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Pesticides in Water, Fish and Shellfish from Littoral Area of Lake Biwa

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Abstract A survey of 29 pesticides were performed for water, fish and shellfish from two littoral areas of Lake Biwa and Yanamune River in 2007. Three insecticides, 5 fungicides and 13 herbicides were detected in the water from the sampling locations, but the insecticides and fungicides were not and the only 9 herbicides were detected in the fish and shellfish from the locations. Bioconcentration factors (BCF) of the 9 herbicides in the fish and shellfish were calculated by the field data obtained from the survey. The average field BCF values of the herbicides in the fish were 8 and 25 for molinate, 5–23 for bromobutide, 4 and 10 for simetryn, 100–214 for esprocarb, 15–41 for pretilachlor, 148 for anilofos, 14 and 79 for mefenacet and 78 for cafenstrole. Those in the shellfish were 6 and 13 for bromobutide, 4 and 8 for simetryn, 67 and 135 for esprocarb, 2 for pretilachlor, 117 for pyributicarb and 57 and 139 for mefenacet. The field BCF data in the fish were evaluated by laboratory BCF data from literatures for molinate, bromobutide, pretilachlor, simetryn and mefenacet.

Keywords Pesticide · Lake Biwa · Water · Fish and shellfish

Pesticide contamination of river and lake waters from agriculture use is a problem of worldwide importance. Many field data on the pesticide contamination of surface waters and aquatic organisms in rivers and lakes have been reported in Japan (Tsuda et al. 1996, 1997, 1998, 1999; Tanabe et al. 2001; Sudo et al. 2002, 2004), in Europe and America (Agradi et al. 2000; Struger et al. 2004; Lekkas et al. 2004), and in other countries (Mansour and Sidky 2003; Abdel-Halim et al. 2006; Leong et al. 2007). In the past, we reported various pesticide contamination of water and fish from rivers flowing into Lake Biwa in Japan (Tsuda et al. 1996, 1997, 1998, 1999). Since 2006, we have again performed extensively the same field survey in water, fish and shellfish from Lake Biwa. About 30 pesticides were detected from the survey of 67 pesticides in Lake Biwa surface waters (18 sites) in 2006 (Nakamura et al. 2008). In this report, the detected 29 pesticides were surveyed for water, fish and shellfish from littoral area of Lake Biwa and Yanamune River in 2007. Field bioconcentration factors (BCF) values of pesticides in the fish and shellfish were calculated from the results of the survey and the field BCF data in the fish were evaluated by laboratory BCF data (Kanazawa 1981, 1983; Tsuda et al. 1988, 1999; Xu et al. 1989; Martin et al. 1992; Tsuda et al. 2009, unpublished data).

Materials and Methods

A standard solution of 68 pesticide mixtures and internal standards (chrysene-d_{12} and phenanthrene-d_{10}) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Florisil PR (60/80 mesh) from Wako Pure Chemical Industries Ltd. (Osaka, Japan) after activation at 130°C for 16 h was used for column clean-up. Pesticide-grade solvents and chemicals were used throughout.

Water, fish and shellfish samples were collected from littoral area of Lake Biwa and Yanamune River (Fig. 1) once or twice every month from April to August in 2007. Fish samples were pale chub (*Zacco platypus*, body length 5.5–11.3 cm and body weight 2.8–25.7 g), Hasu (*Opsariichtys*

