Malformations of the endangered Chinese sturgeon, *Acipenser sinensis*, and its causal agent

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The anadromous Chinese sturgeon (*Acipenser sinensis*) is endangered and listed among the first class of protected animals in China. The possible causes for the decline of this species are the effects of synthetic chemicals, and loss of critical habitat. Chinese sturgeon in the Yangtze River have accumulated triphenyltin (TPT) to 31–128 ng/g wet weigh (ww) in liver, which is greater than the concentrations of tributyltin (<1.0 ng/g ww). Maternal transfer of TPT has resulted in concentrations of 25.5 ± 13.0 ng/g ww in eggs of wild Chinese sturgeon, which poses a significant risk to the larvae naturally fertilized or hatched in the Yangtze River. The incidence of deformities in fry was 7.5%, with 1.2% of individuals exhibiting ocular abnormal development, and 6.3% exhibited skeletal/morphological deformations. The incidences of both ocular and skeletal/morphological deformations were directly proportional to the TPT concentration in the eggs of both the Chinese sturgeon and the Siberian sturgeon (*Acipenser baerii*) in controlled laboratory studies. The rates of deformities in the controlled studies were consistent with the rates caused at the similar concentrations in eggs collected from the field. Thus, TPT is the causal agent to induce the malformation of larvae of Chinese sturgeon. The incidence of deformed larvae of Chinese sturgeon is an indicator of overall population-level effects of TPT on Chinese sturgeon, because TPT at environmentally relevant concentrations can result in significantly decrease both quality and quantity of eggs and spawning frequency of fish.

teratogenesis | fish | triphenyltin | Yangtze River

Human activities have contributed to extinctions of species, and can be a contributing factor to decreases in populations. In particular, some pesticides can adversely affect endangered species (1–3). Sturgeons belong to one of the most ancient groups of the Osteichthyes. Because of their desirability as food, their long-life, and changes in their habitats, populations of sturgeon have declined globally. All extant sturgeon species are listed as "protected" under the Convention on the International Trade of Endangered Species. Among the 25 extant sturgeon species, the Chinese sturgeon (*Acipenser sinensis*) is an anadromous fish that has survived at the edge of extinction, and is listed among the first class of protected animals in China (4).

The Chinese sturgeon inhabits the East China and Yellow Seas, and spawns in the Yangtze River. Loss of critical spawning habitat because of construction of the Three-Gorges Dam and Gezhouba Dam on the Yangtze River is thought to have contributed to a steep population decline (4, 5). To save this endangered species, in the 1980s, the Chinese government began an artificial propagation program. However, this program has not resulted in the recovery of the Chinese sturgeon population. Also, the female:male sex ratio has changed from 0.79 in 1981–1993 (5) to 5.9 in 2003–2004 (6), the motility of sperm has decreased (7), and intersex has been observed (5). These observations have indicated that synthetic chemicals may be having adverse effects that could contribute to the population declines observed for this endangered species. Chinese sturgeon are exposed to relatively great concentrations of synthetic compounds, including musk fragrances and organochlorines, which possibly affect the fertilization and, therefore, affect populations (8). However, until now, there has been no direct evidence that exposure to synthetic compounds was related to adverse effects on the Chinese sturgeon population. Thus, it has been difficult to make appropriate management policies for the protection of Chinese sturgeon.

Both triphenyltin (TPT) and tributyltin (TBT) have been used extensively in paints to prevent fouling of ship hulls and fishnets. In addition to the fact that TPT concentrations measured in marine fish were unexpectedly greater than those of TBT because of the trophic magnification of TPT (9, 10), TPT continues to be used as a contact fungicide to treat crops in China. TPT acetate and TPT hydroxide are registered for use in China especially as molluscicides to eliminate the golden apple snail (*Pomacea canaliculata*) in paddy fields where it has seriously threatened aquatic crops. Based on a questionnaire among the pesticide companies that registered TPT pesticides, ≈200 tons of TPT pesticides are manufactured in China. Although all of the TPT usage in agriculture in Taiwan was completely prohibited in 1999, 27% of the surveyed farmers are still using TPT acetate illegally after the ban (11).

