

Sources and Accumulation of Butyltin Compounds in Ganges River Dolphin, *Platanista gangetica*

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Concentrations of butyltin compounds (mono-, di-, and tri-butyltin) were determined in dolphin (*Platanista gangetica*), fish, invertebrates and sediment collected from the River Ganges, India, in order to understand the contamination levels, sources, and potential for biomagnification in freshwater food chains. Total butyltin concentration in dolphin tissues was up to 2000 ng g⁻¹ wet wt, which was about 5–10 times higher than in their diet. The concentrations in fish and benthic invertebrates, including polychaetes, were 3–10 times greater than in sediment. The biomagnification factor for butyltins in river dolphin from its food was in the range 0.2–7.5. Butyltin concentrations in Ganges river organisms were higher than those reported for several persistent organochlorine compounds. Discharge of untreated domestic sewage was one of the major sources of butyltin residues in Ganges river biota. High concentrations of butyltin compounds in freshwater food chains suggest the need to assess their toxic effects in aquatic organisms and to regulate their use. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

The Ganges river dolphin, *Platanista gangetica* (known locally as 'susu'), is widely distributed in

the Ganges, Brahmaputra and Meghna river systems in India, Bangladesh and Nepal.¹ The population of *P. gangetica* has been roughly estimated to be 4000–5000 animals and is rapidly declining.² The World Conservation Union regards this species as 'vulnerable'.³ The species is threatened by the rapid deterioration of the habitat due to pollution, construction of dams, mining, and directed and incidental catch.^{4,5}

The River Ganges is heavily polluted by the annual usage and discharge of about 2500 tonnes of pesticides and 1.2 million tonnes of fertilizers in its catchment area. Tanneries, textiles, wood and jute mills, sugar mills, distilleries, pulp and paper factories, the synthetic rubber industry, fly ash from coal washeries and DDT factories are the major sources of chemical pollution in the River Ganges and its tributaries.⁶

Earlier studies showed the accumulation of heavy metals, organochlorine pesticides and polychlorinated biphenyls (PCBs) in Ganges river dolphins.⁷ The isomer/congener profile of PCBs, DDTs and HCHs (BHC: hexachlorocyclohexanes) in river dolphin tissues suggested that this species has been vulnerable because of its reduced capacity to degrade xenobiotics.⁸

In recent years, tributyltin (TBT) and its degradation products, monobutyltin (MBT) and dibutyltin (DBT), have received considerable attention due to the high toxicity of TBT at low concentrations. Butyltin compounds are used as poly(vinyl chloride) (PVC) stabilizers, industrial catalysts, industrial and agricultural biocides and wood preserving and antifouling agents. Studies on marine bivalves⁹ and gastropods^{10,11} have demonstrated that TBT exerts chronic toxic effects on susceptible species at water concentrations of a few nanograms per litre. Growth of

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susceptible algae^{12,13} and some zooplankton species^{14,15} was inhibited at a few hundred nanograms per litre or at even lower concentrations. The acute toxicity of TBT in fish lies in the range of a few micrograms per litre.^{16,17} An important effect of TBT on mammals is on the immune system.¹⁸ Thus, the accumulation of high concentrations of butyltin compounds in higher trophic aquatic vertebrates, including cetaceans,^{19,20} is of great concern.

The ecotoxicological impact of tributyltin compounds in marine coastal areas, as a result of their use in antifouling paints, has led to the regulation of their use in many countries in Europe and North America.²¹ In contrast to the marine environment, data on the occurrence of organotins in freshwaters is still scarce, particularly in developing Asian countries. Organotin compounds have been detected in freshwater ecosystems in developed nations.^{22–28} Domestic and industrial wastewater is one of the major sources of organotins in freshwater systems due to their use as biocides in household commodities.^{29–31} The Ganges, tropical Asia's third largest river, is heavily polluted by the discharge of untreated sewage and industrial effluents. Therefore, it is of interest to understand the contamination levels of butyltin compounds in the River Ganges ecosystem. An earlier investigation has reported that butyltin contamination is widespread in developing countries in Asia, including India.³² The present study was conducted to determine the concentrations of butyltin compounds in Ganges river biota in order to understand the sources, contamination levels and biomagnification in freshwater food chains.