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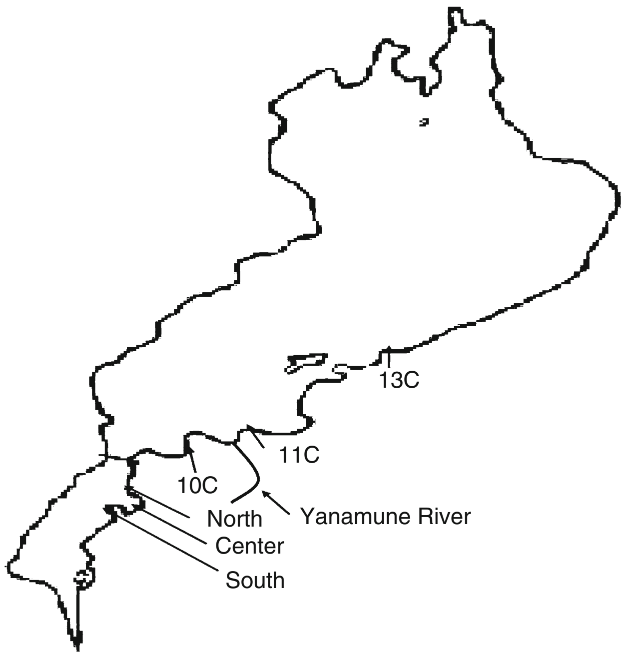

Fig. 1 Map of sampling locations

uncirostris uncirostris, body length 7.0–9.5 cm and body weight 4.0–10.7 g) and bluegill (*Lepomis macrochirus*, body length 5.5–12.2 cm and body weight 6.3–43.0 g), and shellfish samples were Ishigai (*Unio douglasiae nipponensis*, body weight 8.8–21.4 g) and Himetanishi (*Sinotaia quadrata histrica*, body weight 1.0–4.0 g). Water samples were analyzed immediately. Fish samples were homogenized as a mixture of three or four whole body samples, Ishigai as a mixture of three or four soft tissue samples, and Himetanishi as a mixture of 15–30 soft tissue samples for each sampling location and frozen and preserved for analysis.

The concentrations of 29 pesticides in water samples were determined by the following procedure (Nakamura et al. 2008). In brief, a measured volume (500 mL) of water sample was passed through a Aqusis PLS-3 column and eluted with 4 mL of dichloromethane after drying with air stream at room temperature for 40 min. The eluate was analyzed using GC/MS after evaporation to 0.5 mL. Average recoveries (n = 3) were 78%–130% for the 29 pesticides at a spiked level of 0.1 μg/L quantification limits were 0.1 μg/L for propiconazole-1 and propiconazole-2, 0.05 μg/L for cafenstrole, 0.02 μg/L for isoprocarb, fenitrothion, pyrokiron, dimethametryn, pyributicarb, anilofos and mefenacet and 0.01 μg/L for the other 19 pesticides.

Determination of the pesticides in fish and shellfish samples was performed using the method of Tsuda et al. (2008). In brief, about 5 g of the homogenized tissue sample was suspended twice with 30 mL of acetonitrile after addition of 5 g of anhydrous Na_2SO_4, and the organic layer was filtered through anhydrous Na_2SO_4. The combined filtrate was rotary-vacuum evaporated just to dryness at 40°C. The residue was dissolved in 10 mL of hexane and shaken twice with 30 mL of acetonitrile saturated with hexane. The combined acetonitrile layer was rotary-vacuum evaporated just to dryness at 40°C. The residue was dissolved in 2 mL of hexane and passed through a 30 cm × 1.0 cm i.d. glass clean-up column containing 5 g of hexane-rinsed Florisil PR and 1 g of anhydrous Na_2SO_4. The column was eluted with 100 μg of acetone and hexane (10 + 90). The eluate was analyzed using GC/MS after evaporation to 1 μg. Average recoveries (n = 3) were 50%–102% in Japanese smelt (fish) and 64%–122% in corbicula (shellfish) for the 28 pesticides except propiconazole-1 at a spiked level of 100 μg/kg, respectively. Quantification limits were 10 μg/kg for propiconazole-2, 4 μg/kg for mefenacet and cafenstrole and 2 μg/kg for the other 25 pesticides except propiconazole-1. The GC/MS (Finnigan Trace GC Ultra gas chromatograph and Finnigan Polaris Q ion trap mass spectrometer) operating conditions were as follows:

Gas chromatography-GC column: RESTEK Rtx-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness), column temp.: 50°C (2 min) → 20°C/min → 180°C (5 min) → 4°C/min → 300°C (10 min), injection temp.: 250°C, carrier gas: He 1.0 mL/min, injection volume: 1 μL, injection mode: splitless

Mass spectrometry-Ionization voltage: 70 eV, ionization current: 250 μA, interface temp.: 250°C, ion source temp.: 200°C, scan mode: full scan, mass range: 50–550

The field BCF was calculated using the following equation:

$$\text{Field BCF} = \frac{\text{pesticide concentration in whole body of fish or soft tissue of shellfish}}{\text{pesticide concentration in water}}.$$

Calculation was performed at each sampling time when the concentration of each pesticide could be determined for both water and fish or shellfish samples.

