 individuals) were immediately removed and rinsed with filtered seawater. Radioactive faeces produced during the radioactive feeding period were collected and radioassayed. The copepods were then placed in 100 ml of 0.2 μm seawater containing the same species of food and at the same concentration as used during the radioactive feeding period. The faeces were collected at 2, 4, 8, 12 and 24 h during the course of the depuration period. The water was renewed at each time point with the addition of a new batch of food. At the end of the depuration period, all the copepods in each beaker were collected with a mesh and placed in a 5 ml vial. The copepods and the faeces were solubilized with 200 μl of Solvable for 1 day, and then added with 4 ml of cocktail. The radioactivity was finally counted.

The influence of both food concentration and food type on DDT absorption by copepods was examined. In the food concentration experiments, the copepods were fed with ^{14}C -radiolabelled *T. weissflogii* at four different food concentrations: 0.1, 0.5, 2.5, and 5.0 mg L^{-1} . In the food type experiments, *T. pseudonana*, *T. weissflogii* (both exponentially growing and stationary cells), *T. levis*, and *P. minimum* were used as the diets for the copepods. The algal biomass was maintained at 1 mg L^{-1} for the different food type treatments. Each food treatment had three replicates.

The AEs of DDT were similarly measured in the mangrove snappers by pulse feeding on different prey and at different densities of the copepod prey. In the prey type experiment, four prey diets were examined, including copepods and clams (*Ruditapes philippinarum*) radiolabelled from the aqueous phase or dietary phase (by feeding on the radiolabelled diatom *T. weissflogii*). The diatoms were radiolabelled as described above and fed to the copepods or clams for 1 day. The radiolabelled clams were dissected and only the soft tissues (excluding the gills and foot) were fed to the fish. In the prey density experiment, the copepods were radiolabelled by feeding on the radiolabelled *T. weissflogii* for 24 h. The density of copepods presented to the fish was 3, 6, 15 or 30 mg L⁻¹. The fish were fed with the different radiolabelled foods for 60–90 min, after which the fish were removed and immediately placed in non-radioactive seawater and allowed to depurate the ingested food for 32 h. Fresh clam tissues were fed to the fish during the depuration period. The seawater was changed at 24 h of depuration. Any faeces were removed at 4, 7, 9, 19, 24, 28 and 32 h. In each food treatment there were four replicates, each containing two individual fish.

In the mass balance method, the AE of DDT by the copepods and fish was calculated by the following equation:

$$AE = A_{\text{tissue}} / (A_{\text{tissue}} + A_{\text{faeces}}) \quad (2)$$

where A_{tissue} is the radioactivity in the tissues following the depuration, and A_{faeces} is the radioactivity in the faeces collected during the depuration period (cumulative faeces).

In the ratio method, the AE of DDT was calculated by the following equation:

$$AE = 1 - [(^{14}\text{C}/^{59}\text{Fe})_{\text{faeces}}] / [(^{14}\text{C}/^{59}\text{Fe})_{\text{tissue}}] \quad (3)$$

where $(^{14}\text{C}/^{59}\text{Fe})_{\text{faeces}}$ is the ratio of ¹⁴C to ⁵⁹Fe in the cumulative faeces collected during the depuration period, and $(^{14}\text{C}/^{59}\text{Fe})_{\text{tissue}}$ is the ratio of ¹⁴C to ⁵⁹Fe in the tissue measured after the pulse radioactive feeding period. The ¹⁴C radioactivity in the tissue was calculated as the sum of the ¹⁴C radioactivity in the cumulative faeces and soft tissues measured after depuration.

2.4. Elimination of DDT from copepods and fish after aqueous and dietary exposure

To measure the elimination of DDT by the copepods, the animals were exposed to [¹⁴C]DDT either in the aqueous phase or in the dietary phase. In the aqueous exposure treatment, copepods were placed in a 3 L glass beaker and were exposed to 10 μCi [¹⁴C]DDT in the aqueous phase for 12 h. In the food exposure treatment,

copepods were fed on the diatom *T. weissflogii* which had previously been radiolabelled with 20 μCi [¹⁴C]DDT in 40 ml of seawater for 2 h, at different food concentrations (0.1, 0.5, 2.5 and 5.0 mg L⁻¹). After 12 h of feeding, the copepods were filtered and rinsed with filtered seawater. They were then transferred to 300 ml glass beakers (at a density of 0.8 individual ml⁻¹) containing different food concentrations as used during the radioactive feeding. Each food concentration had three replicates. At the beginning of depuration, 20 copepods were collected from each beaker to measure the initial radioactivity in the copepods. During the depuration, 20 individual copepods were collected at 2, 4, 8, 12, 24, 36 and 48 h. The seawater and food was renewed at 2–4 h intervals during the first day and then at 6–12 h intervals during the second day of depuration.

The mangrove snappers were also exposed to [¹⁴C]DDT either in the aqueous phase or in their diets and its elimination was measured. In the aqueous exposure treatment, 21 individual fish were exposed to DDT for 54 h. In the food exposure treatment, 36 individual fish were fed with radiolabelled copepods for 24 h. The copepods had previously been labelled in 3 L of 0.2 μm seawater containing 5 μCi [¹⁴C]DDT for 12 h. After the radiolabelling, the fish were removed and depurated in filtered seawater. The seawater was renewed and the copepods were fed at 6 h intervals for the first day and then at 12–24 h intervals during the remaining depuration days. At different time intervals, three fish were removed and were dissected into gills, viscera, and the remaining tissue. Radioactivity in each tissue was measured as described above after tissue solubilization.

