CATECHOLAMINES IN BLOOD AND BRAIN OF GRADUALLY TO PBT EXPOSED SILVERCARPS

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INTRODUCTION

Neurotransmitters such as Serotonin, L-Adrenalin, L-Noradrenalin and Dopamin belong to an important group of signal transducers. Chemically they represent the Catecholamines which are involved in signal transducing at the synapses and belong to the adregenic, dopamingenic, and serotonigenic neurons. They are inactivated by monoaminooxidases or catecholamine-o-methyltransferases.
Dopamine is formed from L-Dopa by decarboxylation by dopadecarboxylasis and is a product of the Tyrosine-catabolism. Dopamine is the educt for L-Noradrenalin and L-Adrenalin. High doses lead to elevated blood pressure and lack of neurotransmitters cause Parkinson desease in humans.L-Adrenaline and L-Nor-adrenaline are formed in the cortex and in the neurons of the adregenic nervous system
On one hand their dynamic in humans in the presence of pollutants is not throughout understood. In addition neither for mammals nor for other animals such as fish quantitative background data are assessable from literature.
On the other hand data for the quantitative description of neurotransmitter dynamics is hardly required for humans as well as for species in cultivation and natural reservates.
Thirdly the role of sustainable acting chemical stressors such as persistent bioaccumulating toxic substances (PBT) like polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) is completely unknown. Lazyness, aggression, and curiosity- which is needed for learning- is believed to be strongly related to the presence of neurotransmitters and can be possibly triggered by PBT.
Epidemiological studies recently performed in this context with children are lacking from their design and the unability to sample those compounds in blood and especially in brain at vivo[1,2].

Ya-Er Lake is located in the eastern part of Wuhan, Hubei province, China (Figure 1). It is a shallow, eutrophic lake along the middle-lower reach of Yangtze River. The water originally covered 6000 ha, but now only 2000 ha remain. The water depth of the lake is 2-3m and the pH of the water is 7.5-7.9[3].
Around the lake, there are 27,000 ha of farmland with a population of 300,000 people. From 1962-1982, the applied amount of HCH used for agriculture in this area, was up to 22.5 kg/ha. The farmers took the powder formulation of HCH, the mixture of α, β, γ and δ four isomers, to spray it into the agriculture fields. Meanwhile, the water had been continuously polluted with Hexachlorocyclohexane (HCH), chlorinated benzenes (CBs), chlorinated phenols (CPs) and its sodium salts during 1962-1987 by the direct discharge of the effluent from a large chemical factory situated on the bank of this lake. During the time from 1960 till now, the plant has produced parathion, Malathion, HCH , HCB and PCP, etc. 80,000 tons of waste water was daily discharged into the lake. In the course of over 10 years, the lake was seriously polluted and the aquatic ecosystem was badly damaged[3]. The macrophytes vanished completely and the fishery

was ruined. Now, the waste water discharged from another way, then some of biota can be seen more and more in pond 3-5, but pond 5 (see Figure 1) is much better than others.
The original lake consisted of several small sublakes, but now only three big ones of them left. The Ya-Er Lake was the first sublake affected by the wastewater. After Ya-Er Lake, the wastewater flows through a partially drained area to the Wusi Lake into the Yangtze River. A part of Ya-Er Lake was divided into a series of five ponds in 1978 as the oxidation ponds for remediation of the wastewater. Thus before 1962, the Ya-Er Lake was an oligotrophic lake. During 1962-1978, the lake was seriously polluted by the waste effluent from a large chemical plant. In 1979, a series of five ponds were built for the waste effluent treatment by self-oxidative purification.

MATERIALS AND METHODS

Sampling locations in Ya-Er Lake area

The sampling locations for the study are indicated in Figure 1.

