卅二、Biochemical Approaches on the Evaluation of Hazards of Chemicals on Aquatic Organisms

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ABSTRACT

Fish and shellfishes which live in the coastal areas receiving agricultural and industrial wastewaters have been exposed to various chemicals including degradation products. It is quite difficult, however, to identify and determine the various harmful chemicals in the aquatic environment.

Even though the concentrations of harmful chemicals are low so that no mortality of fish and shellfishes occurs, the chemicals may affect biochemically and physiologically on aquatic organisms.

1. Induction of drug-metabolizing enzyme activity in fish and shellfishes

The cytochrome P-450 and monooxygenase (MO) activity in fish are induced by some environmental pollutants such as polycyclic aromatic hydrocarbons, halogenated organic chemicals and so on. The sulfate conjugating activity in bivalaes are also induced by phenolic compounds.

2. Evaluation of marine pollution by drug-metabolizing enzyme activity in fish and shellfishes

The results in the above studies suggest that levels of the MO activity in fish and the sulfate conjugating activity in bivalves may be useful as a biochemical monitor for marine pollution.

By the comparison with the enzyme activity in fish and shellfishes from industrial and non-industrial areas, it has been demonstrated that the cytochrome P-450 content and the activity of aromatic hydrocarbonhydroxylase, O-demethylase and UDPglucuronosyltransferase in flatfish, and also the sulfate conjugating activity in short-necked clam and mussel are quite available as the biochemical monitors for the evaluation of marine pollution.

3. Effects of induced monooxygenase activity on the metabolism of hormone and drug in fish

It has been demonstrated that PCB, a potent inducer of MO activity, reduces the levels of sex steroids (estrone and estradiol) in plasma, resulting in the reduction of the gonadal maturation in fish.

PCB treatment also reduces the maximum concentration and duration

of oxolinic acid in fish tissues after oral administration, which has been used in large quantities as a fish drug in Japan.

Coastal waters are very important areas for fisheries, not only as fishing grounds but as spawning and nursery grounds for fish and shellfishes. However, the abrupt developments in industrialization, agricultural practices and urbanization have given large environmental impacts to the coastal waters.

Fish and shellfishes which live in the coastal areas receiving agricultual, industrial and domestic wastewaters have been exposed to various chemicals including degradation products. It is quite difficult, however, to identify and determine the various harmful chemicals in the environments and also accumulated in aquatic organisms. Even though the concentrations of contaminants in the environments are low so that no mortality of fish and shellfishes occurs, the chemicals may affect biochemically and physiologically on aquatic organisms.

It has been demonstrated recently that the activities of some drug-metabolizing enzymes, especially monooxygenase (MO), in fish livers considerably increase when they are exposed to environmental pollutants, such as polycyclic aromatic hydrocarbons, halogenated organic chemicals and so on. Some studies have been done on the field evaluation of MO induction as a monitor for marine pollution.

This study aims to elucidate the relationship between the activities of various drug-metabolizing enzymes concerning not only oxidation but also conjugation which is easily induced in aquatic organisms and their polluted environments. The study also has been done on the effects of induced MO activity on the metabolism of hormone and drug in fish.

I. Induction of drug-metabolizing enzyme activity in fish and shellfishes

Fig. 1 shows a schematic view of the absorption, biotransformation and excretion of xenobiotics in fish. The xenobiotics absorbed by fish through branchial and oral routes are metabolized by phase I and II reactions and then

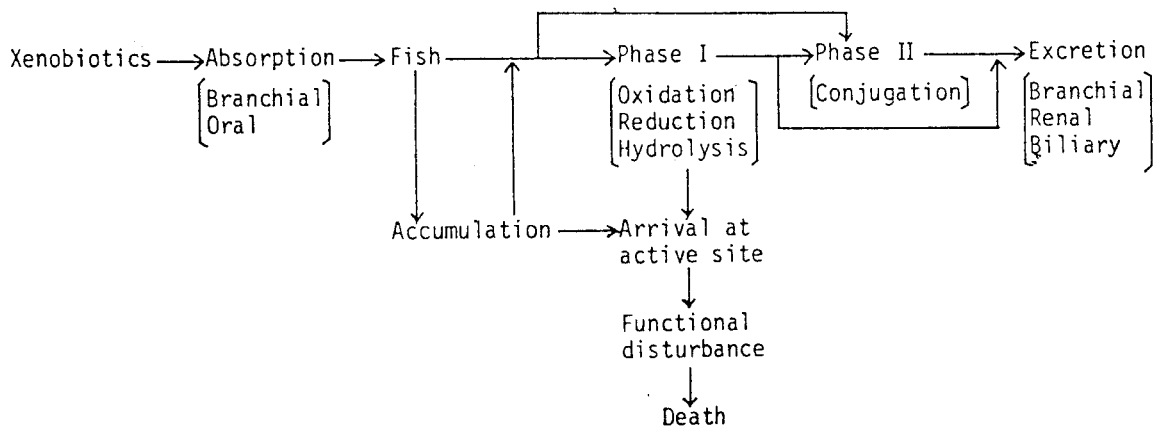

Fig. 1. A schemattic view of the absorption, biotransformation and excretion of xenobiotics in fish.

excreted by the branchial, renal and biliary routes. Lipophilic substances are accumulated in adipose tissues.

After cell fractionation of fish livers, the microsomal preparations were subjected to the determination of cyt. P-450 content and NADPH cyt. c reductase, NADH ferricyanide reductase, aniline and benzo(a)pyrene hydroxylase, *p*-nitroanisole *O*-demethylase and aminopyrine *N*-demethylase activities in the usual way, as shown in Fig. 2.