2.5. Modelling the exposure and trophic transfer factor of DDT

Taking into account DDT bioaccumulation from both the aqueous and dietary phases, the bioaccumulation of DDT in the animals can be described by the following equation (Landrum et al., 1992; Wang and Fisher, 1999):

$$dC/dt = k_u \times C_w + AE \times IR \times C_f - k_e \times C \quad (4)$$

where C is the DDT concentration in the animals at time t , k_u is the uptake rate constant from the dissolved phase (L g⁻¹ d⁻¹), C_w is the DDT concentration in the dissolved phase (μg L⁻¹), AE is the DDT absorption efficiency, IR is the ingestion rate of the animals (g g⁻¹ d⁻¹), C_f is the DDT concentration in the ingested food particles (μg g⁻¹, which can be calculated as the DDT bioconcentration factor, BCF, in the ingested food times C_w), and k_e is the DDT elimination rate constant (d⁻¹).

Thus, the fraction of DDT uptake from the diet R and the food chain transfer factor TTF can be calculated, respectively, by the following equations (Wang and Fisher, 1999; Wang, 2002):

$$R = (AE \times IR \times BCF) / (k_u + AE \times IR \times BCF) \quad (5)$$

$$TTF = (AE \times IR) / k_e \quad (6)$$

3. Results

3.1. DDT aqueous uptake by phytoplankton, copepods and fish

DDT uptake by the phytoplankton, copepods and mangrove snappers exhibited an approximately linear pattern over the period of exposure (Fig. 1). The dry weight concentration factor (DCF) was calculated as the accumulated concentration divided by the aqueous concentration. No steady state or equilibrium was

reached within the first few hours of exposure. Fig. 1 shows the uptake of DDT by different phytoplankton species at the same cell biomass (1 mg L^{-1}). The DCFs for different algal species during the 1.5 h exposure period were $600\text{--}1200 \text{ L g}^{-1}$. The uptake rate constant k_u was calculated from the slope based on the regression of the DCF against the time of exposure. Thus, the k_u was 117 ± 9 (mean \pm SD, $n=3$) $\text{L g}^{-1} \text{ h}^{-1}$ for *Thalassiosira weissflogii* (exponentially growing cells), $168 \pm 36 \text{ L g}^{-1} \text{ h}^{-1}$ for *T. weissflogii* (stationary growing cells), $130 \pm 31 \text{ L g}^{-1} \text{ h}^{-1}$ for *Tetraselmis levis*, $207 \pm 2 \text{ L g}^{-1} \text{ h}^{-1}$ for *Prorocentrum minimum*, and $85 \pm 12 \text{ L g}^{-1} \text{ h}^{-1}$ for *Thalassiosira pseudonana*. Diatom *T. weissflogii* grown to the stationary phase had a k_u 1.44 times higher than cells in the exponential growth phase. The copepods and mangrove snappers had much lower DCF than the phytoplankton cells. The calculated k_u of DDT was $1.20 \text{ L g}^{-1} \text{ h}^{-1}$ for the copepods, and was $0.30 \text{ L g}^{-1} \text{ h}^{-1}$ for the whole individual fish. Among the different fish body tissues, the viscera had the highest DCF and its calculated k_u was $2.17 \pm 0.05 \text{ L g}^{-1} \text{ h}^{-1}$. The k_u was $0.34 \pm 0.12 \text{ L g}^{-1} \text{ h}^{-1}$ for the gills and $0.15 \pm 0.03 \text{ L g}^{-1} \text{ h}^{-1}$ for the remaining tissues, respectively.

