Pesticide Toxicity in the Larvae of Japanese Flounder, *Paralichthys olivaceus*[*1]

Maria S. MENENDEZ[*2] and Atsushi ISHIMATSU

Acute toxicity of diazinon and MEP was assessed for larvae and juveniles of Japanese flounder. The LC_{50} values obtained for diazinon ranged from 0.86 to 2.65 ppm, 0.50 to 1.73 ppm and 0.20 to 0.82 ppm at 24, 48 and 96 hours, respectively. MEP showed similar toxicity to those fish for which the LC_{50} values were 0.74 to 1.89 (24 h), 0.50 to 1.24 (48 h), and 0.26 to 0.86 (96 h). Pre-metamorphosis larvae were generally more susceptible to either pesticide.

Key words: *Paralichthys olivaceus*; diazinon; MEP; acute toxicity; LC_{50}.

Pollutants from industry, agriculture and other human activities are continually coming into the sea through sewage. Their presence in the environment has become increasingly obvious to the extent that mass mortalities of aquatic organisms due to pollution frequently happen all around the world. Large numbers of researches have already been conducted to assess toxicity of environmental pollutants on aquatic organisms, but the results are often complicated by the fact that pollutant toxicity can be largely affected by biological as well as environmental variables.

To evaluate effects of a pollutant on the whole reproductive cycle of a certain species, it is essential to establish which stage of the species is most susceptible to that substance. In spite of its potential vital importance, such notions have not always been taken in the studies of toxicity test on animal species. Without such information, however, one may easily underestimate actual effects of pollutants on wild fauna by selecting animals of more tolerant life stages for practical reasons such as easier accessibility of the material. A few studies have revealed that fish juveniles have a higher sensitivity to pollutants than adults[1-4].

Japanese flounder *Paralichthys olivaceus* is one of the most commercially important fish species in Japan. Like other species of flounders, this fish undergo metamorphosis during its early life history, experiencing transition from pelagic to benthic life style. It is conceivable that sensitivity to pollutants varies during this period. This study was aimed at providing quantitative data concerning possible changes in sensitivity of *P. olivaceus* to two pesticides, diazinon (dimethyl 2-isopropyl-4-methyl-6-pyrimidinyl phosphorothionate) and MEP (dimethyl 4-nitro-*m*-tolyl phosphorothionate), during early life.

Diazinon and MEP are the representative members of organophosphate pesticides. These two chemicals are known to degrade more rapidly in the environment than other pesticides such as organochlorine pesticides. Nevertheless, their environmental levels may be higher because they are more frequently used in larger amounts than the other pesticides[5], making them more liable to contaminate aquatic milieu.

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Materials and Methods

Fish

Fish eggs were generously offered by Environmental Tree Planting Company (Takehara, Hiroshima) and Japan Farming Association, Miyazu Station (Miyazu, Kyoto). Eggs were incubated at 16-18 °C in well aerated running sea water. Feeding started 2-3 days after hatching using rotifers as the initial feed. When fish reached total length of 8 mm, they were fed brine shrimp nauplii. This feed was subsequently replaced by minced sardine and pellet (Kyowa Hakko Kogyo Co., Ltd.) when total length was 15 mm. Fish were reared at Nomo Fisheries Station, Nagasaki University.

Chemicals

Forty percent solution of diazinon and 50 % solution of MEP were purchased from Japan Pharmacological Co. Ltd. and diluted to the concentrations of 1,000 and 10,000 ppm for stock solutions, respectively. These solutions were stored at 4 °C in the dark.

Procedures

Acute toxicity tests were conducted to obtain LC_{50} value up to 96 h. Sea water used for the test was obtained from the coast of the station, filtered, aerated and stored for 24 h before use. About 24 h before experiment, fish were transferred from holding tanks to experimental containers (10 fish per container). Care was taken to avoid unnecessary exposure of the fish to air. We used pipettes for small individuals to keep them in water during transfer. Food was withheld during acclimation and experimental periods. Water volume was adjusted to keep the ratio of fish body weight to water volume below 0.3 g/l. We used one-liter glass beakers as containers for fish below 30 mg of body weight and 10 liter aquaria for larger fish. After 24 h of acclimation, various volumes of the stock solutions were added to containers to make exponential concentration gradient with the ratio of 1.33. Tests were always done in duplicate. In case where mortality of control groups exceeded 10 %, all data were discarded for that particular run. Ninety percent of test water was renewed every 24 h and pollutant concentrations were adjusted appropriately. Dead fish were immediately removed when found. LC_{50} values were estimated by the method of Litchfield and Wilcoxon[6].

