The change in the content of TPP and TTP in the brain after TTFD injection was analyzed by the same procedure as that in the liver, and a slightly increased level of both TPP and TTP was observed at 3 h after the injection (data not shown). However, this increase was not sufficiently distinct to get exact kinetics of these changes. This may be due to a slow penetration of thiamine through the blood-brain barrier when injected in large doses[15,16].

1 P.M. Dreyfus, in: Thiamine, p.229. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
2 R.L. Barch, in: Thiamine, p.283. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
3 Y. Itokawa, Vitamins (Japanese) *49*, 415 (1975).
4 H. Iwata, Vitamins (Japanese) *50*, 1 (1976).
5 G. Rindi and L. de Guiseppe, Biochem. J. *78*, 602 (1961).
6 K. Ishii, K. Sarai, H. Sanemori and T. Kawasaki, Analyt. Biochem. *97*, 191 (1979).
7 K. Ishii, K. Sarai, H. Sanemori and T. Kawasaki, J. nutr. Sci. Vitaminol. *25*, 517 (1979).
8 H. Sanemori, H. Ueki and T. Kawasaki, Analyt. Biochem. *107*, 451 (1980).
9 Y. Itokawa and J.R. Cooper, Biochim. biophys. Acta *158*, 180 (1968).
10 J.H. Pincus, Y. Itokawa and J.R. Cooper, Neurology *19*, 841 (1969).
11 J. Schrijver, T. Dias and F.A. Hommes, Neurochem. Res. *3*, 699 (1978).
12 Y. Itokawa, in: Thiamine, p.361. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
13 I. Sen and J.R. Cooper, Neurochem. Res. *1*, 65 (1976).
14 M. Fujiwara, J. nutr. Sci. Vitaminol. *22*, suppl. 57 (1976).
15 A. Inoue, S. Shim and H. Iwata, J. Neurochem. *17*, 1373 (1970).
16 R. Spector, Am. J. Physiol. *230*, 1101 (1976).

Relationship between the molecular weights of pesticides and their bioconcentration factors by fish

J. Kanazawa

National Institute of Agricultural Sciences, Kannondai, Yatabe, Ibaraki, 305 (Japan), 12 October 1981

Summary. A significant positve correlation was found between the molecular weights (187–412) of 19 pesticides and their bioconcentration factors (4–37,800) in 2 fresh-water fishes; topmouth gudgeon and fathead minnows.

For many non-ionic organic compounds, including pesticides, it has already been shown that the bioconcentration factor (BCF) from water by fish increases as the solubility in water decreases, or as the 1-octanol-water partition coefficient (PC) increases[1–5]. These 2 physico-chemical properties of chemicals were used to predict their bioconcentration potential in living organisms[6]. Zitko and Hutzinaer[7] reported that for both chlorinated and brominated biphenyls, in Atlantic salmon, the accumulation coefficients from water decrease with increasing molecular weights. But, on the contrary, Southworth et al.[8] found that for 3 azaarene homologues, isoquinoline, acridine and benz (a) acridine, the BCFs in *Daphnia pulex* increased dramatically with increase in molecular weight. It is not clear whether this difference is due to the structure of the compounds or the physiological characteristics of the test organisms. The present study is directed towards obtaining a basic understanding of the relationship between the BCFs of 15 pesticides in fresh-water fish; adding to the results for topmouth gudgeon, already reported by the author[5], the BCFs of 4 reference pesticides and their molecular weights.