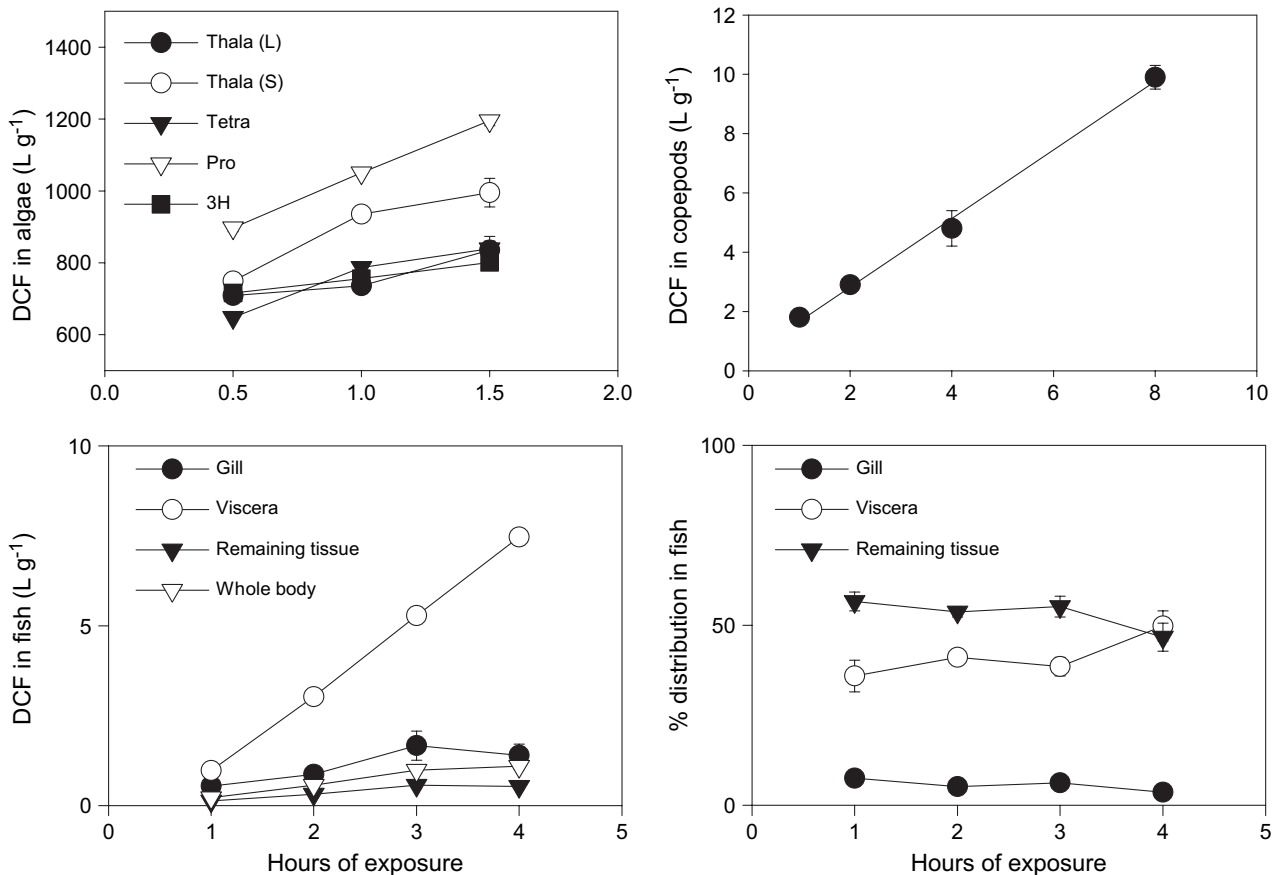


Fig. 1. Uptake of DDT by different species of phytoplankton, copepod *Acartia erythroa*, and mangrove snapper *Lutjanus argentimaculatus* from the dissolved phase quantified as the dry weight concentration factor (DCF). Thala (L), *Thalassiosira weissflogii* at the exponential growth phase; Thala (S), *Thalassiosira weissflogii* at the stationary phase; Tetra, *Tetraselmis levis*; Pro, *Prorocentrum minimum*; 3H, *Thalassiosira pseudonana*. Mean \pm SD ($n=3$).

The relative distribution of accumulated DDT in the fish was roughly constant over the 4 h exposure period, and >50% of the DDT was distributed in the remaining tissues, with a significant distribution in the viscera as well (Fig. 1). The accumulated DDT was least distributed in the gills of the fish.

3.2. Absorption efficiency (AE) of DDT by copepods and fish

The influence of food concentration and type on the AEs of DDT by the copepods and fish were examined using both the mass balance method and the $^{14}\text{C}:$ ^{59}Fe ratio method. The depuration pattern was constructed using radioactivity determined in the copepods after the depuration and the radioactivity determined in the faeces collected at different time points. In the copepods, rapid egestion of unassimilated DDT occurred within the first two hours of depuration, and the egestion rate

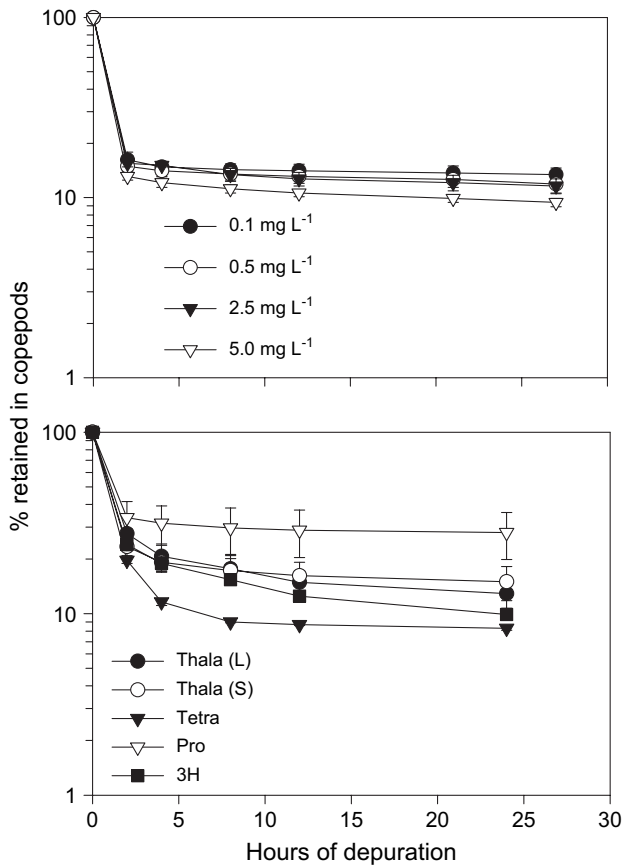


Fig. 2. Retention of DDT by the copepod *Acartia erythroaea* following a pulse ingestion of radiolabelled phytoplankton at different food concentrations (diatom *Thalassiosira weissflogii*, top panel) and for different algal diets (bottom panel). Retention was calculated by the mass balance method based on measurements of radioactivity in copepods after depuration and the radioactivity in faeces. Thala (L), *Thalassiosira weissflogii* at the exponential growth phase; Thala (S), *Thalassiosira weissflogii* at the stationary phase; Tetra, *Tetraselmis levis*; Pro, *Prorocentrum minimum*; 3H, *Thalassiosira pseudonana*. Mean \pm SD ($n=3$).

