Journal of Environmental Sciences Vol. 7, No. 2, pp. 212—217, 1995 ISSN 1001-0742

Relationship between bioaccumulation, distribution of MET and lipid content of aquatic organisms*

Liu Zhengtao, Zhou Fengfan, Jin Hongjun

Department of Environmental Sciences, Nanjing University, Nanjing 210093, China

Abstract—The studies on bioaccumulation and distribution of the plant growth regulator multi-effect triazole (MET) in the fish (*Carassias auratus*) and Daphnia (*D. magna*) showed that the bioaccumulation factor (BCF) was in positive correlation to the lipid content of the organisms, and MET increased the lipid content which bioconcentrated to greater extent than no-MET effected on these organisms, also the bioaccumulation mainly existed in digestive system of the fish. Acute testing on several aquatic species presented that MET belonged to low toxicant chemical, and the valuable estimating equations between lipid content and BCF values have been established.

Keywords: MET; bioaccumulation; distribution; acute toxicity; lipid content.

1 Introduction

Since bioaccumulation of compounds has been expressed on the ecosystem, many quantitative relationships between structure and biological activity of chemicals have been usefully established in aquatic system (Lyman, 1982). Estimation of bioaccumulation factors (BAF) is usually efficient by using two compartments exchange model for description of uptake and depuration rates of chemicals in the aquatic environment. Well known examples are benzol[a] pyrene (BaP), hexachloro-benzene (HCB) and polychlorinated biphenyls (PCB), these organic compounds are concentrated in fatty tissues of organisms. General, the BAF are simply proportional to the hydrophobicity of the chemicals, expressed as octanol/water partition coefficients (*K*ow; Esser, 1986; Geyer, 1985). However, this expression is closely related to the relationship between hydrophobicity and "non specific" aqueous toxicity of compounds, and estimation of BAF via hydrophobicity will result in too high values for chemicals which are subject to metabolic transformation by aquatic organisms (Bruggeman, 1988). Thus, it is important to examine the toxic effects of chemicals in the aquatic environment.

Multi-effect triazole [MET, 2RS+3RS-1-(4-chlorophenyl)-4,4-di-methyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol] is a new classic type of plant growth regulator and fungicide chemical which produced by Impire Chemical Institute Co. (ICI., British, 1981). Although several acute toxicity experiments have been conducted (Lever, 1982), and some reports considered that it had better productive effects on crops (Schwinn, 1984), while the potential ecologic

* This paper was funded by the National Natural Science Foundation of China.

toxicity, chronic effects of lower concentration and residual problems of MET have not been investigated, and making it difficult to estimate accurately the environmental safety and compare with other researches. Therefore, we collected several aquatic organisms as test species, compared the acute toxicities of MET, studied the relationships between lipid content and bioaccumulation, distribution of the chemical in the fish (*C. auratus*) and daphnia (*D. Magna*) to provide some available evidences for the extensive applying and enlargemental safe estimation of MET or the analogous compounds.

2 Materials and methods

2.1 Materials

All MET (purity, 98.25%) were purchased from Jiangsu Agricultural Chemical Institute. ^{3}H - MET were obtained from Beijing Atomic Energy Institute. The specific radioactivities of ^{3}H - MET was 3.6 mCi/ml or 4.5 mCi/mg. The quality characteristics of experimental water were: temperature, 22±1℃; dissolved oxygen, 8.2±0.5 mg/L; pH, 7.5±0.3; alkalinity as $CaCO_3$, 132±7.0 mg/L; hardness as $CaCO_3$, 115±8.0 mg/L; conductivity, 180—250 μS/cm; COD_{Mn}, 1.02—1.20 mg/L.

