Bull. Environm. Contam. Toxicol. 27, 244-249 (1981)

Intake and Excretion of Diazinon in Freshwater Fishes

Koichiro Seguchi and Shiro Asaka

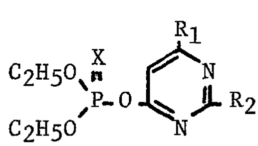
Ageo Research Laboratory, Agrochemicals Division, Nippon Kayaku Co., Ltd., Ageo, Saitama 362, Japan

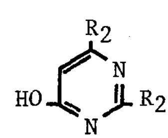
Diazinon is widely used against a variety of agricultural and non-agricultural insect pests. During application for insect control, non-target organisms will also be exposed to this compound. Therefore, it is necessary to clarify the effect of diazinon to fish. Investigations related to the biochemical behavior of diazinon in fish have been conducted by several authors(KANAZAWA 1975, 1978, KAWAAI et al. 1978, HOGAN 1972). Some of these include bioconcentration, intake, excretion and metabolism of diazinon in fish.

In this paper, we report the intake and excretion of diazinon and its metabolites in freshwater fishes, and the relationship between the bioconcentration ratio of diazinon and fat content of fish, as well as the GLC analytical method for these compounds in fish.

MATERIALS AND METHODS

Chemicals : The authentic compounds showed in Fig.1 were synthesized in our laboratory according to the methods of MIYAZAKI et al .(1970) and KATO et al.(1973).





	X	R_1	R_2
Diaz.	S	Me	CH Me_2
M-1	O	Me	CH Me_2
M-2	S	Me	C(Me)=CH_2
M-3	S	Me	C(OH) Me_2
M-4	S	Me	CH(OH)Me
M-5	S	CH_2OH	CH Me_2

	R_1	R_2
M-6	Me	CH Me_2
M-7	Me	C(Me)=CH_2
M-8	Me	C(OH) Me_2
M-9	Me	CH(OH)Me
M-10	CH_2OH	CH Me_2

Fig.1 Structures of diazinon and its metabolites.

0007-4861/81/0027-0244 $01.20

Fish : Carp(Cyprinus carpio) weighing about 8 g/fish, rainbow trout(Salmo gairdneri) weighing about 16 g/fish, loach(Misgurnus anguillicaudatus) weighing about 2.6 g/fish, and shrimp(Penaeopsis joyneri) weighing about 1.2 g/shrimp were obtained from a fish farm pond in Saitama Prefecture, and acclimatized at 22°C for carp and rainbow trout and 25°C for loach and shrimp to the laboratory condition for one week prior to the experiment. Tap water was used after dechlorination with charcoal, and had a pH of 7.4 and a dissolved oxygen concentration of 7.0 ppm. Fish were fed with commercial fish food, UKIMO(Showa Sangyo Co.), once a day through out the experiment.

Administration : 20 fishes were placed in 100-L glass aquaria for each species. A 2 ppm diazinon aqueous solution was continuously diluted hundredfold with 500 mL/min of tap water and introduced into the aquaria. For the experiment of excretion of diazinon and its metabolites in fish, a certain number of fish which had been exposed to continuous flow of water containing 0.02 ppm diazinon for 14 days were transferred to clean flowing water and reared for 7 days.

Determination of diazinon and its metabolites by GLC : Diazinon and related compounds(M-1 - M-5) were directly determined by GLC (FPD). Pyrimidine analogues(M-6 - M-10) were transformed to corresponding derivatives according to the method showed in Fig.2, and then determined by GLC(ECD).

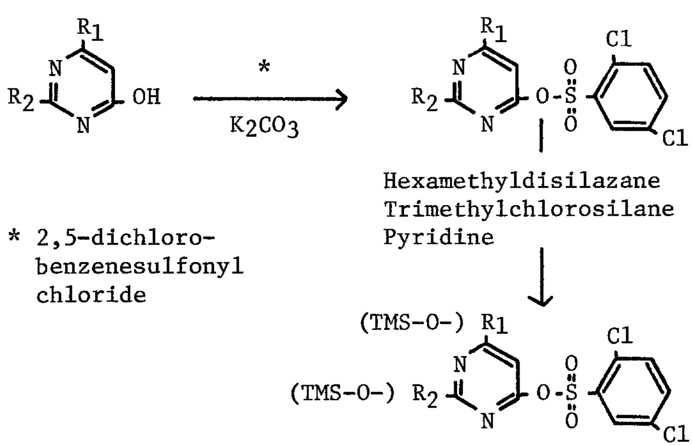
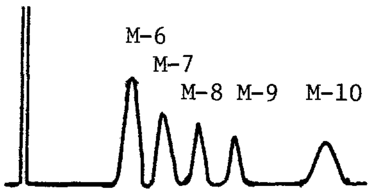