Ocular and morphological malformations have been observed in embryos and larvae of European minnows (*Phoxinus phoxinus*) and zebrafish (*Danio rerio*) after *in ovo* exposure of TPT (12, 13) and in the offspring of medaka (*Oryzias latipes*) maternally exposed to TBT and TPT (14, 15). Also, TBT and TPT can inhibit reproduction (14, 15). Therefore, in the present study, the following questions were addressed. (*i*) Is TPT accumulated by Chinese sturgeon and then transferred to the eggs? (*ii*) Can the malformation be observed in wild Chinese sturgeon population? (*iii*) Can the malformations observed in larvae and fry of wild Chinese sturgeon be caused by TPT under controlled laboratory conditions? Nanoinjection techniques were used to accurately determine the effects of known concentrations including environmentally relevant concentrations of TPT on both Chinese sturgeon and Siberian sturgeon eggs.

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Table 1. Concentrations of BTs and PTs in different tissues (ng/g ww) of the Chinese sturgeon

Tissue	Lipid, %	Value	MBT	DBT	TBT	BTs	MPT	DPT	TPT	PTs
Liver, *n* = 8	12.2 ± 7.4	Min	9.1	11.8	<1.0	20.9	<2.0	5.8	30.8	37.6
		Max	1,115	257	<1.0	1,373	104	324	128	468
		Mean ± SD	293 ± 366	72.1 ± 81.0	—	365 ± 447	33.3 ± 30.9	66.3 ± 105	68.0 ± 31.2	168 ± 134
Heart, *n* = 7	4.2 ± 1.7	Min	5.2	7.6	<1.0	14.6	<2.0	1.9	28.3	31.2
		Max	12.5	15.9	3.8	28.6	4.4	6.4	73.5	79.6
		Mean ± SD	9.3 ± 3.0	10.8 ± 2.9	1.4 ± 1.5	21.4 ± 5.7	1.5 ± 1.3	3.6 ± 1.7	53.0 ± 15.8	58.1 ± 16.9
Muscle, *n* = 8	1.9 ± 1.2	Min	1.5	2.2	<1.0	4.1	<2.0	0.7	17.7	18.4
		Max	8.3	7.5	4.3	20.2	<2.0	3.3	56.7	58.8
		Mean ± SD	3.7 ± 2.3	4.6 ± 1.7	1.3 ± 1.4	9.6 ± 4.8	—	1.8 ± 0.8	38.2 ± 14.9	40.0 ± 15.4
Gill, *n* = 6	2.4 ± 0.6	Min	12.3	3.1	<1.0	18.0	<2.0	<1.0	7.6	8.1
		Max	23.8	14.6	<1.0	37.7	<2.0	8.2	42.6	45.4
		Mean ± SD	18.2 ± 4.7	7.7 ± 3.9	—	25.9 ± 7.5	—	2.4 ± 3.2	25.5 ± 13.0	27.9 ± 14.3
Roe, *n* = 15	33.7 ± 9.8	Min	<1.5	2.2	<1.0	3.4	<2.0	<1.0	7.8	9.1
		Max	10.8	10.8	<1.0	15.8	<2.0	2.4	53.5	55.6
		Mean ± SD	5.9 ± 3.7	4.0 ± 2.0	—	9.9 ± 4.0	—	1.3 ± 0.8	25.6 ± 13.0	26.9 ± 13.4
Gonad, *n* = 6	3.6 ± 1.7	Min	3.4	4.0	<1.0	8.2	<2.0	<1.0	7.1	7.9
		Max	20.0	14.0	<1.0	34.0	<2.0	2.4	32.6	35.0
		Mean ± SD	8.8 ± 6.7	8.9 ± 4.2	—	17.7 ± 10.5	—	1.0 ± 0.7	16.6 ± 9.3	17.6 ± 9.8
Adipose, *n* = 5	66 ± 18	Min	<1.5	<1.0	<1.0	<3.5	<2.0	<1.0	<1.0	<4.0
		Max	3.6	5.3	<1.0	6.2	<2.0	2.5	37.3	39.9
		Mean ± SD	1.3 ± 1.3	1.9 ± 2.1	—	3.2 ± 2.7	—	0.9 ± 0.9	15.8 ± 17.7	16.7 ± 18.3
Intestine, *n* = 7	2.8 ± 1.6	Min	<1.5	2.8	<1.0	3.5	<2.0	<1.0	6.1	6.6
		Max	20.1	9.7	<1.0	26.7	<2.0	1.5	16.3	17.6
		Mean ± SD	12.4 ± 6.1	6.3 ± 2.3	—	18.7 ± 8.0	—	0.6 ± 0.4	11.5 ± 4.4	12.1 ± 4.5
Stomach, *n* = 5	1.3 ± 0.4	Min	<1.5	4.3	<1.0	6.1	<2.0	<1.0	5.5	5.5
		Max	4.4	8.5	<1.0	11.3	<2.0	<1.0	15.1	15.1
		Mean ± SD	2.3 ± 1.3	6.5 ± 1.8	—	8.9 ± 2.4	—	—	10.1 ± 4.0	10.1 ± 4.0
Pancreas, *n* = 2	6.8	Min	2.6	6.4	<1.0	9.0	<2.0	<1.0	22.3	23.1
		Max	10.2	8.3	<1.0	18.5	<2.0	5.1	22.6	27.4
Kidney, *n* = 1	31.5	—	33.5	36.9	4.0	74.4	12.5	40.0	70.0	122
Gallbladder, *n* = 1	23.0	—	9.0	5.7	<1.0	14.7	4.4	7.1	11.0	22.4
Spleen, *n* = 1	ND	—	11.2	13.6	<1.0	24.8	3.4	2.1	40.4	46.0