2.2 Toxicity studies

Organisms used in the static acute toxicity studies were *Selenastrum capricornutum*, *Daphnia magna*, *Ctenopharyngodon iddllus*, *Cipangopaludina chinese*, *Bufo bufo gangarizas*, *Limnodrilus hoffmeisteri*. The green algae(*S. capricornutum*) which served as the food source for daphnia and test organism was cultured in 15 L glass carboys containing 10 L of nutrient media (Quinlan, 1986). The algal cell density in each exposure chamber maintained 2×10^{5} cells/ml, the cell numbers were measured with a hemocytometer and photoperiod was 16 h daylight/8 h dark (3000 lx). All organisms which obtained from local distributors and cultured at laboratory exposured chambers which consisted of 250 ml glass beakers that contained 200 ml test solution. The MET concentration was 0.0 (control), 5.0, 12.5, 25.0, 50.0, 100.0 mg/L, respectively. Conditioned water was used as the diluent for all solutions. 5—10 organisms [*D. magna* less than 24 h old, *C. iddllus* hatched in 12 h, *C. chinese* weight 2.06±0.77 g, tadpoles (*B. gangarizans*) less than 3 days, *L. hoffmeisteri* length 13.7±2.1 mm] were randomly assigned to each of three replicates test vessels at each exposure lever, the test chambers were covered with loosely fitting lids to ratard evaporation and renewed water each 16—24 h (Francis, 1986; Isensee, 1973). Mortality was determined by probing for movement at the end of 24, 48, 72, 96 or 144 h.

2.3 Bioconcentration and distribution studies

The test fish (*C. auratus*) were about 4.0 cm long and 3.0 g weight, after cultured which fed on dry daphnia (15—20 mg/d, one fish) in glass aquariums (30×20×10cm) with the conditioned water for 7 days, these fish exposed in the test water with ^{3}H - MET. The test daphia were transferred in 5000 ml beakers which contained ^{3}H - MET testing solution (4000 ml) at a density of one organism per 50 ml conditioned water. Static effect studies were conducted by placed 4 fish into each of 5L squate jars containing 3L experimental solutions which were changed

1/3 per 24 h, the least MET concentration was approximately 15% of the LC_{50} value (Eastmond, 1984), at 12, 24, 48, 72, 96, 150 h, samples were determined which based on three measurements.

At time intervals, the organisms were blotted and dried at 40℃ for 10 min, then weighted, smashed and homogenized for 15 min with a microtissue glass homogenizer. Each sample was extracted with chloroform/methanol and measured the lipid content (Bligh, 1959). While determined ^{3}H - MET, all test organisms were rinsed with distilled water, bloted, smashed, and then measured the radioactivity of ^{3}H - MET in these mingles. Additionally, obtained about 30—50 mg organs/tissues (gill, liver, intestine, skin, bone, muscle) to determine the distribution of ^{3}H —MET in the fish. The concentration of ^{3}H - MET in both water and organism was measured by liquid scintillation counting techniques (Quinlan, 1986).

Toxicity values (LC_{50}/EC_{50}) and confidence intervals were determined by probit analysis (Exner, 1988) and control mortality was less than 10% for all analysis. The level of statistical significance employed in all cases was $P<0.05$, also each sample was measured three replicates.

3 Results and discussion

3.1 Acute toxicity

The acute toxicity data for MET showed in Table 1. The literature values (Lever, 1982) were: *Rainbow trout* (96 h, LC_{50}), 27.8—33.1 mg/L; Mallard duck, $LD_{50}>7900$ mg/L. In Table 1, LC_{50} values (*S. capriconutum*, 41.5 mg/L; *L. hoffmeisteri*, 35.5 mg/L) agreed with the reported data. Therefore, the MET toxic for fish, algae (*S. capricornutum*) and water worm (*L. hoffmeisteri*) was approximate equal, but it was more toxic for amphibian (tadppoles, 96 h, LC_{50}, 9.0 mg/L) and mollusc (*C. chinese*, 48 h, LC_{50}, 12.8 mg/L). Comparative toxicity of MET between diphnia (*D. magna*) and the other test organisms indicated that MET was more toxic for diphnia than for adult fish (*R. trout*) while less for neonates (*C. iddllus*). According to the critical evaluation (WNO/IMCO/FAO, 1969), MET was lower toxicity.

