— Regular Article —

Cholinesterase-inhibiting Potentials of Amberlite XAD-2 Resin Extracts Collected from River and Drinking Waters in Northwest District of Chiba Prefecture, Japan

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Amberlite XAD-2 resin extracts of river and drinking water sampled in each month during the period from January to December 2008 from the Northwest district of Chiba Prefecture were investigated to characterize and determine their cholinesterase (ChE)-inhibiting potentials and pesticide levels. The XAD-2 extracts from river water collected during the mid-spring to mid-summer periods exhibited strong inhibition effect to horse serum ChE, reflecting the application of organophosphorus and carbamate pesticides to paddy fields. Gas chromatographic-mass spectrometric (GC/MS) determinations of the XAD-2 extracts of the river water collected during spring to summer periods also showed to be comparatively high levels of agricultural chemicals, such as herbicides, insecticides and fungicides, as compared with those detected in the drinking water. Although a considerable reduction in the ChE-inhibiting potentials and in the GC/MS detectable compound levels was observed for the river water samples, it is particularly interest that ChE-inhibiting potentials still remained in the drinking water.

Key words —— river water, drinking water, XAD-2 resin extract, ChE-inhibiting activity, pesticide concentration

INTRODUCTION

Fresh water systems can be contaminated by agrochemicals during their intentional application for crop protection and the control of biting insects, or by unintentional spillages, overspray, field drainage, or spray drift.[1)] Aquatic invertebrates that live in rivers and lakes can therefore be exposed to concentrations of insecticides ranging from sublethal to lethal. Two groups of most often applied pesticides are organophosphorus and carbamate pesticides. These pesticides are preferred in agriculture because of their relatively low persistence in the environment, but some of them exhibit fairly high acute toxicity. They both interact with group of enzymes (cholinesterase, ChE), what may cause effects (cholinergic syndrome). Organophosphorus pesticides additionally react with neuropathy target esterase causing development of a completely different syndrome known as organophosphate-induced delayed polyneuropathy.[2)] ChE activity has been measured *in vivo*, in species such as *Pomatoschistus minutus* (*P. minutus*),[3)] *Rashora caverii*,[4)] and *Cyprinus carpio*.[5)] These studies demonstrated that ChE activity in aquatic organism decreases on exposure to organophophorus and carbamate pesticides. In addition, seasonal variation of ChE activity in Sand Gobies *P. minutus* because of exposure of pesticides was observed.[3)]

In vitro ChE assays have also been developed to detect organophosphorus and carbamate pesticides and to evaluate the enzymatic toxicity of aquatic environments. Onodera *et al.*[6)] performed ChE assays for several organophosphorus and carbamate pesticides, by using horse serum, and measured the pH changes induced by the production of acetic acid from acetylcholine iodide (AChI). Henmi *et al.*[7)] and Makihata *et al.*[8)] developed an acetylcholinesterase (AChE) assay for several organophosphorus and carbamate pesticides by using human red blood cells. To detect pesticides and

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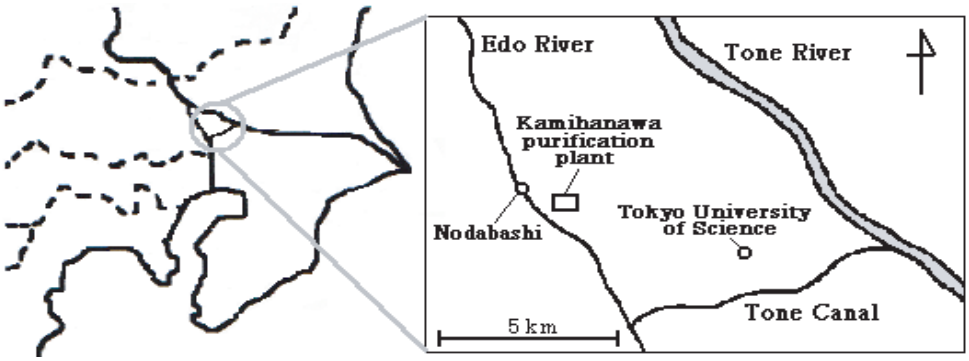

Fig. 1. Location of the Area Investigated, Sampling Sites on the Edo River, and Our University
Left, the Kanto plain; right, the Edo River, water treatment plant, and our university.

organophosphoric acid esters that also inhibit AChE assay, Kinugasa *et al*.[9] developed the AChE assay using AChE derived from the brain of the electric ell. Although the validity of those studies as tool for monitoring organophosphorus and carbamate pesticides has been investigated, few previous studies have monitored changes of ChE activity in aquatic environments by using *in-vitro* assays. Therefore, it is necessary to monitor the ChE-inhibiting activity using *in-vitro* assay and the concentrations of inhibitor determined by instrumental analysis techniques such as gas chromatography-mass spectrometry (GC/MS) for evaluation of variation, human health effects, and the eco-toxicology of aquatic environments.

