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.

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Gluconolactonase: a zinc containing metalloprotein

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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

Table 2. Kinetic constants

°C	K_m (mM)[a]		V_m (lCO_2/min)[a]		V_m/K_m	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
20	12.9(±0.3)	6.6(±0.1)	3.8(±0.9)	5.9(±0.8)	0.29	0.89
30	10.5(±0.3)	6.2(±0.2)	5.5(±1.5)	7.7(±1.1)	0.53	1.24
37	6.7(±0.2)	5.8(±0.1)	6.5(±1.0)	9.0(±0.9)	0.97	1.54

[a]Mean value ± SD.

Table 3. Transition state thermodynamic constants for gluconolactonase reaction

	20 °C		30 °C		37 °C	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
ΔG^{+} (kcal/mole)	12.4	9.4	12.5	9.5	12.6	9.7
ΔH^{+} (kcal/mole)	10.4	4.7	10.4	4.7	10.4	4.7
ΔS^{+} (e.u.)	−6.6	−16.0	−6.9	−15.8	−7.2	−16.1

was 8.5 units or 4.55×10^{-3} µM as determined by the pH method[1,2], using a standard assay mixture containing 50 mM tris, pH 7.5, 1.3 mM $MnCl_2$ and gluconolactonase. The kinetics reported herein were determined manometrically in $NaHCO_3$ buffer as described below.

Reaction mixtures were prepared as follows: All the reactants except the substrate, 1,5-gluconolactone, were placed in the main compartment such that the final volume was 4.0 ml of solution. The $NaHCO_3$ buffer concentrations were 24.0 mM at 20 °C, 20.6 mM at 30 °C and 18.6 mM at 37 °C. All metal ion concentrations were 0.5 mM. The sidearm contained 100 µl of DMSO as a solvent for the lactone, as the latter is hydrolyzed to gluconic acid by water. Tests with and without DMSO showed no appreciable effect on either the enzymatic or the nonenzymatic hydrolysis of 1,5-gluconolactone.

Dialysis of gluconolactone solutions was accomplished by dialyzing 5 ml each of 2 enzyme samples against 4 l of 1 mM EDTA, pH 9.0, for 24 h at 4 °C. The buffer solution was changed 4 times during this period.

Atomic absorption measurements were performed on duplicate samples as described previously[6] using a Varian Model475 and a Perkin Elmer Model460 atomic absorption spectrometer. Flame methodology was used for Ca, Mg, Zn and Cu. A graphite furnace was used for Mn.

Results and discussion. The results of a metal analysis by atomic absorption is contained in table 1. The results are an average of duplicate analyses of 2 bovine hepatic gluconolactonase samples obtained from separate preparations. The 1st analysis was done on 2 highly purified, homogeneous samples each of whose sp. act. was approximately 1000 units per mg of protein. The 2nd metal analysis was completed on the same samples after they were subjected to dialysis against 1 mM EDTA, in an attempt to remove any losely bound metal ions. Similar treatment at an earlier stage in purification process lowers catalytic activity which can then be completely restored only by the addition of Mn^{2+}. Addition of Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+} only partially restores the enzyme's activity[1,2].

As is shown in table 1, gluconolactonase contains the equivalent of at least 1 zinc atom per subunit. Of the remaining divalent metals, only Mg^{2+} and possibly Ca^{2+} are present to any extent. Ca^{2+} is the only ion that varies considerably in metal to enzyme ratio from sample to sample. Furthermore, of the ions tested, Ca^{2+} is most easily removed from the enzyme, and it is relatively ineffective in restoring catalytic activity to a highly purified sample of gluconolactonase[1,2]. Zn^{2+} is the only metal ion present in abundance after both the purification process and the EDTA treatment. A conclusion based upon these and other results[1,2] is that the zinc is required for both catalytic activity and possibly structural integrity. Similar cases have been observed including superoxide dismatase[7] where zinc and copper are required, and thermolysin[8] where zinc and calcium are necessary.

In view of our previous results[1,2] and the metal analysis, we compared the relative effects of either 0.5 mM Mn^{2+} or 0.5 mM Mg^{2+} on gluconolactonase kinetics in bicarbonate buffer (pH 7.5) at 20, 30 and 37 °C. Kinetic constants obtained from Lineweaver-Burk[9] plots are given in table 2. 1,5-gluconolactone concentrations were 0.49, 0.77, 1.1, 1.98, 5.05, 7.5 and 11.0 mM. Examination of table 2 suggests that although Mn^{2+} is preferred at lower temperatures, Mg^{2+} is a possible substitute at 37 °C.

Plots of log v vs 1/T at varying lactone concentrations give activation energies of 11.0±1.4 kcal/mole and 5.3±0.4 kcal/mole for the Mg^{2+} and Mn^{2+} containing reactions, respectively. In addition, plots of log (V_m/K_m) vs 1/T give E_a-values of 12.6±1.1 kcal/mole and 5.8±0.5 kcal/mole for the Mg^{2+} and Mn^{2+} containing reactions.

Finally, equations 1, 2 and 3 have been used to calculate the transition state thermodynamic constants given in table 3 for both the Mg^{2+} and Mn^{2+} lactonase reactions. The rate constants were determined from $V_m/(E)$ and the E_a's used were 11.0 kcal/mole for the Mg^{2+}-lactonase reaction and 5.3 kcal/mole for the Mn^{2+}-lactonase reaction. As is expected, the enthalpies

$$E_a = \Delta H^{+} + RT \tag{1}$$

$$k = \frac{kT}{h} \exp \frac{-\Delta H^{+}}{RT} \exp \frac{\Delta S^{+}}{R} \tag{2}$$

$$\Delta G^{+} = \Delta H^{+} - T\Delta S^{+} \tag{3}$$

and entropies of activation reflect the difference in activation energy. Comparable results were obtained for the hydrolysis of benzoyl-L-tyrosinamide and benzoyl-L-tyrosine ethyl ester by chymotrypsin at 25 °C[10].

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