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Effect of Biphenyl Ether Herbicides on the Formation of Mutagenic Intermediates from Procarcinogens by Rainbow Trout

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Increasing frequencies of tumors among aquatic animals in polluted waters have been reported (Sindermann 1979). Experiments have also shown that exposure of fish to certain well-known and widely distributed xenobiotics causes them to develop tumors in a relatively short time (Harshbarger 1977). Formation of mutagenic intermadiates from procarcinogens by fish liver homogenates had been reported (Balk et al. 1982; Miyauchi 1984). It is well established that fish have the ability to biotransform xenobiotics in a manner similar to that of mammalian species. These biotransformations include cytochrome P-450-dependent monooxygenase systems (Elcombe & Lech 1979). In fish as well as mammals, cyt. P-450 systems are known to be induced by environmental pollutants. These inducers of cyt. P-450 systems are thought to influence the appearance of toxicity of chemicals. Biphenyl ether herbicides have been widely used all over the world, and particularly in Japan, they are indicated as environmental pollutants (Watanabe et al. 1983). Judging from their structures like as PCB, they are suspected to induce cyt. P-450 systems in fish. Considering the increased fish tumors in polluted waters, it is very interesting to examine the variation of mutagenicity of procarcinogens caused by biphenyl ether herbicides. In this study, the effects of biphenyl ether herbicides and related compounds on the formation of mutagenic intermadiates from procarcinogens by the S-9 fractions from rainbow trout were examined by using the Salmonella/microsome test.

MATERIALS AND METHODS

2-Aminoanthracene (2-AAT), 2-acetylaminofluorene (2-AAF), benzo(a)pyrene (BaP), Kaneclor-500 (PCB), 3-methylchoranthrene (3-MC) and α-naphthoflavone

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(α -NAF) were purchased from Wako Chemical Industry Co., Ltd (Osaka, Japan). D-Glucose-6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH were from Oriental Yeast Co., Ltd (Tokyo, Japan). Metyrapone was from Japan Ciba-Gaigy Company. All other chemical reagents were of the highest commercial quality available. Biphenyl ether herbicides and related compounds were prepared by the procedures described in the previous paper (Miyauchi et al. 1981).

Rainbow trout with an average weight of 85-110 g were purchased from a commercial source. Fish were maintained in basins with aerated, filtered and recirculated dechlorinated-water at a temperature of 10± 2°C. Under these conditions the fish were acclimatized for a week and starved during the period of chemical injection.

At days 0, 2 and 4 the fish were injected i.p. with the chemicals dissolved in olive oil or olive oil alone at 0.4 ml per dose. The total dosage levels were: biphenyl ethers, 0.63 mmol and 1.89 mmol/Kg (two dosage levels were used); PCB, 500 mg/Kg; 3-MC, 80 mg/Kg. After the first injection the fish were transferred to separate basins filled with noncirculating but aerated dechlorinated tap water at a temperature of 10± 2°C. A daily half-volume change of water was performed. Injections with biphenyl ethers, PCB and 3-MC produced no toxic effect in the fish.

After 6 days from the last injection the fish were killed and their livers were immediately removed, taking care not to puncture the gall bladder, weighed and rinsed twice in ice-cold 0.15 M KCl. The S-9 fractions were prepared according to Ames et al. (1975) and stored at -80℃. The protein content of the S-9 fractions was measured by the method of Lowry et al. (1951).

Tests for mutagenicity were carried out mainly according to the Ames method as modified by Yahagi (1975) with tester strain TA 98. Titrations were performed with three concentrations of each of three procarcinogens (1, 5 and 10 μg/plate for 2-AAT and BaP; 10, 25 and 50 μg/plate for 2-AAF) and with three separate protein concentrations (0.5, 2.0 and 5.0 mg/plate) for each of the three procarcinogen concentrations. The mixture of procarcinogen, S-9 fraction, bacteria and cofactors in a test tube was preincubated at a temperature of 25℃ and for a time of 60 min in a shaking water bath. The plates were incubated in the dark for 48-72 h at 37℃. Each determination was carried out in parallel on three or four

different plates. In the case of the observation of the effect of P-450 inhibitors on the formation of mutagenic intermediates from procarcinogens, α-NAF or metyrapone dissolved in 50 μl dimethylsulfoxide was added to the test tube at a concentration of 10^{-4} M and 10^{-3} M per plate, respectively. This concentration of the inhibitors had no toxic and mutagenic effect (with and without S-9 fraction) on the tester strain. Statistical tests for differences were performed using Student's t-test.

RESULTS AND DISCUSSION

Table 1 summerizes maximum ratios of the number of revertant colonies observed on the test plate (at optimal protein and procarcinogen concentrations for each set of titrations) to the number of revertant colonies appearing on the corresponding control (enzyme blank) plate; i.e. ratio of test to background. The S-9 fraction from control fish formed mutagenic intermediates from 2-AAT and 2-AAF, but not BaP. Treatments of rainbow trout with 3-MC and PCB

Table 1. Formation of mutagenic intermediates from procarcinogens by the S-9 fractions from the liver of rainbow trout.