Three major rivers drain into Tokyo Bay, namely the Edo, Ara, and Tama Rivers. Each has been used for agricultural and cosmetic purposes as well as a source of potable water. Currently, the Edo and Ara Rivers are major sources of water for many million people in the Tokyo Metropolitan area. The Edo River is located in East of Tokyo Metropolitan; it arises from the Tone River, the biggest river in Japan, and flows to the Tokyo Bay, covering a distance of approximately 60 km, mostly passing through urban areas and some paddy fields. Thus, domestic and agricultural wastewaters flow into this river continuously. However, large quantities of water are taken for domestic and agricultural purposes from the upper and middle regions of this river via water treatment plants in Saitama Prefecture, Chiba Prefecture, and the Tokyo Metropolitan area (Fig. 1). A number of surveys in this river have reported the presence of chemical contaminants, such as pesticides, pharmaceutical chemicals, and personal care products, by using instrumental analysis techniques for evaluating of the eco-toxicology of aquatic environments and the resultant effects on human health.[10–13] However, there is little information with regard to monitoring of the chemicals by bioassays in this river.

The present study aimed to investigate and compare the potentials for ChE-inhibiting activity by organophosphorus and carbamate pesticides in the river water and drinking water. For this purpose, the horse ChE-inhibition assay was applied. An Amberlite XAD-2 resin column was also used for fractionation of the water samples. Furthermore, the presence of organophosphorus and carbamate pesticides were determined by GC/MS in order to investigate possible relations between the ChE-inhibiting potentials in water concentrates and their concentrations in the river water and drinking water.

MATERIALS AND METHODS

Chemicals —— The organic solvents (acetone, *n*-hexane, diethyl ether and methanol) used were of analytical-reagent grade for pesticide residue analysis (Wako Pure Chemical Industries, Osaka, Japan). High-purity water obtained from a Milli-Q purifier system (Japan Millipore Inc., Tokyo, Japan) was used throughout. Agricultural chemicals such as herbicides, insecticides, and fungicides were commercially available (Table 1). Chloropyrifos-oxon, diazoxon, Sumioxon, and isoxaoxon, which are oxidation products (P = O type) from the parent organothiophosphorus pesticides (P = S type) with ozone or chlorine were also obtained from Wako Pure Chemical Industries. Standard solutions of these compounds both alone and as mixture were prepared by dissolving the compound in methanol or acetone, with subsequent serial dilutions.

Table 1. Usage, Chemical Names, GC/MS Data, and Limits of Detection (LOD) of Agrochemicals Tested in This Study

Usage	Chemical name (Abbrevation)	Chemical formula	Retention time (min)	Monitor ion	LOD (μ/l)	
					RW[a)]	DW[b)]
Herbicide	Benthiocarb	$C_{12}H_{16}ClNOS$	20.26	100, 125	0.01	0.01
	Bromobutide	$C_{15}H_{22}BrNO$	19.3	232, 119	0.03	0.01
	Butamifos	$C_{13}H_{21}N_2O_4PS$	22.18	152, 202	0.03	0.01
	Dichlobenil (DBN)	$C_7H_3Cl_2N$	12.51	171, 136	0.03	0.01
	Esprocarb	$C_{15}H_{23}NOS$	20.18	222, 162	0.01	0.01
	Metolachlor	$C_{15}H_{22}NClO_2$	21.87	238, 162	0.01	0.01
	Oxadiazon	$C_{15}H_{18}N_2Cl_2O_3$	25.11	302, 258	0.01	0.01
	Pretilachlor	$C_{17}H_{26}ClNO_2$	22.31	176, 238	0.01	0.01
	Pyributicarb	$C_{18}H_{22}N_2O_2S$	24.49	165, 108	0.02	0.01
	Simazine (CAT)	$C_7H_{12}ClN_5$	17.56	201, 186	0.03	0.01
	Simetryn	$C_8H_{15}N_5S$	19.43	213, 170	0.03	0.01
Insecticide	Fenobucarb (BPMC)	$C_{12}H_{17}NO_2$	16.23	150, 121	0.03	0.01
	Chloropyrifos	$C_9H_{11}Cl_3NO_3PS$	20.39	199, 197	0.03	0.01
	Dichlorvos (DDVP)	$C_4H_7Cl_2O_4P$	11.29	220, 185	0.01	0.01
	Diazinon	$C_{12}H_{21}N_2O_3PS$	18.38	137, 179	0.02	0.01
	Fenitrothion	$C_9H_{12}NO_3PS$	20.13	125, 277	0.03	0.02
	Isofenphos	$C_{15}H_{24}NO_4PS$	21.3	213, 121	0.04	0.02
	Isoxathion	$C_{13}H_{16}NO_4PS$	22.56	105, 208	0.03	0.02
	Pyridaphenthion	$C_{14}H_{17}N_2O_4PS$	24.58	340, 188	0.04	0.03
Fungicide	Iprobenfos (IBP)	$C_{13}H_{21}O_3PS$	19.05	91, 204	0.02	0.01
	Isoprothiolane	$C_{12}H_{18}O_4S_2$	22.26	162, 290	0.02	0.01

a) River water, *b*) drinking water.