Water temperature of the holding tanks gradually increased from 16 °C to 25 °C during the experimental period of April to June. Water temperature of experimental containers was kept to the temperature of the holding tank at that time. Dissolved oxygen concentration always remained high (4.7-8.1 mgO_2/l) due to frequent water renewal. Salinity was 33 ‰ throughout the study.

Results

Fish Growth

Figure 1 shows changes in body weight (top)

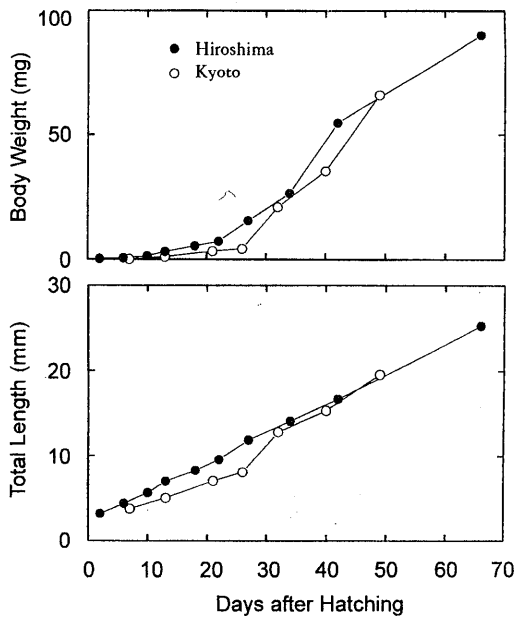

Fig. 1. Growth of *P. olivaceus* used in this study. Solid circles indicate fish hatched from eggs obtained from Hiroshima, open ones are from Kyoto.

and total length (bottom) of *P. olivaceus* after hatching. The two groups of fish showed nearly same growth during experimental period of about 70 days. Metamorphosis began 10-12 days after hatching and was completed 48-49 days after hatching.

Changes in LC_{50}

Figure 2 demonstrates changes in 24h (top), 48h (middle) and 96h (bottom) LC_{50} values of diazinon with fish growth. For 24h and 48h values, LC_{50} showed a slight transient decline prior to metamorphosis. The values then increased during metamorphosis and subsequently leveled off. These trends could not be seen for 96h values because of the limited data available. Pre-metamorphosis larvae were in general more susceptible to diazinon with 24h LC_{50} values being 60% of post-metamorphosis values.

Figure 3 illustrates the results for MEP. Similar, though less pronounced, patterns of changes in LC_{50} were found for this pesticide as for diazinon in that lower values were obtained for pre-metamorphosis fish.

Figures 4 and 5 show relationships between LC_{50} values and exposure period at each fish size for diazinon and MEP, respectively. It can be seen that LC_{50}'s had become stabilized by 96 h at most of the fish size tested.

Reproducibitiy of the test

Differences in estimated LC_{50} values obtained from duplicate runs at each fish size were 0.06 ± 0.048 (SD, N=26) for diazinon and 0.09 ± 0.046 (N=29) for MEP, overall difference being 0.08 ± 0.067 (N=55).

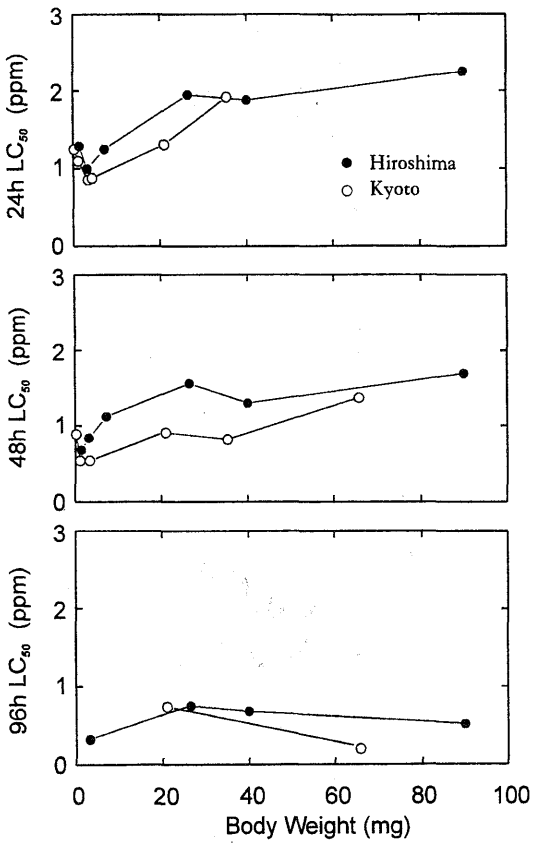

Fig. 2. Changes in median lethal concentration (LC_{50}) of diazinon with growth of *P. olivaceus*. Symbols are the same as in Fig. 1.