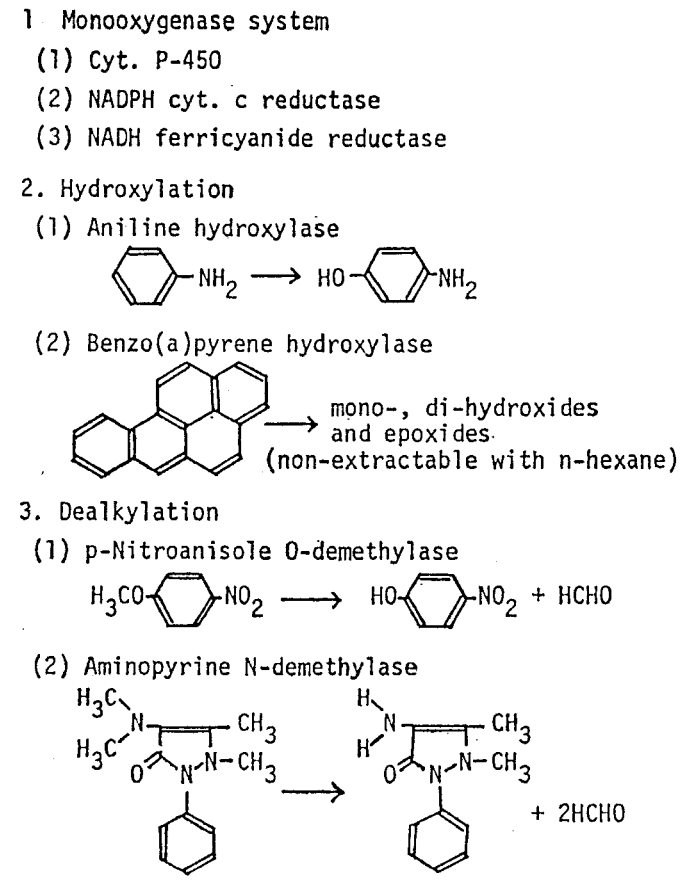

Fig. 2. Assay of xenobiotic oxidation activity in microsomes.

Fig. 3 shows the procedures for the assay of the activities of glycerophosphate and *p*-nitrophenyl phosphate hydrolysis, and glucuronide, sulfate and glutathione conjugations.

Although the mixed function oxidases (MFO) activity in fish liver is usually very low compared with that in mammals, it is induced by some chemicals, as mentioned previously. Payne and Penrose reported that the aryl hydrocarbon hydroxylase (AHH) activity in brown trout liver increased *ca.* 3.5 times after exposure to 1 ppm petroleum for 16-17 days, and that in capelin liver increased twice and five times after one and two weeks, respectively (Table 1).

They also reported that the AHH activity in trout from oil polluted lake was approx, 13 times that in trout from non-polluted lake (Table 2). Then, Payne

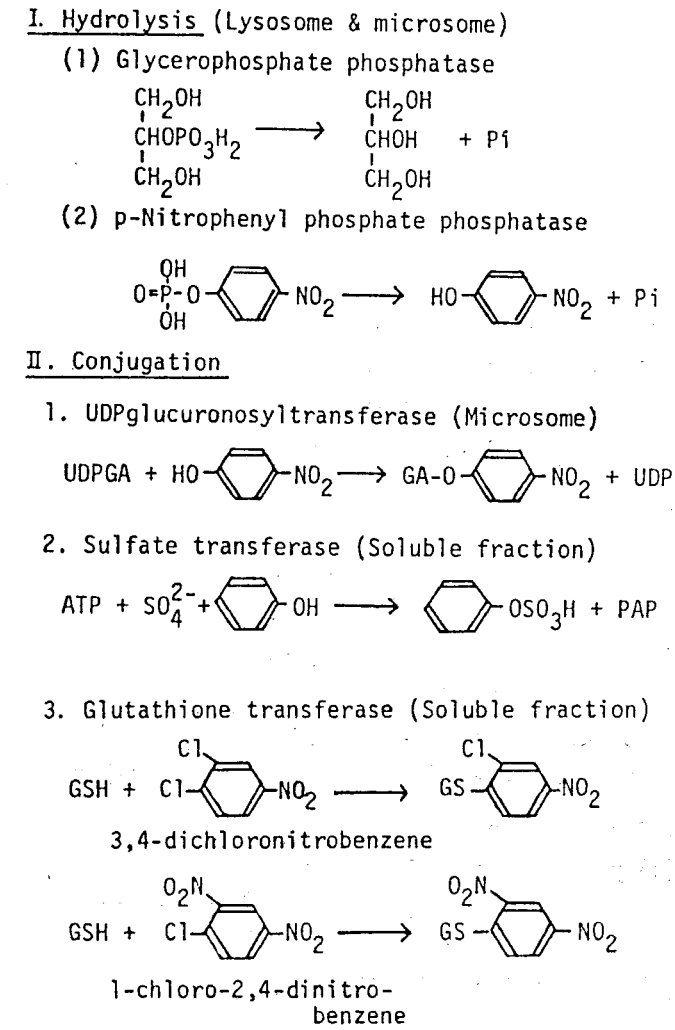

Fig. 3. Assay of xenobiotic hydrolysis and conjugation activity.

Table 1. Induction of aryl hydroxylase (AHH) in fish by petroleum (ca. 1 ppm)

Species	Exposure (days)	AHH specific activity in liver (U/mg protein)	
		Control	Oil treated
Trout	0	102	
	16-17	68±14	240±88
Capelin	0	29	
	7-8	27±17	58±17
	15-16	27±11	131±34

(1976) applied the induction of AHH activity in fish to monitoring marine petroleum pollution. The availability of AHH activity as a monitor for oil pollution has been confirmed by several scientists.