Table 1

Absorption efficiency (AE) of DDT by the copepod *Acartia erythroaea*

Treatment	AE (%)	
	Mass balance method	Ratio method
Food concentration (<i>Thalassiosira weissflogii</i>)		
0.1 mg L ⁻¹	14.1 \pm 2.4	11.8 \pm 1.6
0.5 mg L ⁻¹	13.1 \pm 1.1	10.9 \pm 1.3
2.5 mg L ⁻¹	12.7 \pm 1.1	10.2 \pm 1.4
5.0 mg L ⁻¹	10.6 \pm 0.5	8.9 \pm 0.5
Food type		
<i>Thalassiosira weissflogii</i>	14.9 \pm 2.4	8.5 \pm 2.2
<i>Thalassiosira weissflogii</i> (stationary phase)	16.2 \pm 3.0	10.8 \pm 3.6
<i>Tetraselmis levis</i>	8.7 \pm 0.1	6.1 \pm 5.7
<i>Prorocentrum minimum</i>	28.8 \pm 8.4	23.2 \pm 10.9
<i>Thalassiosira pseudonana</i>	12.5 \pm 0.3	ND

Mean \pm SD ($n=3$). Both the mass balance method and the ratio method ($^{14}\text{C}:$ ^{59}Fe) were used to calculate the AE. ND, not determined.

then decreased slowly with the depuration time (Fig. 2). There was very little loss of ingested DDT from the copepods into the faeces after the initial depuration. In the food density experiments (with the diatom *T. weissflogii*), the AEs of DDT ranged between 9% and 14% over the diatom concentration range examined, and decreased slightly with increasing diatom concentration (Table 1, but not statistically significant, $p=0.12$, one-way ANOVA). The AEs determined by both the mass balance method and the $^{14}\text{C}:$ ^{59}Fe ratio method were comparable. In the food type experiments, the AEs of DDT from different algal species were 6–29%. The highest AE was 29% from *P. minimum* and the lowest AE was 9% from *T. levis* (mass balance method). The AE quantified for *T. weissflogii* in the food type experiment was comparable to the AE quantified in the food concentration experiment. There was no significant difference in the AEs from *T. weissflogii* between the two growth phases ($p > 0.05$).

In mangrove snappers, the unassimilated DDT was slowly egested throughout the 36 h depuration period (Fig. 3). The majority of ingested dietary DDT were retained by the fish. The calculated AEs of DDT in the fish using both the mass balance method and the $^{14}\text{C}:$ ^{59}Fe ratio method were extremely high (72–99%) (Table 2). In the prey (copepods) density experiment, the AEs of DDT were in the range of 97–99% and were not influenced by the food density due to the very efficient absorption of dietary DDT by the fish. In the food type experiment, DDT from copepods was similarly highly assimilated by the fish (>94%), whereas the DDT from the clam tissues was absorbed with a somewhat lower efficiency (72%, mass balance method). Similarly, the AEs quantified using both the mass balance method and the $^{14}\text{C}:$ ^{59}Fe ratio method were rather comparable. The original exposure route of the prey (aqueous or dietary exposure) did not affect the AEs of DDT by the fish.

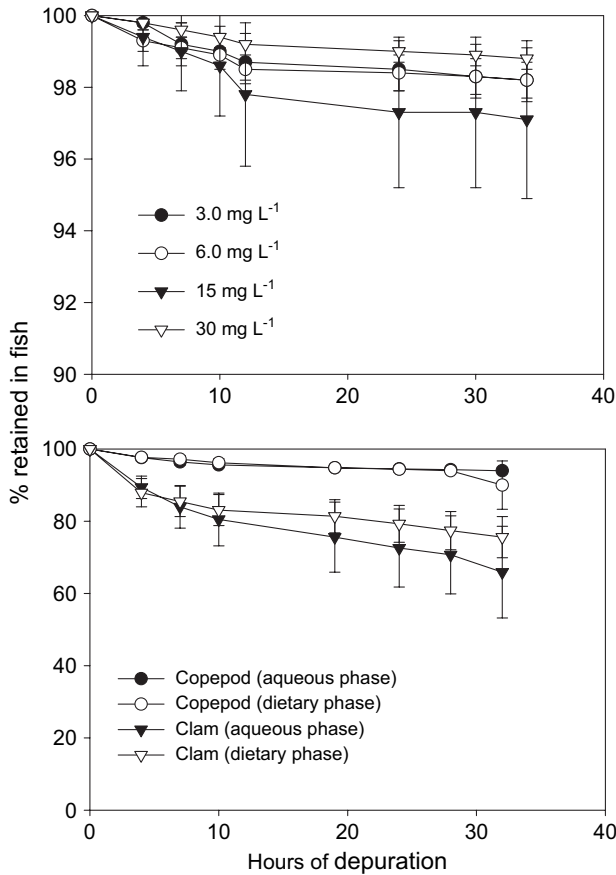


Fig. 3. Retention of DDT by the mangrove snapper *Lutjanus argentimaculatus* following a pulse ingestion of radiolabelled preys at different copepod prey concentrations (top panel) and for different preys (bottom panel). Retention was calculated by the mass balance method based on measurements of radioactivity in fish after depuration and the radioactivity in faeces. Mean \pm SD ($n=4$).

3.3. Elimination of DDT by copepods and fish

The elimination of DDT by the copepods and fish was examined at different prey densities following

Table 2
Absorption efficiency of DDT (AE) by the mangrove snapper *Lutjanus argentimaculatus*

Treatment	AE (%)	
	Mass balance method	Ratio method
Copepod density		
3 mg L ⁻¹	96.8 \pm 1.4	ND
6 mg L ⁻¹	97.1 \pm 0.9	ND
15 mg L ⁻¹	96.9 \pm 2.2	ND
30 mg L ⁻¹	98.6 \pm 0.3	ND
Prey type		
Copepods (dissolved)	94.5 \pm 0.6	95.6 \pm 0.5
Copepods (dietary)	94.4 \pm 0.8	94.4 \pm 3.3
Clam (dissolved)	72.6 \pm 10.8	80.9 \pm 3.6
Clam (dietary)	71.8 \pm 8.3	82.0 \pm 1.7

Mean \pm SD ($n=43$). Both the mass balance method and the ratio method (¹⁴C-⁵⁹Fe) were used to calculate the AE. ND, not determined.