MATERIALS AND METHODS

Sample collection

The Ganges river dolphins studied were found entangled in fishing nets or were drowned. Blubber, muscle, kidney and liver were obtained from six animals collected in Patna (25°N, 85°E), India, during 1988–1992. Data on age, sex and length of the dolphins are shown in Table 1. The normal length of Ganges river dolphins at the time of birth is 70 cm and they attain 199 cm at 28 yr.³³ The age determinations for dolphins collected in this study were based on the above

Table 1. Sample details of Ganges river dolphins

Sample	Sex	Growth stage	Length (cm)	Age	Collection date
MC1	M	Neonatal	70	A few days	24 January 1988
MC2	M	Immature	95	2 months	14 July 1992
FC1	F	Immature	95	2 months	2 July 1992
IM1	M	Immature	104	1 yr	6 October 1991
IF1	F	Immature	115	1.3 yr	21 July 1991
AF1	F	Adult	233	30 yr	27 March 1992

estimates. We also collected the milk of dolphins from the stomach of a neonatal female. Other food items of dolphins, such as finfish (*Mastacembellus pancalus*, *Chela laubuca*, *Puntius sophore*, *Puntius* sp., *Colisa fasciatus*, *Chanda ranga*, *Glossogobius giuris* and *Nangra* sp.), gastropods (*Bellamya crassa*, *B. bengalensis*, *Thiara scabra*, *T. tuberculata*, *T. granifera*, *Lymnaea acuminata*, *Gyraulus convexiusculus*, *Indoplanorbis exustus* and *Brotia costula*) and *Gomphus* sp. (larvae of dragon flies, Order Odonata, Class Insecta) were collected from the River Ganges in Patna during 1988–1992. Similarly, bivalves (*Novaculina gangetica*, *Parreysia olivaria*, *P. caerulea*, *Corbicula bensoni* and *C. striatella*) and polychaetes (*Nephtys oligobranchia*, *N. polybranchia* and *Nemalycastis indica*) were also collected. A sample of surface sediment obtained in the River Ganges near Patna was homogenized and air-dried. Fish, benthic invertebrates and sediment were collected near the sites where dolphins had been caught. For food items, individuals belonging to the same group were pooled and analysed. A species of the mud-frequenting fish, *Mastacembellus pancalus*, the most preferred food of the dolphin, was separately analysed. The length of fish and polychaetes was <10 cm. All the samples, except sediment and milk, were preserved in 10% formalin and were stored at 4 °C in darkness until analysis. Raw sewage sludge was collected at three different drains close to the point of discharge in the River Ganges in Patna in May 1995, dried in shade and stored at –20 °C until analysis. In the case of fish, the whole body was used for analysis whereas only the soft tissues were examined for gastropods and bivalves. Analysis of formalin, used for preserving the muscle, showed no butyltin (<1 ng l⁻¹) residues, suggesting that butyltin compounds from tissues were not leached out by formalin preservation.

Analysis

Butyltin compounds were analysed following the method described by Kannan *et al.*³² Approximately 4–5 g of tissues was homogenized and extracted twice with 40 ml of 0.1% tropolone–acetone and 10 ml of 1 M HCl. The combined extract was transferred to 100 ml of 0.1% tropolone–benzene and 500 ml of hexane-washed water. After shaking and partitioning, the organic layer was eluted through a glass column packed with 35 g of anhydrous Na₂SO₄ to remove moisture. The benzene extract was rotary-evaporated at 40 °C almost to dryness and the volume was made up to 5 ml with hexane. An aliquot of the benzene extract was used to determine fat content gravimetrically. The extract was derivatized by the addition of 5 ml of propylmagnesium chloride (in diethyl ether) as the Grignard reagent. The excess Grignard reagent was decomposed with 20 ml of 1 N H₂SO₄ and the derivatized extract was passed through a 20 g Florisil®-packed dry column to remove lipids and then cleaned by eluting through a 6 g Florisil®-packed wet column. Sediment and sewage sludge samples were also analysed following the same procedure by eliminating the Florisil dry-column step.

Sample extracts were analysed for MBT, DBT and TBT using a gas chromatograph equipped with flame photometric detector (GC–FPD). Chromatographic separation was performed on a Hewlett-Packard 5890 Series II gas chromatograph with a 30 m × 0.25 mm (i.d.) DB-1 capillary column coated at 0.25 µm film thickness. The column oven temperature was programmed from 80 °C (1 min hold) at 160 °C at a rate of 15 °C min⁻¹ and then at a rate of 5 °C min⁻¹ to a final temperature of 260 °C with a 5 min final hold time. Injector and detector temperatures were held at 200 and 270 °C, respectively. The flame photometer was operated using a hydrogen–air–nitrogen flame and was equipped with a 610 nm bandpass filter selective for tin-containing compounds.