However, they confined their attention to the AHH induction. Therefore, we

Table 2. AHH in trout from non-polluted and polluted lakes

	AHH in trout liver (U/mg protein)
Non-polluted lake	27±19
Oil polluted lake	362±51

[J. F. Payne & W. R. Penrose: Bull. Environ. Contam. Toxicol., **14**, 112-116 (1975)]

tried to confirm the availability of other enzyme activities including conjugation as a monitor for marine pollution.

Fig. 4 shows the induction of the cyt. P-450 content in hepatic microsomes of carp by dietary administration (0.1 mg/100 g-b. w./day) of PCB, 3-methylcholanthrene (3-MC), furazolidone (FZD), BHC, DDT, pentachlorophenol (PCP) and fenitrothion (MEP) for 10 days. Among the seven chemicals used in this experiment, PCB showed the highest induction of cyt. P-450 content, followed by 3-MC and FZD, BHC, DDT, PCP and MEP did not induced.

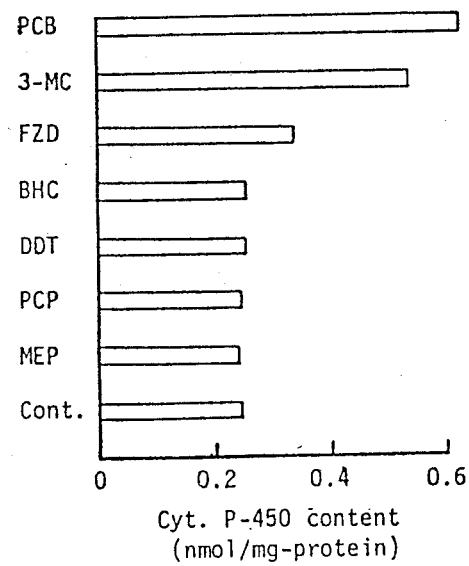

Fig. 4. Induction of the cytochrome P-450 content in hepatic microsomes of carp by dietary administration (0.1 mg/100 g-body weight/day) of octachlorobiphenyl (PCB), 3-methylcholanthrene (3-MC), furazolidone (FZD), γ-BHC, p,p'-DDT, pentachlorophenol (PCP) and fenitrothion (MEP) for 10 days.

Then we studied on the induction of drug-metabolizing enzymes by a long-term administration of PCB-diet and also the duration of their induced activities in the hepatopancreas of carp.

Fig. 5 shows induction of the cyt. P-450 content and the activities of benzo(a)pyrene hydroxylase and *p*-nitroanisole O-demethylase, and duration of the induced content and activities after discontinuance of PCB administration. The cyt. P-450 content in PCB-group increased *ca.* 2 times that in control at 2-

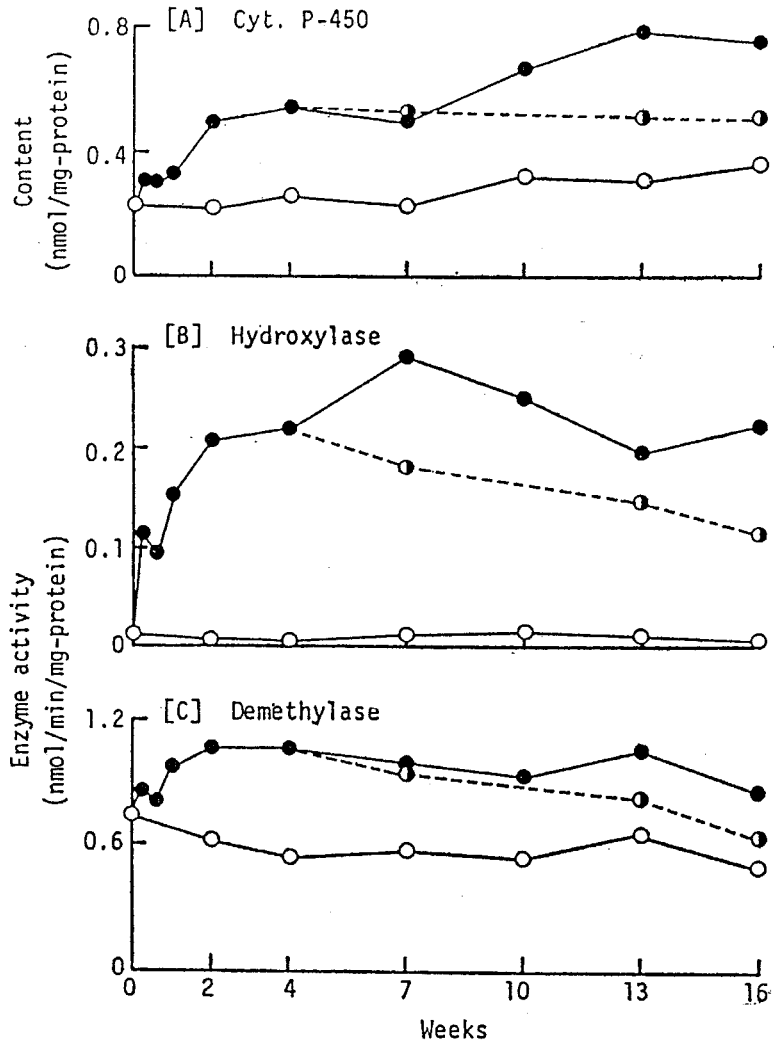

Fig. 5. Induction of the cytochrome P-450 content [A] and the activities of benzo (a) pyrene hydroxylase [B] and *p*-nitroanisole *O*-demethylase [C] in hepatic microsomes of carp by dietary administration of PCB (0.05 mg/100 g body weight/day), and duration of the induced content and activities after discontinuance of PCB administration.
—●— PCB-group ---◐--- Retention-group —○— Control-group

week, and this ratio continued until the end of this experiment. The retention-group which was discontinued PCB administration after 4 weeks, also maintained the induced level at 4-week until the end of this experiment (Fig. 5-A).