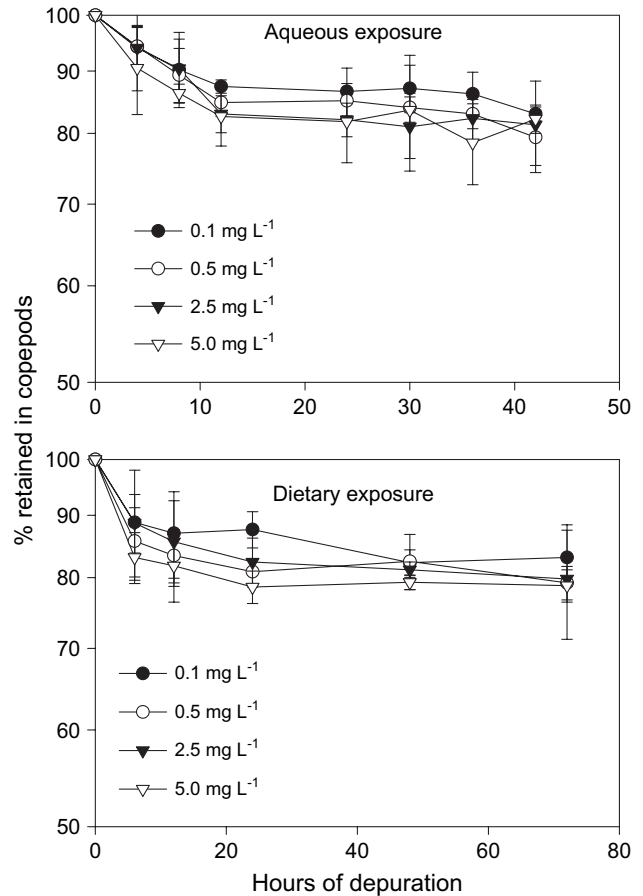


Fig. 4. Elimination of DDT by the copepod *Acartia erythraea* at different diatom food concentrations (*Thalassiosira weissflogii*) following aqueous (top panel) and dietary (bottom panel) exposure for 12 h. Mean \pm SD ($n=3$).

accumulation from the aqueous and food sources. In experiments with the copepods, the amount of DDT retained by the copepods decreased within the first 12 h, followed by a slower loss pattern (Fig. 4). The elimination rate constant (k_e) was calculated from the regression of the natural log of the percentage retained in copepods against the depuration time (12 h onward, Table 3). The k_e was very low and ranged between 0.01 and 0.05 d⁻¹. The calculated biological half-life of DDT in the copepods was 1.5–2.0 months. Generally, the

Table 3
Elimination rate constant (d⁻¹) of DDT by the copepod *Acartia erythraea* following uptake from the dissolved phase and food phase (diatom *Thalassiosira weissflogii*) for 12 h

Treatments	Dissolved exposure	Food exposure
Diatom concentration during elimination		
0.1 mg L ⁻¹	0.034 \pm 0.009	0.024 \pm 0.010
0.5 mg L ⁻¹	0.048 \pm 0.019	0.014 \pm 0.010
2.5 mg L ⁻¹	0.014 \pm 0.010	0.024 \pm 0.007
5.0 mg L ⁻¹	0.017 \pm 0.014	0.010 \pm 0.010

Mean \pm SD ($n=3$).