Known amounts of butyltin trichloride, dibutyltin dichloride and tributyltin chloride (0.1 µg of each) were concurrently run through the whole analytical procedure and the propylated mixture was used as an external standard. Only freshly derivatized external standards prepared along with samples were used to estimate concentrations. Concentrations were quantified by comparing peak heights of butyltins in

samples with those in the external standards. Hexyltributyltin was used as an internal standard. Procedural blanks were included with every batch of four samples to check for interfering compounds and to correct sample values. Monobutyltin, probably originating from commercial solvents or reagents that came into contact with PVC containing this compound as a stabilizer, was found at trace levels (*ca* 1 ng) in reagent blanks (Fig. 1). The values obtained for MBT in samples were, therefore, corrected for blank concentrations. However, blanks never contained TBT. The detection limits of MBT, DBT and TBT in tissues were 3.0, 1.0 and 0.13 ng g⁻¹ wet wt, respectively. The average recovery rates for monobutyltin trichloride, dibutyltin dichloride and tributyltin chloride dissolved in hexane, spiked into the muscle of cod (*Gadus morhua*) and passed through the whole analytical procedure were 85 ± 10, 106 ± 11, and 93 ± 5% (*n* = 8), respectively. The recoveries of matrix spikes were calculated based on freshly propylated mixture of external standards. All results refer to butyltin species as the ion and they were corrected for the recovery by the internal standard. Representative chromatograms of standard, procedural blank, dolphin, fish and sediment extracts are shown in Fig. 1.

It has been suggested that degradation of TBT to MBT can occur during storage of samples. Mussel tissue stored at 4 °C for over one year did not show any reduction in the total butyltin concentration, while the composition of TBT decreased with a concomitant increase in MBT.³⁴ Since the samples analysed in this study were stored at 4 °C for over 2–3 years prior to analysis, part of the TBT was assumed to have been converted to MBT during storage (Fig. 1). Therefore, we present only the results of total butyltin (BTs = MBT + DBT + TBT) concentrations. As the population of the dolphins is small, the animals were not killed deliberately to obtain fresh tissues for this monitoring.

RESULTS AND DISCUSSION

Concentrations and sources of butyltin compounds

BTs (MBT + DBT + TBT) concentrations in the liver, kidney, blubber and muscle of river dolphins were in the range 61–2000 (mean: 890), 52–1400 (800), 360–1800 (950) and 38–1300

(770) ng g^{-1} wet wt, respectively (Table 2). BTs concentration in different tissues seemed to be comparable. Earlier studies indicated that the butyltin compounds accumulate in liver with

relatively lower concentrations in blubber, kidney and muscle of marine cetaceans exposed to TBT.¹⁹ The river dolphins were exposed primarily to MBT and DBT (Fig. 1) rather than