Results and Discussion

Results of the survey are summarized in Table 1 for east littoral zone (C_{10}, C_{11} and C_{13}) of northern basin of Lake Biwa and in Table 2 for littoral zone (North, Center and South) of Akanoi Bay in southern basin of Lake Biwa and Yanamune River. Two insecticides, 4 fungicides and 10 herbicides in the water, 4 herbicides in the fish (Hasu and pale chub) and 4 herbicides in the shellfish (Ishigai and Himetanishi) were detected from the east littoral zone of northern basin of Lake Biwa. Two insecticides, 5 fungicides and 12 herbicides in the water and 8 herbicides in the bluegill were detected from the littoral zone of Akanoi Bay in southern basin of Lake Biwa. Three insecticides, 5 fungicides and 13 herbicides in the water and 6 herbicides in Himetanishi were detected from Yanamune River. The 3 insecticides, 5 fungicides and 13 herbicides were detected in the water from the two littoral areas of Lake Biwa and Yanamune River, but the insecticides and fungicides were not and the only 9 herbicides were detected in the fish and shellfish from the locations. An example of concentration changes of the 9 herbicides in the water and bluegill from the littoral zone of Akanoi Bay (Center) in southern basin of Lake Biwa is shown in Fig. 2 throughout the survey from May to August in 2007. The concentrations of molinate, bromobutide, simetryne and mefenacet in the water were high in May and June. This result corresponds to the maximum use of the herbicides in paddy fields of Japan. Detections of the 9 herbicides in the fish corresponded well to those in the water, but the order of the herbicide concentrations in the fish was different from that in the water. For example, the concentration of esprocarb was low in the water but high in the fish. This is probably because bioconcentration potential of esprocarb is higher

Table 1 Pesticide concentrations in fish and shellfish from east littoral zone of northern basin of Lake Biwa

Pesticides	Use	Water (µg/L)	Fish (µg/kg)		Shellfish (µg/kg)	
		(n = 21)	Hasu (n = 5)	Pale chub (n = 7)	Ishigai (n = 2)	Himetanishi (n = 1)
Isoprocarb	Insecticides	<0.02–<0.02	<2–<2	<2–<2	<2–<2	<2
Fenobucarb		<0.01–0.02	<2–<2	<2–<2	<2–<2	<2
Diazinon		<0.01–0.01	<2–<2	<2–<2	<2–<2	<2
Fenitrothion		<0.02–<0.02	<2–<2	<2–<2	<2–<2	<2
Fenthion		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Dichlobenil	Fungicides	<0.01–0.01	<2–<2	<2–<2	<2–<2	<2
Pyrokiron		0.02–0.37	<2–<2	<2–<2	<2–<2	<2
Iprobenfos		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Fthalide		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Flutolanil		<0.01–0.02	<2–<2	<2–<2	<2–<2	<2
Isoprothiolane		0.01–0.06	<2–<2	<2–<2	<2–<2	<2
Propiconazole-1		<0.1–<0.1	–	–	–	–
Propiconazole-2		<0.1–<0.1	<10–<10	<10–<10	<10–<10	<10
Molinate	Herbicides	<0.01–0.53	<2–<2	<2–7	<2–<2	<2
Simazine		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Propyzamide		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Bromobutide		0.03–1.90	<2–14	<2–29	<2–<2	15
Simetryn		0.03–1.11	<2–<2	<2–<2	<2–<2	6
Alachlor		<0.01–0.02	<2–<2	<2–<2	<2–<2	<2
Esprocarb		<0.01–0.07	<2–7	<2–10	<2–5	<2
Thiobencarb		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Dimethametryn		<0.02–0.06	<2–<2	<2–<2	<2–<2	<2
Dimepiperate		<0.01–<0.01	<2–<2	<2–<2	<2–<2	<2
Pretilachlor		<0.01–0.23	<2–<2	<2–<2	<2–<2	<2
Thenylchlor		<0.01–0.03	<2–<2	<2–<2	<2–<2	<2
Pyributicarb		<0.02–<0.02	<2–<2	<2–<2	<2–<2	<2
Anilofos		<0.02–<0.02	<2–<2	<2–<2	<2–<2	<2
Mefenacet		<0.02–0.57	<4–<4	<4–14	<4–20	108
Cafenstrole		<0.05–0.08	<4–<4	<4–<4	<4–<4	<4