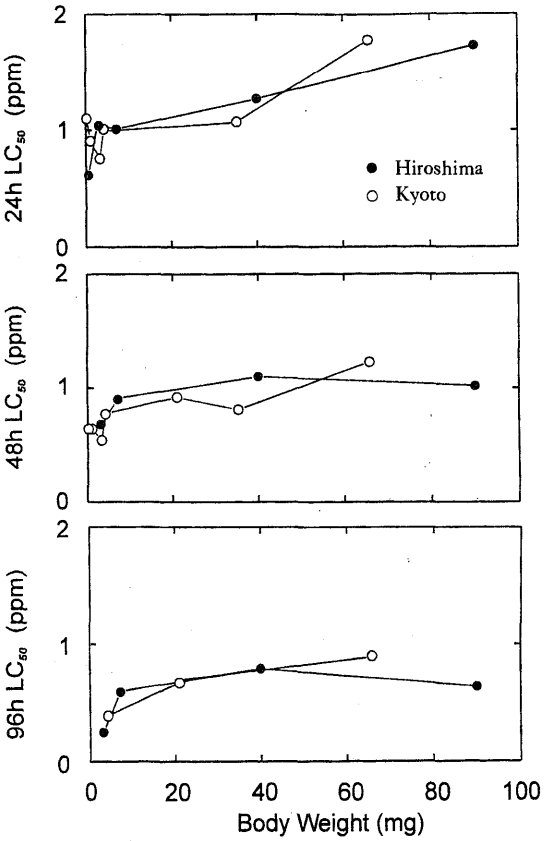

Fig. 3. Changes in median lethal concentration (LC_{50}) of MEP with growth of *P. olivaceus*.

Discussion

Organophosphates have been known to cause reduction in cholinesterase activity by short-term exposure, acting as nerve poisons by blocking synaptic transmission in the cholinergic neurons[7]. Symptoms like uncoordinated swimming and convulsions were observed in the course of this study. Kanazawa[8] reported that fish quickly take up diazinon but only slowly metabolize this pesticide, resulting in deformities like spinal curvature. Hirose and Kitsukawa[9] also reported a similar vertebral damage in fish exposed to diazinon. We found vertebral abnormalities in the larvae exposed to either pesticide while small size of the fish made it sometimes difficult to detect vertebral damages.

In general, smaller larvae of *P. olivaceus* showed higher sensitivities to both diazinon and MEP than larger post-metamorphosis fish. Similar trends were reported for other fish species as well. Thus, larvae of *Acanthopagrus schlegeli, Casmichthys dolichognathus, Girella punctata*, and *Pagrus major* showed lower LC_{50} values than larger fish for MEP, while those of *A. schlegeli, C. dolichognathus, G.punctata*, and *Mugil cephalus* exhibited same trends for cadmium (Cd)[4]. *P. major* showed a transient decrease in LC_{50} of Cd at body weight of 200-500 mg while larger values were found for smaller larvae[4]. These results emphasize the importance of finding life stages most susceptible to toxicants when one is willing to evaluate influences of toxicants on whole reproductive cycles of fish.

On the other hand, using very small fish

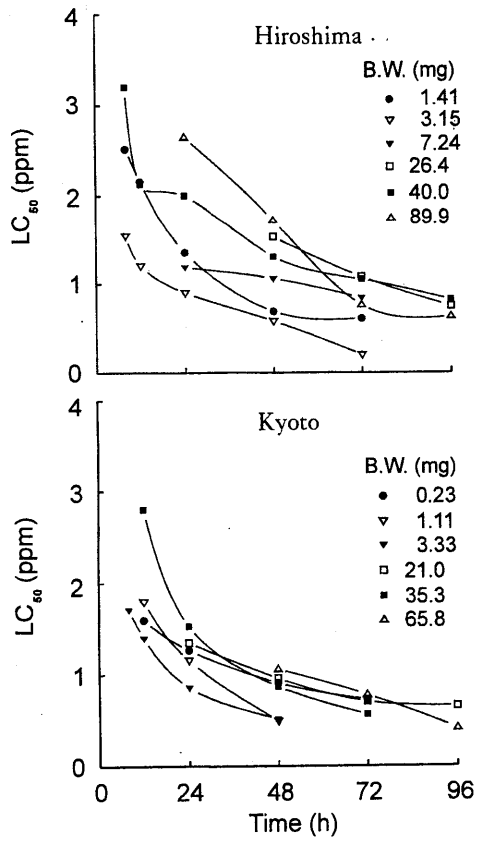

Fig. 4. Relationship between LC_{50} of diazinon and exposure period at different body weights of *P. olivaceus*.

