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Accumulation and Depuration of DDTs in the Food Chain from Artemia to Brook Trout (*Salvelinus fontinalis*)

J. S. Wang,[1] K. L. Simpson[2]

[1]Department of Food Health. Chia Nan College of Pharmacy, Tainan 71710. Taiwan, Republic of China
[2]Department of Food Science and Nutrition, University of Rhode Island. Kingston, Rhode Island 02881, USA

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The persistent organochlorine pesticides such as DDT are lipophilic compounds and tend to build up in different segments of the environment, specially in aquatic biota through the food chain. Although the widespread use of DDT has been banned in the US and many other countries since 1972, residues of DDT and its metabolites are still detected in many aquatic samples gathered in many different regions of the world (Eisenberg and Topping 1985; Teran and Sierra 1986; Ober *et al.* 1987).

Direct partitioning or adsorption of these contaminants from the aqueous medium plays the major role in the uptake of lipophilic compounds by the lower level of planktons (Hoke *et al.* 1994). Transport of these organochlorines is thought to be by adsorption, in an intermediate step, onto the surfaces of particulate materials then by absorption in small organisms through the cell wall because of the relatively greater surface to volume ratio (Harding 1986; Opperhuizen and Stokkel 1988). Concentrations of DDT pollutants (DDT's = sum of *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT) on a wet whole-body-weight basis have been shown to be increased in higher-order aquatic predators (Tanabe and Tatsukawa 1984). However, it is important to study the mechanism of DDT's accumulation in the lower level of zooplanktonic biota and assess the role of these biota in transporting these contaminants to other larval stages of aquatic fish in the food chain system. In particular, when *Artemia* (a good diet source for the growth of larvae in the aquaculture) encountered different degrees of contamination, a higher contaminant accumulation in the larval predators might be present.

The objective of the present study was to assess the extent to which the contaminated *Artemia* nauplii serve as the source of organochlorine pesticides for accumulation or dietary transfer in a predatory fish, brook trout. Also, the levels of these organochlorine compounds in this fish species were measured after exposure to a clean *Artemia* diet for the deputation study.

Correspondence to: J. S. Wang

MATERIALS AND METHODS

Four standard organochlorine compounds (> 99.7% of purity) used in this study, *p,p'*-DDE (1,1-dichloro-2,2-bis(4-ethylphenyl)ethane), *p,p'*-DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), *o,p*-DDT (1,1,1-trichloro-2-(2-chlorophenyl)2,2-(4-chlorophenyl)ethane) and *p,p'*-DDT (1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane), were purchased from Ultra Scientific Co., Rhode Island, USA. Each organochlorine was weighed and dissolved in a series of dilutions with acetone to achieve 100 ng/mL of stock solution.

Brook trout (*Salvelinus fontinalis*) at 1-mon-old (40 ± 5 mg) were obtained from Lafayett State Hatchery, Rhode Island, USA. Hatching of the *Artemia* cysts (*Artemia salina*, San Francisco Bay Brand) was performed in a separatory funnel containing 2 L of 0.45 µm filtered seawater (30 ‰ of salinity) under continuous strong aeration for 24 hr (20 ± 2°C).

In the present study, 1 and 2 mL each of 100 ng/mL prepared stock organochlorine mixtures were pipetted into Erlenmeyer flasks containing 200 mL of filtered seawater (0.45 µm) to achieve 0.5 ng/mL and 1.0 ng/mL concentrations, respectively. Freshly *Artemia* nauplii were harvested under a 106 µm sieve and exposed to 0.5 ng/mL and 1.0 ng/mL prepared mixtures for another 24 hr in order to accumulate DDTs.

Forty five brook trout larvae were randomly collected and transferred to each fiberglass tank (60 cm x 30 cm x 30 cm) containing 40 L of aerated fresh water. One gram of uncontaminated or contaminated nauplii was collected and fed to each tank every 2 d. At day 12, fifteen fish were collected at random from each tank. The feeding experiment was terminated on day 24 and then 15 fish were collected at random from each tank.

From day 24, all fish in each tank were fed 2% g of uncontaminated *Artemia* nauplii every 2 d. Every time before feeding, 5 L of water were siphoned to remove fecal detritus and dead nauplii. The depuration experiment was terminated on day 48. Another treatment of uncontaminated *Artemia* was set up as the control group throughout the experiment. All collected samples of brook trout and *Artemia* were freeze-dried and placed at -20°C before chemical extraction.