Horse serum was obtained from Gibco (product of New Zealand, Invitrogen Corporation, Carlsbad, CA, U.S.A.). The horse serum, which was thawed immediately before preparation of the enzyme solution, was mixed with 0.01 mol/l Tris-HCl buffer (pH 8.0, 1 : 8, v/v) to prepare the enzyme solution. AChI, 5,5′-dithiobis [2-nitrobenzoic acid (DTNB)], and neostigmine bromide were obtained from Wako Pure Chemical Industries, Sigma-Aldrich Japan (Tokyo, Japan), and Nakalai Tesque (Kyoto, Japan), respectively. Solutions of 0.1 mol/l AChI, 0.04 mol/l DTNB, and 0.01 mol/l neostigmine bromide in 0.01 mol/l Tris-HCl buffer (pH 8.0) were prepared immediately before the ChE assay.

The adsorbent, Amberlite XAD-2 resin (20–50 mesh, Rohm and Hass, Philadelphia, PA, U.S.A.) was commercially available. Fines were removed by decanting after slurrying in water. The resin was cleaned in a Soxhlet extractor with acetone/*n*-hexane (50 : 50, v/v) for 24 hr, in order to remove interferences from the resin. During the cleaning, a portion of the solvent was evaporated and checked for interferences by GC. If necessary, the washing in the Soxhlet extractor was repeated. When the blank chromatogram showed no interferences, the resin was removed from the extractor. The solvent remaining on the adsorbent was then evaporated completely in a vacuum disiccator for 24 hr. The purified adsorbent was stored under methanol.

Collection and Preparation of XAD-2 Resin Extract —— River water as source water was collected from the Nodabashi of the Edo River, during the period from January to December 2008. This water is typically to the Kamihanawa purification plant and then distributed to the Northwest district of Chiba Prefecture, including to our university (Fig. 1). The distance from the plant to our campus is about 6 km. Great care was taken to avoid contaminations during collection: all utensils and containers were scrupulously cleaned and rinsed with high-purity water before use. A Heyroth water sampler was employed, and all 20 l samples were obtained from the middle layer below the water surface, in the middle river. In the laboratory, all these samples were filtered through a membrane filter (0,45 μm; Advantic, Tokyo, Japan) to avoid biodegradation, and stored at 4°C until XAD-2 resin extraction.

A glass column [3.2 cm internal diameter (i. d.), 30 cm length] quipped with a No. 2 glass filter and a polytetrafluoroethylene (PTFE) stop-cook, which was packed with 200 ml of each cleaned adsorbent,

was used for isolation of trace organic pollutants in river and drinking water samples, in order to study their ChE-inhibiting activity and chemical characteristics. Before processing the water samples, the column was washed with 5 l of high purity water to remove the residual methanol on the resin. Each column was connected directly to a water tank of 20 l, which was completely filled with river water. Drinking water was also introduced bottom-to-top into the XAD-2 resin column, which was connected directly to the water tap in the laboratory. Water samples were continuously passed through the resin column in the cooler room at 4°C, at a flow-rate of 2 bed volumes/min during each sampling period.

After processing either 20 l or 5000 l water sample, the column was washed with 2 l of high-purity water, and then the residual water was removed from the column using dry nitrogen gas for 10 min. The XAD-2 resin adsorbent was removed from the column and the resin extractable organic substances on the resin were then extracted by sonication with 200 ml of acetone/*n*-hexane (50 : 50, v/v). The sonication extractions were repeated twice or more with a fresh 200 ml volume of the mixed solvent. These extracts were dried over anhydrous sodium sulfate and evaporated to dryness by means of a rotary evaporator at 40°C. The dry concentrate was dissolved, as rapidly as possible, in 0.5 (river water) or 5 ml (drinking water) of methanol, producing a concentration factor of 2.5×10^5 or 1×10^6, respectively, and stored in a deep freezer at −80°C until the subsequent bioassay and chemical analysis were performed.

Assay of ChE-inhibiting Activity —— Assays of ChE-inhibiting activity were carried out according to procedure described by Ellman *et al.*[14] with some modification. Twenty five, fifty, and one hundred twenty five microliters of the river water concentrates dissolved in methanol were placed in glass tubes and the 25-, 50-, and 125-μl samples were diluted to 200-μl with methanol. Similar procedures were also performed for the drinking water concentrates. These samples corresponded river-water sample volumes of 100, 200, and 500 ml and also drinking water sample volumes of 1, 2, and 5 l. The ChE-inhibiting activity was measured for each sample and the dose response curves were plotted. Two hundred microliters of the sample dissolved in methanol was mixed with 1.8 ml enzyme dilution and incubated at 37°C for 30 min. After incubation, 200 μl of this mixture was mixed with 200 μl AChI solution, 600 μl DTNB solution, and 3 ml Tris buffer. The absorbance of this solution at 412 nm was then immediately measured by use of a Hitachi UV-1100 spectrometer (Hitachi, Tokyo, Japan), and changes of absorbance during 3 min were recorded using an electronic polyrecorder (EPR-100A, TOA Denpa, Tokyo, Japan). The ChE-inhibiting activity was calculated as follows:

$$Inhibition(\%) = 100 \times [1 - (\Delta Abs_{\text{sample}} - \Delta Abs_{\text{neo}})/(Abs_{\text{blank}} - \Delta Abs_{\text{neo}})] \quad (1)$$

where, $\Delta Abs_{\text{sample}}$ is the absorbance slope of the sample, ΔAbs_{neo} is the absorbance slope of 0.01 mol/l neostigumine bromide, which represents substrate decomposition, and $\Delta Abs_{\text{blank}}$ is the absorbance slope of methanol, which represents non-inhibition conditions.

Analytical Methods —— The XAD-2 resin extracts of river and drinking water were analyzed using an HP model 5890 II gas chromatograph (Yokogawa-Hewlett-Packard Ltd., Tokyo, Japan) equipped with a JEOL Automass 50 mass spectrometer (JEOL Ltd., Tokyo, Japan). A Zebron capillary GC column ZB-5, 30 m × 0.25 mm i.d., and 0.25 μm film thickness (Phenomenex, Torrance, CA, U.S.A.) was programmed from 40 to 280°C at a rate of 10°C/min. Splitless injection of sample (1 μl) into the GC/MS was performed at 280°C. The electron ionization conditions were as follows: ion energy, 70 eV; ion source temperature, 200°C; and m/z = 50–600 full scan for qualitative analysis. Retention times, quantifying and qualifying ions, detection limits, are listed in Table 1.

RESULTS AND DISCUSSION

An Outline of the Water Sample and Control Tests

The XAD-2 resin extracts were prepared from river water obtained from the Nodabashi of the Edo River, as raw water, and from the laboratory tap water sampled in each month during winter (January, February, and March 2008), spring (April, May, and June), summer (July, August, and September), and fall (October, November, and December). The drinking water in this area is distributed from the Kamihanawa purification plant (Northwest of Chiba Prefecture), which draws water from the midstream of the Edo River. In this treatment plant, the raw water is generally purified by employing clarification, flocculent precipitation, and sand filtration, and final chlorine disinfection techniques, and sometimes

Table 2. Data on Sampling Period, Volume of Sample, Water Temperature, Water Level, and pH Value in the Edo River and Drinking Waters

River water					Drinking water			
Sampling date	Volume of sample (l)	Water temp.(°C)	Water level (m)	pH	Sampling period	Volume of sample (l)	Water temp.(°C)	pH
2008/01/17	20	5.4	0.4	6.2	2007/12/29–01/08	5100	16.9	6.4
2008/02/22	20	6.1	0.15	7.0	2008/01/27–02/08	5000	14.8	6.5
2008/03/29	20	13.8	0.45	6.6	2008/02/13–03/22	5950	17.0	7.0
2008/04/17	20	15.9	1.10	6.7	2008/03/31–04/07	5270	14.9	7.6
2008/05/19	20	19.2	0.60	6.3	2008/04/30–05/09	5100	17.8	6.7
2008/06/18	20	23.0	1.15	7.3	2008/05/30–06/07	5070	19.1	6.5
2008/07/22	20	27.2	0.85	6.4	2008/07/04–07/14	5700	23.1	6.4
2008/08/26	20	20.4	3.60	6.9	2008/07/30–08/09	4000	22.2	7.3
2008/09/16	20	22.3	1.60	7.3	2008/09/01–09/11	4500	22.1	6.9
2008/10/20	20	19.4	0.70	6.8	2008/10/01–10/13	4300	19.7	7.3
2008/11/23	20	10.0	0.50	7.4	2008/11/05–11/18	3800	18.6	6.4
2008/12/21	20	9.0	0.40	7.3	2008/12/02–12/15	2900	17.8	7.0

by using activated carbon adsorption for removing organic contaminants, if necessary. The water level in the river is generally low in the winter and high in the summer. Data on sampling periods, volume of samples, water level depth), water temperature, and pH values are summarized in Table 2.

XAD resins are commonly used to recover and to concentrate organic pollutants from raw and drinking waters. In these experiments, however, it is important to differentiate between contaminants, including ChE inhibitors, present in the original samples and those produced as artifacts during such concentration procedures. To examine the possibility of such artifacts existing in our clean XAD-2 resin, control experiments were carried out using a small-scale column (50 ml) packed with resin and processing with non-chlorinated and chlorinated water (20 l) containing 0.5 mg/l of active chlorine. No artifacts and ChE-inhibiting activity were apparent from the *n*-hexane/acetone (50 : 50, v/v) extract from the non-chlorinated water. However, very low concentrations of artifacts and weak ChE inhibitory effects were observed when testing the extract from the chlorinated water by GC/MS analysis and ChE-inhibiting assay. However, the ChE-inhibiting assays of these samples did not exceed the negative control (inhibition of 3–5%) in the tested range (0.5–2.0 l equivalents). These findings indicate that small amounts of ChE inhibitors are produced as a result of the reaction of active chlorine with XAD resin. However, it appears that the contribution of these inhibiting artifacts is relatively small as compared with the ChE-inhibiting activity of the organic residues from the drinking water.