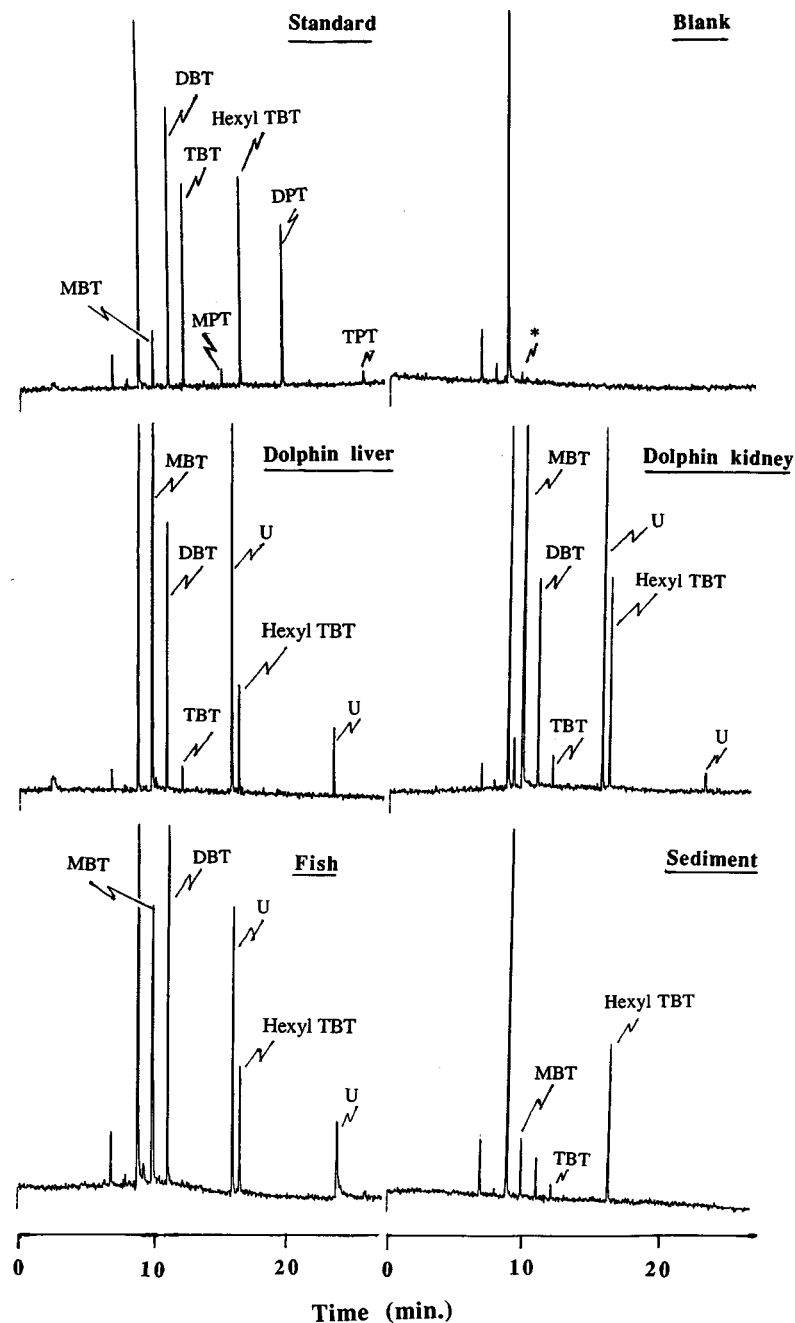


Figure 1 GC-FPD chromatograms of the propylated mixture of butyltin standard, procedural blank, river dolphin liver and kidney, fish and sediment extracts from the River Ganges. Hexyl TBT was the internal standard. *Peak appearing at the same retention time as MBT. U, unidentified organotin.

TBT, as evidenced from the analysis of sewage sludge discharged into the River Ganges. MBT and DBT might partition rapidly into several body tissues and organs because of their increased hydrophilicity. There were only small variations in BTs concentration between various size groups of dolphins (Table 2), although one adult female dolphin contained somewhat lower concentrations than the immature ones. Accumulation of butyltin compounds in river dolphin tissues was not related to lipid content (Table 2), as has been observed in fish.³⁵

Despite the small number of samples, the concentrations of butyltin compounds in river dolphins were comparable with those observed in bottlenose dolphins (*Tursiops truncatus*) collected from Italian coastal waters,²⁰ higher than those observed in harbour porpoise (*Phocoena phocoena*) from Puck Bay, Poland,³⁶ and 3–4 times lower than those of finless porpoise (*Neophocaena phocaenoides*) collected from Japanese coastal waters in 1994.¹⁹ The concentration of butyltin derivatives in the milk of the

Ganges river dolphin was low (Table 2), implying that the locational transfer of these compounds to young ones may be of little significance. However, larger numbers of samples need to be analysed.