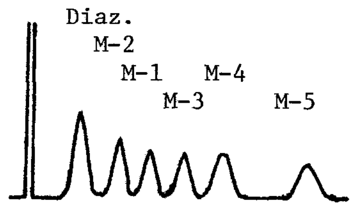
Fig.2 Derivatization procedure of pyrimidines.

The chromatograms of these compounds and operating conditions of the GLC are shown in Fig.3 and Fig.4.



Condition : ^{63}Ni ECD
Column : 3% SE-30/Chromosorb-W
(3 mm X 1 m)
Column temp. : 190°C
Det. temp. : 270°C

Fig.3 Gas chromatogram of derivatives of pyrimidines.



Condition : FPD
Column : 2% QF-1/Gaschrom-Q
(3 mm X 1 m)
Column temp. : 150°C
Det. temp. : 180°C

Fig.4 Gas chromatogram of diazinon and related compounds.

Analytical procedure : Diazinon and its metabolites in fish were extracted, separated, derivatized, and determined according to the scheme outlined in Fig.5.

Fish : Extraction
- Homogenized and extracted with chloroform/2-propanol(1/1)
- Dried with sodium sulfate

Cleanup with silica gel Col.Chrom.
- Washed with ether/n-hexane(1/1)
- Eluted with chloroform/2-propanol (1/1)

Separation by silica gel TLC
- Developed with benzene/ethyl acetate/chloroform/2-propanol(2/2/1/1)

Derivatization
- with 2,5-dichlorobenzenesulfonyl chloride
- with TMS

Determination by GLC [Pyrimidines]

Cleanup with alumina Col. Chrom.
- Eluted with ethanol/ n-hexane(3/7)

Determination by GLC [Diazinon and related compounds]

Fig.5 Analytical method for diazinon and its metabolites in fish.

RESULTS AND DISCUSSION

Analysis : GLC determination was performed successfully for the intact molecule for diazinon and related compounds or by derivatization for pyrimidine analogues. The derivatization of pyrimidine analogues by the present method was performed quantitatively at the microgram level, and the derivatives produced were stable and highly sensitive to GLC(ECD). The minimum detection limit of these compounds were 0.5 - 0.01 ng. This method was applied to the analysis of residual diazinon and its metabolites in fish. The average recoveries of the respective compounds from fish fortified at 0.5 ppm were 72 - 105 %.

Intake and excretion of diazinon and its metabolites in fish : Table 1 presents the data obtained from water and fish analyses.

Table 1 Content of diazinon and its metabolites in fish.

Species	Day after exposure	Content of diaz. in flow water (ppm)	Content of diaz. and its metabolites in fish (ppm)			Bioconcentration ratio of diazinon
			Diaz.	M-2	M-6	
Carp	1	0.018	1.0	0.019	0.22	58
	3	0.018	2.4	0.054	0.25	130
	7	0.018	2.1	0.045	0.39	120
	14	0.018	2.1	0.033	0.21	120
	R-7		0.006			
Rainbow trout	1	0.014	0.80	0.025	0.15	57
	3	0.012	1.2	0.026	0.28	92
	7	0.012	0.81	0.023	0.33	64
	14	0.015	0.82	0.019	0.29	62
	R-7		0.008			
Loach	1	0.016	0.18		0.17	11
	3	0.014	0.36		0.12	24
	7	0.015	0.36		0.20	24
	14	0.014	0.42		0.24	28
	R-7		0.007			
Shrimp	1	0.016	0.050		0.018	3
	3	0.021	0.066		0.014	4
	7	0.020	0.053		0.021	3
	14	0.021	0.054		0.020	2
	R-7		0.004			

R-7 : 7 days after return to clean water

The concentration of diazinon in water was kept fairly constant, although it was a little lower than the pre-set value except for shrimp. In the exposure to continuous flow water containing 0.02 ppm of diazinon, the concentration of diazinon in fish rapidly increased, and it reached to the maximum after 3 days for exposure

. Thereafter, the diazinon concentration slightly decreased, and remained at equilibrium. The bioconcentration ratios of carp, rainbow trout, loach and shrimp at the equilibrium were 120, 63, 26 and 3, respectively.