Table 1 The acute toxicity (LC_{50}, mg/L) of MET

Time,h	24	48	72	96	120
D. magna	47.0	28.7	N.A	N.A	N.A
S. capricornutum	N.A	N.A	N.A	41.5*	33.5*
L. hoffmeisteri	N.A	50.8	42.2	35.5	N.A
C. chineses	14.2	12.8	N.A	N.A	N.A
B. bufo(tedpole)	15.6	14.4	11.0	9.1	N.A
C. iddllus(neonate)	19.4	N.A	N.A	N.A	N.A

*: EC_{50} N.A: information is not available

3.2 Distribution of ^{3}H - MET in the fish

The accumulated doses of ^{3}H - MET in the organs/tissues of the fish (*C. auratus*) in test times are shown in Table 2. Indicating that the accumulated doses in the organs/tissues were dif-

ferent at same time . The ^{3}H - MET in the intestine was highest, then the ranges of radio - activity dose were: liver's > gill's > skin's > bone's > muscle's.

Table 2 Distribution of ^{3}H - MET in the fish

Time,h	Radioactivity, dpm/mg					
	Gill	Skin	Liver	Intestine	Muscle	Bone
12	49.36	17.28	229.92	159.23	36.89	26.44
24	54.57	28.12	232.03	238.52	38.76	31.27
48	59.78	40.26	272.01	311.07	39.87	37.83
96	64.99	46.27	286.73	414.82	44.04	39.64
144	68.04	58.36	299.04	432.30	44.59	46.78

The distribution of ^{3}H - MET in the fish are presented in Fig. 1. There also showed that the ^{3}H - MET in intestines increased faster than that of livers', and of all these test organisms, the accumulated does in the intestines and skins raised faster than those of the others; it might relatives of these organs direct contacting with ^{3}H - MET . However, as the same direct tactile organ, the dose in the intestine were higher than that of gill, and in indirect tactile liver, the ^{3}H - MET does also were higher than that of gill (Fig. 1). So, these information indicated that the bioconcentration of ^{3}H - MET in the fish mainly existed in digestive system.

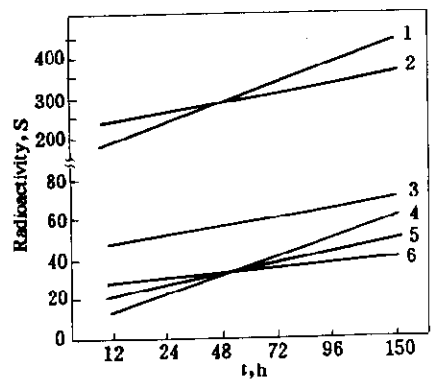

Fig. 1 Distribution of ^{3}H - MET in the fish
1: intestine 2: liver 3: gill 4: skin 5: bone 6: muscle

The radioactivity (S) of ^{3}H - MET in all organs/tissues were better correlated to the test time (T). The regression equations were follows:

liver: $S=148.18+30.35 \ln T$, $r=0.97$

intestine: $S=-125.02+114.39 \ln T$, $r=0.98$

gill: $S=30.68+7.52 \ln T$, $r=0.97$

skin: $S=-21.57+15.64 \ln T$, $r=0.98$

bone: $S=7.67+7.55 \ln T$, $r=0.97$

muscle: $S=28.42+3.25 \ln T$, $r=0.97$

3.3 Bioaccumulation and lipid content

The kinetic changes of lipid content and accumulation of ^{3}H - MET in the fish and the daphnia are shown in Table 3.

Table 3 Lipid content and accumulation of ^3H - MET in the organisms

Group	Time, h	Lipid, %		Radio - organism, dpm/mg		Radio - water, dpm/ml	BAF	
		Fish	Daphnia	Fish	Daphnia		Fish	Daphnia
Control	12	4.63	4.18	57.89	93.12	1436.4	40.34	64.82
	24	5.27	4.34	61.92	103.6	1057.7	58.52	98.01
	48	5.60	4.87	68.77	108.5	967.3	71.08	112.2
	72	6.03	5.05	72.54	117.9	951.2	76.24	123.9
	96	7.04	5.30	84.77	128.3	940.1	90.17	136.4
	120	7.28	5.62	88.25	156.1	918.3	96.12	169.8
	150	7.55	5.67	100.4	158.6	825.6	121.5	192.1
Treat	12	4.86	4.15	53.47	90.25	1451.8	36.81	62.16
	24	5.32	4.72	72.55	124.1	1245.5	58.23	99.68
	48	7.45	5.14	91.72	141.2	1222.5	75.01	115.5
	72	8.33	5.42	102.8	150.4	1167.9	80.04	128.7
	96	8.97	6.03	110.1	167.6	1026.1	107.4	163.2
	120	9.64	6.54	117.7	190.2	958.7	122.7	198.4
	150	9.94	6.87	123.2	196.1	804.4	153.2	143.7

