Bull. Environ. Contam. Toxicol. (1999) 63:297-304

Bulletin of Environmental Contamination and Toxicology

Significant Organotin Contamination of Sediment and Tissues of Milkfish in Brackish Water Ponds

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Received: 3 March 1999/Accepted: 12 July 1999

Organotin pesticides, triphenyltin acetate or hydroxide (Brestan, Aquatin or Telostan), have long been used as an inexpensive method to control the population of brackish water snails *Cerithidea cingulata* in the pond culture of milkfish (*Chanos chanos* Forsskal), an important food fish in the Philippines (Coloso et al. 1998). The use of organotin pesticides has been banned for several years now because the chemical renders the soil sterile, is non-biodegradable and bioaccumulates, and is hazardous to humans. Despite the ban, the clandestine use of the pesticide in milkfish ponds continues to threaten the environment and humans.

Organotin pesticides such as butyltins and phenyltins and their degradation products have been detected in sediment and biosamples from many areas of organotin use. The composition of organotin compounds in these samples differs depending on the species being sampled and sampling point. Elevated concentrations of tributyltin (TBT), the most toxic to aquatic life of the butyltin compounds, were found in molluscs and triphenyltin (TPT) was the major organotin in red mullet liver found along the western Mediterranean coast (Morcillo et al. 1997). Elevated levels of total butyltins were also found in the liver of bottlenose dolphins found along the southeastern U. S. Atlantic and Gulf coasts and levels of butyltins in the fish muscle indicated recent inputs (Kannan et al. 1997). Butyltins were also detected in muscle of fish collected in many countries in Asia and Oceania indicating widespread contamination (Kannan et al. 1995a). The increased proportions of monobutyltin (MBT) over dibutyltin (DBT) and TBT in the samples indicate degradation of TBT to MBT in fish tissues. In blue mussels collected along Tokyo Bay, DBT concentrations were highest among the butyltins and TPT concentrations were highest among phenyltins (Higashiyama et al. 1991). The detectable levels of TBT, DBT, and TPT in many fish and shellfish from retail markets in Japan suggest the health hazards posed by environmental organotin contamination on humans (Tsunoda 1993).

In vivo and *in vitro* studies have shown that organotins are readily taken up by fish. The red sea bream readily accumulate TBT and TPT from seawater by gill uptake and oral intake simultaneously (Yamada et al. 1994). In addition,

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TPT appears to be more easily accumulated by dietary uptake than TBT. Among tissues and organs of the red sea bream, TBT and TPT are least accumulated by muscle and no correlation exists between lipid content and accumulated TBT or TPT content (Yamada and Takayanage 1992). Rainbow trout hatchlings bioconcentrate TBT oxide from seawater (Triebskorn et al. 1994). Exposure of isolated rainbow trout hepatocytes to TBT results in tin recovery in the cellular fraction (Reader et al. 1996).

TBT and TPT and their degradation products are highly toxic to fish. Organotins can also alter fish gill morphology (Pinkney et al. 1989), affect the lymphatic system and spleen (Kalbfus et al. 1994), liver, thymus, kidney, and intestines (Shimizu and Kakuno 1994). They can also affect the reproductive performance of mature fish (Shimizu and Kimura 1992) and cause physical abnormalities in young fish (Fent and Meier 1994).

The persistent use of organotin compounds especially TPT to control the brackish water snail population in milkfish ponds in the Philippines threaten the environment and humans who consume fishery products that may be contaminated with organotins. In this study we found evidence of triphenyltin contamination of sediment and tissues of milkfish collected from brackish water ponds that have previously been treated with organotins. Total tin and triphenyltin levels were used as indicators of organotin contamination.

MATERIALS AND METHODS

Samples of sediment and milkfish tissues were obtained from commercial milkfish ponds in Dumangas, Iloilo and Kalibo, Aklan, Philippines. TPT was last applied in the Dumangas pond one year prior to sampling and six months prior in the Kalibo pond. Fishfarmers use an application rate of 1 kg/ha.

Duplicate sediment samples (I kg) were taken by scraping 5 cm of top sediment from an area of 1 sq m. The wet sediment was then brought to the laboratory and air dried for ten days. The dried samples were then pulverized, mixed thoroughly, and random subsamples (100 g) were taken for analysis. Milkfish (250 g body wt) were obtained from each pond and immediately placed on ice. Milkfish reared from the juvenile stage in concrete tanks at the Tigbauan Main Station of the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC) served as reference. Pond-reared milkfish were not used as reference because most milkfish ponds in the Philippines had, in one time or another, used organotin pesticides to control brackish water snails and contamination is highly possible. Duplicate samples of whole fish, intestine, liver, depot fat, and flesh were obtained, weighed, minced, and dried to constant wt at 60°C.