The benzo(a)pyrene hydroxylase (AHH) activity abruptly increased by PCB treatment and reached a level of 22-fold with that in the control at 2-week. After 2-week period, however, almost no further increase of this enzyme activity was observed in spite of the continuous administration of PCP-diet until 16-week. The result suggests some limitation of the induction of this enzyme. Although the induced AHH activity in the retention-group gradually decreased after discontinuance of PCB administration, it was still at a high level as much as 13-fold with that in control even after 12 weeks (Fig. 5-B).

The activity of *p*-nitroanisole *O*-demethylase was also increased in a pattern similar to that in cyt. P-450 content (Fig. 5-C). The glucuronide and glutathione conjugating activities were also induced by PCB treatment at a similar ratio to that in *O*-demethylase activity. However, the apparent induction of NADPH cyt. c reductase, NADH cyt. b5 reductase and aminopyrine *N*-demethylase was not observed.

All the subcellular fractions of bivalve mid-gut glands did not show any MFO activity, but its cytosol fraction displayed the sulfate and glutathione conjugating activities at the comparable levels to that of fish livers.

Fig. 6 shows the induction of the phenol-sulfate conjugating activity in the cytosol of short-necked clam mid-gut gland by 3-day exposure to pentachlorophenol (PCP), resorcinol, *p*-cresol, *p*-chlorophenol, *p*-nitrophenol and phenol at 0.1 ppm, respectively. Among the tested phenols, PCP was the most effective inducer of this conjugating activity of the clam, resulting in an increase of the activity by approx. 2.4-fold with that in the control. Therefore, a subsequent experiment was performed to elucidate the induction of that activity in the clam by a long-term exposure to PCP and also the duration of its induced activity.

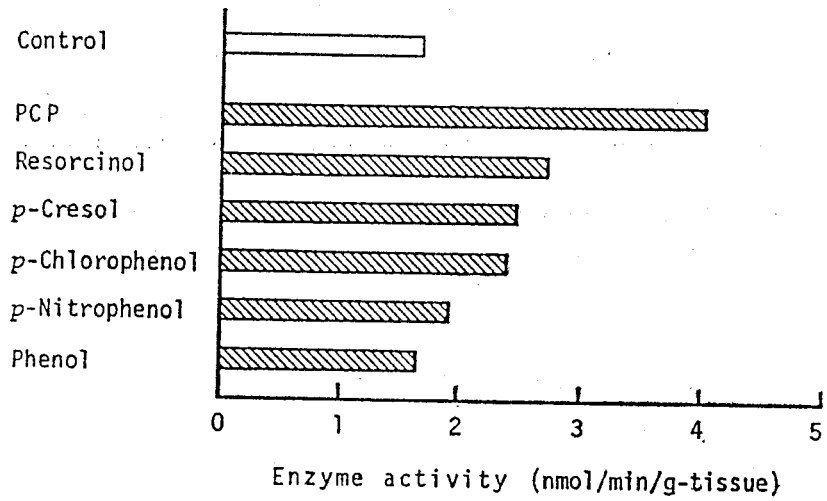

Fig. 6. Induction of the phenol-sulfate conjugating activity in the cytosol of short-necked clam mid-gut gland by 3-day exposure to pentachlorophenol (PCP), resorcinol, *p*-cresol, *p*-chlorophenol, *p*-nitrophenol and phenol at 0.1 ppm, respectively.

As shown in Fig. 7, the conjugating activity in the PCP-group clams increased almost linearly with the exposure time for 5 weeks and reached finally *ca.* 7-fold with that in the control. The activity in the retention-group clams which had been induced to a level of 24 nmol/min/g-tissue by 2-week exposure, gradually decreased after transferring the clams to PCP-free sea water, resulting in 18, 11 and 10 nmol/min/g-tissue at 1-, 2- and 3-week periods, respectively.

However, the duration of the sulfate conjugating activity induced by PCP-exposure was much longer as compared with the biological half-life of PCP

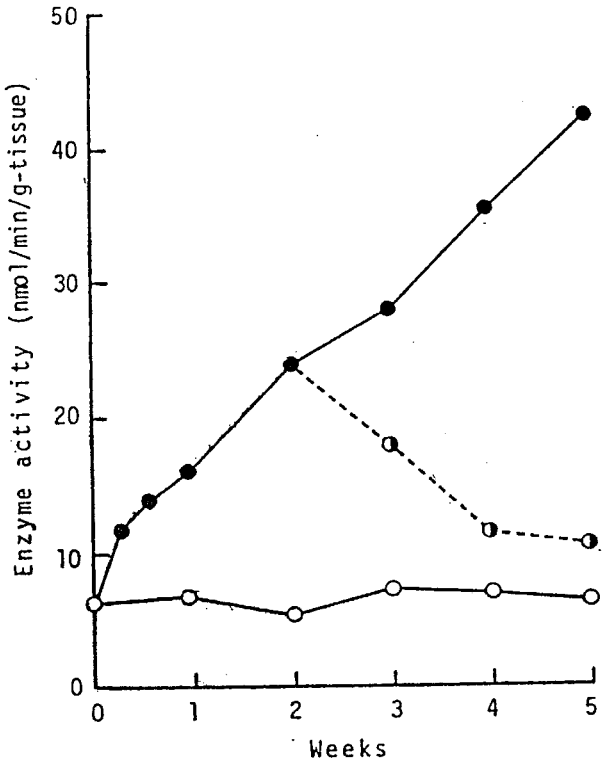

Fig. 7. Induction of the phenol-sulfate conjugating activity in the cytosol of short-necked clam mid-gut gland by exposure to 0.1 ppm PCP for 5 weeks, and duration of its induced activity after discontinuance of PCP-exposure.
—●— PCP-group —◑— Retention-group —○— Control-group

accumulated in the clam, because the time for decrease to one-half the conjugating activity was *ca.* 2 weeks, while the biological half-life of PCP in the clam was *ca.* 1 hour.

