

2023年 3月 19日

YYYY/MM/DD

独立行政法人日本学術振興会理事長 殿

To: President, Japan Society for the Promotion of Science

研究活動報告書

Research Report

1. 受入研究者/ Host researcher

受入研究機関・部局・職

Name of Host Institution, Department and Title

神戸大学・内海域環境教育研究センター・教授

受入研究者氏名

Host Researcher's Name

岡村 秀雄

2. 外国人招へい研究者/ Fellow

所属研究機関・部局・職

Name of Institution, Department and Title

Bharathidasan University, School of Environmental Sciences, Professor

外国人招へい研究者氏名

Fellow's Name

Babu Rajendran Ramaswamy

3. 採用期間/ Fellowship Period

2022年 5月 20日

～

2023年 2月 1日

4. 研究課題/ Research Theme

Environmental forensics and ecotoxicity of Non Phthalate Plasticizers in Japanese coastal waters.

5. 研究活動報告/ Research Report

(1) 研究活動の概要・成果/ Summary of Research Results

Phthalates have been used for about hundred years as plasticizers in number of commercial and industrial products. Due to their massive and widespread use they are easily leached in to the environment. Number of studies reported their ubiquitous presence in all the spheres of the planet earth, and in humans. It is obvious from the toxicological and epidemiological reports that exposure to phthalates leads to reproduction failure, neurotoxicity, carcinogenicity, attention disorders and autism in organisms. Due to some regulations in the use of phthalates have led to the introduction of non-phthalate plasticizers (NPPs). In this study environmental monitoring and toxicity risk evaluation of Plasticizers (phthalate and non-phthalate) were performed.

The Liquid Chromatograph with Mass spectrometer (LC/MS) was used to determine the levels of phthalate and non-phthalate plasticizers in seawater and sediment from the Osaka Bay, Japan. The extraction and analytical methods were standardized for the plasticizers such as Dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl)phthalate (DEHP), Diisobutyl adipate (DIBA), Acetyl tributyl citrate (ATBC), Di-(2-ethylhexyl)adipate (DEHA), Di-(2-ethylhexyl)sebacate (DEHS) and Trioctyl trimellitate (TOTM). The analytes extraction from seawater was made by using solid-phase extraction and with suitable solvent for analyte's elution. Recovery of each analyte was performed in triplicate and the average was taken. The obtained recoveries of the analytes were ranged between 68 and 105 % for natural seawater using the MAX column. Further, among solvents, acetone (10 ml) gave good recovery than

(注) 採用期間終了後3ヶ月以内に提出

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methanol. Seawater samples from Osaka Bay, Japan was analysed using this standardized analytical method. High concentrations of non-phthalate plasticizers were found in seawater samples than DEHP. Whereas, in sediment DEHP levels were found predominant.

The Toxicity risk assessment of plasticizers by exposing an important model organisms, Japanese medaka (*Oryzias latipes*) was performed in the laboratory. The effects of bis-(2-ethylhexyl) phthalate (DEHP) and bis-(2-ethylhexyl) adipate (DEHA) were investigated. DEHP significantly increased the expression of thyroid stimulating hormone beta subunit (tsh β -like), tsh β , deiodinase 1 (dio1), deiodinase 2 (dio2), and thyroid hormone receptor alpha (tr α) and beta (tr β). However, DEHA only significantly increased tsh β at 7.4 μ g/L but significantly decreased dio2 expression at 25.8, 111.1, and 412.6 μ g/L, while other genes were not significantly affected. Both plasticizers reduced eye size and total body length, however did not affect embryo development, hatching time and rate, and swimming performance. DEHA alone affected swim bladder inflation and not DEHP. This is the first report that not only DEHP but also DEHA disrupt thyroid hormone activity in fish (Horie et al., 2022; *Aquatic Toxicology*, doi.org/10.1016/j.aquatox.2022.106312).

We also evaluated the expression levels of thyroid hormone, reproduction and estrogen responsive genes of Japanese medaka (*Oryzias latipes*) exposed to bis(2-ethylhexyl) sebacate (DEHS). The gene expression levels of thyroid-stimulating hormone subunit beta (tsh β), deiodinase 1 (dio1), and thyroid hormone receptor alpha (tr α) did not change. Whereas, DEHS suppressed dio2 expression, did not induce swim bladder inflation, and eventually reduced the swimming performance of Japanese medaka. These findings indicate that DEHS can potentially disrupt the thyroid hormone-related gene expression and fish metabolism. This is the first report providing evidence that DEHS can disrupt thyroid hormone-related metabolism in fish (Horie et al., 2023; *Comparative Biochemistry and Physiology, Part C*, doi.org/10.1016/j.cbpc.2022.109531).

In another study, we exposed Japanese medaka to diisobutyl adipate (DIBA) to assess the development toxicity during fish embryogenesis. At 11.57 mg/L, an increased embryonic mortality was witnessed. While abnormal development, including body curvature, edema, and lack of swim bladder inflation, were observed at 3.54 and 11.57 mg/L. Growth inhibition and reduced swimming performance were also observed. In addition, DIBA exposure increased the levels of thyroid-stimulating hormone beta-subunit (tsh β) and deiodinase 1 (dio1) but decreased the levels of thyroid hormone receptor alpha (tr α) and beta (tr β). These results indicate that DIBA has low estrogenic activity and does not disrupt the endocrine reproduction system in fish. Overall, this is the first report indicating that nonphthalate DIBA plasticizer is embryotoxic and disrupt thyroid hormone activity in fish (Horie et al., 2023; *Journal of Applied Toxicology*, in press).

(2) 主な研究発表 (雑誌論文、学会、集会、知的財産権等) / Main Research Publications

1. Yoshifumi Horie, Babu Rajendran Ramaswamy, Juan Manuel Ríos, Chee Kong Yap, Hideo Okamura (2023). Effects of plasticizer diisobutyl adipate on the Japanese medaka (*Oryzias latipes*) endocrine system. *Journal of Applied Toxicology* (accepted) (IF 3.446).
2. Yoshifumi Horie, Miho Nomura, Babu Rajendran Ramaswamy, Hiroya Harino, Chee Kong Yap, Hideo Okamura (2023). Effects of non-phthalate plasticizer bis(2-ethylhexyl) sebacate (DEHS) on the endocrine system in Japanese medaka (*Oryzias latipes*). *Comparative Biochemistry and Physiology, Part C* 264: 109531 (IF 4.52)
3. Yoshifumi Horie, Miho Nomura, Babu Rajendran Ramaswamy, Hiroya Harino, Chee Kong Yap, Hideo Okamura (2022). Thyroid hormone disruption by bis-(2-ethylhexyl) phthalate (DEHP) and bis-(2-ethylhexyl) adipate (DEHA) in Japanese medaka *Oryzias latipes*. *Aquatic Toxicology* 252: 106312 (IF 5.202).

(3) その他/Remarks

1. Research presentation on 'Mass Spectrometric Determination of Emerging Chemical Contaminants from the Indian Rivers' in 'The 25th JSWE Symposium' held at University of Tokyo, Japan during September 6-7, 2022.
2. Delivered a Special Lecture on 'Environmental Forensics of Emerging Chemical Contaminants' at Kobe University Fukae Campus, Japan on September 28, 2022.
3. Awarded International Associate Member of the Japan Society on Water Environment (JSWE) for the years 2022 and 2023.

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