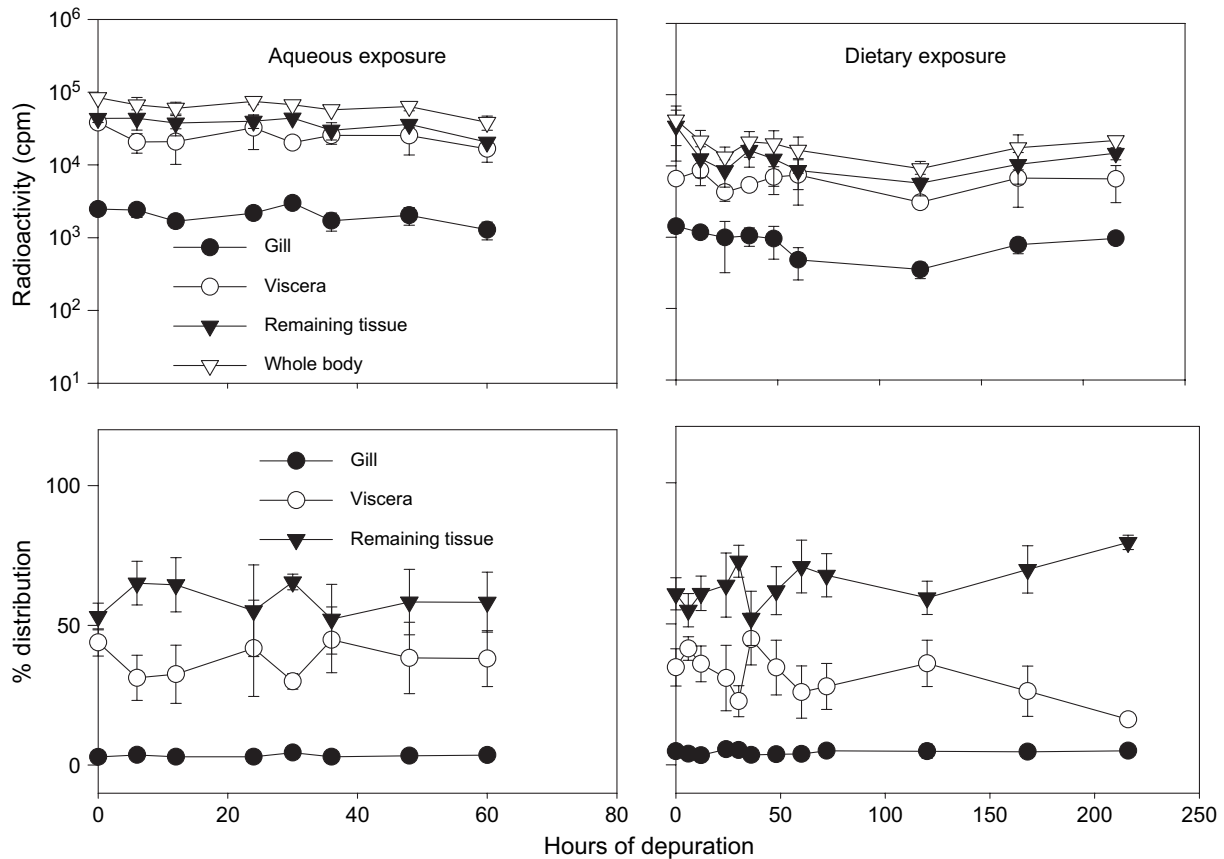


Fig. 5. Radioactivity of [¹⁴C]DDT retained in the mangrove snapper *Lutjanus argentimaculatus* following aqueous (left panel) and dietary (right panel) exposure, and the relative distribution of DDT in different tissues of fish during depuration. Mean \pm SD ($n=3$).

source of uptake (aqueous vs. dietary) did not significantly affect DDT elimination by the copepods ($p > 0.05$, two-way ANOVA), but DDT was lost at a significantly faster rate with increasing prey density (diatom *T. weissflogii*) ($p < 0.05$, two-way ANOVA). The k_e differed by 2.0–2.4-fold between the lowest and the highest food concentration examined.

In contrast to the copepods, DDT was little depurated from the fish following uptake from either the aqueous or dietary phase (Fig. 5). Since the radioactivity was quantified by sacrificing individual fish at different time intervals, there were considerable variations in the radioactivity retained among different individuals. The k_e was calculated from the slope of the regression between the natural log of the mean % retention of DDT against the time of depuration (between 6–60 h for aqueous exposure and 12–216 h for dietary exposure). Thus, the calculated k_e was 0.028 d⁻¹ and 0.002 d⁻¹ for the aqueous and dietary exposure, respectively. In these experiments, the percentage of DDT distribution in different tissues was rather constant during the depuration period. Very little DDT was detected in the gills, and a higher fraction of DDT was detected in the remaining tissues compared to that in the viscera.

3.4. Modelling the exposure of DDT

The potential exposure of copepods and fish to DDT from both the aqueous and food sources and the food chain transfer factor (TTF) were assessed using Eqs. (5) and (6), respectively. Values of k_u , AE, k_e , and BCF were derived from this study, whereas the IR was derived from the literature (Table 4). Based on our measurement, the BCF for phytoplankton is assumed to be 5×10^5 – 5×10^6 (L kg⁻¹) (Fig. 1). The BCF for the copepods can be calculated as the k_u divided by k_e , and was 6×10^4 (L kg⁻¹). However, we allow the BCF to vary by over 1 order of magnitude to take into account its uncertainty (10^4 – 10^5 L kg⁻¹). Using Eq. (5), calculations indicated that >58–80% of DDT in copepods is indeed derived from the dietary phase at the median BCF (10^6) (Fig. 6). At the low end of BCF (10^5), aqueous uptake of DDT appears to be the dominant pathway for DDT accumulation in copepods. In contrast, at the median BCF of fish prey (6×10^4), 4–43% of DDT in fish is predicted to be from the ingestion of prey (Fig. 6). Under most conditions, >50% of DDT in fish is derived from the aqueous uptake. The calculated TTF is 1–9 in copepods fed on phytoplankton, and 4–45 in mangrove snappers fed on copepod prey.

Table 4
Numeric values of parameters used in modeling the transfer of DDT in marine copepods and fish

	Copepods		Fish		Sources
	Range	Mean	Range	Mean	
IR ($\text{g g}^{-1} \text{d}^{-1}$)	0.2–0.6	0.4	0.01–0.10	0.05	Wang and Fisher, 1998; Zhao et al., 2001
k_u ($\text{L g}^{-1} \text{h}^{-1}$)		1.20		0.30	This study
AE (%)	10–30	20	70–100	90	This study
k_e (d^{-1})	0.01–0.05	0.02		0.002	This study
BCF in prey (L kg^{-1})	5×10^5 – 5×10^6	10^6	10^4 – 10^5	6×10^4	This study

IR, ingestion rate, k_u , uptake rate constant from aqueous phase, AE, absorption efficiency, k_e , efflux rate constant, BCF, bioconcentration factor in prey.

4. Discussion

The uptake of DDT by different phyla and sizes of phytoplankton was quantified in our study. The smallest size examined was 3–5 μm (for *T. pseudonana*) and the largest was 13–16 μm (for *P. minimum*) or 10–20 μm (for *T. weissflogii*). There were some differences in the uptake of DDT by these different phytoplankton species with similar cell sizes (e.g. *P. minimum*, *T. weissflogii*, and *T. levis*) at the same biomass, thus it is unlikely that

cell size is an important factor in determining the uptake of DDT by the cells. Fan and Reinfelder (2003) found that organic pollutants first partitioned on the cell surfaces and reached pseudoequilibrium within a relatively short period (2–20 min). Afterwards, the organic compounds diffused into the cell interior. This may imply that physical adsorption is not the main mechanism for the uptake of DDT by the algae.