Accumulation of butyltin residues in river dolphin tissue suggests the presence of potential sources and inputs of these compounds into the River Ganges. Tributyltin contamination from antifouling paints was not expected to make a major contribution due to the boating activities in the River Ganges. Moreover, in the freshwater environment, the fouling problem is mainly restricted to algal and periphyton attachment. Most of the boats used by local fishermen are painted with coal tar, and therefore tributyltin sources from boats may be unimportant. Other sources of tributyltin (TBT) compounds are from the use in paint manufacturing factories and as a slimicide and biocide in paper manufacture. MBT and DBT are used as stabilizers and catalysts for polyurethane foams and silicones, and TBT as a disinfectant in textiles including diaper covers and sanitary panties.³⁷ The principal commercial use of butyltin compounds is in the stabilization of PVC. About 95% of the worldwide organotin production is used to produce diorganotin antioxidants used in plastics manufacture.²¹ India is one of the major producers of plastics with an annual consumption of PVC plastics in 1994 accounting for 470 000 tonnes. At present there are 20 registered small- and medium-scale plastic industries and some of these are located along the River Ganges. Wastewater from PVC processing industries and normal leaching and weathering of PVC pipes used for portable water and wastewater and from other plastics materials containing organotins may be the major sources of MBT and DBT into the River Ganges. Leaching of organotins from PVC pipe into the flowing water has been reported previously.^{38,39} The widespread application of butyltin compounds in household articles explains their presence in municipal and industrial wastewater.^{29–31,37}

Concentrations of MBT, DBT and TBT in sewage sludge collected from local discharge points were 170 ± 40 , 140 ± 31 and 28 ± 7 ng g⁻¹ dry wt ($n = 3$), respectively. This suggests that the discharge of municipal sewage has been a significant source of butyltin derivatives in the riverine environment. The River Ganges, along its 2525 km course, receives sewage discharges from more than 50 cities and about 48 towns

Table 2. Concentrations of butyltins (BTs = MBT + DBT + TBT) (ng g⁻¹ wet wt) in various tissues and organs of Ganges river dolphin

Sample ^a	Tissue	Fat (%)	BTs
MC1	Liver	4.9	2000
	Kidney	1.2	1200
	Blubber	34	1100
	Muscle	10	130
MC2	Liver	7.6	1800
	Kidney	5.6	1400
	Blubber	10	1800
	Muscle	5.0	1300
FC1	Liver	8.3	820
	Kidney	8.9	1100
	Blubber	44	1200
	Muscle	5.5	1100
	Milk	13	4.6
IM1	Liver	6.9	380
	Kidney	1.3	580
	Blubber	31	520
	Muscle	19	1200
IF1	Liver	6.0	250
	Kidney	2.7	500
	Blubber	41	700
	Muscle	16	860
AF1	Liver	4.3	61
	Kidney	1.7	52
	Blubber	74	360
	Muscle	1.4	38

^aRefer to Table 1 for details.

(having a population greater than 50 000). The total urban sewage discharged in the River Ganges in India in 1985 was 450 mgd (million gallons per day), of which 200 mgd enters upstream of Patna. Industrial effluents contribute only a smaller fraction (10–20%) of the wastewater discharged into the River Ganges.⁶

Municipal and industrial wastewater has an increased proportion of MBT and DBT relative to TBT.²⁹ The River Ganges samples, including sediments, contained an increased ratio of MBT and DBT to TBT (Fig. 1). This pattern is consistent with those observed in municipal sewage sludge despite the possibility of the alteration in butyltin species composition during storage.

Butyltin compounds in benthos and biomagnification

To examine the biomagnification of butyltin compounds in Ganges river dolphins, we estimated the concentrations in fish, gastropods, molluscs, polychaetes and sediment (Table 3). In general, total butyltin concentrations in fish and other benthic organisms were higher than in sediments. Similarly, the concentrations in fish were up to an order of magnitude lower than those in dolphin tissues, indicating the bioaccumulation of butyltin compounds in a sediment–benthos–fish–dolphin food chain. Since higher trophic aquatic organisms such as dolphins accumulate persistent contaminants via food, we determined the biomagnification factor (BMF: concentration in dolphins/concentration in food) for BTs in river dolphins. For comparison, we estimated BMFs for persistent

organochlorines in the same river dolphin samples based on the average concentration values reported in our previous study.⁸ The ranges of BMFs for PCBs, DDTs, chlordanes, HCHs and BTs were 20–31, 29–81, 0.3–2.5, 2.5–7.9 and 0.2–7.5, respectively (Table 4). Generally, the BMFs for BTs in river dolphins were in the ranges observed for the relatively more polar organochlorine insecticides such as HCH and chlordanes. The log K_{ow} (K_{ow} : octanol–water partition coefficient, which is a measure of lipophilicity or hydrophobicity) for TBT has a similar range of values to those of HCHs and chlordanes (Table 4). The mean BMF of BTs of 3.1 (0.2–7.5) in river dolphins, estimated on the basis of average concentrations in various tissues, was higher than in red sea bream (*Pagrus major*) with (0.38)⁴⁴ and finless porpoise (1.8).¹⁹ Whole-body analysis of dolphins might lower the average concentrations of butyltins, which would further reduce the biomagnification factor. Similar conclusions, on a lower biomagnification potential of butyltins, were reached for the phytoplankton–*Mytilus edulis* and the *M. edulis*–*Nucella lapillus* food chains.⁴⁵ Despite high accumulation of butyltins in river dolphins due to the increased exposure by feeding a contaminated diet, these compounds are probably metabolized and excreted rapidly.