ND, not determined.

Results and Discussion

Concentrations of Organotins in Chinese Sturgeon Tissues. Concentrations of both TPT and TBT and their metabolites were measured in tissues of wild Chinese sturgeon (Table 1). TBT was detected in 2 heart samples [*n* = 7, < limit of quantification (LOQ)-3.8 ng/g wet weight (ww)], 3 muscle samples (*n* = 8, <LOQ-4.3 ng/g ww), and 1 kidney sample (4.0 ng/g ww), whereas dibutyltin (DBT) and monobutyltin (MBT) were detected in all tissues, but were more prevalent in liver (*n* = 8, 11.8–257 ng of DBT/g ww, 9.1–1115 ng of MBT/g ww) and kidney (*n* = 1, 36.9 ng of DBT/g ww, 33.5 ng of MBT/g ww). Although TPT was found in all of the tissues except for 2 adipose samples (*n* = 5, <LOQ-37.3 ng/g ww), diphenyltin (DPT) and monophenyltin (MPT) occurred with the greatest prevalence and concentrations in liver (5.8–324 ng of DPT/g ww, <LOQ-104 ng of MPT/g ww), kidney (*n* = 1, 40.0 ng of DPT/g ww, 12.5 ng of MPT/g ww), and gallbladder (*n* = 1, 7.1 ng of DPT/g ww, 4.4 ng of MPT/g ww). The greatest concentration of TPT (*n* = 8, 30.8–128 ng/g ww) was found in liver. This concentration was greater than those in fishes from Inner Danish waters (16), and comparable with those in marine fishes from the Japanese market (17), but less than those from the Mediterranean (10), Taiwan (15), Netherlands (18), and Japan (19). Although concentrations of total BTs in liver (365 ± 447 ng/g ww) were greater than those of PTs (168 ± 134 ng/g ww), concentrations of TPT (68.0 ± 31.2 ng/g ww) were greater than those of TBT (<1.0 ng/g ww). This observation suggests different toxico-kinetic behaviors of TPT and TBT in Chinese sturgeon. Because the Chinese sturgeon is an anadromous fish and lives most of its life in the deep ocean, where concentrations of organotins are less than in coastal areas, these results suggest that Chinese sturgeon have a greater capacity to accumulate TPT relative to TBT than do other fishes.