These results indicated that the accumulated ^3H - MET were rising with the increasing of lipid contents. In controlled groups, the changeable ranges both lipids and ^3H - MET doses were less than that of treated group. Such as the lipids changes from 4.86% to 9.94% in treated, compared to that of 4.63% to 7.55% in the controlled groups at same intervals, and it was such changes of the ^3H - MET concentration in the organisms. Therefore, the concentrated MET were better correlated with the lipid contents of the test organisms, and MET could affect the lipid contents, then the lipids reversely affected the bioconcentration of MET in the organisms.

The expression equations between lipid contents (X) and BAF (Y) of MET in the fresh water species were:

controlled groups, fish, $Y=\exp(2.38+0.30X)$, $r=0.96$

daphnia, $Y=\exp(1.82+0.59X)$, $r=0.96$

treated groups, fish, $Y=\exp(2.62+0.22X)$, $r=0.95$

daphnia, $Y=\exp(2.34+0.45X)$, $r=0.98$

These equations showed that the BAF was higher positive correlated to the lipid contents. We also notices that the BAF data of the daphnia were higher than that of fish while the lipid contents were lower (Table 3), suggesting that MET was not wholly distributed in the lipid. Because the coefficients of correlation were strongly between lipid and BAF ($r>0.95$), so lipid content was the mainly determinative factor of BAF, and may be no biomagnifical effects on the test organisms. Chiou (Chiou, 1985) presented that the BAF of chemical could be estimated according to lipid content of organisms, and Geyer (Geyer, 1985) indicated that BAF based on

lipids of aquatic organisms were simple proportional to the hydrophobicity of the chemicals, expressed as octanol - water partition coefficients (Kow). In fact, this express was chiefly related to the relationship between hydrophobicity and non biological effects of chemicals. Some reports indicated that the BAF of some higher molecular weight compounds and compounds metabolized by organisms did not correlated with Kow (Barry, 1985), according to the results, the MET increased the lipid contents which effected the accumulation of ^{3}H - MET in the fish. So, by no considering the biological effects such as toxic, physiologic or biochemical effects of compounds, estimating BAF based on the octanol/water partition coefficients was not complete to the aquatic organisms. While, these similar researches needed to be investigated more extensively.

Acknowledgements—We thank Professor Wang Liansheng for his helpful reviews and suggestions.

References

Barry GO, Arthur JN. Environ Sci Technol, 1985; 19:842

Blight EG, Biochem Phys, 1959; 37:911

Bruggeman WA. Manual on aquatic ecotoxicology. India: Kluwer Academic Pub, 1988: 149

Chiou CT. Environ Sci Technol, 1985; 19:57

Esser HO. Pestic Sci, 1986; 17:265

Eastmond DA. Aech Environ Contam Toxic, 1984; 13:105

Exner O. Correlation analysis of chemical data. New York: Plenum Press, 1988:48

Francis PC. Bull Environ Contam Toxic, 1986; 36:730

Gardner WS. Environ Toxic Chem, 1990; 9:1269

Geyer II. Chemosphere, 1985; 14:545

Isensee AR. Environ Sci Technol, 1973; 7,841

Lever BG. Proceeding British crop protection conference, 1982, 1:3

Lyman WJ. Handbook of chemical property estimation methods. New York: McGraw - Hill Book Co., 1982:52

Quinlan GD. Acta Horticulturae, 1986; 179:443

Neujahr HY. Anal Biochem, 1964, 8:847

Schwinn FJ. Pestic Sci, 1984; 15:40

WHO/IMCO/FAO, Water Research, 1969; 3:995

(Received October 18, 1993)