As for the metabolites, M-6 was found in all fish species, but M-2 was found only in carp and rainbow trout. The concentration of these metabolites reached to the maximum after 3 - 7 days exposure to diazinon. Diazinon was metabolized to diazoxon in the channel catfish liver microsomal enzyme system(HOGAN 1972), but it was not found in any fish species.

When the fish was transferred from diazinon containing water to clean water, diazinon and its metabolites were rapidly excreted from fish. Seven days after transferring to clean water, the diazinon concentration decreased to 0.3 - 8.0 % of the equilibrium concentration during exposure period and the metabolites decreased below the detection limit. Similar results about the excretion of diazinon from fish have been observed on topmouth gudgeon by KANAZAWA(1975, 1978) and rainbow trout by KAWAAI(1978).

A plot of the bioconcentration ratio of diazinon in fish vs the fat content of fish, which was referred to the list of standard component of food in Japan(1976), is shown in Fig.6. A satisfactory linear relationship was observed between the bioconcentration ratio and fat content in fish. The regression equation is $Y = 11.2X - 2.9$ with the correlation coefficient of 0.93. Therefore, the bioconcentration ratio of diazinon by fish was closely related to the fat content of fish. Similar relation has been recognized by ROBERT et al.(1977) with bioaccumulation of the chlordane by northern redhorse suckers. On the other hand, the correlation of diazinon bioconcentration ratio and body weight of topmouth gudgenon, and DDT residues in the whole body and the size of the fish have been explained by KANAZAWA(1978) and BUHLER et al.(1959), respectively.

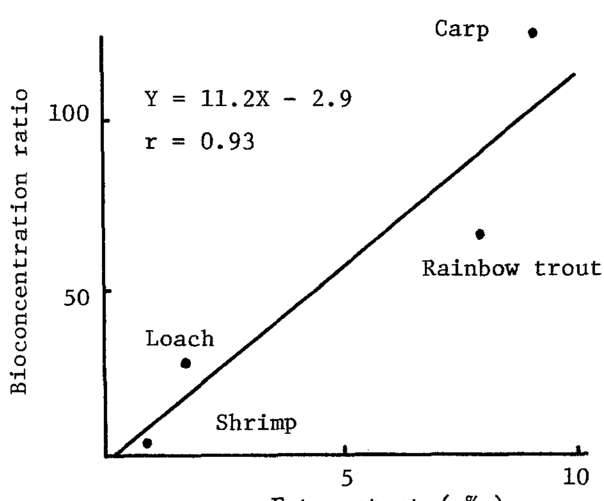


Fig.6 Relationship between bioconcentration ratio of diazinon and fat content of fish.

Acknowledgments. We thank I.Tozimbara for the advice on fish breeding and J.Kanazawa for the revision of the manuscript.

REFERENCES

BUHLER D.R., M.E.ROSMUSSON, W.E.SHANK : Toxicol. Appl. Pharmacol. 14, 535 (1959)

Compiled by National Institute of Resources : The list of standard component of food in Japan (1976)

HOGAN,J.W. : Bull. Environm. Contam. Toxicol. 8, 61 (1972)

KANAZAWA,J. : Bull. Environm. Contam. Toxicol. 14, 346 (1975)

" : " " " " 20, 613 (1978)

" : J. Pestic. Sci. 3, 185 (1978)

KATO,T., H.YAMANAKA, M.NODA : J. Pharmaceut. Soc. Japan 93, 1437 (1973)

KAWAAI,S., M.FUKUSHIMA, K.ODA, G.UNO : Kankyo Gijutsu 7, 668 (1978)

MIYAZAKI,H., I.TOZIMBARA, K.WATANABE, T.OSAKA, S.OKUI : Proc. 1st Symp. Drug Metabo. Action. Chiba. Japan. Pharmaceutical Society of Japan, Tokyo, 135 (1970)

ROBERTS J.R., A.S.W.DEFRIETAS, M.A.J.GIDNET : J. Fish Res. Bd. Can. (CAN) 34, 89 (1977)

Accepted May 26, 1981