II. Evaluation of marine pollution by drug-metabolizing enzyme activity in fish and shellfishes

The results in the above studies suggest that the levels of the MO activity in fishes and the sulfate conjugating activity in bivalves may be useful as a biochemical monitor for marine pollution.

Fig. 8 shows a map of the sampling stations of flatfish (Makogarei; *Limanda yokohamae*), short-necked clam (Asari; *Ruditapes philippinarum*) and mussel (Murasakiigai; *Mytilus edulis*) which were selected as the suitable species for this investigation in northern Kyushu, because of their low migration and wide distribution.

As shown in Fig. 9, the cyt. P-450 content and NADPH cyt. c reductase activity in fish from Hakata Harbor polluted with petroleum and the industrial aseas such as Ube and Oita were significantly higher than those in fish from non-industrial areas such as Hakata Bay and Buzen, where are considered as non-contaminated sites.

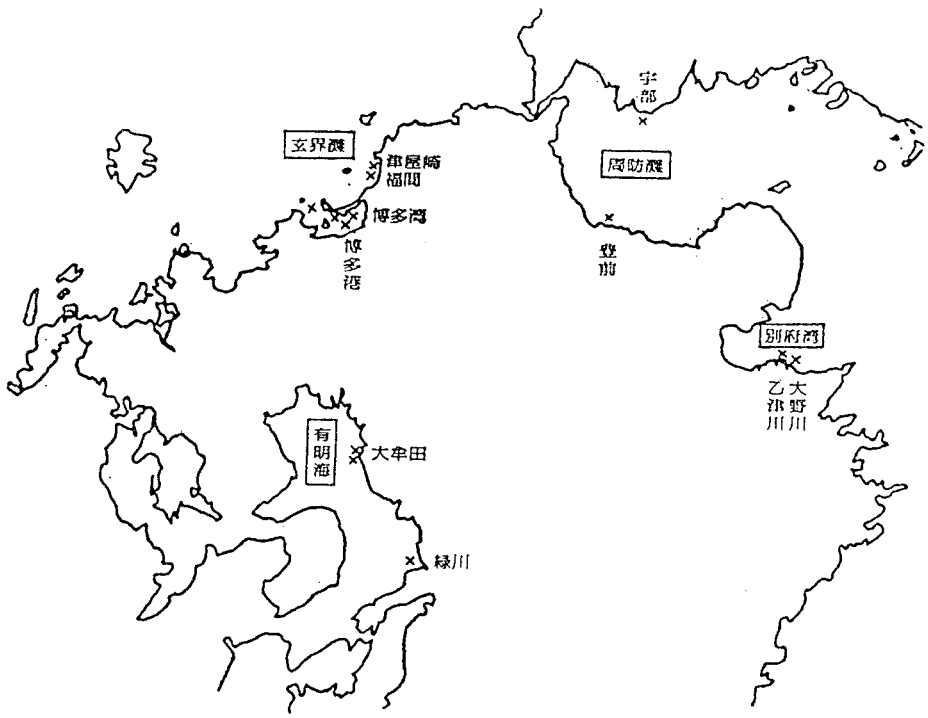

Fig. 8. Sampling stations of flatfish, short-necked clam and mussel for evaluation of marine pollution by their drug-metabolizing enzyme activity. (Northern Kyushu)

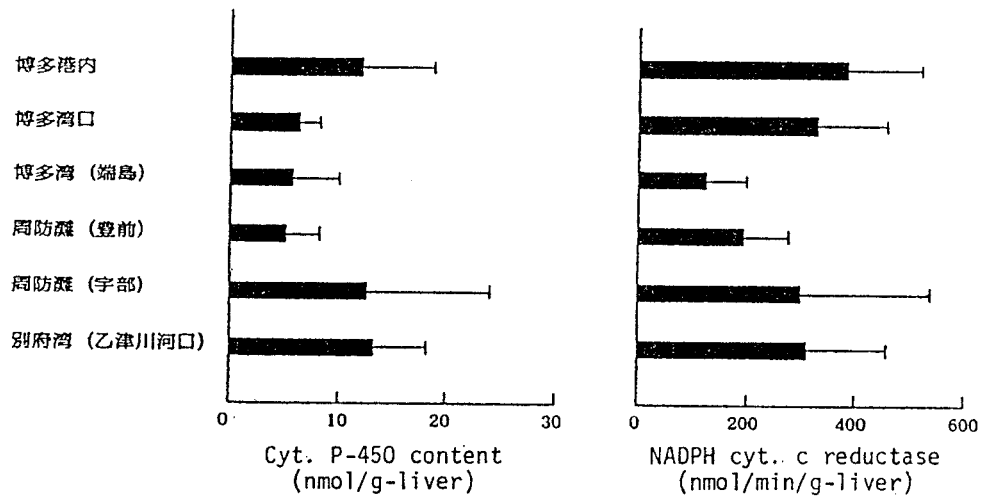

Fig. 9. Regional difference in cyt. P-450 content and NADPH cyt. *c* reductase activity in flatfish liver (Nov., 1984-Jan., 1985).

Fig. 10 shows the activities of benzo(a)pyrene hydroxylase and *p*-nitroanisole *O*-demethylase in the same fish shown in Fig. 9. The regional difference between the industrial and non-industrial areas is much distinguishable in the hydroxylase and *O*-demethylase activities compared with the cyt. P-450 content and NADPH cyt. c reductase activity shown in Fig. 9.

