Toxicity of Zinc and Cygon, Applied Singly and Jointly, to Zebrafish Embryos*

by R. R. ROALES and A. PERLMUTTER

Laboratory of Aquatic Biology, Department of Biology, Graduate School of Arts & Science, New York University, New York, N.Y. 10003

The toxic effects of zinc and pesticides on adult fishes has been the subject of much study in recent years. However, relatively few papers on the effects of zinc (AFFLECK 1952; PICKERING and VIGOR 1965; SKIDMORE 1965, 1966) and pesticides (KATZ and CHADWICK 1961; HILTIBRAN 1967; ABEDI and MCKINLEY 1967; MATTON and LAHAM 1969) on fish embryos or larvae exist.

Since it is likely that many cases of environmental contamination result from the interaction of two or more pollutants, several studies have been concerned with the interaction of various contaminants (JONES 1938; WEISS 1959; LLOYD 1961; HERBERT and SHURBER 1964; SPRAGUE et al. 1965; BROWN et al. 1968; CAIRNS and SCHEIER 1968; SOLON and NAIR 1970). However, little work has been concerned with the interaction of contaminants, especially zinc and organic phosphorus pesticides, on fish embryos.

The purpose of this study was to determine the toxicity of zinc and Cygon (O,O-dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate), singly, and the type of toxic interaction between the two contaminants on zebrafish (Brachydanio rerio) embryos.

Materials and Methods

Adult zebrafish were obtained commercially, and random pairs of fish were spawned using lights to control the time of daily spawning (LEGAULT 1958). Only embryos after the

*Supported in part by Grant No. UI-00327-02 U.S.P.H.S., and submitted in part by the senior author in partial fulfillment of the requirements for the degree of Master of Science at New York University.

Bulletin of Environmental Contamination & Toxicology, Vol. 12, No. 4 © 1974 by Springer-Verlag New York Inc.

late blastula or early gastrula stage (HISAOKA and BATTLE 1958) were used, since mortality can be extensive at the earlier stages. The criteria for death were lack of body movement, circulation and visible heartbeat. Embryos exhibiting a slow, irregular heartbeat or exhibiting body movements, but no heartbeat or circulation were considered to be alive.

The toxicities of zinc ($ZnSO_4 \cdot 7H_2O$) and Cygon (Technical Grade 99% pure) to the embryos were obtained by determining the 24, 48 and 72 hour median tolerance limit (Tl_m) for each compound using probit analysis (BLISS 1937, 1952). A total of 40 embryos were used at each dose tested, and 100 embryos were used as controls. The type of interaction between zinc and Cygon was determined using a modification of OSTERHOUT's (1914a, 1914b) method. In the present study, 80 embryos each were exposed for 72 hours to combinations of varying percentages of the respective 72 hour Tl_m dosages for zinc and Cygon, and the mortality was recorded.

In an effort to remove as many variables as possible, e.g. water hardness, calcium and magnesium hardness, glass distilled water was used as the dilution water. At no time did the dissolved O_2 levels fall below 6 ppm, nor did the dissolved CO_2 levels exceed 2 ppm. The biomass per test solution volume was 0.45 g/liter.

Results and Discussion

The results of the 24, 48 and 72 hour Tl_m experiments are shown in Table I. The zinc results are, generally, in agreement with those of SKIDMORE (1965), although he did not determine the precise values at 24, 48 and 72 hours.

TABLE I

Tl_m data for zebrafish embryos exposed to zinc and Cygon

Toxicant	Tl_m (ppm ± S. E.)		
	24 hrs.	48 hrs.	72 hrs.
Zinc	6,714 ± 570	136 ± 18.5	19 ± 2.2
Cygon	--------	940 ± 13.2	259 ± 10.9

The embryos exposed to Cygon appeared to be retarded in development, as noted by lack of heartbeat and little movement of the embryos at 24 hours. Pigment formation was

also retarded in these animals, and high doses of Cygon (800 ppm) produced an abnormal enlargement of the heart region. Due to the retardation and subsequent lack of heartbeat at 24 hours, no Tl_m was obtained at 24 hours. The data for 48 and 72 hours, however, seems to indicate that the embryos were more resistant to Cygon as compared with zinc.