Samples of freeze-dried fish and *Artemia* nauplii were first extracted by using hexane:acetone (1:1 by volume; 5 mL) in a Polytron homogenizer to obtain crude lipid. The lipid extracts were then cleaned-up by 6% deactivated alumina oxide adsorption column (Al_2O_3, 80-120 mesh, 20 cm x 0.5 cm i.d.) with anhydrous sodium sulfate on top and eluted with hexane (20 mL) for the elimination of lipid and polar compounds. The eluted hexane containing these organochlorines were concentrated and dissolved in hexane (1.0 mL) for gas chromatography analysis.

A gas chromatography (GC, Tractor MT-220) equipped with a ^{63}Ni electron-capture detector (ECD) and a programming integrator (Spectra-Physics 4270)

connected to the GC was used for the analysis of these chlorinated compounds. The glass column was 1.5% SP-2250/1.9% SP-2401 (100-120 mesh, 2.4 m x 3.175 mm i.d.) on Supelcoport®. The temperatures operated for injector, column and detector were kept at 220°C, 200°C and 275°C, respectively. Ultra-high-pure-grade argon:methane (95:5) was used as the carrier gas with a flow rate of 25 mL/min. Quantification of the concentrations was calculated by comparison of peak areas and standard injection. The recovery of these organochlorines after these extraction and clean-up procedures was 80-90%. The detection limit of each compound was 1.0 ng/g (wet wt basis) in this study.

RESULTS AND DISCUSSION

The concentrations and bioconcentration factors (BCFs = organochlorine concentration in *Artemia* : organochlorine concentration in water) of four organochlorines found in the 24-h treated *Artemia* nauplii are presented in Table 1. Organochlorine residues in the nauplii were found all below detection limits on the control (0.0 ng/mL) group. Apparent organochlorine concentrations were determined in the nauplii after 24-h contamination from two different treatment

Table 1. Organochlorine concentrations (ng/g, wet wt basis) and bioconcentration factors (BCF) in *Artemia* nauplii after 24 hr of exposure to each of 0.0 ng/mL, 0.5 ng/mL and 1.0 ng/mL concentration groups individually.

	Concentrations (ng/g) (± SD)[a] *in Artemia nauplii*		
	0.0 ng/mL	*0.5 ng/mL*	*1.0 ng/mL*
p,p'-DDE	ND[b]	24.10 (±1.42)	41.42 (±3.75)
p,p'-DDD	ND	28.02 (±1.98)	53.51 (±4.66)
o,p -DDT	ND	58.89 (±3.26)	127.82 (±8.98)
p,p'-DDT	ND	116.46 (±8.77)	247.94 (±9.69)
	Bioconcentration Factors (BCF)[c]		
	0.0 ng/mL	*0.5 ng/mL*	*1.0 ng/mL*
p,p'-DDE	ND	48	41
p,p'-DDD	ND	56	54
o,p -DDT	ND	118	128
p,p'-DDT	ND	233	248

[a] n = two determinations. [b] ND = Not detected.

[c] $BCF = \frac{\text{Concentration (ng/g) of organochlorine in } Artemia}{\text{Concentration (ng/mL) of organochlorine in seawater}}$

groups. Among four organochlorines tested in the nauplii after 24 hr of exposure, *p,p'*-DDT showed the highest levels, 116.46 ng/g and 247.94 ng/g (wet wt basis) in the 0.5 ng/mL and 1.0 ng/mL treatment groups, respectively. The lowest concentrations in the nauplii were determined to be *p,p'*-DDE, 24.10 ng/g and 41.42 ng/g in the 0.5 ng/mL and 1.0 ng/mL treatment groups, respectively.

The concentrations of these organochlorine compounds in the *Artemia* from 1.0 ng/mL aqueous were consistently about twice that from 0.5 ng/mL aqueous. Evidently, this followed the direct partitioning process that caused a significant result on the transfer of the organochlorines between the water and tested zooplankton (McLean *et al.* 1987). Two BCF values of each organochlorine in two different treatment groups were about the same magnitude (Table 1). No data so far was reported for the DDT and its metabolites bioconcentrated in the *Artemia* from aqueous solution. In a similar study, McLean *et al.* (1987) demonstrated that experimental *Artemia* were found to have 10.8 ng/g and 92.7 ng/g *cis*-chlordane (a chlorinated insecticide) after 24 hr contamination from the 0.5 ng/mL and 1.0 ng/mL treatment groups, respectively. The organochlorine values in *Artemia* from that study also follow the order of magnitude in the aqueous concentrations.