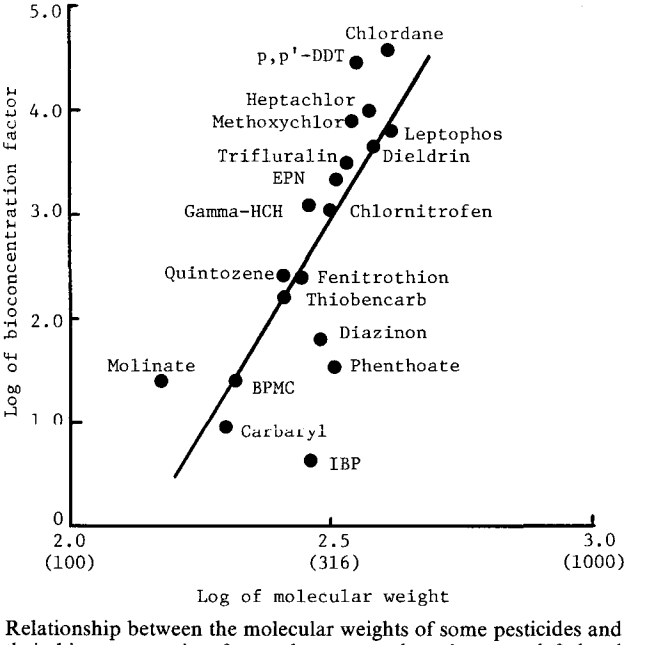
Material and methods. For the BCFs for topmouth gudgeon *(Pseudorasbora parva)* from water using a flow-through test at equilibrium, and the PCs of 15 pesticides, the values already reported by the author[5] were used. The BCFs for methoxychlor, p,p'-DDT, heptachlor and chlordane were the values in fathead minnows *(Pimephales promelas)* reported by Veith et al.[4].

Results and discussion. The molecular weights, BCFs and PCs of 19 pesticides including 6 organochlorine insecticides, 5 organophosphorus insecticides, 2 carbamate insecticides, 2 fungicides and 4 herbicides are shown in the table. A plot of log (BCF) vs log (molecular weight) is shown in the figure. As shown in the figure, for the 19 pesticides tested, which had a wide range of structures, the BCF increased as the molecular weights increased from 187 to 412. A satisfactory linear relationship is observed between the log of BCFs and the log of molecular weights. The regression equation shows:

$$\text{Log } Y = 10.9 \log X - 24.2$$

Where Y is the BCF by fish and X is the molecular weight of the pesticides. The correlation coefficient was 0.846 which was significant at the 1% probability level. Therefore, it is possible to predict the bioconcentration potential of a pesticide by the molecular weight.

It is already well known that a significant correlation is present between the PC values of many non-ionic organic compounds and their BCFs from water by fish[1,2,4,5]. Therefore, it can be presumed that the partition of chemicals in water through the gills into the blood of fish, and the rate



Relationship between the molecular weights of some pesticides and their bioconcentration factors by topmouth gudgeon and fathead minnows. Parenthesis shows an integral number.

of uptake, are dependant on the PC of chemicals. On the other hand, a linear relationship between the molecular weights of the 15 pesticides tested and their PCs was also obtained. This correlation coefficient was 0.671, which was significant at the 1% probability level. It can therefore be presumed as a matter of course that a significant positive correlation exists between the molecular weights of pesticides and their BCFs in fish. The result of this study suggests that the lipid solubility of chemicals plays a much greater role in determining the movement of chemicals across the gills of fish and the resulting bioconcentration than the structure of the molecule. However, as pointed out by Zitko and Hutzinaer[7], the uptake of chlorinated paraffins by Atlantic salmon from water takes place only up to a molecular weight limit of 600. Therefore, the correlation obtained by this study may be present only within the range of pesticides tested. This subject should be further investigated on pesticides with a higher molecular weight.

Molecular weights, bioconcentration factors and partition coefficients of the pesticides tested

Pesticide	Molecular weight	BCF	PC
Molinate	187.3	26	1628
Carbaryl	201.2	9	197
BPMC[a]	207.3	26	1500
Thiobencarb	257.8	170	2650
Quintozene	259.4	238	16552
Fenitrothion	277.2	246	2767
IBP[b]	288.3	4	1630
Gamma-HCH	290.9	1246	4611
Diazinon	304.4	152	1386
Chlornitrofen	318.6	1109	4709
Phenthoate	320.3	36	781
EPN	323.3	2346	7027
Trifluralin	335.3	3142	9328
Methoxychlor	345.7	8300	
p,p'-DDT	354.5	29400	
Heptachlor	373.3	9500	
Dieldrin	380.9	4430	4611
Chlordane	409.8	37800	
Leptophos	411.8	6058	20833

[a] 2-sec-butylphenyl methylcarbamate; [b] S-benzyl O,O-di-isopropyl phosphorothioate.