The distributions of BTs and PTs among tissues are described in *SI Materials and Methods*. Organotins did not accumulate in sturgeon in proportion to lipid content as do many neutral organochlorine compounds. This phenomenon may be because of the close affinity of trialkyltin compounds with some amino acids, peptides, and proteins (20). As shown by the tissue distributions of BTs and PTs (Fig. S1), TPT was transferred from female Chinese sturgeon to their eggs, in which concentration ranged from 7.8 to 53.5 ng of TPT/g ww and there was an age-related accumulation (Fig. 1), whereas TBT was not detected in eggs. Maternal transfer was described by the ratio of the concentration of TPT in eggs to that in the liver of females (21). The value for this ratio was 0.34 ± 0.11, which was comparable with those of organochlorines such as hexachlorobenzene (HCB; 0.61) and total 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDTs including *o,p*′-DDD, *p,p*′-DDD, *o,p*′-DDT, *p,p*′-DDT, *o,p*′-DDE, and *p,p*′-DDE, 0.27) in the same Chinese sturgeon (8).

Malformation of Wild Chinese Sturgeon Larvae. To determine whether malformations in Chinese sturgeon occurred in the Yangtze River, ≈2- to 3-day-old larvae of Chinese sturgeon were captured on 26, 27, 28, 30 November, and 1 December 2007 from the spawning area below Gezhouba Dam, which is located 38 km downstream from the Three-Gorges Dam in Yangtze River. The larvae or embryos of Chinese sturgeon were cultured in con-

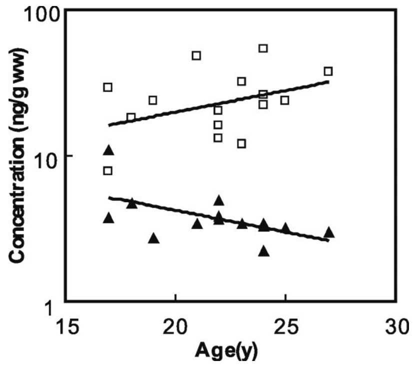

Fig. 1. Relationships between age of adult female Chinese sturgeon and concentration (ng/g ww) of TPT (□) and DBT (▲) in their eggs. Chinese sturgeon were captured from the spawning location,Yichang of Yangtze River, and died during the artificial propagation in each year between 2003 and 2006. TPT: log [TPT] = 0.0294 × age + 0.7127, r^2 = 0.1496, *P* = 0.154. DBT: log [DBT] = −0.0295 × age + 1.2142, r^2 = 0.3250, *P* = 0.026.

trolled facilities at the Chinese Sturgeon Hatchery, Jingzhou, Hubei, until ≈18 d posthatch, and then were inspected for deformities. The incidence of skeletal/morphological deformations was 6.3% (65/1039), and 1.2% (12/1039) had no eyes or only 1 eye. At the same time, 2 adult female and 2 adult male Chinese sturgeon were captured from the Yangtze River for artificial propagation. Spawning was induced, and mature eggs were artificially fertilized. A subsample of eggs was retained for subsequent quantification of organotins. Of the larvae artificially propagated from the 2 wild Chinese sturgeon, 3.9% (40/1075) of juveniles 18 d posthatch exhibited skeletal/morphological deformations, whereas 1.7% (18/1075) had only 1 eye or no eyes (Fig. 2 *A* and *B*). Alternatively, TPT concentrations in the eggs of the 2 wild Chinese sturgeon were 20.0 and 23.7 ng of TPT/g ww, whereas the concentrations of TBT were both under detection limit (<1.0 ng/g ww). A single metabolite of TBT, MBT, was detected at concentrations of 3.0 and 4.0 ng of MBT/g ww.

***In Ovo* Exposure of Sturgeon by Nanoinjection.** Eighty Chinese sturgeon eggs for each exposure group were exposed to TPT *in ovo* via nanoinjection. The background concentration of TPT in Chinese sturgeon eggs was 19.3 ng of TPT/g ww, and for other organotins, were 8.7 ng of MBT/g ww, 1.6 ng of DBT/g ww, <1.0 ng of TBT/g ww, <2.0 ng of MPT/g ww, and 1.1 ng of DPT/g ww, respectively. Injection of the eggs with control (triolein), 30 or 150 ng/g ww TPT during the period just after fertilization until mid to lategastrula formation resulted in 4.0% (2 of 50), 11.1% (5 of 45), and 22.6% (7 of 31) skeletal/morphological deformations, and 0% (0 of 50), 4.4% (2 of 45), and 9.7% (3 of 31) ocular deformations, respectively. Skeletal/morphological deformation were significantly related to concentration of TPT (χ^2 = 4.244, 1° of freedom, *P* = 0.039). Even thought a dose-dependant response was also observed for ocular deformation, because of the limited sample size, no statistically significant (χ^2 = 1. 917, 1° of freedom, *P* = 0.166) relationship with TPT concentration was observed.