Dried sediment and tissue samples were sent to Lonza, Inc., Basel, Switzerland for analysis of total tin as a convenient index of any organotin contamination of the sediment and biosamples. Samples were prepared using a procedure that prevents the formation of SnO_2 which is difficult to dissolve with normal dissolution procedure (Caricchia 1994). Sediment samples were stored at -70° C before analysis. The samples were then dissolved by microwave digestion with a mixture of HNO_3, HCI and HF. After digestion, boric acid was added. Fish tissue samples were also stored at -70° C prior to digestion. To obtain a homogeneous material for analysis, samples were mixed with about the same amount of dry ice and then milled thoroughly with a laboratory mill. After milling the CO_2 was allowed to escape and the sample was dissolved by microwave digestion with a mixture of HNO_3, HCI and HF. After digestion, the solution was transferred to a plastic volumetric flask and boric acid was added to complex the hydrofluoric acid. The measurement of total tin concentration was carried out by using a JOBIN YVON ICP-OES (inductively coupled plasma-optical emisssion spectrometer) model 36. The spectrometer chamber was flushed with nitrogen to allow measurement at short UV range. HF resistant cross-flow nebulizer, nebulisation chamber and torch were used.

Dried sediment and tissue samples were sent to the Nutrition and Food Sciences Department, University of Santiago de Compostela, Spain for the analysis of triphenyltin. Samples were subjected to simultaneous microwave-assisted extraction and derivatization to obtain ethylated organotin compounds. The isooctane extracts were then passed through alumina, eluted with diethyl ether to remove very polar compounds, and the final extracts subsequently concentrated under nitrogen. The clean samples were then analyzed by gas chromatography - atomic emission spectroscopy (GC-AES) using both external (triphenyltin) and internal (tripropyltin chloride) standard additions to obtain quantitative results (Rodriguez et al. 1997).

Data were subjected to analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) using StatView (Abacus Concepts, Inc. Berkeley, CA) for the IBM-PC. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The new and important finding in this study is the evidence for triphenyltin contamination of milkfish tissue samples from brackish water ponds that had previously been treated with organotin compounds to eradicate the snails *Cerithidae cingulata* (Table 1). The total tin content of sediment from milkfish ponds ranged from less than 0.5 to 1.4 mg Sn/kg dry matter, with pond A (Dumangas) sediment containing more tin than that of pond B (Kalibo). Pond A had a smaller area (1 ha) compared with pond B (10 ha). The exact amount of pesticide applied to each pond could not be accurately determined from the interviews with the operator-owner. Although organotin was last applied to pond A one year and to pond B six months prior to the sampling, pond A

Table 1. Residues of tin and triphenyltin in sediment and milkfish tissue samples from brackish water ponds

Source	Total tin or triphenyltin, mg Sn/kg dry matter *					
	S	WF	DF	F	L	I
Pond A , Dumangas, Iloilo						
Total tin	1.4 ± 0[b]	0.2 ± 0[b]	0.3 ± 0.1[b]	0.4 ± 0.1[b]	2.3 ± 0[b]	1.2 ± 0.1[a]
TPT	n. d.	0.18±0.01[b]	0.20±0.01[c]	0.34±0.04[b]	0.60±0.01[b]	0.02±0[a]
Pond B, Kalibo , Aklan						
Total tin	<0.5[a]	0.2 ± 0[b]	0.2 ± 0[b]	0.4± 0[b]	2.8 ± 0.1[c]	2.1 ± 0[b]
TPT	n.d.	0.21±0.01[b]	0.14±0.02[b]	0.40±0.01[b]	0.73±0.03[c]	0.02±0.01[a]
Concrete tank (Control)						
Total tin	n.d.	< 0.1[a]	< 0.1[a]	<0.1[a]	0.4 ± 0.1[a]	0.8 ± 0.1[a]
TPT	n.d.	<0.01[a]	<0.01[a]	<0.01[a]	<0.01[a]	<0.01[a]

* Means ± standard error (n=2) in a column with the same superscript are not significantly different at $p < 0.05$. Abbreviations: S - sediment; WF - whole fish; DF - depot fat; F - flesh; L - liver; I - intestine; n. d. - not determined; TPT - triphenyltin

could have accumulated more organotin compounds than pond B from prolonged pesticide use.