Different levels of the organochlorine in *Artemia* were due to different liposolubility and selectivity of the compounds partitioned in the lipid of the zooplanktonic organisms. Veith *et al.* (1979) suggested that the polarity of the chemical compound could also reduce the BCF of the compound. Similarly, McLean *et al.* (1987) demonstrated that dieldrin (a polar organochlorine pesticide) could concentrate as greatly as *cis*-chlordane (a less polar organochlorine pesticide) in *Artemia* maintained in water containing 0.1 ng/mL and 1.0 ng/mL concentrations. Among the four test organochlorines, *p,p'*-DDT with highest polarity was found to have highest BCFs, 233 and 248 in 0.5 ng/mL and 1.0 ng/mL treatment groups, respectively. Adsorption of high *p,p'*-DDT residues in the surface of the nauplii may be the reason that high BCFs of *p,p'*-DDT were found in this small size of zooplankton with large biomass.

The four organochlorine concentrations in brook trout accumulated and depurated from two different contaminated *Artemia* diets during the 48-d feeding experiment are shown in Figure 1. No fish mortality was found during the entire experiment period. On the initial day (day 0) of the experiment, *o,p*-DDT was the only residue that was not found on the fish body, however *p,p'*-DDE (2.67 ng/g) showed the highest level compared to *p,p'*-DDD (1.01 ng/g) and *p,p'*-DDT (1.31 ng/g). After feeding with contaminated *Artemia* diets for 12 d and 24 d, brook trout were found to have different extents of linear increase of body organochlorine concentrations. During the feeding period, *Artemia* pre-contaminated with 1.0 ng/mL organochlorines had significantly higher accumulation rate in the fish than those pre-treated with 0.5 ng/mL organochlorines. Various studies have revealed that the significance of the dietary organochlorine contaminants in top predators is due to food chain transfer. Larvae of winter flounder, *Pseudopleuronectes americanus*, fed the contaminated

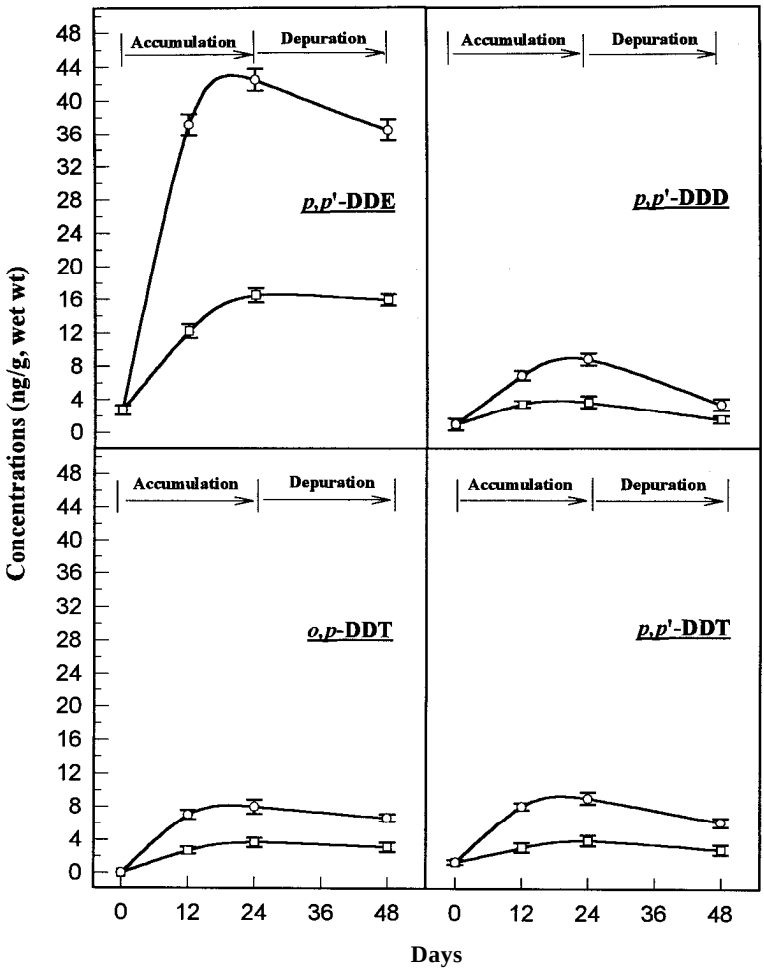

Figure 1. Concentrations of four organochlorines accumulated in brook trout when fed 0.5 ng/ml(□—□) and 1.0 ng/mL(○—○) each of pre-contaminated *Artemia* for 24 d (day 0-24) then fed uncontaminated *Artemia* for 24 d (day 24-48).