Recovery tests were also performed by spiking water with agrochemical compound at 0.1 μg/l level and carrying out the entire procedure, in order to evaluate the total analytical precision for individual agrochemicals. Small-scale column packed with clean XAD-2 resin (50 ml) was used for the recovery tests. After processing with 50 l of the above spiking water at a flow rate of 100 ml/min, the residual water was blown from the column with dry nitrogen. The agrochemical compounds adsorbed on the XAD-2 resin were then extracted by sonication with acetone/*n*-hexane (50 : 50, in volume, 3×50 ml). The resulting extracts were concentrated using a rotaly evaporator at 40°C for GC/MS analyses. Although drastic precautions were taken during the evaporation steps, loss of the more volatile and thermally unstable compounds such as benthiocarb and esprocarb occurred, with exception of other agrochemical compounds tested in the present procedure.

ChE-inhibiting Potentials of XAD-2 Resin Extracts from River and Drinking Waters

The organic concentrates obtained from river and drinking waters by XAD-2 resin adsorption and subsequent sonification extraction with *n*-hexane/acetone (50 : 50, v/v) were also tested at three or more doses for their ChE-inhibiting activity. Dose response relationships between sample volume and ChE-inhibiting activity were clearly apparent in this study (Fig. 2). Substantially considerable ChE-inhibiting activity was observed for the river water concentrates collected on July 22 as compared with those observed for the drinking water concen-

trates collected during the similar period. Thus the ChE inhibitors were definitely present in both the river and drinking water samples collected from the Edo River and the laboratory tap. To compare the results conveniently, the ChE-inhibiting activity in XAD-2 resin extracts were expressed as the inhibition percent inhibited by the water concentrates in 0.5 l equivalent of river water and 5 l equivalent of drinking water, respectively, because a plot of sample volume *vs*. inhibition percent gave a straight line in the range from 10 to 90% (Fig. 2).

Results of ChE-inhibiting activity in the river and drinking water samples collected during January to December 2008 are shown in Fig. 3. ChE-inhibiting activity in river water increased from the early spring to summer, and decreased from the mid-summer to fall and winter seasons, with the maximum activity appearing for the June sample (73%). In contact, two maxima activities in the inhibition activity of the drinking water concentrates were observed for the July (94%) and October samples (58%) during the investigation period. A previous study investigated the AChE-inhibiting activity, measured by using human red blood cells, of paddy water and rural river water, and reported it range from 39 to 70% in the paddy water, and from 29–34% in rural river water.[15] Another previous study, reported the AChE-inhibiting activity of water samples from urban and rural rivers to range from 0 to 56% and from 0 to 22%, respectively, and the most of water samples from rural rivers did not have inhibiting activity.[9] However, in these studies, sampling was carried out one, and the reports were inadequate with regard to the periodic tendency of AChE inhibition and the origin of the AChE inhibitors.

The trends in the variations of ChE-inhibiting activity were consistent with the variation of pesticide concentrations observed in this study and with the results of previous studies.[16,17] The ChE-inhibiting activity of the water samples collected on June 18 from the Edo River was relatively high. Therefore, the samples collected on June 18 from the Edo River were replicated on the basis of the chemical concentrations recorded, excepted for dichlorvos (DDVP) standard, and the ChE-inhibition activity of these samples was measured. However, no ChE-inhibiting activity was observed in the replicated samples. AChE-inhibiting

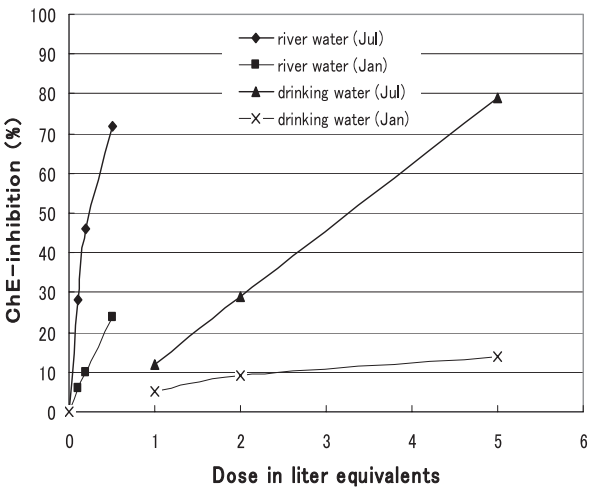

Fig. 2. Dose-response Relationships between Sample Volume and ChE-inhibiting Activity of XAD-2 Resin Extracts Collected from the Edo River and from the Laboratory Tap during Summer and Winter Seasons