Table 2 Pesticide concentrations in fish and shellfish from littoral zone of Akanoi Bay in southern basin of Lake Biwa and Yanamune River

Pesticides	Use	Littoral zone of Akanoi Bay (n = 3)		Yanamune River	
		Water (μg/L) (n = 21)	Bluegill (μg/kg) (n = 14)	Water (μg/L) (n = 7)	Himetanishi (μg/kg) (n = 2)
Isoprocarb	Insecticides	<0.02–<0.02	<2–<2	<0.02–<0.02	<2–<2
Fenobucarb		<0.01–0.04	<2–<2	<0.01–0.04	<2–<2
Diazinon		<0.01–0.28	<2–<2	<0.01–0.02	<2–<2
Fenitrothion		<0.02–<0.02	<2–<2	<0.02–0.04	<2–<2
Fenthion		<0.01–<0.01	<2–<2	<0.01–<0.01	<2–<2
Dichlobenil	Fungicides	<0.01–0.01	<2–<2	<0.01–0.02	<2–<2
Pyrokiron		0.02–0.53	<2–<2	0.03–0.55	<2–<2
Iprobenfos		<0.01–<0.01	<2–<2	<0.01–<0.01	<2–<2
Fthalide		<0.01–0.02	<2–<2	<0.01–0.30	<2–<2
Flutolanil		<0.01–0.06	<2–<2	<0.01–0.02	<2–<2
Isoprothiolane		0.01–0.12	<2–<2	<0.01–0.08	<2–<2
Propiconazole-1		<0.1–<0.1	–	<0.1–<0.1	–
Propiconazole-2		<0.1–<0.1	<10–<10	<0.1–<0.1	<10–<10
Molinate	Herbicides	<0.01–1.40	<2–14	0.01–0.93	<2–<2
Simazine		<0.01–<0.01	<2–<2	<0.01–<0.01	<2–<2
Propyzamide		<0.01–<0.01	<2–<2	<0.01–<0.01	<2–<2
Bromobutide		0.02–5.77	<2–32	0.06–7.21	<2–70
Simetryn		0.03–3.44	<2–6	0.04–2.53	<2–10
Alachlor		<0.01–0.02	<2–<2	<0.01–0.03	<2–<2
Esprocarb		<0.01–0.44	<2–59	<0.01–0.69	<2–30
Thiobencarb		<0.01–0.06	<2–<2	<0.01–0.01	<2–<2
Dimethametryn		<0.02–0.13	<2–<2	<0.02–0.21	<2–<2
Dimepiperate		<0.01–<0.01	<2–<2	<0.01–<0.01	<2–<2
Pretilachlor		<0.01–0.46	<2–6	<0.01–1.98	<2–4
Thenylchlor		<0.01–0.13	<2–<2	<0.01–0.34	<2–<2
Pyributicarb		<0.02–<0.02	<2–<2	<0.02–0.06	<2–7
Anilofos		<0.02–0.10	<2–7	<0.02–0.04	<2–<2
Mefenacet		<0.02–2.65	<4–29	<0.02–2.30	<4–180
Cafenstrole		<0.05–0.09	<4–9	<0.05–0.26	<4–<4