Because the Chinese sturgeon is endangered, it was possible to obtain only a few eggs from only few adults. Therefore, a hybrid study was conducted, in which larger numbers of eggs of Siberian sturgeon (*Acipenser baerii*) were used as a surrogate species to obtain more robust statistics by nanoinjection studies of all of the organontim compounds. Siberian sturgeon eggs were propagated and exposed to TPT *in ovo* via nanoinjection at concentrations of 0 (control, triolein), 27, 136, or 681 ng of TPT/g ww. The background concentration of TPT in Siberian

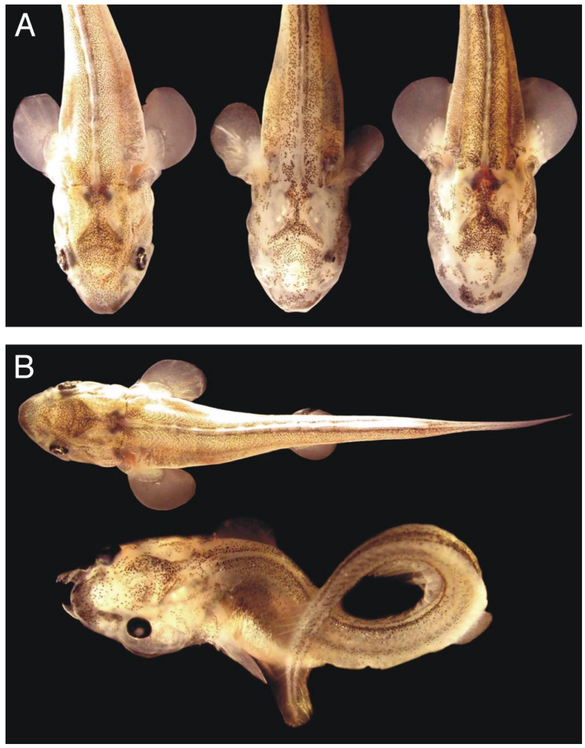

Fig. 2. Malformations of 18 d posthatch wild Chinese sturgeon (*A. sinensis*) larvae. (*A*) Abnormal ocular development (left to right, normal larva, single eye larva, and no eye larva). (*B*) Skeletal/morphological deformation (*Upper*, normal larva; *Lower*, curved larva).

sturgeon eggs was <1.0 ng/g ww. Both ocular and morphological malformations were observed (Fig. 3*A* and *B*), and their frequencies were directly proportional to exposure concentration (Table 2). At concentrations that were similar to those observed in wild Chinese sturgeon from the Yangtze River (27 ng of TPT/g ww), malformation incidences for ocular and morphological deformation were 2.7 (17/625) and 4.2% (26/625), respectively. The association between malformation incidences and exposure TPT concentration was statistically significant (for morphological malformation: χ^2 = 15.85, 1° of freedom, *P* = 0.000; for abnormal ocular malformation: χ^2 = 16.85, 1° of freedom, *P* = 0.0).

To further clarify the potential for organotin compounds to cause malformations, *in ovo* exposure to other organotins (MBT, DBT, and DPT) detected in the eggs of Chinese sturgeon were investigated individually, by injecting them into Siberian sturgeon eggs at concentrations of 0.0 (control; triolein), 30, 150, or 750 ng/g ww. In all of the *in ovo* exposure groups, no ocular deformation was observed, and the rates of skeletal/morphological deformation for MBT and DBT were 1.17%–1.72%, but no dose-dependant response was observed for both organotins. The rate of skeletal/morphological deformation for DPT was 1.03%–2.03%, and dose-dependant response was observed, but no statistically relationship was obtained (Table S1). The skeletal/morphological deformation rates in blank and injection control groups were 0.70 and 0.92%, respectively. This result indicates organotin compounds other than TPT that were detected in eggs of Chinese sturgeon would not cause the observed malformations of wild Chinese sturgeon.