The results of the zinc/Cygon interaction experiments are shown in Table II, and are represented graphically in Figure 1. In the present method, a horizontal line indicates an additive effect; a bell-shaped curve shows synergism, while an inverted-bell curve indicates antagonism. The shape of the curve (Figure 1) seems to indicate that low doses of zinc (20% of the 72 hour Tl_m) are antagonistic to the toxicity of Cygon. Since all dosages, except for 20% zinc/80% Cygon, are not statistically different (Table III), the possibility

TABLE II

Percent kill of various zinc/Cygon combinations during 72 hours

zinc dose: 19 ppm
Cygon dose: 259 ppm

Test solution composition		% kill ± S. E.
% zinc	% Cygon	
100	0	78.0 ± 6.8
80	20	71.8 ± 7.2
60	40	40.2 ± 18.8
40	60	40.5 ± 6.7
20	80	1.2 ± 1.0
0	100	56.8 ± 15.2
Control		0.0

of an additive effect between the two toxicants might be indicated. The results at the 20% zinc/80% Cygon level are not due to dilution of the Cygon, since dilution of Cygon to this level (207 ppm) yielded a mortality of 30 ± 5.8%. Thus, the reduction in mortality is probably due to the action of zinc.

Organic phosphorus pesticides have been shown to be inhibitors of cholinesterase activity in fishes (HENDERSON and PICKERING 1957; WEISS 1959). However, whether or not this is the particular mode of action of Cygon on fish embryos is not known at this time. Various theories exist as to the exact mechanism of zinc toxicity in fishes (SKIDMORE 1964), however, the precise mechanism involved in embryos remains to be determined.

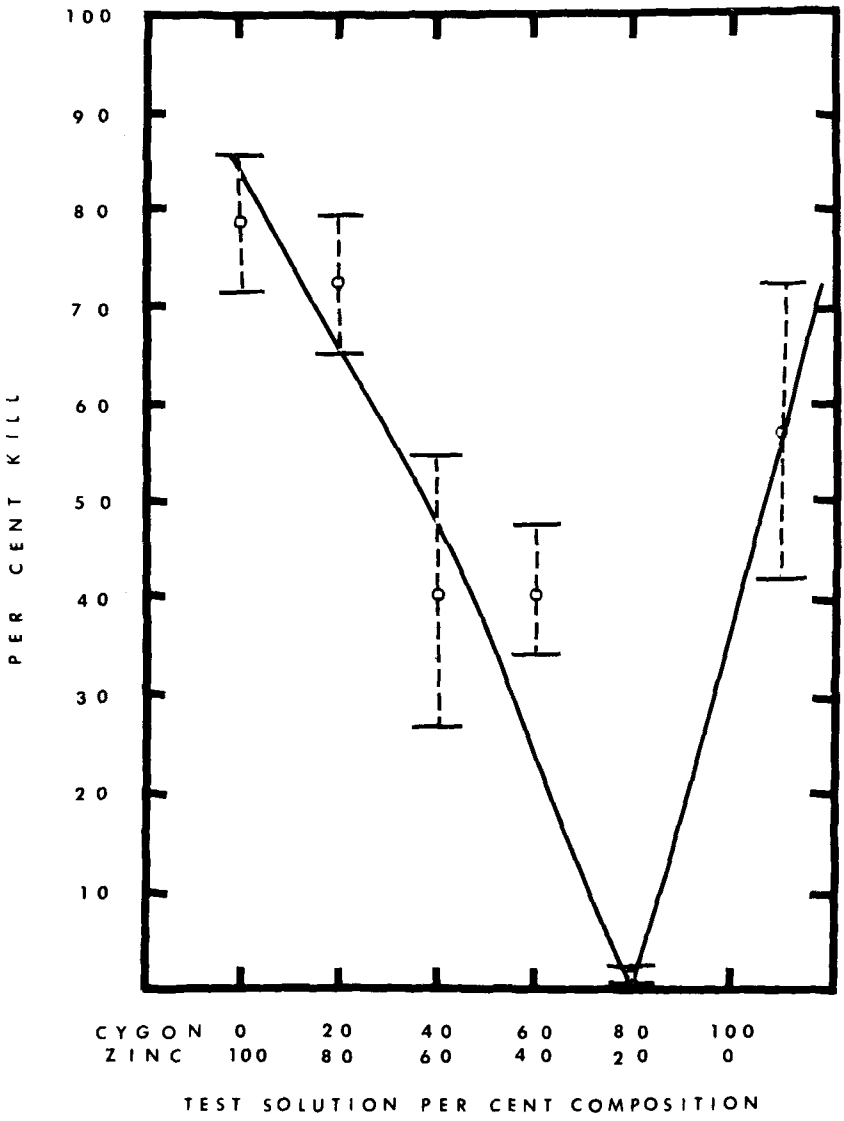

Figure 1. Dosage-mortality curve for zebrafish embryos exposed to varying percentages of the respective 72 hour Tl_m dosage for Cygon and zinc.