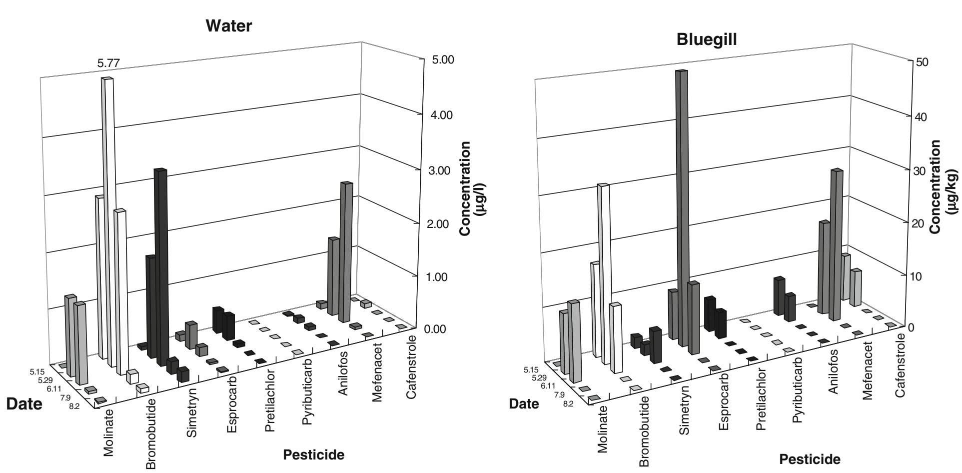

Fig. 2 Concentration changes of the 9 herbicides in the water and fish from the littoral zone of Akanoi Bay (Center) in southern basin of Lake Biwa throughout the survey from May to August in 2007

than the other herbicides. Average BCF values of the herbicides in the three kinds of fish and the two kinds of shellfish were calculated from the field data (Tables 1, 2) and are shown as field BCF data in Fig. 3. The field BCF values of the herbicides in the fish were 8 and 25 for molinate, 5–23 for bromobutide, 4 and 10 for simetryn, 100–214 for esprocarb, 15–41 for pretilachlor, 148 for anilofos, 14 and 79 for mefenacet and 78 for cafenstrole. Those in the shellfish were 6 and 13 for bromobutide, 4 and 8 for simetryn, 67 and 135 for esprocarb, 2 for pretilachlor, 117 for pyributicarb and 57 and 139 for mefenacet. The BCF values of bromobutide and simetryn were low and those of esprocarb were high in both of the fish and the shellfish. For the other herbicides, the BCF values of mefenacet were not so high in the fish but high in the shellfish and those of pretilachlor were middle in the fish but very low in the shellfish. Field BCF data in the fish from this study and laboratory BCF data (Kanazawa 1981, 1983; Tsuda et al. 1988, 1999, 2009; Xu et al. 1989; Martin et al. 1992, unpublished data) are shown in Fig. 4 for molinate, bromobutide, simetryn, pretilachlor and mefenacet. The average field BCF values were nearly equal to the average