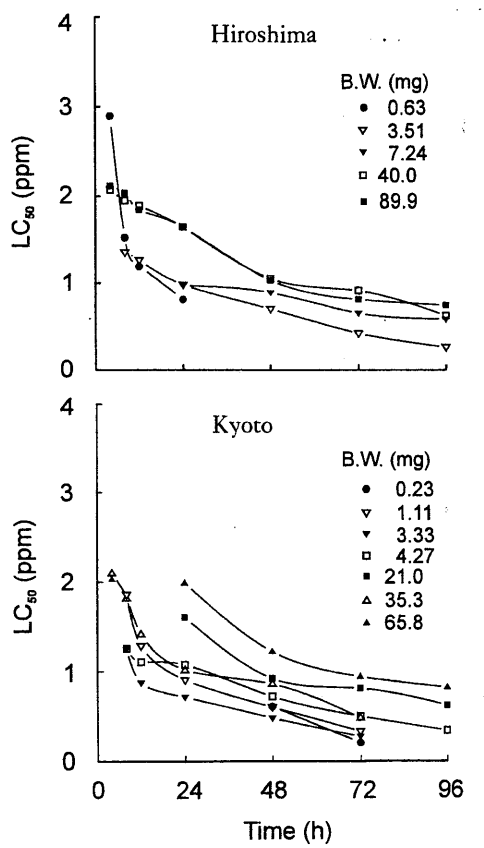

Fig. 5. Relationship between LC_{50} of MEP and exposure period at different body weights of *P. olivaceus*.

(larvae and early juveniles) is not always feasible at many laboratories, particularly if seawater species is considered. For that purpose, one must maintain many small fish in good conditions, which requires at least plenty supply of good quality sea water and stable supply of feed organisms. These requirements cannot always be met due to, for example, location of the laboratory and unavailability of facilities. Moreover, these smaller fish are generally more sensitive to stresses like handling, rendering experimental results less accurate and reproducible. We experienced technical difficulties in handling larvae and juveniles during preliminary experiments, and it was only after some experience that our results became reasonably reproducible. Thus, although it is important to realize that fish larvae and juveniles are generally more sensitive than larger ones that are usually used for toxicity tests, using fish of the most susceptible stage is not always practical and recommendable. Therefore, we suggest that relationship between susceptibility to a pollutant and fish size should be established for test fish species in experienced laboratories and the ratio of LC_{50} value for the most sensitive stage and that for more commonly used size is to be taken into consideration to assess overall toxicity to the species.

Toxicity of organophosphates is known to positively correlate with temperature[10-12]. The present results obtained for the two groups of fish agreed well for both diazinon and MEP in spite of temperature difference of about 3 °C between the tests (Kyoto > Hiroshima) (Figs. 2 and 3). This suggests that temperature dependence of these pesticides was moderate and temperature fluctuation of that magnitude exerted little, if any, effect on the result.

Figures 4 and 5 demonstrate that LC_{50} values more or less stabilized as exposure period approached 96 h. Although chronic effects of these pesticides on *P. olivaceus* should be evaluated by long-term observation, these 96 h values can be used as a first approximation for chronic LC_{50} values for this species.

It is generally accepted that crustaceans like crabs and shrimps are more susceptible to the pesticides than fish[13-15]. Therefore, threat to a local ecosystem can be substantial even if environmental concentrations of the toxicants are low enough for fish. For the better control of environmental pollution, we must further investigate overall impacts of pollutants considering these ecological relationships.

Acknowledgments

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ヒラメ仔魚に対する2種農薬の急性毒性

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孵化直後から約70日齢までのヒラメ仔魚に対する有機燐系農薬2種，ダイアジノンおよびMEPの急性毒性について検討した．ダイアジノンによる半数致死濃度(LC_{50})は，0.86－2.65 ppm (24時間値)，0.50－1.73 (48時間値)，0.20－0.82 (96時間値) であった．MEPのLC_{50}値もほぼ同じ値を示した．いずれの農薬についても変態前仔魚は，変態後の個体よりも低いLC_{50}値を示した．これらの結果より，通常毒性試験に使用されるサイズの個体を用いた毒性試験のみによっては，種の生活史全体に対するこれら汚染物質の影響を過小評価する危険性があることが指摘された．