TPT has been observed to inhibit osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway (22). Because TPT can bind to the retinoid X receptor (RXR) with even greater affinity than the endogenous ligand, 9-*cis* retinoic acid (23), and RXRα null mice exhibited an ocular

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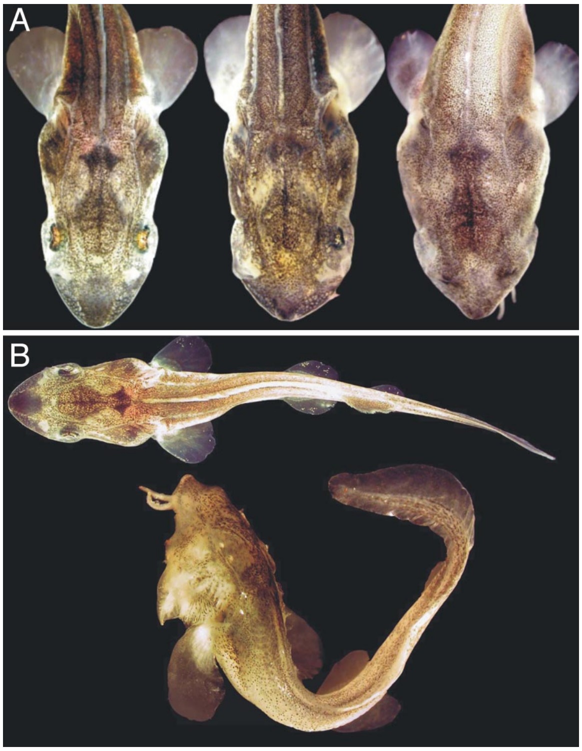

Fig. 3. Malformations of Siberian sturgeon (*A. baerii*) larvae exposed to TPT via nanoinjection of eggs. (*A*) Abnormal ocular development (left to right, normal larva from control, single eye larva, and no eye larva). (*B*) Skeletal/morphological deformation (*Upper*, normal larva from control; *Lower*, curved larva with no eyes).

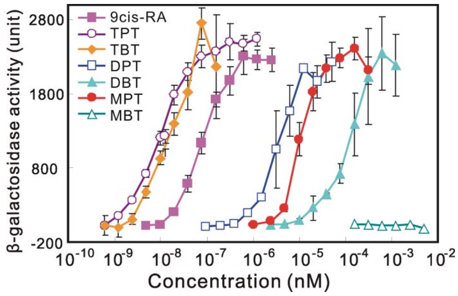

Fig. 4. Dose-response curves of triphenyltin chloride (TPT), diphenyltin dichloride (DPT), monophenyltin trichloride (MPT), tributyltin chloride (TBT), dibutyltin dichloride (DBT), and monobutyltin trichloride (MBT). A natural RXR ligand, 9-*cis*-retinoic acid (RA), was used as positive control.

abnormality (24), it could modulate this receptor in a way that could lead to the observed deformities. Therefore, in addition to the *in ovo* studies, the relative potencies of the 6 organotins to interact with the RXR were determined by use of a 2-hybrid yeast assay system with RXRα. The relative potencies of DPT, MPT, TBT, DBT, and MBT relative to TPT were estimated to be 2.8×10^{-3}, 9.4×10^{-4}, 0.48, 8×10^{-5}, and $<2 \times 10^{-6}$, respectively (Fig. 4; Table S2). This result suggests that none of the organotins except TPT were sufficiently potent to cause deformities at the concentrations that were detected in the eggs of Chinese sturgeon.

Together, these multiple lines of evidence were consistent with the hypothesis that TPT was the likely cause of the malformations observed in larvae of wild Chinese sturgeon, although other contaminants may be present that could produce similar effects. Polychlorinated biphenyls (PCBs) are widespread in the environment, and a relatively great concentration of PCBs (5810 ng/g ww) has been detected in the eggs of Shovelnose sturgeon from the Mississippi River (25). In Chinese sturgeon eggs, the mean total concentration of PCBs was 95.1 ng/g ww (16.8–229 ng/g ww) (Fig. S2 and Table S3). When eggs of Siberian Sturgeon were injected with 100 or 300 ng/g ww Aroclor 1254 (a commercially manufactured PCB product), 1.13 (9 of 798 larvae) and 1.33% (7 of 526 larvae) had skeletal/morphological deformities, respectively, and no ocular deformations were observed (Table S1).