	Procarcinogens (μ g/plate)		
Treatment	2-AAT(10)	2-AAF(50)	BaP(5)
Control (Olive oil)	34"	5.0'	1.0
3-MC	153"	23"	28"
PCB	116"	15"	4.6'
4-NO_2			
0.63 mmol	79"	20"	1.1
1.89 mmol	52"	22"	1.0
4-CNO_2			
0.63 mmol	94"	17"	0.9
1.89 mmol	49"	19"	1.1
2,4-$DCNO_2$			
0.63 mmol	113"	13"	1.2
1.89 mmol	102"	18"	1.1
2,4,6-$TCNO_2$			
0.63 mmol	78"	11"	0.9
1.89 mmol	65"	12"	0.8

Statistical significance at ' $P < 0.05$ and " $P < 0.001$.

resulted in about 3.0-5.0-fold increases in the mutagenicity of both 2-AAT and 2-AAF, and caused the formation of mutagenic intermediates from BaP resulting in 28-fold and 4.6-fold increases in the number of

revertant colonies, respectively. While, treatments of rainbow trout with biphenyl ethers resulted in about 1.5-3.0-fold and 2.0-4.0-fold increases in the mutagenicities of 2-AAT and 2-AAF, respectively. These increases in the mutagenicities of 2-AAT and 2-AAF were observed at both low and high dosage levels of biphenyl ethers. The increase in the mutagenicity of 2-AAT was greater at low dosage level and was not related to the number of chlorine in biphenyl ethers; however, that of 2-AAF was greater at high dosage level and was related to the number of chlorine. 4-Nitrobiphenyl ether (4-NO_2) was the most effective treatment followed by 4'-chloro, 2',4'-dichloro and 2',4',6'-trichloro 4-nitrobiphenyl ethers (4-CNO_2, 2,4-$DCNO_2$ and 2,4,6-$TCNO_2$). BaP was not converted to mutagenic intermediates by the S-9 fractions from the liver of rainbow trout treated with biphenyl ethers. As it has been reported that biphenyl ethers used in this study had the mutagenic activity (Miyauchi et al. 1983), biphenyl ethers in the S-9 fractions are suspected to show the mutagenic activity. But this suspectable point would be denied because biphenyl ethers were base-change type mutagens (Miyauchi et al. 1983), and there were no revertant colonies on the blank plate (contained only the S-9 fraction from biphenyl ethers-treated rainbow trout). In the mammalian liver, there is an enzyme system of utmost importance in the detoxication and activation of xenobiotics. This cyt.P-450 system is believed to be responsible for the activation of numerous compounds, including several carcinogenic ones (Heiderberger 1975). The existence of cyt.P-450 system in fish is now well established and is thought to be responsible for the activation of carcinogenic compounds as well as mammals. 3-MC and PCB are known to induce cyt.P-450 system in fish, and this induction would result the acceleration of metabolisms of 2-AAT, 2-AAF and BaP to mutagenic intermediates. One of the biphenyl ether herbicides, 2,4,6-$TCNO_2$, induces cyt.P-450 system in mammals (Burke et al. 1983). In fish there is no report on the induction of cyt.P-450 system by biphenyl ether herbicides; however, the results in Table 1 would indicate that biphenyl ethers induced cyt.P-450 system which concerned with the metabolisms of 2-AAT and 2-AAF. The failure of formation of mutagenic intermediates from BaP by the treatments with biphenyl ethers would imply that the induction pattern of cyt.P-450 system by biphenyl ethers was different from those by 3-MC or PCB.

Inhibitors of cyt.P-450 system were used to characterize the cyt.P-450 relating to the formation of mutagenic intermediates from procarcinogens in the S-9

fractions of the liver of rainbow trout treated with biphenyl ethers, 3-MC or PCB. Inhibition rates of mutagenicity of procarcinogens by inhibitors (α -NAF and metyrapone) are indicated in Table 2. Metyrapone, which acts an inhibitor of the activity of cyt.P-450 in untreated or phenobarbital treated mammals (Gaujon et al. 1972), had little effect on the mutagenicity of procarcinogens with any of S-9 fractions. α -NAF, inhibitor of cyt.P-448 system, strongly inhibited the mutagenicity of 2-AAT and BaP with the S-9 fractions from 3-MC or PCB treated rainbow trout, but didn't in-

Table 2. Inhibition rates (%) of revertant colonies by α -NAF and metyrapone.

