General Summary (English)

学 位 論 文 の 要 旨

論文題目　**Occurrence, dynamics, spatio-temporal variations and risk assessment of pesticide residues in Kurose River and Seto Inland Sea, Japan**

（黒瀬川および瀬戸内海での残留農薬の存在、動態、時空間分布、リスクアセスメントに関する研究）

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Pesticides are natural or synthetic agents commonly used worldwide in agriculture, industries and homes (FAO, 1996; US-EPA, 2000). They are used as mixtures called formulations which contain inert substances and active ingredients. In agriculture, pesticides play a significant role in maintaining agricultural productivity and ensuring increased and quality products (US-EPA, 2000). They are generally classified based on target pest such as insects (insecticides), weeds (herbicides), fungi (fungicides), bacteria (bactericides), and mites (miticides) (FAO, 1996; Prieto *et al.*, 2012). Some pesticides like diuron and irgarol 1051 are also used in the marine industry as antifouling agents. However, when flushed into aquatic ecosystems, pesticide residues may cause undesirable effects on the environment and cause great harm to benthos and other aquatic organisms (Bloomfield *et al.*, 2006; Ohyama *et al.*, 1987; JPPA, 2000). Furthermore, pesticides can be toxic and harmful to non-target organisms and to humans even at trace levels (US-EPA, 2000). For example, cyanazine is moderately toxic to most fauna and flora and may cause adverse health effects if ingested as it is a neurotoxin. Diazinon is a neurotoxicant in humans and other mammals, and it is toxic to most aquatic organisms, honeybees and birds (PPDB, 2017).

Japan is one of the developed countries that use large quantities of pesticides (EMECS, 2007; NIES, 2017). From 2012 to 2016 the annual tonnage (ranges) of 0.24–1.05, 28–79.4, 25–

40, 76–104, 83–182, 330–355, and 379–478 were recorded for fenarimol, cyanazine, simetryn, isoprothiolane, diuron, diazinon, and fenitrothion, respectively (NIES, 2017).

Various pesticide groups including triazines and organophosphates have been detected in Seto Inland Sea and many surrounding rivers like Kurose in Japan (Tsunemasa and Okamura, 2011; Okamura *et al*., 2003; Derbalah *et al*., 2003; EMECS, 2007; Balakrishnan *et al*., 2012; Kaonga *et al*., 2015). Due to the environmental dynamics and continued use of pesticides in Japan, it is imperative to monitor and study their residues in the environment, and evaluate their ecotoxicological and human health risks.

In this study eight pesticides namely cyanazine, simetryn, fenarimol, isoprothiolane, diazinon, fenitrothion, diuron and irgarol 1051 were studied. They are all listed as important compounds under Agricultural Chemical Regulation Law in Japan (FAMIC, 2017). Their selection was based on usage trends in Japan, toxicological properties, chemical properties, compatibility with solid-phase-extraction (SPE) High-Performance Liquid Chromatography-Ultraviolet visible (SPE-HPLC-UV Vis) and their previous detection in Japan and elsewhere. This Thesis is divided into six chapters. Chapter 1 gives the general introduction and significance of the study. The last chapter (6) gives general discussions and conclusions along with recommendations and future line work. The major objectives of this study were:

- To conduct SPE-HPLC-UV/Vis method validation for measurement of pesticide residues in various natural water matrices. (Chapter 2)
- To study spatio-temporal variations of selected pesticide residues in the Kurose river in Higashi-Hiroshima city, Japan. (Chapter 3)
- To perform ecotoxicological and human health risk assessment of the selected pesticides in Kurose river (Chapter 4)
- To study contamination, dynamics and distribution of pesticide residues in marine samples (seawater, planktons, sediments, fish and marine animals), from Seto Inland Sea, Japan. (Chapter 5)

Chapter 2 involved method validation of the SPE HPLC-UV-Vis system for assessment of pesticide residues in different matrix. Five pesticides (cyanazine, simetryn fenarimol, isoprothiolane, and diazinon) used in the initial experiments recorded good recoveries (70–102%) and the limits of quantification at levels required by the US-EPA, EU Legislation (Directive 2013/39/EU) and Japan Ministry of the Environment. Generally, the method demonstrated good sensitivity, robustness and specificity for analysis of wider groups of pesticides in natural water without significant chromatographical and matrix interferences.

