MUSSEL WATCH PROGRAM: MONITORING OF DIOXIN RELATED COMPOUNDS IN ASIAN COASTAL WATERS USING MUSSELS AS BIOINDICATORS

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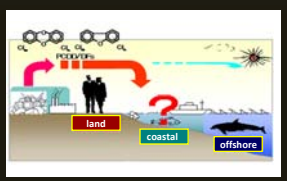
Introduction

Dioxin related compounds (DRCs) such as PCDDs, PCDFs, and DL-PCBs are lipophilic-stable contaminants of great concern with respect to their toxic effects on humans and wildlife (1). In developed countries, it is estimated that release into the environment and human exposure to DRCs have generally decreased in recent years because of highly efficient incinerators and strict regulations on production and usage of CNP, PCP and PCBs (2). In contrast, only very few studies have reported contamination status of DRCs in developing countries, and so information on these chemicals is still limited.

With this background, we conducted investigations on DRCs in soil and human breast milk collected from Asian developing countries and found that the residue levels of DRCs were higher in soil and human breast milk from the open dumping sites than from the agricultural and urban sites far from these areas, indicating that the dumping sites are significant pollution sources for DRCs and that residents there are exposed to relatively higher levels of these contaminants (3). We have also conducted a survey of DRCs in skipjack tuna collected from the Indian Ocean and the North Pacific Ocean and demonstrated that PCDD/Fs concentrations of offshore specimens in the Asian region are significantly low (4). This result indicates that dioxins (PCDDs and PCDFs) could cause regional pollution due to their higher absorption to particles. By a series of such investigations, contamination status on land and offshore areas in Asian developing countries has been well understood. However, our previous studies focused only on land and offshore areas of the Asian developing countries, and so it is unknown whether coastal areas in these regions are polluted by high levels of DRCs.



In order to elucidate the contamination status by DRCs in coastal waters of Asian countries, the present study analyzed PCDD/Fs and DL-PCBs in mussel samples collected from Asian coastal waters using GC-HRMS. In addition, we conducted Dioxin Responsive-Chemical Activated LUciferase eXpression (DR-CALUX) assay to provide important information on the overall dioxin-like potency of complex mixtures. These CALUX-TEQ values were compared with the chemical TEQs obtained from PCDD/Fs and DL-PCBs measurements in mussels.

Conclusion

1) **DRCs were detected in all mussel samples analyzed,** indicating widespread contamination by these compounds in coastal waters of Asia (Figure 1).

2) Apparently higher PCDD/Fs and DL-PCBs concentrations were found in mussels from coastal waters of industrialized/urbanized locations of all the countries, suggesting that significant sources of these compounds exist in and around these regions (Figure 1).

3) In Asian developing countries such as **Cambodia, Indonesia and the Philippines, TEQ concentration values were comparable to developed countries** such as Japan and Korea (Figure 2).

4) In DR-CALUX assay, dioxin-like activities were detected in all samples and those values were significantly higher than chemical analysis TEQs, indicating that the probable presence of other Ah-receptor agonists might have contributed greatly to DR-CALUX activities in mussels collected from Asian coastal waters (Figure 3).

To our knowledge, this is the first comprehensive report on the presence of dioxins in coastal waters of Asian developing countries. Based on our observations, we suggest that further investigations on pollution and sources of DRCs in Asian coastal areas are needed to assess human health risk.

Results and Discussion

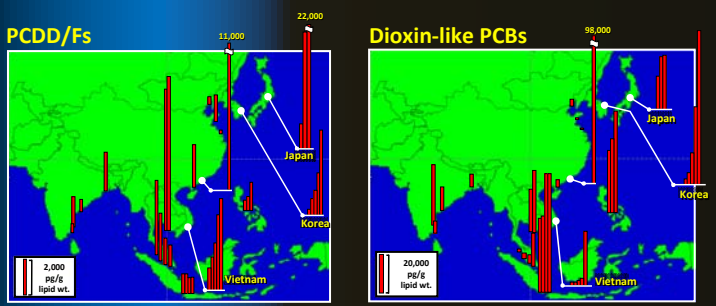


Figure 1. Geographical distribution of DRCs concentrations in mussels from coastal waters of Asia.

DRCs were detected in all the samples analyzed indicating a widespread contamination by these compounds in coastal waters of Asia . Average concentrations of PCDD/Fs in mussels from coastal waters of Hong Kong, Japan, Vietnam, Malaysia, Korea, India, China, Indonesia and the Philippines were 11,000, 10,000, 3000, 2400, 2,000, 1,300, 1,100, 1000, 990 pg/g lipid wt., respectively. Most of the samples contained a number of PCDD/Fs isomers mainly characterized by a complex mixture of TCDFs, TCDDs and OCDD, including both toxic and nontoxic isomers. Apparently higher PCDD/Fs concentrations were found in mussels from coastal waters of industrialized/urbanized locations in each country, which suggests that significant sources of these compounds exist in and around these regions. Similarly, DL-PCB concentrations in mussels were also higher in industrialized/urbanized locations than rural areas. However, distribution patterns of PCDD/Fs are different between DL-PCBs and PCDD/Fs, showing that pollution sources of these compounds were different.