Treatment	Procarcinogens	α -NAF	Metyrapone
Control	2-AAT	49.1	3.2
	2-AAF	35.5	2.2
3-MC	2-AAT	91.3	6.7
	2-AAF	61.1	4.7
	BaP	92.6	1.7
PCB	2-AAT	80.0	28.9
	2-AAF	55.2	12.3
	BaP	75.9	6.2
4-NO_2	2-AAT'	38.7	1.2
	2-AAF"	30.2	1.6
4-CNO_2	2-AAT'	47.7	0.5
	2-AAF"	29.4	5.2
2,4-$DCNO_2$	2-AAT'	49.7	1.5
	2-AAF"	29.1	1.8
2,4,6-$TCNO_2$	2-AAT'	44.2	4.5
	2-AAF"	20.0	2.0

' S-9 fraction treated with 0.63 mmol/Kg of biphenyl ether was used.
" S-9 fraction treated with 1.89 mmol/Kg of biphenyl ether was used.

hibit severely the mutagenicity of 2-AAF. Using the S-9 fractions from biphenyl ethers treated rainbow trout, inhibition rates of the mutagenicity of 2-AAT and 2-AAF by α -NAF were not so severe and similar to those of control. 3-MC and PCB are known to induce new cyt.P-450(s) whose catalytic reactions are mainely inhibited by cyt.P-448 inhibitors (Stegeman et al. 1981). Considering the effect of inhibitors on the mutagenicity of procarcinogens with the S-9 fractions from biphenyl ethers treated rainbow trout, biphenyl ethers would not induce new cyt.P-450(s) but enhance the activities of cyt.P-450(s) related to the metabolisms of 2-AAT and 2-AAF. To characterize the biphenyl ethers-induced cyt.P-450, it will be required

to examine the activities of cyt.P-450 systems by using various substrates, for example, aniline hydroxylase, ethylmorphine N-demethylase, BaP hydroxylase, ρ-nitroanisole deethylase, etc..

This study revealed that biphenyl ether herbicides and their related compounds enhanced the mutagenicity of procarcinogens (2-AAT and 2-AAF). Biphenyl ether herbicides are used widely and are pollutants in the aquatic environment and animals. Biphenyl ether herbicides are dechlorinated in the environment by various factors, such as the sunlight (Nakagawa & Crosby 1974), and therefore their dechlorinated forms, 4-NO_2 and 4-CNO_2 would be detected. When the fish were polluted with biphenyl ehters and encountered procarcinogens (aromatic amines and amides) the risk of carcinogenicity would increase.

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REFERENCES

Ames, B.N., J. McCann and E. Yamasaki: Methods of detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31, 347-364 (1975).

Balk, L., J.W. Depierre, A. Sundvall and U. Rannug: Formation of mutagenic metabolites from benzo(a)pyrene and 2-aminoanthracene by the S-9 fraction from the liver of the northern pike: inducibility with 3-methylcholanthrene and correlation with benzo(a)pyrene monooxygenase activity. Chem. biol. Interactions 4, 1-13 (1982).

Burke Hurt S.S., J.M. Smith and A. Wallace Hayes: Nitrofen: A review and prespective. Toxicology 29, 1-37 (1983).

Elcombe, C.R. and J.J. Lech: Induction and charaterization of hemoprotein(s) P-450 and monooxygenation in rainbow trout. Toxic. app. Pharmac. 49, 437-450 (1979).

Gaujon, F.M., D.W. Nebert and J.E. Gielen: Genetic expression or aryl hydrocarbon hydroxylase induction. IV. Interaction of various compounds with different forms of cytochrome P-450 and the effect on benzo(a)pyrene metabolism in vitro. Molec. Pharmac. 8, 667-680 (1972).

Harshberger, J.C.:Role of the registry of tumors in lower animals in the study of environmental carcinogenesis in aquatic animals. Ann. N.Y. Acad. Sci. 298, 280-289 (1977).

Heiderberger, C.: Chemical carcinogenesis. Ann. Rev. Biochem. 44, 79-121 (1975).
Lowry, O.H.: Protein measurement with folin phenol reagent. J. biol. Chem. 193, 265-275 (1951).
Miyauchi, M., M. Koizumi and T. Uematsu: Studies on the toxicity of chlorinated p-nitrobiphenyl ether. I-Methemoglobin formation in vitro and in vivo induced by nitroso and amino derivatives of chlorinated biphenyl ether. Biochem. Pharmacol. 30, 3341-3346 (1981).
Miyauchi, M., M. Haga, Y. Takou and T.Uematsu: Mutagenic activity of 4-nitrobiphenyl ethers and their nitroso- and amino-derivatives. Chem. biol. Interactions 44, 133-141 (1983).
Miyauchi, M.: Conversion of procarcinogens to mutagens by the S-9 fraction from the liver of rainbow trout: Inducibility with PCB, 3-methylcholanthrene and phenobarbital and inhibition by metyrapone and a-naphthoflavone. Comp. Biochem. Physiol. 79C, 363-367 (1984).
Nakagawa, M. and D.G. Crosby: Photodecomposition of nitrofen. J. agric. Fd. Chem. 22, 849-853 (1974).
Sindermann, C.J.: Pollution-associated diseases and abnormalities of fish and shellfish: A review. Fishery Bulletin 76, 717-749 (1979).
Stegeman, J.J., A.V. Klotz, B.R. Woodin and A.M. Pajor: Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in scup. Aquatic Toxicology 1, 197-212 (1981).
Watanabe, S., S. Watanabe and K. Ito: Investigation on the contamination of freshwater fish with herbicides (CNP, chlomethoxynil, benthiocarb and molinate). J. Pesticide Sci. 8, 47-53 (1983).
Yahagi, T.: Screening methods using microbes for the environmental carcinogens. Protein Nucl. Acid Enzyme. 20, 1178-1189 (1975).

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