Chapter 3 involved a spatio-temporal assessment of selected pesticide residues (cyanazine, simetryn, fenarimol, isoprothiolane, and diazinon) in the Kurose river for one year (February 2016 to March 2017). Water samples were collected from seven sites namely Namitakiji (K1), Tokumasa (K2), Izumi (K3), Ochiai (K4), Kanekiyo Bashi (K4T), Hinotsume (K5), and Kawasumi Bashi (K6). The maximum cyanazine, simetryn, fenarimol, isoprothiolane, and diazinon were 282, 391, 60, 1086, and 1194 ng/L, respectively. Cyanazine was most frequently detected (64% of samples, n = 12), followed by simetryn (58%), and diazinon (57%). Pesticides were frequently detected in spring (May–June). Except for cyanazine and simetryn, all pesticides were below detection in winter. The three principal components (PCs) obtained linked the majority of pesticides to total suspended solids (TSS), rainfall, and dissolved organic carbon (DOC). There were no clear correlations between pesticides and other water quality parameters (EC, Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, NO_3^-, Cl^-, and SO_4^{2-}). Diazinon and isoprothiolane were consistent with their usage trends in Hiroshima Prefecture. The maximum diazinon obtained is concerning because of bioconcentration potential (Log K_{ow}=3.81) of its residues in edible fish and other marine animals.

Chapter 4 is a study on an integrated approach combining monitoring and ecotoxicological and human health risk assessment of pesticides in Kurose river. The investigated pesticides and protocol are similar to those described in Chapter 3. Ecotoxicological risk assessment was based on the Risk Quotient ($RQ = {}^{MEC}/_{PNEC}$) with respect to three trophic levels namely algae, aquatic invertebrate and fish. Pesticides were also measured in freshwater invertebrate red algae (*Audouinella sp.*) and diatoms (*Cocconeis placentula*) sampled from Kurose river. The maximum diazinon (1194 ng/L) detected was higher than the human safety level of 100 ng/L set by EU (Council Directive 98/83/EC) for a single pesticide. Furthermore, diazinon showed the highest ecotoxicological risk (RQ_m = 3.614). Generally, non-acceptable ecotoxicological risks were obtained mainly for four compounds (diazinon, cyanazine, simetryn and fenarimol). The highest *RQs* (6.65–29.21) were reported in spring and summer (May and June) while the lowest (0–1.16) was in winter (December, January and February). Diazinon showed the highest human health risk for both an adult *(HQ* = 0.0080–0.0120) and a child (*HQ* = 0.0172–0.0260) on assumptions of drinking raw water. In contrast, isoprothiolane showed the lowest human health risk (*HQ* = 0.0001–0.0002). Children were subject to 2-times higher risks than adults, and this is consistent with studies elsewhere (Shi *et al.*, 2011 and Hu *et al*., 2011; Papadakis *et al*., 2015). All the five studied pesticides posed no carcinogenic threats to people.

Chapter 5 involved studies on contamination, dynamics and distribution of pesticide residues in marine samples (seawater, sediment, plankton, and edible fish and marine animals) from Seto Inland Sea, Japan. Bioconcentration Factors (BCF) in planktons, edible fish and marine animals and the associated human health risks were also assessed. Eight pesticides (cyanazine, simetryn, fenarimol, isoprothiolane, diazinon, fenitrothion, diuron and irgarol 1051) were investigated in this study. Marine samples were collected during four sea cruises in 2016 and 2017 at different locations (Hiroshima bay, Hiuchi-nada, Bisan Seto, Harima-nada, Aki-nada, Osaka bay and Kii Channel). The pesticides were detected in marine samples, highest mostly in coastal areas. The most contaminated sites were in Osaka bay attributed to effluent and wastewater from Yodo river and other streams and contaminants from the shipping industry where some pesticides are used as antifouling agents and to treat weeds along roadsides and railway lines. The BCF in the orders of 10^3–10^4 (planktons) and 10^2–10^3 (fish and marine animals) depict high hydrophobicity of the pesticides to accumulate in the marine samples, and this is consistent with previous studies. Cyanazine, simetryn and diazinon were detected in fish samples (bd – 385 ng/g dw) caught from Seto Inland Sea, hence a need for concerted efforts in marine ecosystem protection and pesticide management practices.

Chapter 6 gives a general discussions and conclusion of the study. The study generally revealed that agriculture, urban activities and marine industry activities are contributing to contamination of water in Kurose river and Seto Inland Sea. The results obtained in this study are comparable to previous studies in Kurose river, Seto Inland Sea and other areas in Japan and elsewhere. The study recommends that risk assessment studies be extended to other major rivers in Japan used for drinking water abstraction. Due to continued use of pesticides in Japan, there is need to measure the pesticide residues in other marine animals from Seto Inland Sea consumed by humans to ascertain their safety. This study generated valuable data on pesticide residues in river water and marine samples. Pesticides detection in the sediments shows that the Seto Inland sea is an important sink of various contaminants, hence a need for integrated marine ecosystem protection and management practices. Furthermore, the data generated is significant for modeling (mass balance), understanding the behavior and fate of pesticide residues in the environment, policy formulations, and integrated pesticide management practices