In this study, we observed malformations in wild Chinese sturgeon, and then correlated the response with the putative causative agent, TPT. We isolated the putative causative agent and introduced it into eggs of both Chinese and Siberian sturgeon, and were able to reproduce the same deformities at the similar proportions for similar doses in the laboratory; these rates were similar to those observed in embryos spawned in the wild. Thus, we have completed Kock's postulates, and concluded that TPT was the most likely cause of the observed deformities. The deformities observed in Chinese sturgeon in the Yangtze River are a measurable indicator of the adverse effects of TPT, but the rates of deformities observed in the larvae of Chinese sturgeon alone would be unlikely to have severe adverse effects on the population. In our study, it was observed that exposure of medaka to TPT at environmentally relevant concentrations could also inhibit reproduction (15). In particular, the quality and quantity of eggs and spawning frequency were significantly decreased (Table S4), because TPT inhibited the induction of vitellogenin, which is essential material for vitellogenesis, oocyte maturation, and yolk biosynthesis in fish (15). As an overall result, exposure to TPT at environmentally relevant concentrations can reduce the capacity to produce offsprings. The signal inducing deformations by TPT exposure are much weaker than that on decrease of capacity to produce viable offspring. When rates of ocular and skeletal/morphological deformities reached 1.2 and 6.3, the capacities to produce viable offspring would be reduced by 58.4% and 75.9%, respectively. Even though the effects of TPT on the capacity to produce viable offspring are unknowable under field conditions, because the same types of

Table 2. Malformations of Siberian sturgeon (*A. baerii*) larvae developed from eggs exposed via nanoinjection to TPT

	No.					Frequency, %	
Exposure, ng TPT/g ww	Eggs injected	Hatched larvae	Survived larvae*	Abnormal skeletal larvae	Abnormal ocular larvae	Abnormal skeletal larvae	Abnormal ocular larvae
Blank	5,287	3,431	3,226	19	0	0.59	0
Control	1,048	660	606	4	0	0.66	0
27	1,090	717	625	26	17	4.16	2.72
136	1,190	701	600	37	26	6.17	4.33
681	1,044	450	316	42	24	13.3	7.59

*Eighteen days posthatch larvae.

deformities were observed in both Chinese sturgeon and medaka exposed to the same concentrations of TPT, suggesting that the concentrations of TPT in Chinese sturgeon would likely contribute to reduced overall fecundity and, thus, the declined fitness of the Chinese sturgeon population in the Yangtze River.

Materials and Methods

Eggs of Chinese Sturgeon for Artificial Fertilization. Two female and 2 male Chinese sturgeon were captured from the Yichang region of the Yangtze River in 2007. The female individuals were both 24 years old with body weights (BWs) of 228 and 242 kg, and body lengths 336 and 332 cm, respectively. The 2 male individuals were 63.5 and 103.5 kg, and 210 and 260 cm, respectively. After injecting luteinizing hormone releasing hormone A2 (LHR-A2) (Ningbo second hormone factory) of 9 μg/kg BW for female, and 4.5 μg/kg BW for male, fish spawned in the following 16 h at 18 °C. Egg and sperm were collected, and the eggs were artificially fertilized by the female with BW 228 kg of to the male with BW 63.5 kg, and the 242.2-kg female was spawned with the 103.5-kg male. The fertilized eggs were cultured in active carbon-treated water until ≈18 d old, and then were inspected for malformation. Also, 2 samples of wild Chinese sturgeon eggs were collected before propagation for analyzing to determine concentrations of TPT. Concentrations of TPT in eggs of each fish were determined in triplicate and expressed as the mean ± SD ($n = 3$).

Sample Collection. The Chinese sturgeon is a typical anadromous fish that lives in the sea and returns to spawn in rivers, primarily the Yangtze River. As a valuable, ancient fish species, the Chinese sturgeon is protected by the Chinese government (4), and the capture for these individuals was done under a permit that authorized collection strictly for scientific purposes. During the 1980s, the population of Chinese sturgeon declined rapidly (5). Since then, artificial propagation has begun to save this endangered species, and 8 to 10 Chinese sturgeon were allowed to be captured for this study. After propagation, surviving sturgeon are released back into the Yangtze River. However, some do not survive. In this study, the eggs and other tissues were collected for quantification of TPT before propagation, and the other organs and tissues came from 17 sturgeon that had died during the artificial propagation between 2003 and 2006. After collection, samples of tissues were frozen immediately at −20 °C and kept at that temperature until analysis. The ages of fish were determined by counting growth layers in the cleithrum, as described (5, 6). The details of the samples analyzed in this study are shown in Table S5.