Biosamples of whole milkfish and fish tissues from the brackishwater ponds contained significantly elevated tin and TPT levels compared with the reference samples of fish from the concrete tank. In general, high total tin levels positively correlated with levels of TPT except for the intestinal samples where the levels of TPT were low and those of total tin remained high. This indicates that oral intake was the primary route of entry of TPT. Highest total tin and TPT residues were found in fish livers. Total tin levels were six- to seven-fold higher while TPT levels were 60- to 70-fold higher in livers of fish from pond A and B than those in livers of reference samples. Depot fat of fish from pond A had significantly higher TPT content than those of fish from pond B although total tin levels are similar. In contrast, the livers of fish from pond A contained significantly lower total tin and TPT levels than those of fish from pond B. The data suggest that the higher levels of TPT and total tin in livers of fish from pond B reflect more recent inputs, while the higher levels of TPT in depot fat of fish from pond A reflect more extensive assimilation and metabolism from earlier inputs. The rank order of milkfish tissues according to total tin content is as follows: liver > intestine > flesh > depot fat > whole fish; and according to TPT content is as follows: liver > flesh > depot fat > whole fish > intestine. Lowest total tin and TPT content were found in tissues of reference milkfish samples.

Based on our data, the estimated concentration of TPT in milkfish flesh is about 230 ng TPT / g wet tissue which is in the same order of magnitude of organotin concentrations found in contaminated biosamples elsewhere. For example, the highest concentrations of TBT, DBT, and TPT reported in fish and shellfish from retail markets of Niigata, Japan were 669, 674, and 186 ng/g, respectively (Tsunoda 1993). Commercial freshwater fish cultured in Saitama Prefecture in Japan had TBT levels of 30 - 300 ng/g (Iijima et al. 1991). High butyltin contents (up to 1400 ng/g) were also found in the livers of bottlenose dolphins found stranded in the U.S. Atlantic and Gulf coasts (Kannan et al. 1997) and those of fish caught off the waters of Sydney, Australia and Honiara, the Solomon Islands (Kannan et al. 1995b). Similarly, milkfish livers had TPT concentrations of about 400 ng/g wet tissue. Although flesh is more important than the liver from the human consumption viewpoint, in most rural communities in the Philippines practically the whole fish including the visceral organs is eaten as part of the diet. The high TPT concentrations found in milkfish livers in addition to that found in the flesh may be a source of human exposure to organotins. Furthermore, the liver could be a suitable organ for monitoring TPT contamination in various marine and freshwater fishes.

Human dietary exposure to butyltins is greatest in marine foods (Kannan et al. 1995b) and the same may be true for TPT. Thus far no adverse effect on human health due to exposure to organotin contaminated foods is known. The FAO/WHO (1991) has established an acceptable daily intake of 0.5 µg Sn/kg body weight. In other mammals such as hamsters and rats, most of the tin compounds in the brain of TPT treated animals were TPT and TPT induces diabetogenic effects in hamsters but not in rats (Ohhira and Matsui 1996). TPT has also been shown to be cytotoxic to mouse thymocytes (Bollo et al. 1996) and thus possesses the ability to compromise the mammalian immune system. Furthermore, TPT causes failure in implantation of the fetus during early pregnancy in rats (Ema et al. 1997).

Milkfish production accounts for about 30% of total aquaculture production in the Philippines. The widespread use of organotin pesticides for many years to control brackish water snails in milkfish ponds has jeopardized the milkfish industry and put the public at risk. The high levels of total tin and the contamination of whole fish and fish tissues including flesh with TPT indicate a significant environmental hazard to humans through the consumption of contaminated fishery products from these ponds. Because TPT and its degradation products are more toxic than elemental tin, information on the relative amounts of organotins particularly TPT and elemental tin in sediments and biosamples is important. In addition, a wider study involving organotin analyses of samples from different milkfish ponds from the three geographical locations , i. e., northern, central, and southern Philippines, as well as of milkfish and other fishery products in the wet markets should be investigated to assess the extent of organotin contamination in seafood. The average daily intake of organotin compounds from fish and perhaps shellfish consumption should be studied to determine the relative risk of organotin toxicity in the population. Lastly, the ban on organotin usage in milkfish ponds should be strictly implemented to reduce the threat of this pesticide to the environment, natural resources, and the human population. Viable alternative methods to the use of organotins in brackish water ponds should be explored.

Acknowledgments We thank Lucia Jimenez for excellent technical assistance and Dr. Ronaldo Ferraris and Dr. Rene Blum for their comments and suggestions to improve the manuscript.

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