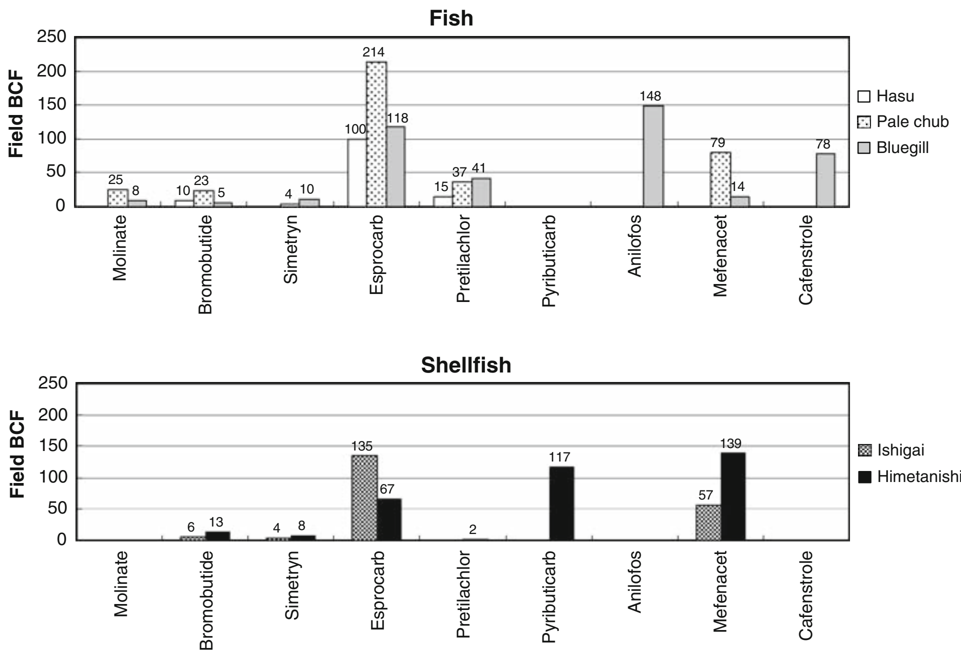

Fig. 3 Average BCF values of the 9 herbicides in the three kinds of fish and the two kinds of shellfish from the field data

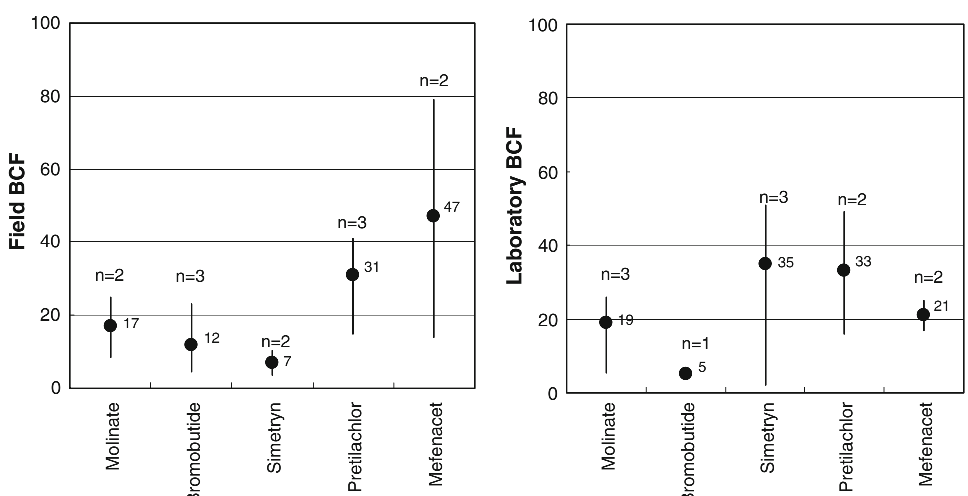

Fig. 4 Field BCF data in the fish from this study and laboratory BCF data from literatures

laboratory BCF values for molinate, bromobutide and pretilachlor but slightly lower for simetryn and slightly higher for mefenacet. The differences in the field and laboratory BCF values of simetryn and mefenacet are not wide, so both of the field and laboratory BCF data are considered to be the same levels for all of the 5 herbicides. From the comparison shown in Fig. 4, it was clarified that the concentrations of the 5 herbicides in the fish from Lake Biwa and Yanamune River could be approximately estimated by the laboratory BCF of the herbicides in fish.

References

Abdel-Halim KY, Salama AK, El-khateeb EN, Bakry NM (2006) Organophosphorus pollutants (OPP) in aquatic environment at Damietta Governorate, Egypt: implications for monitoring and biomarker responses. Chemosphere 63:1491–1498. doi:10.1016/j.chemosphere.2005.09.019

Agradi E, Baga R, Cillo F, Ceradini S, Heltai D (2000) Environmental contaminants and biochemical response in eel exposed to Po river water. Chemosphere 41:1555–1562. doi:10.1016/S0045-6535(00)00067-9

Kanazawa J (1981) Measurement of the bioconcentration factors of pesticides by freshwater fish and correlation with physiochemical properties or acute toxicities. Pestic Sci 12:417–424. doi:10.1002/ps.2780120408