Artemia nauplii for 25 d were found to bioaccumulate organochlorine compounds in all 25 treatment groups (Scura and Theilacker 1977). Body burdens of striped bass (*Morone saxatilis*) were also shown to have the highest accumulation rates for DDT's when the larvae fed on *Artemia* nauplii containing apparent organochlorine residues (Westin *et al.* 1985).

In the present study, concentrations of *p,p'*-DDD, *o,p*-DDT and *p,p'*-DDT in the fish when fed two different pre-contaminated *Artemia* diets were found to be about the same order of magnitude (day 0-24). After feeding with *Artemia* diets containing lowest amounts of *p,p'*-DDE, brook trout were detected to have their highest concentrations (42.47 ng/g and 16.40 ng/g from 1.0 ng/mL and 0.5 ng/mL

treatment groups, respectively) compared to the other three organochlorines during the 24-d feeding period. This abnormal accumulation pattern of *p,p'*-DDE on the fish suggests that the mixed-function oxidase (MFO) may have been induced for the dechlorination of *p,p'*-DDT to *p,p'*-DDE. For the organochlorine residues determined in freshwater fish, Schmitt *et al.* (1985) concluded that *p,p'*-DDE constituted approximately 70% of the *p,p'*-DDT residues. Addison and Zinck (1977) also described an experiment in which brook trout were injected with ^{14}C-*p,p'*-DDT to yield a few mg/kg tissue concentrations after which the rate of dehydrochlorination of *p,p'*-DDT was converted to *p,p'*-DDE and measured by a linear regression of percent on time. The MFO may be responsible for the resulting lower *p,p'*-DDT concentrations found in brook trout after feeding high residues of *Artemia* diets in this study.

The concentrations of these organochlorines in brook trout after feeding with uncontaminated *Artemia* diets for the depuration study (day 24-48) are shown in Figure 1. During the 24-d depuration period (day 24-48) shown in Fig 1, all organochlorines detected in the fish were depurated to some extent. After 24 d depuration, concentrations of organochlorine in the fish were found to reach a state of equilibrium after feeding with 0.5 ng/mL pre-contaminated *Artemia*. According to Khan (1977) the rate of depuration depends on the water solubility and liposolubility of the chlorinated compounds, where 50-90% of the accumulated organochlorines in the aquatic fish can be depurated within 4 wk after being transferred to clean water or being fed uncontaminated food. From Table 2, we can find *p,p'*-DDD had the highest depuration percentages, 62.1% and 57.1% for the fish fed 1.0 ng/mL and 0.5 ng/mL pre-contaminated *Artemia*,

Table 2. Percentage of depuration of the chlorinated organochlorines in brook trout on two treatment groups after feeding uncontaminated *Artemia* diets for 24 d (day 24-48).

	Depuration (%) in brook trout (± S.D.)[a]	
	0.5 ng/mL	*1.0 ng/mL*
p,p'-DDE	3.48 (±0.21)	14.25 (±1.09)
p,p'-DDD	57.10 (±4.58)	62.10 (±3.17)
o,p -DDT	14.63 (±2.06)	16.52 (±1.28)
p,p'-DDT	26.08 (±3.35)	31.98 (±2.71)

a n = two determinations.

respectively. Lowest depuration percentages were found to be *p,p'*-DDE, 14.25% and 3.48% for fish fed *Artemia* pre-contaminated under 1.0 ng/mL and 0.5 ng/mL organochlorines, respectively. All fish groups fed with 0.5 ng/mL pre-contaminated *Artemia* diets showed higher degrees of depuration magnitude than

that fed with 1.0 ng/mL pre-contaminated diets.

In conclusion, the present study demonstrated that the persistent chlorinated compounds were accumulated in the *Artemia* nauplii through aqueous environments depending on different magnitudes of contamination conditions, and the BCFs in this small zooplankton were consistent with the contamination levels. In addition, following 24-d feeding with contaminated diets, brook trout larvae accumulated different extents of these organochlorines in the body through the food-chain phenomenon. After feeding uncontaminated *Artemia* diets, the fish larvae showed lower degrees of depuration, in comparing with those contaminated by higher concentrations of organochlorines.

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