Nanoinjection. Because there were few eggs of Chinese sturgeon available to conduct statistically robust studies, under controlled laboratory conditions where rates of deformities could be accurately measured, Siberian sturgeon eggs propagated from adults in artificial culture were exposed to TPT and other organotins *in ovo* via nanoinjection. Eggs of Siberian sturgeon were obtained from the Yangtze River Fisheries Research Institute. Detailed information on the procedure for nanoinjection is given in a previous article (26). Briefly, aluminosilicate capillary tubes (1.0-mm outer diameter and 0.58-internal diameter; Sutter Instrument) were used to make injection needles with 5- to 10-μm internal-diameter tips. Approximately 7 nL (<0.1% of egg volume; egg weight, 16 mg ± 0.2 mg/egg) of trioline (control) or TPT stock solution was injected directly into the fertilized egg within 8 h using a picoinjector (PLI-90; Harvard Apparatus) and Stereomicroscope (Zeiss Stemi 2000; Diagnostic Instruments). Exposure concentrations of TPT were 0 (control, trioline), 27, 136, or 681 ng/g ww. After injection, the eggs were incubated in flow-through containers (30 × 30 × 16 cm), suspended in stainless steel barrels (120-cm i.d. × 80-cm height), supplied with activated-carbon treated water renewed daily at 16 °C–18 °C, and dead embryos were removed daily and hatched larvae number were recorded. Finally, ≈18 d posthatch, larvae were inspected for malformation. No obvious effect was observed in mortality and development in control (trioline) compared with blank (uninjected groups).

The methods used for *in ovo* exposure of Siberian sturgeon to other organotins and PCBs were the same as described above. Injected concentrations of DBT, MBT, DPT were 0 (control, trioline), 30, 150, or 750 ng/g ww, and those of PCBs (as Aroclor 1254, a commercial PCBs product) were 100 and 300 ng/g ww. Details of the methods used for nanoinjection of Chinese sturgeon eggs are provided in *SI Materials and Methods*.

Quantification of TPT and Its Related Chemicals. The method used to quantify the 6 organotins was based on a previous article (9) with some modifications, and a detailed method description is provided in *SI Materials and Methods*. The fortification experiments were conducted by adding a comparable amount of the 6 deuterium-labeled surrogate analogues with the typical concentrations in Chinese sturgeon samples, which ranged from 10 to 50 ng/g ww. Recoveries of the 6 deuterium-labeled surrogates were calculated by response relative to that of the internal standard TeBT-d_{36}. Recoveries were 56 ± 7% for MBT-d_9, 113 ± 3% for DBT-d_{18}, 115 ± 5% for TBT-d_{27}, 112 ± 4% for DPT-d_{10}, and 109 ± 3% for TPT-d_{15}, but the recovery of MPT-d_5 was limited to 47% ($n = 6$). Limits of quantification for MBT, DBT, TBT, MPT, DPT, and TPT at $S/n = 3$ were 1.5, 1.0, 1.0, 2.0, 1.0, and 1.0 ng/g ww, respectively.

Analysis for PCBs. A detailed method description is provided in *SI Materials and Methods*.

Pathological Examination and Statistics. An external inspection of larvae was observed under a microscope with an ocular micrometer each day. The malformations were sorted into skeletal/morphological deformation and ocular deformation. The skeletal/morphological deformation included shortening of the notochord, deletions of pinna, and curvature of body or tail, and the ocular deformation was those with no eyes and only 1 eye. Most of larvae with skeletal/morphological deformation lost normal swimming behavior. A χ^2 test was used to test the differences in morphological malformation or abnormal ocular malformation between the control and the TPT exposure group, and difference was considered significant if $P < 0.05$. The statistical analyses were performed with SPSS 15.0.

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