Because the lipophilicity of organic pollutants critically determines their bioavailability, the amount of

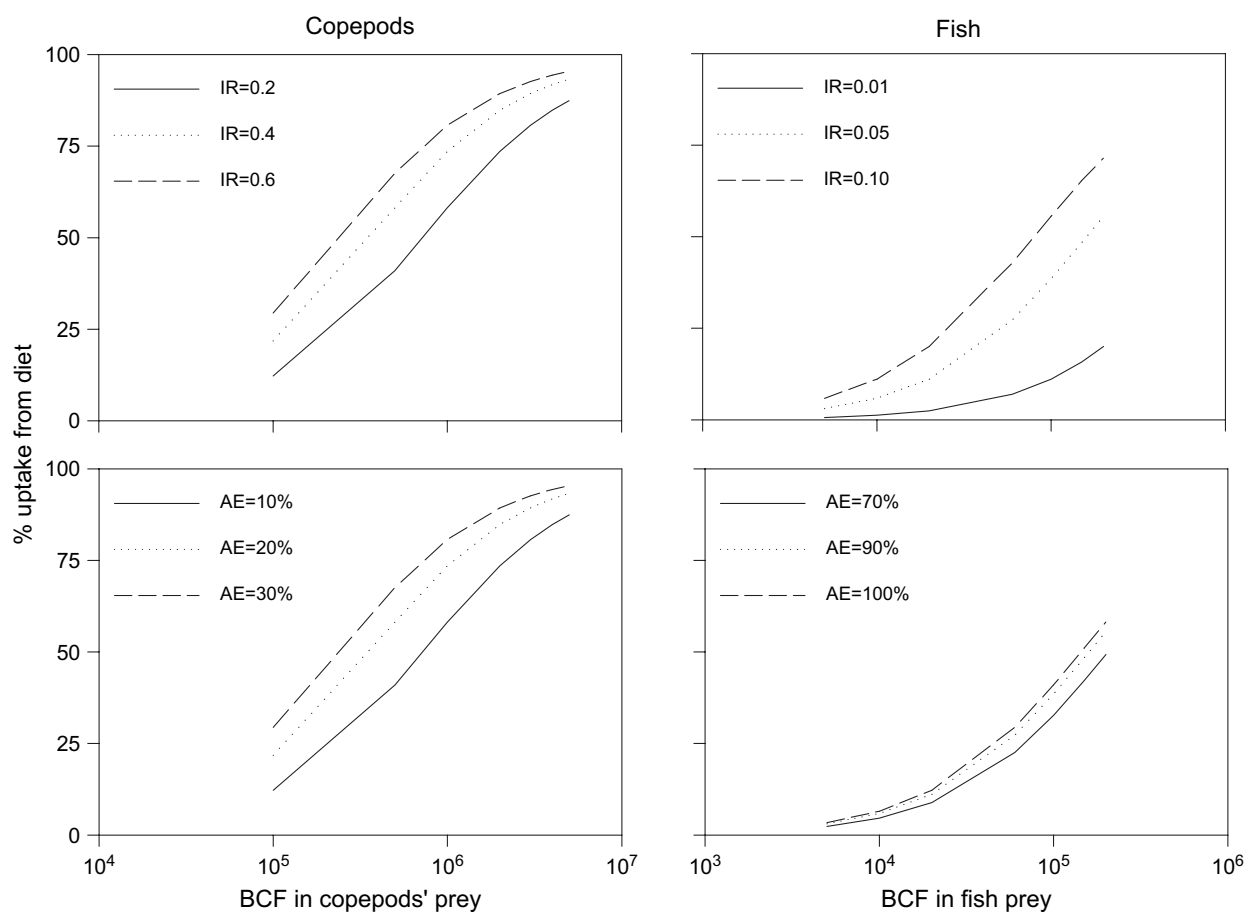


Fig. 6. The model-predicted percentage of DDT uptake from trophic transfer in the copepods *Acartia erythroa* and mangrove snappers *Lutjanus argentimaculatus* as a function of the DDT bioconcentration factor in the prey (BCF, L kg^{-1}). IR, ingestion rate ($\text{g g}^{-1} \text{d}^{-1}$); AE, absorption efficiency.

lipids within the cells is a major determinant of DDT accumulation. The composition of lipid in these four algal species has been examined in previous studies. *P. minimum* contained three major sterols in the free sterol fraction, which collectively represented >85% of the sterols: C28:2, C29:2, and C30:1 (Volkman et al., 1999). The major fatty acids of green alga *Codium* (same class as *T. levis*) were C16:0, C18:1 ω 9, C18:3 ω 3, and C16:3 ω 3, C18:2 ω 6 and C20:4 ω 6 (Xu et al., 1998). *T. weissflogii* contained five major sterols, all of which had methyl or ethyl substitutions at C22 or C24 (Harvey and Macko, 1997). *T. pseudonana* contained significantly greater amounts of C16:0 and C16:3 (Thompson et al., 1996). Because DDT can easily combine with polar compounds (e.g. high molecular weight sterols), the uptake rate of DDT should be in the order of *P. minimum* > *T. levis* > *T. weissflogii* > *T. pseudonana* based simply on the sterol composition. Our experimental results were consistent with this trend. In addition, our study found that DDT uptake by diatoms in their logarithmic phase was lower than by cells in the stationary phase, which may be related to a higher fraction of pectin produced by the aged diatoms.

DDT uptake may be affected by its physicochemical properties which control its deposition in fatty tissues. DDT can diffuse into the cells through the concentration gradient after it is associated with a suitable set of lipomolecules on the external membrane. At a high concentration, DDT is accumulated by different aquatic organisms through passive partitioning between the aqueous environment and the organism compartment (Leblanc, 1995). In fish, water is processed through the gills and DDT is selectively diffused into the fatty tissue, whereas the major route of intake for small organisms such as phytoplankton and zooplankton is not only controlled by their respiratory surface but also by their lipid composition/content. Fish lipids are rich in n-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA, 22: 6n-3) and eicosapentaenoic acid (EPA, 20: 5n-3). The lipid content of fish was about 0.17–0.20% dry weight (Rainuzzo et al., 1997), as compared to 4–43% for the copepods (Fisk et al., 2001; Buhning and Christiansen, 2001). Our data demonstrated that the k_u of DDT decreased from phytoplankton to copepods and to fish. Such difference between copepods and fish may also be accounted for by their difference in lipid content.