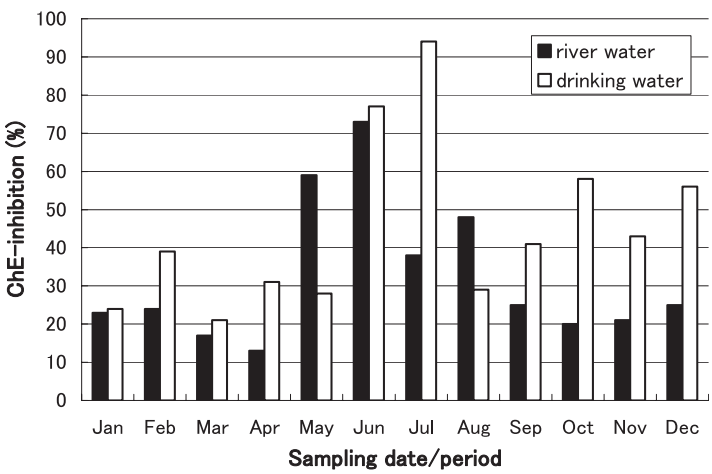

Fig. 3. Seasonal Variation in the ChE-inhibiting Activity of XAD-resin Extracts Collected from the Edo River and Drinking Water during January to December 2008

The inhibiting activity shown corresponds to 0.5 l of the river water and to 5 l of the drinking water.

Table 3. Concentrations of Pesticides in XAD-2 Resin Extracts Collected from River Water during January to December, 2008

Compounds examined	Concentration (μg/l)											
	Jabuary	February	March	April	May	June	July	August	September	October	November	December
Herbicide												
Bentiocarb	ND	ND	ND	0.01	0.02	ND	0.02	0.02	0.02	ND	0.03	0.02
Bromobutide	ND	ND	ND	0.07	0.11	0.18	0.09	0.08	0.08	0.07	0.07	0.07
Butamifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.08	ND	ND
Diclobenil	ND	ND	ND	0.03	0.04	0.03	0.03	0.02	ND	ND	ND	ND
Esprocarb	ND	0.04	0.03	0.03	0.04	0.06	0.09	0.03	0.04	0.03	ND	ND
Metolachlor	ND	ND	ND	0.03	0.04	0.03	0.03	0.02	0.02	0.01	ND	ND
Oxadiazon	ND	ND	ND	0.01	0.02	0.03	0.02	0.02	0.01	ND	ND	ND
Pretilachlor	ND	ND	0.02	0.03	0.05	0.1	0.03	ND	ND	ND	ND	0.03
Pyributicarb	ND	ND	ND	ND	0.03	0.03	0.03	ND	ND	ND	ND	ND
Simadine	ND	ND	ND	0.05	0.06	0.08	0.05	0.05	ND	ND	0.05	ND
Simetryn	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Insecticide												
Fenobucarb	ND	ND	ND	ND	ND	0.18	0.08	0.09	0.11	ND	0.09	0.08
Chloropyrifos	ND	0.05	0.05	0.05	0.04	ND	ND	0.05	ND	ND	ND	ND
Diazinon	ND	ND	0.1	0.09	0.12	0.11	0.11	0.11	0.12	0.11	0.12	ND
Dichlorvos	ND	ND	0.14	0.12	0.12	0.21	0.16	0.15	0.28	0.34	0.16	0.2
Fenitrothion	0.12	0.13	0.13	0.13	0.14	0.14	0.13	0.13	0.16	0.14	0.15	0.14
Isofenphos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isoxathion	0.1	0.36	0.29	0.28	0.25	0.53	0.29	0.21	0.35	0.5	0.53	0.45
Pyridaphenthion	0.13	0.15	0.12	0.12	0.14	0.16	0.12	0.13	0.14	0.12	ND	0.12
Fungicide												
Irobenfos	ND	ND	ND	0.07	0.05	0.05	0.05	0.08	0.08	ND	ND	ND
Isoprothiolane	ND	ND	ND	0.03	0.04	0.09	0.06	0.03	0.04	0.03	0.03	0.03

activity of the surfactants dodecyl benzyl sulfonate and sodium dodecyl sulfate have been reported in *in-vivo* and *in-vitro* assays[18] and it has also been reported that some polycyclic aromatic hydrocarbons had AChE-inhibiting activity and contributed in an additive effect.[19] Thus, the ChE-inhibiting activity observed in this study might not only because of the organophosphorus and carbamate pesticides studied but also because of other chemicals.