1 W.B. Neely, D.R. Branson and G.E. Blau, Envir. Sci. Technol. *8*, 1113 (1974).
2 C.T. Chiou, V.H. Freed, D.W. Schmedding and R.L. Kohnert, Envir. Sci. Technol. *11*, 475 (1977).
3 W.B. Neely, Envir. Sci. Technol. *13*, 1506 (1979).
4 G.D. Veith, D.L. Defoe and V. Bergstedt, J. Fish Res. Bd Can. *36*, 1040 (1979).
5 J. Kanazawa, Pestic. Sci. *12*, 417 (1981).
6 E.E. Kenaga, Ecotoxic. envir. Safety *4*, 26 (1980).
7 V. Zitko and O. Hutzinaer, Bull. envir. Contam. Toxic. *16*, 665 (1976).
8 G.R. Southworth, J.J. Beauchamp and P.K. Schmieder, Envir. Sci. Technol. *12*, 1062 (1978).

Gluconolactonase: a zinc containing metalloprotein

W.R. Carper, Aruna S. Mehra, D.P. Campbell and J.A. Levisky

Department of Chemistry, Wichita State University, Wichita (Kansas 67208, USA), and Frank J. Seiler Research Laboratory, USAF Academy (Colorado 80840, USA), 22 February 1982

Summary. A metal analysis of bovine hepatic gluconolactonase indicates the presence of at least 1 atom of tightly bound zinc per enzyme subunit in this hexameric protein. Other divalent metals are present in lesser quantities and are subject to removal by EDTA. Activation energies for the manganese and magnesium catalyzed reactions are 5.3 and 11.0 kcal/mole, respectively.

Gluconolactonase (E.C.3.1.1.17) catalyzes the hydrolysis of 1,5-gluconolactone to gluconic acid, and the hydrolysis of 6-phospho-1,5-gluconolactone to 6-phosphogluconic acid. This protein consists of 6 similar subunits and is located in the cytosol[1,2]. Gluconolactonase requires a divalent metal for catalytic activity similar to other lactonases[3-5] with Mn^{2+} generally preferred over Mg^{2+}. In view of the lack of information concerning the metal content of this protein, we have subjected bovine liver gluconolactonase to a metal analysis by atomic absorption, the results of which are contained herein. We also report the results of a kinetic study in which either Mn^{2+} or Mg^{2+} are used with this enzyme to catalyze the hydrolysis of 1,5-gluconolactone.

Materials and methods. D-glucono-1,5-lactone, M 155°, was purchased from Sigma Chemical Company and purified by recrystallization from ethanol. DMSO was purified by alternately freeezing and thawing to remove water immediately prior to use. Gluconolactonase was purified from beef liver according to the method of Bailey et al.[2]. The amount of enzyme used in each of the kinetic experiments

Table 1. Results of metal analysis for both native and EDTA-treated enzyme

Metal	Enzyme concentration (mg/ml)	Metal concentration (mg/ml)		Number of atoms per mole of enzyme	
		Native	EDTA-treated	Native	EDTA-treated
Ca (II)	1.07	0.110	0.062	0.60	0.34
	0.33	0.220	0.148	3.90	2.62
Mg (II)	1.07	0.250	0.183	2.25	1.65
	0.33	0.120	0.112	3.54	3.30
Zn (II)	1.07	2.24	2.19	7.48	7.30
	0.33	0.93	0.94	10.2	10.2
Cu (II)	1.07	0.210	0.212	0.72	0.73
	0.33	0.070	0.072	0.79	0.81
Mn (II)	1.07	0.037	0.029	0.15	0.15
	0.33	0.012	0.010	0.16	0.17