TABLE III

Significance of difference between various doses in the zinc/Cygon interaction experiments

Between % zinc		% Cygon	t	P*
100		0		
	and		0.20	>90< 80%
80		20		
80		20		
	and		2.03	>20< 10%
60		40		
60		40		
	and		0.02	<90%
40		60		
40		60		
	and		5.81	>1 < 0.1%
20		80		
20		80		
	and		3.63	>5 < 2%
0		100		
100		0		
	and		1.27	>30< 20%
0		100		

*N= 4; P = 0.05 when t = 2.57

Zinc has been shown to reduce the mitotic index of fish cells in culture (RACHLIN and PERLMUTTER 1968), and to exert action on the cell nucleus (KROEGER 1964; STUDZINSKI 1965). HILLER and PERLMUTTER (1971) have shown that zinc can alter the net charge of fish cell surfaces. HOSKIN and ROSENBERG (1967) postulated that charges surrounding the cholinesterase-containing systems may prevent compounds from inhibiting the enzyme. Thus, it is possible that low doses of zinc may enhance this phenomenon, while higher doses may inhibit it. However, whether or not zinc acts on the nucleus of the cell or on the cell surface, altering its charge, remains to be determined.

Summary

The zinc 24, 48 and 72 hour Tl_m and the Cygon 48 and 72 hour Tl_m were determined for zebrafish embryos. In addition, zinc, at low doses, was found to be antagonistic to the toxic action of Cygon on these embryos. At other levels tested, zinc and Cygon may interact additively.

Literature Cited

ABEDI, Z.H. and W.P. MCKINLEY: Nature 216:1321-1322 (1967).
AFFLECK, R.J.: Australian J. Mar. & Freshwater Res. 3: 142-164 (1952).
BLISS, C.I.: Ann. Appl. Biol. 24:815-852 (1937).
BLISS, C.I.: The statistics of bioassay. New York: Academic Press 1952.
BROWN, V.M., V.V. MITROVIC and G.T.C. STARK: Water Res. 2:255-263 (1968).
CAIRNS, JR., J. and A. SCHEIER: Not. Nat. Acad. Nat. Sci. Phil. 299:1-12 (1968).
HENDERSON, C. and Q.H. PICKERING: Trans. Am. Fish. Soc. 87:39-51 (1957).
HERBERT, D.W.M. and D.S. SHURBER: Ann. Appl. Biol. 53: 33-41 (1964).
HILLER, J.M. and A. PERLMUTTER: Water Res. 5:703-710 (1971).
HILTIBRAN, R.C.: Trans. Am. Fish. Soc. 96:414-416 (1967).
HISAOKA, K.K. and H. BATTLE: J. Morph. 102:311-328 (1958).
HOSKIN, F.C.G. and P. ROSENBERG: Science 156:966-967 (1967).
JONES, J.R.E.: J. Exp. Biol. 15:394-407 (1938).
KATZ, M. and G.C. CHADWICK: Trans. Am. Fish. Soc. 90: 394-397 (1961).
KROEGER, H.: Chromosoma 15:36-70 (1964).
LEGAULT, R.: Copeia 1958:328-330 (1958).
LLOYD, R.: Ann. Appl. Biol. 49:535-538 (1961).
MATTON, P. and Q.N. LAHAM: J. Fish. Res. Bd. Canada 26: 2193-2200 (1969).
OSTERHOUT, W.J.V.: Bot. Gaz. 58:178-186 (1914a).
OSTERHOUT, W.J.V.: Bot. Gaz. 58:272-276 (1914b).
PICKERING, Q.H. and W.N. VIGOR: Prog. Fish-Cult. 27:153-157 (1965).
RACHLIN, J.W. and A. PERLMUTTER: Water Res. 2:409-414 (1968).
SKIDMORE, J.F.: Quart. Rev. Biol. 39:227-248 (1964).
SKIDMORE, J.F.: Ann. Appl. Biol. 56:47-53 (1965).
SKIDMORE, J.F.: J. Fish. Res. Bd. Canada 23:1037-1041 (1966).
STUDZINSKI, G.P.: J. Histochem. Cytochem. 13:365-375 (1965).
SOLON, J.M. and J.H. NAIR, III: Bull. Environ. Con. & Toxicol. 5:408-413 (1970).
SPRAGUE, J.B., P.F. ELSON and R.L. SAUNDERS: Int. J. Air & Water Pollut. 9:531-543 (1965).
WEISS, C.M.: Sew. & Ind. Wastes 31:580-593 (1959).