Characteristics of Agrochemicals and Their Levels in River and Drinking Water

GC/MS determinations of pesticides in XAD-2 resin extracts collected from river and drinking waters in each month during January to December 2008 are summarized in Tables 3 and 4, and presented graphically in Fig. 4 as a plot of total pesticide concentrations against each sampling date or period. Several herbicides, such as benthiocarb, bromobutide, esprocarb, metholachlor and oxadiazon, applied to paddy fields were frequency detected in the river water during the investigation period. The total herbicide levels in the river water ranged from ND (not detected) to 0.54 μg/l with a mean concentration of 0.18 μg/l (Table 3). High concentrations of these compounds were detected in river water collected during the spring to summer seasons, whereas the low values were observed for the fall and winter samples (Fig. 4). We considered these periodic variations to be due to periodic application of the herbicides described in the previous studies.[10, 16, 17, 20, 21]

Several insecticides, such as diazinon, DDVP, fenitrothion, isoxathion, and pyridaphention, applied to paddy fields were also frequently detected in river water during the investigation period, and others were either not or detected in traces. The total insecticide levels in the river water ranged from 0.26 to 1.33 μg/l with a mean value of 0.91 μg/l (Table 3). The concentrations of these insecticides were relatively low as compared with the concentrations reported in previous studies.[10, 16, 17] Although the data given in Table 3 and Fig. 4 showed seasonal scatter in the river water, it appears that the absolute amounts of total insecticides (their concentrations × each water level) during the spring to summer seasons, except for the July sample, are higher than those during the fall and winter seasons. The varia-

Table 4. Concentration of Pesticides in XAD-2 Resin Extracts of Drinking Water Collected in Each Month during January to December, 2008

Compounds examined	Concentration (μg/l)											
	Jabuary	February	March	April	May	June	July	August	September	October	November	December
Herbicide												
Bentiocarb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bromobutide	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
Butamifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dichlobenil	ND	ND	ND	ND	0.01	0.02	0.01	0.01	0.01	ND	ND	ND
Esprocarb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	ND	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01
Oxadiazon	ND	ND	ND	0.01	0.02	0.02	0.01	0.01	ND	ND	ND	ND
Pretilachlor	ND	ND	ND	ND	0.01	0.01	0.01	ND	ND	ND	ND	ND
Pyributicarb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Simazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Simetryn	ND	ND	ND	ND	ND	ND	ND	ND	0.01	0.01	ND	ND
Insecrticide												
Fenobucarb	ND	ND	ND	ND	0.01	0.01	0.01	0.01	0.01	0.01	0.01	ND
Chloropyrifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dichlovos	ND	0.02	ND	0.02	ND	ND	ND	ND	ND	ND	ND	ND
Fenitrothion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isoenphos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isoxathion	ND	ND	ND	ND	ND	ND	ND	0.03	0.04	ND	ND	ND
Pyridaphenthion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fungicide												
Ibrobenfos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isoprothiolane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

tions in the spring samples might be because of timing of the pesticide application to paddy fields, and the changes in the late summer and early fall samples might be because of application to upland field and non-cultivated land, including gardens.[16, 17)]

In addition, two fungicides, Ibrobenfos (IBP) and isoprothiolane, were detected in the river water samples during the investigation period. The total fungicide levels in the river water samples ranged from ND to 0.14 μg/l with a mean value of 0.06 μg/l. High concentrations of these fungicides were observed during the mid-spring to early summer for isoprothiolane and from mid-summer to late summer for IBP, whereas low values was detected for the fall and winter samples. The variations in these pesticides levels might be because of application timing to paddy field.

A number of herbicides, such as bromobutide, dichlobenyl, metholachlor, oxadiazon and pretiachlor, were also detected in drinking water samples taken from the laboratory tap during the investigation period. The total herbicide concentrations in the drinking water ranged from 0.01 to 0.08 μg/l with a mean value of 0.03 μg/l. A comparison of total herbicide concentrations in the river water and drinking water showed that the mean removal rate of total herbicides from the river after processing them in the water treatment plant was approximately 84%. This value was also lower as compared with results observed in our previous paper,[22)] demonstrating a high removal rate (95%) of dissolved organic contaminants after processing it in the same water treatment plant. In contrast to the herbicides, a marked reduction in the insecticide and fungicide concentrations in the river water was observed during the investigation period, and over 97% of total insecticides in the river water were eliminated after processing in the water treatment plant (Table 3). Similar observations on the decreased these pesticide levels in river water after processing them in water treatment plants have been reported in our previous paper.[10)]

As Table 3 and Fig. 4 show, almost all organophosphorus and carbamate pesticides, as representative cholinesterase inhibitors, in the river water, except for fenobucarb (BPMC), DDVP and isoxathion, could be eliminated completely by the processing in the water treatment plant. On the ba-