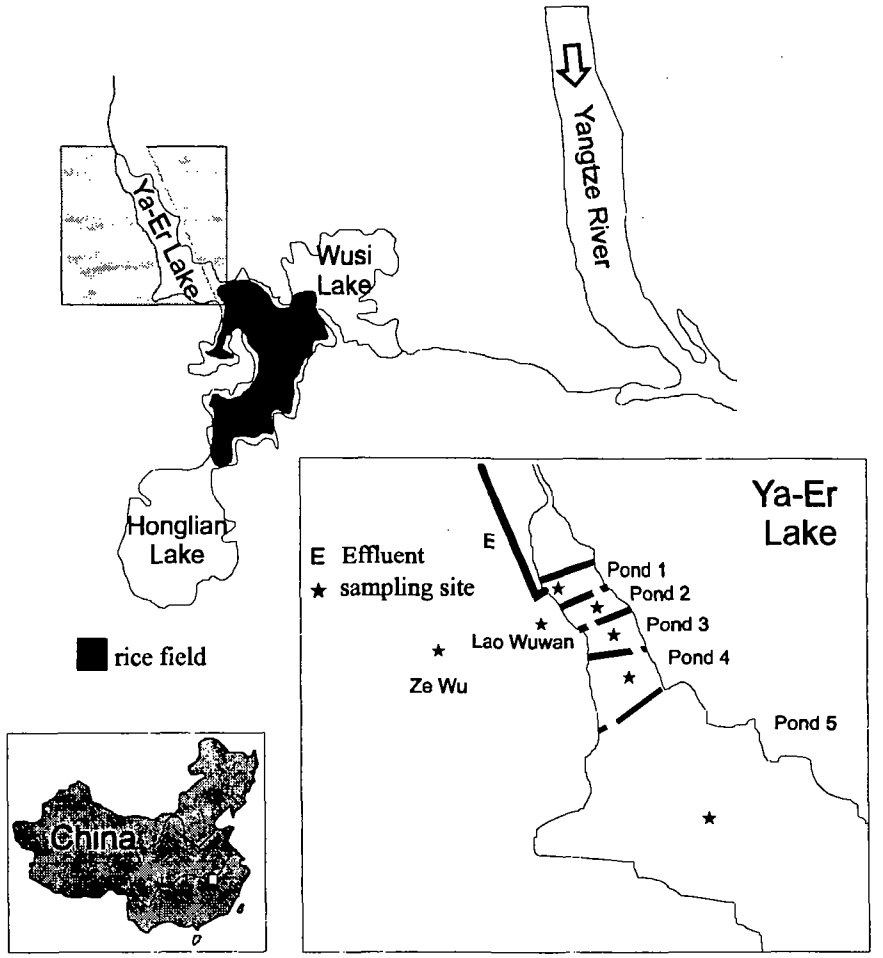

Figure 1: Ya-Er lake and sampling locations

The sediment samples were collected by a Kajak sediment sampler (made in Australia) in the usual manner at depth of 20-40cm. The soil sampler was equipped with a stainless tube at a depth of 10-30cm.
Each sample was an aliquot of the mixture from 5 locations. Afterwards, the samples were transported to the laboratory and freeze dried. The samples were examined for PCDD/F.

Sampling method for blood of fish
Silver carps were taken of five ponds of Ya-Er Lake during September 1999. The fish were collected by net just before sampling. The body of the fish was carefully opened and the heart was cut out with a pair of operation scissors. The blood was slowly pumped out with a syringe at one time. Then the blood was slowly pumped into a tube and put in ice bath. After the sampling, the samples were stored at 4 °C for a whole night. The samples were centrifuged under 4000 rpm for 5 minutes. The supernatant was collected as serum. The serum was stored at -20°C.
Extraction and analysis of catecholamines was performed following the procedure of Chromsystems[4]. Final detection and quantification has been achieved by employing High Performance Liquid Chromatography (HPLC) and Electro-Chemical-Detection (ECD). DHBA was used as internal standard.