Kanazawa J (1983) A method of predicting the bioconcentration potential of pesticides by using fish. Jpn Agric Res Q 17:173–179

Lekkas T, Kolokythas G, Nikolaou A, Kostopoulou M, Kotrikla A, Gatidou G, Thomaidis NS, Golfinopoulos S, Makri C, Babos D, Vagi M, Stasinakis A, Petsas A, Lekkas DF (2004) Evaluation of the pollution of the surface waters of Greece from the priority compounds of list II, 76/464/EEC directive, and other toxic compounds. Environ Int 30:995–1007. doi:10.1016/j.envint.2004.04.001

Leong KH, Tan LLB, Mustafa AM (2007) Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor River, Malaysia between 2002 and 2003. Chemosphere 66:1153–1159. doi:10.1016/j.chemosphere.2006.06.009

Mansour SA, Sidky MM (2003) Ecotoxicological studies. 6. The first comparative study between Lake Qarun and Wadi El-Rayan wetland (Egypt), with respect to contamination of their major components. Food Chem 82:181–189. doi:10.1016/S0308-8146(02)00451-X

Martin JF, Bennett LW, Anderson W (1992) Off-flavor in commercial catfish ponds resulting from molinate contamination. Sci Total Environ 119:281–287. doi:10.1016/0048-9697(92)90269-X

Nakamura T, Inoue A, Tanaka K, Tsuda T (2008) Results of pesticide surveys in water from Lake Biwa in 2006. Report of Lake Biwa Environmental Research Institute 3:205–212

Struger J, L'Italien S, Sverko E (2004) In-use pesticide concentrations in surface waters of the Laurentian Great Lakes, 1994–2000. J Great Lakes Res 30:435–450

Sudo M, Kunimatsu T, Okubo T (2002) Concentration and loading of pesticide residuues in Lake Biwa basin (Japan). Water Res 36:315–329. doi:10.1016/S0043-1354(01)00196-8

Sudo M, Kawachi T, Hida Y, Kunimatsu T (2004) Spatial distribution and seasonal changes of pesticides in Lake Biwa, Japan. Limnology 5:77–86. doi:10.1007/s10201-003-0115-0

Tanabe A, Mitobe H, Kawata K, Yasuhara A, Shibamoto T (2001) Seasonal and spatial studies on pesticides residues in surface waters of the Shinano River in Japan. J Agric Food Chem 49:3847–3852. doi:10.1021/jf010025x

Tsuda T, Aoki S, Kojima M, Harada H (1988) Bioconcentration and excretion of benthiocarb and simetryne by willow shiner. Toxicol Environ Chem 18:31–36. doi:10.1080/02772248809357306

Tsuda T, Inoue T, Kojima M, Aoki S (1996) Pesticides in water and fish from rivers flowing into Lake Biwa. Bull Environ Contam Toxicol 57:442–449. doi:10.1007/s001289900210

Tsuda T, Kojima M, Harada H, Nakajima A, Aoki S (1997) Herbicides in water and fish from rivers flowing into Lake Biwa. Toxicol Environ Chem 61:243–249. doi:10.1080/02772249709358490

Tsuda T, Kojima M, Harada H, Nakajima A, Aoki S (1998) Pesticides and their oxidation products in water and fish from rivers flowing into Lake Biwa. Bull Environ Contam Toxicol 60:151–158. doi:10.1007/s001289900603

Tsuda T, Takino A, Kojima M, Harada H (1999) Accumulation and excretion of molinate, bromobutide and their degradation products in fish. Toxicol Environ Chem 73:237–246. doi:10.1080/02772249909358862

Tsuda T, Nakamura T, Inoue A, Tanaka K (2008) Investigation on comprehensive analysis of pesticides in fish and shellfish by GC/MS. J Environ Chem 18:227–235

Xu Y, Lay JP, Zhang Y (1989) Uptake and transfer of 14C-simetryne through the laboratory freshwater food chain. Chin J Oceanol Limnol 7:10–16. doi:10.1007/BF02842653