Many studies have examined DDT concentrations in copepods and fish in the field conditions, but their AE has not yet been determined. We used both the mass balance method and the ^{14}C : ^{59}Fe ratio method to determine the DDT AE in the copepods and fish. We used ^{59}Fe as an inert tracer for food passage because of its insignificant absorption by the animals and faster passage through the digestive system than DDT. In general, the AEs of DDT from the dietary source

calculated by the mass balance and ratio methods were rather comparable.

Food concentration did not significantly affect the AE of DDT by the copepods, whereas the AEs were more variable when the copepods fed on different food types. Among the four algal species examined, the AE of DDT was the highest for *P. minimum* > *T. weissflogii* > *T. pseudonana* > *T. levis*. DDT was also absorbed by the fish at a higher efficiency from the copepods than from the clams. In our experiments, the copepods' AEs were much lower than those of fish. In fish, the ingested food passed through the gut, and any polar contaminants may have accumulated in the liver with its high lipid content, thus facilitating the direct absorption of organic contaminants. The metabolites of DDT such as DDD and DDE are not easily detected in fish because of their lipid solubility and high octanol-water coefficient ($\log K_{ow} > 6$). Because the radiotracer technique used in this study detected the total radioactivity of ^{14}C , the proportion of the DDT and its metabolites in the copepods and fish tissues was not measured in our study, but biotransformation was assumed to be small within the relatively short period of depuration (<2 days).

Fish can eliminate chemicals across the gills and by egestion in faeces or by metabolic transformation. Small amounts of chemicals can also be eliminated in the urine. McKim et al. (1986) found that after the rainbow trout had been exposed to pentachlorophenol in water, 50% of the dose was eliminated through the gills, 30% in the faeces and bile, and 20% in the urine. The gills are the primary sites of passive diffusion of lipophilic chemicals both into and out of the fish. The absorbed contaminants are then distributed among the lipid compartments within the body. Metabolic transformation may also facilitate the elimination of DDT from the body. The metabolism of very hydrophobic, halogenated aromatic chemicals is usually considered negligible because these compounds are only slowly metabolized into hydrophobic metabolites. For example, Livingstone (1992) concluded that the high concentrations of organochlorine compounds in zooplankton and fish was consistent with the limited metabolic capacities of these organisms. Thomann (1989) suggested that compounds with high $\log K_{ow}$ values (5–7) tended to have low elimination rates, which may also be applied to DDT with a $\log K_{ow} > 6$. Borga et al. (2001) compared the concentrations and patterns of organochlorines in pelagic crustaceans, copepods, euphausiids, amphipods, fish, and seabirds, and found that the relative concentrations of DDT and PCBs along the food chain increased whereas HCHs, HCB and chlordane decreased. This indicated that DDT had a lower potential of elimination and was very resistant to metabolic breakdown in fish than HCHs and other compounds.

Contaminants are transferred from the environment to biota by two major routes: direct contact with the

contaminated water, and ingestion of contaminated food. In bioaccumulation studies, it is difficult to separate the uptake from water alone or from the total combined dietary and water uptake. It has been generally supposed that uptake via food rather than from the dissolved phase is the dominant exposure route for compounds with $\log K_{ow} > 5$ (Randall et al., 1998). Randall et al. (1998) showed that in the trout (*Salmo trutta*), the largest proportion of tetrachlorobenzene was taken up through the gills. They concluded that intake from food was only a minor pathway. In our study, the bioenergetic-based kinetic model was used to assess the DDT accumulation in copepods and fish. We found that dietary uptake was more important than aqueous uptake in the copepods at the median BCF for marine phytoplankton (10^6), whereas the aqueous uptake appears to be more important than the dietary uptake in the fish. The importance of dietary intake in marine copepods is presumably related to the high bioconcentration potential of marine phytoplankton as well as the ingestion activity of the copepods, despite that dissolved uptake is also high in the copepods. Conversely, the lower weight-specific ingestion rate of fish and bioconcentration potential in fish prey may account for the decreasing significance of dietary intake in fish, even though the DDT AE in fish is exceedingly high.

The TTF was 1–9 in copepods feeding on phytoplankton and 4–45 in fish feeding on copepods, indicating that the DDT was biomagnified in the marine food chain. Even though the aqueous intake appears to be more important for the overall DDT accumulation in fish, trophic transfer is still a significant process in marine fish as a result of the high dietary AE. In the Baltic Sea, DDT concentrations in the predators were higher than those in their prey (Strandberg et al., 1998a,b). Ruus et al. (1999) studied organochlorine contaminants in a local marine food chain (the lesser sand eel, cod, harbour seal, and grey seal) in Jarfjord, northern Norway, and found that the concentrations of the pollutants generally increased with trophic level. The highest biomagnification factor was up to 37 from sand eel to harbour seals. Borga et al. (2001) investigated the biomagnification of organochlorines in a marine food chain near Svalbard consisting of copepods, euphausiids, amphipods, and cod, and found that the biomagnification factor for DDT was 1.7 from the copepods to the cods. Similarly, Gray (2002) concluded that over two thirds of the DDT field measurements indicated a biomagnification of DDT. The predicted TTF values from the laboratory measurements of this study are rather comparable to these field measurements of zooplankton and fish, suggesting that the parameters used to quantify the TTF can realistically be used to predict the trophic biomagnification of DDT in aquatic food chains. Clearly, there were variations in the TTFs due to the variability of AE, IR and k_e in the animals.

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