The concentrations of butyltins in River Ganges fish and dolphins were higher than those of several persistent organochlorine contaminants except DDT.^{7,8} This implies that the butyltin contamination due to sewage disposal in the River Ganges may have a potential impact on lower trophic aquatic organisms. In freshwater ecosystems, much less is known about highly susceptible organisms, but they also probably exist. Toxicological studies and the evidence from field data have shown that shellfish, particularly bivalve molluscs and gastropods, are vulnerable to the toxic effects of tri-butyltin (TBT) compounds.^{9–11} As to tin compounds other than TBT, predictions of the effects of long-term exposure on aquatic life cannot be made at present because of the scarcity of data. It is noteworthy that MBT and DBT are prevalent in the River Ganges and that they are released from a common source such as municipal sewage. Therefore studies on the effects of butyltins from sewage on benthic organisms in the River Ganges deserve consideration.

This study indicates that butyltin contamination can be widespread in riverine systems in

Table 3. Concentrations of butyltins (BTs = MBT + DBT + TBT) (ng g⁻¹ wet wt) residues in fish, invertebrates and sediment from the River Ganges

Sample ^a	Fat (%)	BTs
Fish (pooled)	4.4	400
Fish: <i>Mastacembellus pancalus</i>	3.4	100
<i>Gomphus</i> sp.	4.9	290
Gastropods	2.5	280
Bivalve molluscs	1.5	350
Polychaetes	3.9	250
Sediment ^b	—	35
Sewage sludge ^b	—	340

^aRefer to text for details.

^bConcentrations are expressed on a dry weight basis.

Table 4. Ranges of biomagnification factors (BMFs) for butyltins and organochlorines in Ganges river dolphins and the log K_{ow} values for chemicals

Compound	BMF	log K_{ow}
BTs	0.2–7.5 ^a	3.7–3.9 ^b
HCHs	2.5–7.9	3.7–3.8 ^c
Chlordanes	0.3–2.5	2.8–6.0 ^d
DDTs	29–81	5.9–7.0 ^c
PCBs (hexa- and hepta-chlorobiphenyls)	20–31	6.7–7.3 ^e

^aRefers to total butyltin (MBT+DBT+TBT). ^bRefers to TBT from Ref. 40. ^cRef. 41. ^dRef. 42. ^eRef. 43.

developing countries. It appears that the use of articles or processes containing MBT and DBT releases substantial amounts of these contaminants into the environment. Further studies are needed to assess the origin, pathways and the amount of input of these compounds into the River Ganges. Since tributyltin (TBT) compounds exert immune toxic effects in fish,^{16,17} accumulation of high concentrations of these contaminants in river dolphins might have serious implications for their survival. Studies have documented that resistance to bacterial infection decreased in fish exposed to butyltin compounds, even at the concentration with the lowest effect.⁴⁶ A high rate of nematode infection in river dolphins may be one of the manifestations of pollution.⁴⁷ Gastropods and bivalve molluscs in the Ganges river ecosystem should be examined for 'imposex' (development of male sex characteristics in females, caused by the presence of high TBT levels) and growth abnormalities.

Attempts to determine phenyltin compounds in the Ganges river biota indicated that their levels were low ($<50 \text{ ng g}^{-1}$ wet wt). It is noteworthy that several unidentified organotin compounds were also found in the tin-specific GC–FPD chromatograms of the Ganges river samples (Fig. 1). These peaks were not noticed in reagent blanks. The use of a wide variety of organotin compounds in commercial products as well as in agriculture⁴⁸ might possibly contaminate the riverine systems receiving domestic wastewater and agricultural run-off. Further studies are needed to characterize the accumulation potential and toxic effects of these compounds.

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