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Bioconcentration and Toxicity Effect on Lipid Content of Aquatic Organisms

Z. T. Liu, Z. M. Kong, F. Zhou, L. S. Wang

Department of Environmental Science and Engineering, Nanjing University, Nanjing 210093, People's Republic of China

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Since bioconcentration of compounds have been determined in the environment, there are many quantitative relationships between structure and biological activity of chemicals established in aquatic systems (Esser 1986). The estimation of bioconcentration factors (BCF) is usually efficient by using two compartments exchange model for description of uptake and depuration rates of chemicals in the aquatic environment. Some well known examples are benzol[a]pyrenes, hexachlorobenzene and polychlorinated biphenyls (PCB) ; these organic compounds are concentrated in fatty tissue of organisms. Generally, BCF is simply proportional to the hydrophobicity of the chemical, and be expressed as octanol/water partition coefficients (Kow) (Geyer 1985). However, this expression is closely related to the relationship between hydrophobicity and " non specific" aqueous toxicity of compounds, the estimation of BCF via hydrophobicity will result in too high values for chemical acting by specific mechanism (Bruggeman 1988). So, it is important to examine the toxic or ecological effects of chemicals in the aquatic ecosystem.

Multi-Effect Triazole [MET, 2RS + 3RS - 1 - (4-chlorophenyl) - 4. 4-dimethyl - 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. (British 1981). Although several acute toxicity experiments have been conducted (Sugavanam 1984), and some reports showed that it had better productive effects on crops (Deas 1982). While the potential ecologic toxicity, chronic effects of lower concentration and

Correspondence to: Z. T. Liu

residual problems of MET have not been investigated, thence it is difficult to estimate accurately the environmental safety and compare with other research. Therefore, we have collected several aquatic organisms as test species, compared the acute toxicites of MET, studied the relationships between lipid content and bioconcentration of the chemical in the fish (*Carassias auratus*) and the *daphnia magna,* these provide some available evidences for the extensive applying and safe estimation of MET or analogous compounds.

MATERIALS AND METHODS

All MET were purchased from Jiangsu agricultural chemical institute, the purity was 98.25 %. ^{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 source of diluent water were: temperature, 22 ± 1 C; 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 uS/cm; COD_{Mn}, 1.02 - l.20 mg/L.

Organisms used in the static acute toxicity studies were *Selenastrum capricornutum, Daphnia magna, Ctenopharyngodon idellus, Cipangopaludina chinese, Bufo bufo gangarizas, Limnodrilus hoffmeisteri,* The green algae (*S. capricornutum*) which served as food source for *daphnia* and test organism was cultured in 15-L glass carboy containing 10 L of nutrient media (Price 1990). The algal cell density in each expoure chamber maintained 2 x 10^{5} cells /mL, the cell numbers were measured with a hemocytometer and photoperiod was 16 hr daylight / 8 hr dark (about 3000 LX). All organisms 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 group), 5. 0, 12. 5, 25. 0, 50. 0, 100. 0 mg/L. The source was used as the diluent for all solutions, 5 organisms of each species [*D. magna* less than 24 hr, *C. idellus* hatched in 12 hr, *C. chinese* weight 2.06 ± 0.77 g, *B. gangarizans* less than 3 days, *L. hoffmeisteri* length 13.7 ± 2.1 mm] were randomly assigned to each of three replicate test vessels at each exposure level, the test chambers were covered with loosely fitting lids to ratard evaporation and renewed water each 16-

24 hr (Francis 1986). EC_{50} of the algal was observed by determining the concentration reducing algal productivity by 50% (Coler 1989). Mortality was determinted by probing for movement at the end of 24, 48, 72, 96 or 120 hr.

The test fish (*C. auratus*) were about 4.0 cm long and 3.0 g weight, after cultured which fed on dry daphnia (15-20mg/day, one fish) in glass aquariums (30 x 20 x 10 cm) with the conditioned water for 7 days, these fish exposed in the test water with ^{3}H-MET which radioactivity was about 6000 dpm/mL. The test *daphnia* were transferred in 5000 mL beakers which contatined same dose of ^{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 5 L squate jars containing 3 L experimental solutions, the lest MET treated concentration was approximately 15 % of the LC50 value (Eastmond 1984). In the controlled groups of the bioconcentration test, there was ^{3}H-MET and no MET treatment in the experimental solution. At 12, 24, 48, 72, 96, 120, 150 hr, samples were determinted which based on three measurements.

At time intervals, the organisms were blotted, then weighted, homogenized for 15 min with a microtissue glass homogenizer. Each sample was extracted with chlorofrom / methanol and measured the lipid content (Folch 1957). While determinted ^{3}H-MET, all test organisms were rinsed with distilled water, blotted, smashed, and then measured the radioactivity of ^{3}H-MET in these samples. Additionally, obtained about 30-50 mg organs / tissues (gill, liver, intestine, skin, bone, muscle) to determint the distribution of ^{3}H-MET in the fish. The concentration of ^{3}H-MET in both water and organism was measured by the liquid scintillation counting techniques (Neujahr 1964).

Toxicity values (LC_{50}/ EC_{50}) and confidence intervals were determinted 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.