Fig. 11 shows the regional difference in phenol-sulfate conjugating activity in

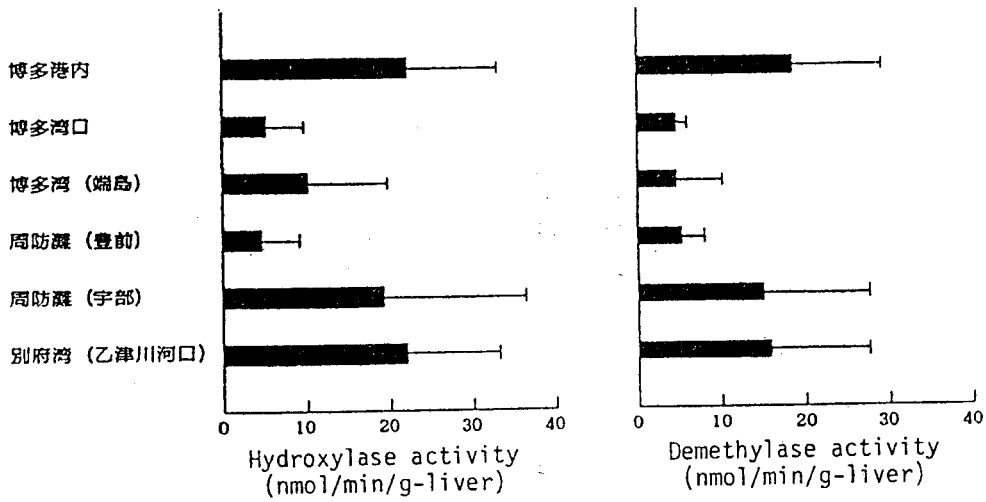

Fig. 10. Regional difference in activity of benzo(a)pyrene hydroxylase and *p*-nitroanisole *O*-demethylase in flatfish liver (Nov., 1984-Jan., 1985).

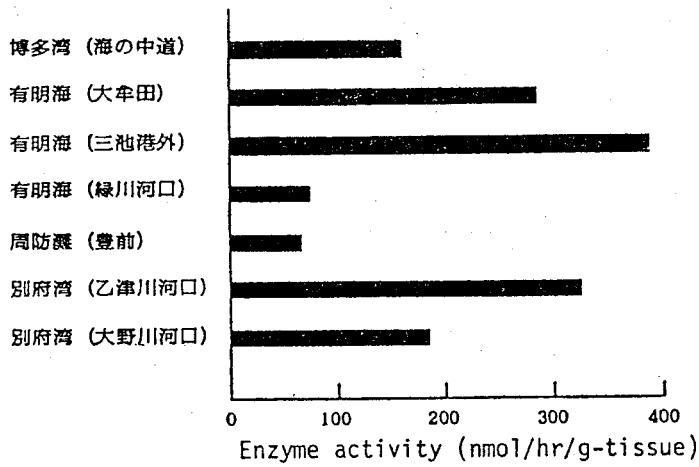

Fig. 11. Regional difference in phenol-sulfate conjugating activity in clam mid-gut gland (Oct.-Nov., 1984).

clam. The clams obtained from tidelands nearby chemical factories in Omuta, the outside of Miike Harbor and Otozu River, showed high sulfate conjugating activities, while the clams from non-industrial coasts such as Midori River and Buzen showed low activities. The high activity in clams suggests that their living area might be polluted with phenolic compounds.

In non-tideland coasts such as reclaimed seasides and harbors, mussel was employed as a test bivalve instead of clam. As shown in Fig. 12, mussels obtained from a petroleum polluted harbor and an industrial area showed a high sulfate conjugating activity as well as clam.

By the comparison with the drug-metabolizing enzyme activity in fish and shellfishes from industrial and non-industrial areas as showh in Figs. 9-12, it has beed demonstrated that the cyt. P-450 content and the activities of AHH and *O*-demethylase in flatfish, and also the sulfate conjugating activity in shortnecked

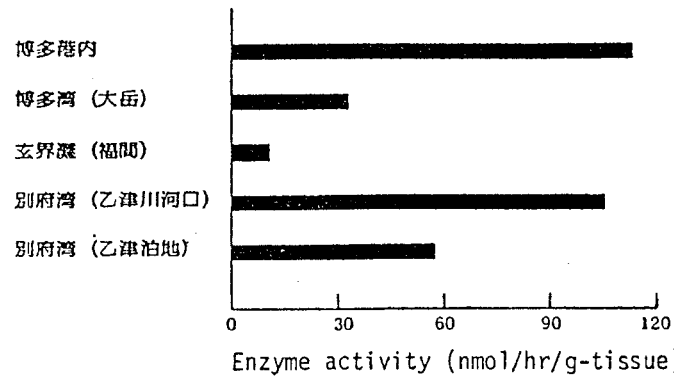

Fig. 12. Regional difference in phenol-sulfate conjugating activity in mussel mid-gut gland (Jun., 1984).

clam and mussel are quite available as the biochemical monitors for the evaluation of marine pollution.

III. Effects of induced monooxygenase activity on the metabolism of hormone and drug in fish

Although a number of studies have been done on the induction of MO activity in fish, the biochemical and physiological effects of the Induced MO activity on fish have been obscured.

Table 3 shows the effects of PCB on cyt. P-450 content and drug-metabolizing enzyme activities in goldfish liver microsomes. PCB was given to goldfish intraperitoneally in corn oil at a dose of 25 mg/100 g b. w., and the fish were sacrified

Table 3. Effect of PCB (Aroclor 1248) pretreatment on cytochrome P-450 content and enzyme activities in goldfish liver microsomes

	Control	PCB-treated
Microsomal protein (mg/g liver)	9.5±1.0	10.2±3.4
Cytochrome P-450 (nmol/mg protein)	0.19±0.07	0.41±0.11
Aminopyrine *N*-demethylase (nmol HCHO produced/mg protein/min)	0.16±0.04	0.34±0.06
p-Nitroanisole O-demethylase (nmol *p*-nitrophenol formed/mg protein/min)	0.24±0.06	0.60±0.13
Bemzo (a) pyrene hydroxylase (nmol/mg protein/min)	0.017±0.006	0.077±0.027
Progesterone hydroxylase	0.054±0.012	0.082±0.035
Estradiol-17β hydroxylase	0.068±0.007	0.085±0.011
Testosterone hydroxylase	0.089±0.006	0.134±0.059
Cortisol hydroxylase	0.0062±0.0008	0.009±0.0027

1) PCB was given i. p. in corn oil at the dose of 25 mg/100 g, and goldfish were sacrificed 6 days after injection.
2) The activity of each steroid hydroxylase is expressed as nmol of polar metablites formed per mg protein per min.
3) Values are mean±SD obtained from 8 to 10 fish.