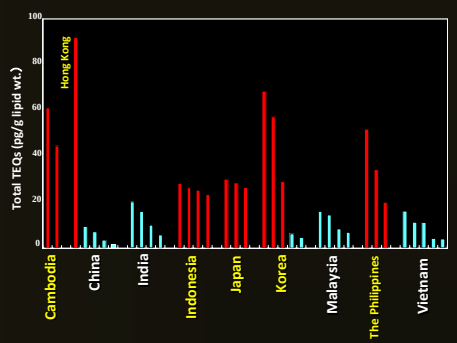


Figure 2. TEQ concentrations (pg/g lipid wt.) in mussels from coastal waters of Asia.

WHO-TEQ concentrations in mussels varied from 1.3 to 97 pg-TEQ/g fat wt. depending on countries and regions. The highest concentration of TEQs was detected in the sample from Hong Kong, and relatively higher concentrations of these compounds were observed in Cambodia, Indonesia, Japan, Korea and the Philippines. A point deserving special mention is that in Asian developing countries such as Cambodia, Indonesia and the Philippines, the concentrations of TEQs were comparable to developed countries such as Japan and Korea. It can be anticipated that pollution by DRCs in developing countries may further increase in the future, because even now the sources of these contaminants are not regulated yet.

Materials and Methods

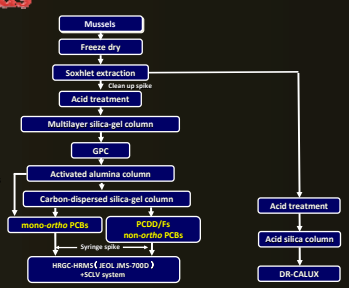


Fig. 5. Sampling locations and analytical methods.

Green mussels (*Perna viridis*) and blue mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) were collected from various locations in Cambodia, China, Hong Kong, India, Indonesia, Japan, Korea, Malaysia, the Philippines, and Vietnam from 1997 to 2005 (*n*=35). After shucking, the whole soft tissues of mussels from each location were pooled separately, homogenized, and stored in the Environmental Specimen Bank (*es*-BANK) for Global Monitoring at Ehime University at -25 ˚C until chemical analysis. Chemical analysis of PCDD/Fs and DL-PCBs were performed following the procedure described previously (5) with slight modification. Identification and quantification of PCDD/Fs and DL-PCBs were conducted using GC-HRMS. TEQs were calculated using 2005 WHO-TEFs (6). In a part of the mussel samples (n=17), AhR-mediated luciferase activity (as dioxin-like activity) was measured using DR-CALAX assay with a rat hepatoma H4ⅡE cell line with AhR-regulated luciferase gene construct (H4ⅡE-luc), obtained from BioDetection Systems B.V. (The Netherlands) according to culture conditions and assay procedures described elsewhere (7). The contribution of PCDD/Fs and DL-PCBs, determined by chemical analysis, to DR-CALUX response was evaluated by calculating their CALUX-TEQs using REP values (EC50 based) established by Behnisch *et al* (8).

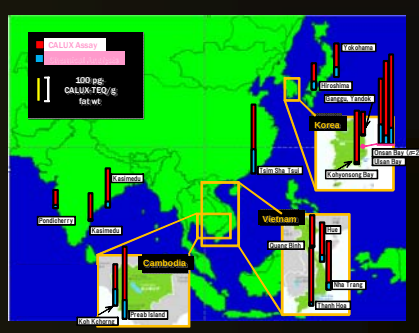


Figure 3. Comparison of DR-CALUX TEQ and Chemical analysis-TEQ values in mussels from Asian coastal areas. Chemical analysis TEQs were recalculated using DR-CALUX REP values.

As a result of DR-CALUX assay, dioxin-like activities were detected in a range of 99-370 pg CALUX-TEQ/g fat wt. in mussels collected from Asian coastal waters. To understand the extent of contribution by PCDD/Fs and DL-PCBs to DR-CALUX TEQ values (i.e. total dioxin activities), the DR-CALUX TEQ values were compared to chemical analysis data and recalculated using CALUX-REP values and found that the DR-CALUX and PCDD/F TEQ values measured by GC-HRMS are not significantly correlated. Moreover, the ratio of CALUX results to chemical results varies between 2.5 to 31, which implies that contribution of PCDD/Fs and DL-PCBs to total dioxin like activities was only 40% at the best in mussels collected from Asian coastal areas. These results indicate that the probable presence of other acid-resistant Ah-receptor agonists such as brominated dioxins might have contributed greatly to DR-CALUX activities in mussels collected from Asian coastal waters.

References

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Acknowledgments

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