RESULTS AND DISCUSSION

The acute toxicity data for MET showed in table 1. The

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

organism	Time (hr)				
	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	16.2	N.A	N.A	N.A

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

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

Time (hr)	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
150	68.04	58.36	299.04	432.30	49.78	44.59

literature values (Sugavanam 1984) were *Rainbow trout* (96 hr, LC_{50}), 27.8 - 33.1 mg/L. In table 1, LC_{50} values (*S. capricornutum*, 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 aquatic oligochaete (*L. hoffmeisteri*) was approximate equal, but it was more toxic for amphibian (*B. bufo*, 96 hr; LC_{50}, 9.1 mg/L) and mollusc (*C. chinese*, 48 hr, LC_{50}, 12.8 mg/L). Comparative toxicity of MET between *Daphnia* (*D. magna*) and the other test organisms indicated that MET was more toxic for *Daphnia* than for adult fish (*R. trout*) while less for neonates (*C. iddllus*).

The accumulated doses of ^{3}H-MET in the organs/tissues of the fish (*C. auratus*) changed with the time were showed in table 2. Such as at 96 hr, the radioactivity doses were: bone's, 39.64 dpm/mg; muscle's 44.04 dpm/mg; liver's, 286.73 dpm/mg; intestine's, 414.82 dpm/mg. Indicating that the accumulated doses in organs/tissues of the fish were different at same

Table 3. Lipid content and concentration of ^{3}H-MET in the organisms*

Group	Time (hr)	Lipid (%) F	Lipid (%) D	Radioactivity (dpm/mg) F	Radioactivity (dpm/mg) D	BCF (dpm/mL) Water	BCF F	BCF D
A	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	90.25	156.1	918.3	98.12	171.8
	150	7.35	5.67	96.42	158.6	825.6	105.5	182.1
B	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	208.4
	150	9.72	6.87	123.2	196.1	884.4	134.2	221.7

* F= *Carassias auratus* . D = *Daphnia magna.*
BCF = radioactivity in the organism / radioactivity in the water.
A = control group, B = MET treatment group.

time intervals. The ^{3}H-MET in the intestine was highest, then the ranges of the radioactivity dose were liver's > gill's > skin's > muscle's > bone's.

The distribution of ^{3}H-MET in the fish 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 relativize 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 of gill's. Thus, these informations indirected that the bioconcentration of ^{3}H-MET in the fish mainly existed in digestive system.

Table 4. Regression equations between radioactivity in the organism and the test time

Organ/Tissue	Equations	r^2
liver	S= 148.18 + 30.351nT	0.95
intestine	S= -125.02 + 114.391nT	0.96
gill	S= 30.68 + 7.521nT	0.95
skin	S= -21.57 + 15.641nT	0.93
bone	S= 7.67 + 7.551nT	0.95
muscle	S= 28.42 + 3.251nT	0.95

r^2, coefficient of correlation.
S, radioactivity units. T, test time units.

Table 5. Expression equations between lipid content and BCF

Group	Organism	Equation	r^2
control	fish	Y= EXP (2.38 + 0.31 X)	0.95
	Daphnia	Y= EXP (1.82 + 0.59 X)	0.95
treated	fish	Y= EXP (2.62 + 0.22 X)	0.95
	Daphnia	Y= EXP (2.34 + 0.45 X)	0.96

r^2, coefficient of correlation.

The radioactivity of ^{3}H-MET in all organs/tissues were better correlatived to the test time. The regression equations were listed in Table 4. The kinetic changes of lipid content and concentration of ^{3}H-MET in the fish and the daphnia were showed in table 3. These results indicated that concentrated 3H-MET were rising with the increasing of lipid contents in the growth period of the test organisms. In control groups, the changeable ranges both lipids and ^{3}H-MET doses were less than that of treated groups. Such as the lipids changed from 4.86 % to 9.72 % in treated group, compared to that of 4.63 % to 7.55% in control groups at the same time intevals, and it was such change of ^{3}H-MET concentration in the test organism. Therefore, the concentrated MET were better correlated with the lipid contents of test organisms, and MET could affect the lipid content, then the lipids reversely affected the bioconcentration of MET in the organisms.

Expression equations between lipid content (X) and BCF (Y) of MET in the fresh water species were given in Table 5. These results have presented that the BCF was higher positive correlated to the lipid contents. We also notices that BCF data of the Daphnia were higher than that of fish while the lipid contents were lower (Table 3), suggesting that MET may be not wholly distributed in the lipid or uptake of MET is faster in *Daphnia* due to its larger surface to volume ratio. Because the coefficients of correlation were strongly between lipid and BCF ($r^2 > 0.95$), so lipid content was the mainly determinative factor of BCF. Chiou (1985) presented that the BCF of chemricals could be estimated according to lipid contents of organisms, and Geyer (1985) indicated that BCF 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 expression was chiefly related to the relationships between hydrophobicity and non specific biological effects of chemical. Some reports indicated that the BCF value of some higher molecular weight compounds and compounds metabolized by organisms did not correlated with Kow (Oliver 1985), according to the results, the MET increased the lipid contents which effected the accumulation of ^{3}H-MET in the fish. While, the similar researches need to be investigated extensively.

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