[H. Matsuyama & T. Yano: Sci. Bull. Fac. Agr., Kyushu Univ., **42**, 1-7 (1987)]

at 6 days after injection. Both the cyt. P-450 content and the demethylase activity increased approx, twice, and benzo(a)pyrene hydroxylase activity *ca.* 5 times by PCB treatment. The steroid hydroxylase activity also increased *ca.* 1.5 times.

Table 4 shows the plasma steroid levels in the same group fish, as shown in Table 3. The levels of progesterone, estradiol and testosterone except cortisol reduced to 40, 40 and 73% of control, respectively.

Table 4. Effect of PCB (Aroclor 1248) pretreatment on plasma steroid levels of goldfish

	Control	PCB-treated
Progesterone (pg/ml plasma)	387±190	156±75
Estradiol-17β (pg/ml plasma)	128±57	50±18
Testosterone (ng/ml plasma)	1.32±0.51	0.96±0.40
Cortisol (ng/ml plasma)	159±39	166±64

1) PCB was given i.p. in corn oil at a dose of 25 mg/100 g, and goldfish were sacrificed 6 days after injection.
2) Values are mean±SD obtained from 10 fish.
[H. Matsuyama & T. Yano: Sci. Bull. Fac. Agr., Kyushu Univ., **42**, 1-7 (1987)]

Fig. 13 shows the effect of PCB administration on estrone and estradiol excretion in medaka (*Oryzias latips*). The amount of estradiol excreted into water remarkably decreased in PCB-treated fish. The decrease of estradiol excretion might be due to its lowered plasma level in fish, as shown in Table 4.

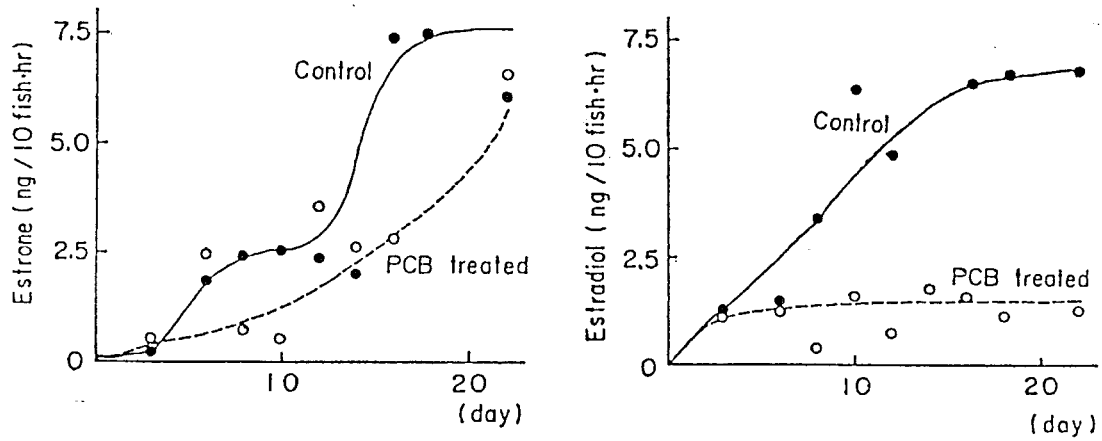

Fig. 13. Time course of effect of PCB administration on estrone and estradiol excretion in medaka. [H. Ando & T. Yano: Sci. Bull. Fac. Agr., Kyushu Univ., **36**, 79-82 (1982)]

Fig. 14 shows the reduction of GSI (gonado-somatic index) in medaka by PCB administration. This suggests that the induction of MO activity by environmental pollutants presumably affects on the fish reproduction, resulting in the decrease of fisheries resources.

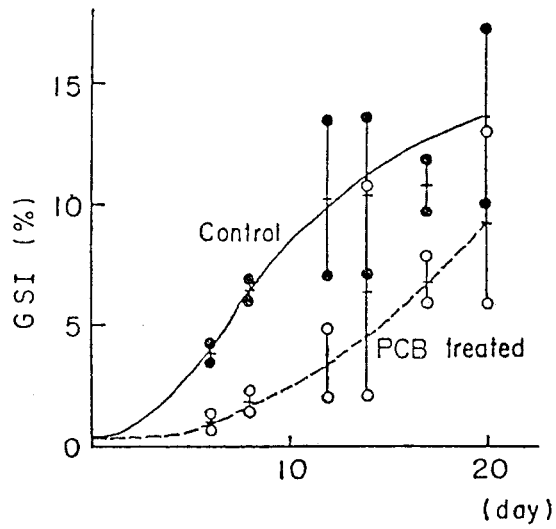

Fig. 14. Effect of PCB administration on gonadal maturation in medaka. [H. Ando & T. Yano: Sci. Bull. Fac. Agr., Kyushu Univ., **36**, 79-82 (1982)]

Table 5 shows the induction of drug-metabolizing enzyme activity in carp by dietary administration of PCB (0.1 mg/100 g-b. w./day) for 14 days. The cyt. P-450 content, benzo(a)pyrene hydroxylase and glutathione conjugating activities increased *ca.* 3, 18 and 2.4 times respectively those of control by PCB administration.