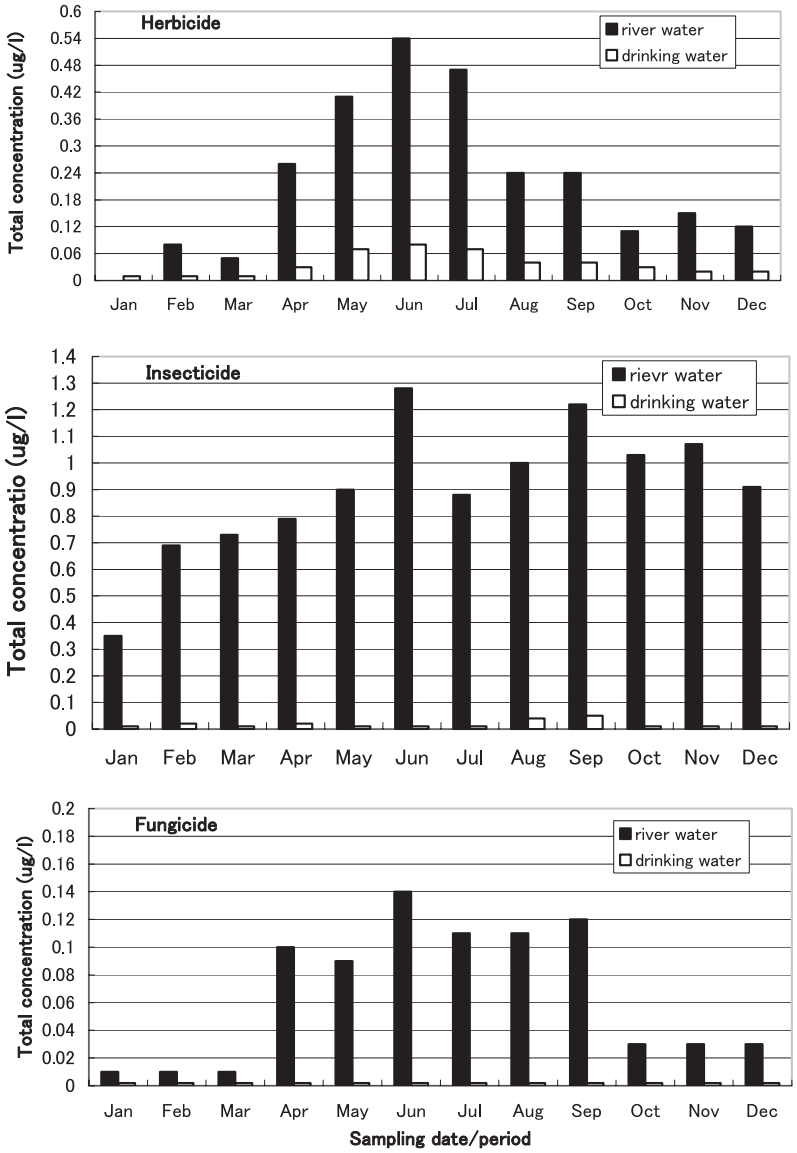

Fig. 4. Variation of Pesticide Concentrations in XAD-2 Resin Extracts Collected from River Water and Drinking Water during January to December 2008

sis of these results, it, therefore, is expected that the ChE-inhibiting potentials of XAD-2 resin extracts collected from the drinking water might be very low. However, comparative high ChE-inhibiting potentials were observed for the drinking water concentrates, particularly collected during the summer and fall periods (Fig. 4). Onodera *et al.*[6] and Makihata *et al.*[8] added organophosphorus pesticides (P = S types) to river water samples and reported the ChE-inhibiting activity of these samples increased by chlorination. Furthermore, the phenomenon of increased inhibiting activity as a results of chlorination has been reported for almost all organophosphorus pesticides (P = O structure).

Previous studies have also reported that the P = S structure of organophosphorus pesticides was easily converted to P = O structures on oxidation with chlorine during the water treatment process.[6,8,23,24] In this study, diazoxon and chloropyriphos-oxon were not quantitatively detected, but their presence at trace levels was confirmed by GC/MS. Thus, the increased ChE-inhibiting activity observed in the drinking water concentrates might be because of organophosphorus pesticides with P = O structure. On the basis of these results described above, it is necessary to use the ChE-inhibiting potentials of both river and drinking water concentrates, together with instrumental analysis of pesticide concentrations in water samples for evaluating the eco-toxicity of environ-

mental water and the human health effect of drinking water.

In conclusion, the XAD-2 resin extracts of river and drinking water sampled in each month during January to December 2008 from the Northwest district of Chiba Prefecture, Japan, were investigated to characterize and determine their pesticide levels and ChE-inhibiting potentials. The GC/MS determinations showed the periodic variations of pesticide levels in both river and drinking waters, reflecting the application of pesticides to paddy fields. The trends in the variations of ChE-inhibiting activity were consistent with the variations of these pesticide concentrations observed in this study. However, results of the ChE-inhibiting assays for both river and drinking waters could not be explained on the basis of GC/MS determinations of pesticide concentrations. Thus, the combined method of the ChE-inhibiting assays with GC/MS determinations for the cholinesterase inhibitors in water samples is necessary to evaluate the eco-toxicity of environmental water and the human health effect of drinking water.

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