RESULTS AND DISCUSSION

The PCDD/F pollution of Ya-Er lake sediments is shown in Table 1. The data are given depth dependent. I can be assumed that the top horizon of the sediment correspond to the presence of PCDD/F in the water. Regarding I.TEQ-values the pollution decreases from pond 1 to pond 5 by a factor of 10. The sediment values of pond 1 are extraordinary high. In addition the pattern of the PCDD/F congeners is changing from pond 1 to 2 due to a change of discharge quality. However this does not strongly affect the I-TEQ. Besides the PCDD/F the PCB, chlorinated benzenes and phenols as well as chlorinated cyclohexanes are present in the sediments and follow the same spatial trend than PCDD/F.

Table 1: The results of PCDD/F concentrations, toxic equivalent (TEQ), 2,3,7,8-substituted congeners.

	depth (cm)	ΣPCDD/F (ng/kg)	I-TEQ (ng/kg)
Pond 1	0-12	13845	420
	12-24	11664	369
	24-36	7474	297
	36-48	10212	377
	48-60	14840	370
	60-72	4754	118
Pond 2	0-12	10325	62
	12-24	4552	13
	24-36	180	1.3
	36-48	101	0.58
	48-60	38	0.18
	60-72	23	0.15
Pond 3	0-12	3918	29
	12-24	5774	24
	24-36	90	0.55
	36-48	59	0.31
	48-60	40	0.19
	60-72	45	0.21
Pond 4	0-12	6744	24
	12-24	2127	14
	24-36	67	0.32
	36-48	38	0.32
	48-60	35	0.16
	60-72	19	0.16
Pond 5	0-12	5713	10
	12-24	1760	6.7
	24-36	127	0.64
	36-48	129	0.46
	48-60	80	0.44
	60-72	82	0.25

Serotonin could not be determined with this method in all samples at a LOD of 1 microg./L for plasma and brain homogenate (Table 2)

Table 2: Levels of Dopamin, L-Adrenalin, and L-Noradrenalin in blood and brain of fish sampled in the gradually polluted Ya-Er lake.

	Noradrenalin		Adrenalin		Dopamin	
POND NO.	Blood	Brain	Blood	Brain	Blood	Brain
	Plasma	Homogenate	Plasma	Homogenate	Plasma	Homogenate
	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)
1	29	37206	<10	169	656	2831
2	<10	16208	43	62	<10	666
3	<10	14926	<10	282	<10	829
4	460	36262	66	268	<10	2752
5	1231	40420	725	454	62	5974

The concentration of neurotransmitters in blood and brain seem to correspond in some cases. Adrenalin and Noradrenalin is increasing from pond No. 2 to pond No. 5 with decreasing pollution. Although Dopamin cannot cross the blood –brain-border in humans it can be found in all brains of the silver carp.
In addition pond No. 1 is an exception. The values for brain are similar to pond No. 5 which is not polluted. However in case of blood the values of Nordrenalin and Adrenalin are following the extend of pollution.
These preliminary results indicate an relationship between PBT-exposure and concentrations of neurotransmitters in blood and brain of silver carp. Further investigations and confirmations are required.

REFERENCES

1) Weisglas-Kyperus, N.: Effects of Perinatal Environmental Exposure to Polychlorinated Biphenyls and Dioxins on Developmewnt in Dutch Children. In: Dioxins and Dioxin-like Chemicals (Eds. Schlumpf, M., Lichtensteiger, W.), Zurich, pp13-18 (2000)

2) Winneke, G.: Development Neurotoxicity of Polychlorinated Bipheyls (PCBs): Recent findings from the Düsseldorf Cohort. In: Dioxins and Dioxin-like Chemicals (Eds. Schlumpf, M., Lichtensteiger, W.), Zurich, pp19-28 (2000)

3) Schramm K-W, Henkelmann B, Kettrup A, Xu, Y, Wu W (Report), Sources, Fate, Bioaccumulation and Sinks of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in the People's Republic of China. Especially Ya-Er Lake Aerea, near Wuhan, GSF-Forschungszentrum, ISSN 0721-1694 (1999)

4) N.N.: Reagenzienkit für die HPLC-Analytik der Katecholamine im Plasma/Serum. CHROMSYSTEMS application sheet, www.chromsystems.de, (1999)