Table 5. Induction of drug-metabolizing enzyme activity in carp hepatopancreas by dietary administration of PCB (0.1 mg/100 g-b. w./day) for 14 days

Enzyme	Control	PCB-treated
Cyt. P-450 (nmol/mg-mic. protein)	0.11	0.34
Benzo (a) pyrene hydroxylase (nmol/min/mg-mic. protein)	0.04	0.70
Glucuronide conjugation (nmol/min/mg-mic. protein)	0.067	0.052
Sulfate conjugation (nmol/min/g-liver)	0.12	0.09
Glutathione conjugation (nmol/min/g-liver)	30.7	73.1

After PCB treatment, fish were subjected to the oxolinic acid metabolism test. Oxolinic acid is a synthetic antimicrobial drug which has been used in large quantities in Japan as a fish drug in aquaculture.

Fig. 15 shows the changes in concentrations of oxolinic acid in control and PCB-treated carp tissues after oral administration at a dose of 20 mg/kg-b. w. The maximum concentration and duration of oxolinic acid in PCB-treated fish tissues reduced to 1/2-1/3 levels of those in control. This suggests that the efficacy of drugs is presumably reduced by water pollution with some contaminants which induce the MO activity in fish.

In short, some pollutants induce the activities of durg-metabolizing enzymes in fish and shallfishes. The induction of the MO activity in fish and the sulfate

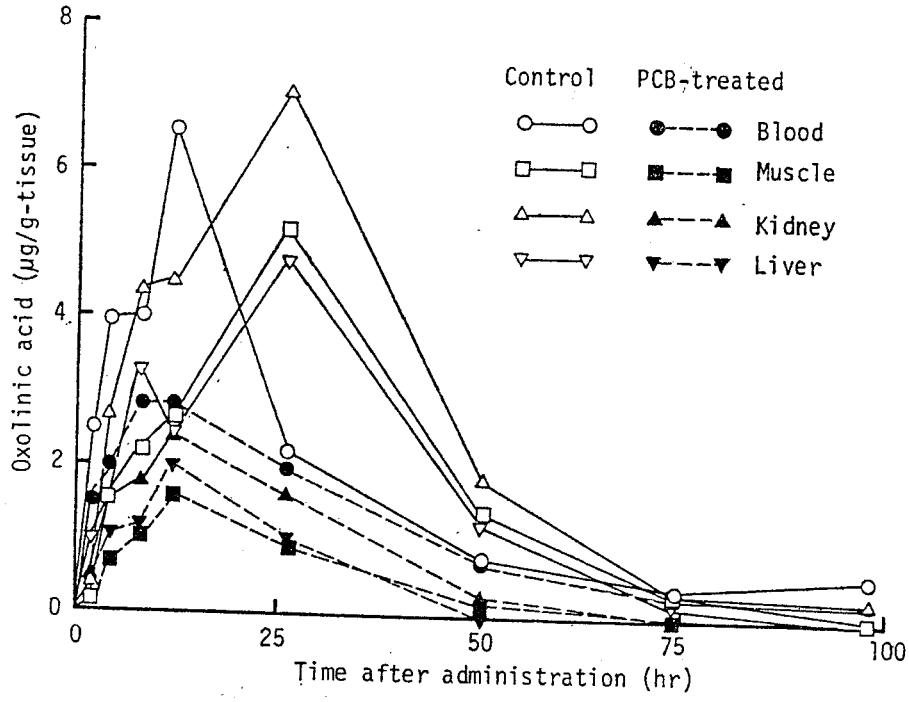

Fig. 15. Changes in concentrations of oxolinic acid in control and PCB-treated carp tissues after oral administration at a dose of 20 mg/kg-b. w.

conjugating activity in shellfishes is available as a biochemical monitor for the evaluation of marine pollution. Although the effects of the induced MO activity on fish have not been demonstrated clearly, it affects at least on the metabolism of hormones and drugs in fish, resulting in the reduction of fish reproduction and drug efficacy.

化學汚染物對水中生物危害之生化學手段評估

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摘 要

沿岸海洋生物經常受到農業和工業廢水之侵害，長期曝露於各種不同化學物質及其衰變產物中，但鑑定或測定不同有毒化學物質對海洋生物之影響至爲困難。有毒化學物質在海水中之濃度，一般並不至於高到可使魚貝類大量死亡，可是對海洋生物之生化和生理却有所影響，本文僅就下列主題加以敍述。

1. 誘導魚貝類藥物代謝酵素活性之增加。
   魚類之細胞色素 P-450 (cytochrome P-450) 和單加氧酶 (monooxygenase) 活性受某些環境汚染物如多環芳香族碳氫化合物和鹵化有機化合物等刺激而增加。貝類之硫酸鹽結合活性受酚類刺激而增加。
2. 以魚貝類藥物代謝的酵活性作爲評估海洋汚染之指標。
   如同上述，魚類之單加氧酶和貝類之硫酸鹽結合等活性可做爲判估海洋汚染之生化學指標。其中比較工業地區和非工業地區之魚貝類的酵素活性，得知鮃鰈魚之細胞色素 P-450 和三種酵素，以及貝類之硫酸鹽結合等活性，皆可做爲海洋汚染之生化學指標。
3. 誘導之單加氧酶活性對魚體激素和藥物代謝之影響。
   已知 PCB 爲單加氧酶活性之良好誘導劑，但會減少血中性激素 (estrone 和 estradiol) 之量，導致魚類卵巢成熟之延慢。

在日本 oxolinic acid 爲大量使用之魚病藥物，當同時餵食該藥物和 PCB 時，PCB 對魚體組織 oxolinic acid 之最高含量及保存性，皆有降低之作用。（黃登福譯）