Hiroshima Journal of Medical Sciences
Vol.34, No.3, 327～330, September, 1985
HIJM 34-47

Acute Toxicity of 14 Different Kinds of Metals Affecting Medaka Fry

Yukio HIRAOKA[1], Shoichi ISHIZAWA[2], Toshihiko KAMADA[3] and Hisanori OKUDA[1]

1) Department of Hygiene, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan
2) Hiroshima City Institute of Public Health, Shoko-center, 4-1-2, Nishi-ku, Hiroshima 733, Japan
3) Hiroshima Bunka Women's Junior college, Gion-cho, Nagatsuka 174-170, Asaminami-ku, Hiroshima 731-01, Japan

(Received June 19, 1985)

Key words: Acute toxicity, Metals, Medaka fry

ABSTRACT

We examined the acute toxicity of 14 different kinds of metals affecting medaka fry and from the results, arranged them according to the order of intensity level of acute toxicity. The most toxic metals to medaka fry were Ag, Hg, Cu, and Cd, the second most toxic were Zn, As, Cr, Ni, and Pb, and the third most toxic were Se, V, Fe, and Co. The metal least toxic to medaka fry was Mn.

In most metal exposure experiments the effects of from one to several kinds of metals on a test organisms are usually examined. There are therefore many reports about the medical and biological effects of various kinds of metals, for example, acute or chronic toxicity[5], absorption[4], excretion[4], accumulation[2], and so on. Therefore, the effects of some metals on test organisms seem to be moderately elucidated. However, to the best of our knowledge, a comparison of the biological effects of many different kinds of metals on a test organism at the same time and under the same conditions has not yet been carried out. We therefore exposed medaka fry to 14 different kinds of metals at the same time independently and the toxic level was compared among those metals and discussed in terms of the order of toxic level.

MATERIALS AND METHODS

Red medaka, red variety of *Oryzias latipes*, were purchases from Ito Fish Farm, reared carefully in indoor aquariums, and made to spawn. The fertilized eggs were reared and hatched out under carefully managed conditions. The fry were fed on the primary bait for *Ayu* *(Plecoglossus altivelis)* (Oriental Yeast Co., Ltd; *Ayu* fish starter No. 0) until 6 days after hatching but were not fed on the 7th day after hatching. Around 500 of the 8-day-old fry were prepared at the same time and used in the following experiments. Blank tests, using a solution comprised of 50 ml tap water and 50 ml deionized water, were carried out twice. The test solution (100 ml) consisted of 50 ml of tap water and 50 ml of deionized water containing one concentration of a certain metal ion, as shown in Table 1. Ten fry were put into each beaker containing each test solution and after 24 hr the numbers of dead fry were counted. Judgement of the effects was made on the number of dead fry, death of fry being defined as a lack of any perceptible heart beat. The test solution pH was 6.9, total hardness ($CaCO_3$) was 10.5 mg/liter, C1 was 6.5 mg/liter, and water temperature was 25°C. The 14 different kinds of metal ion concentration in the tap water are shown in Table 2.

Table 1. Addition of 14 different kinds metal ions, their concentrations and the number of deaths of medaka fry exposed to each metal ion

Ion	Added as	Added metal ion concentration (ppm)					
		0.01	0.1	1.0	10	100	1000
Mn(II)	$MnCl_2$	—	—	0	1	7	9
Fe(II)	$(NH_4)_2Fe(SO_4)_2$	—	—	0	7	9	10
Cr(VI)	$K_2Cr_2O_7$	—	—	0	10	—	—
Zn(II)	$ZnCl_2$	—	0	2	10	—	—
As(III)	$NaAsO_2$	—	—	0	9	10	—
Se(IV)	Na_2SeO_3	—	—	0	3	10	—
Ni(II)	$NiCl_2$	—	—	0	10	—	—
Co(II)	$CoCl_2$	—	—	0	8	9	—
Pb(II)	$Pb(CH_3COO)_2$	—	—	0	10	—	—
V(V)	NH_4VO_3	—	—	0	1	10	—
Hg(II)	$HgCl_2$	0	9	10	—	—	—
Cu(II)	$CuSO_4$	0	8	10	10	—	—
Cd(II)	$CdCl_2$	0	5	10	10	—	—
Ag(I)	$AgNO_3$	0	10	10	10	—	—

—: Not tested

Table 2. Metal ion concentrations in tap water (ppm)

Zn	0.22	Cu	N.D.
Fe	0.60	Mn	0.006
Cr	N.D.	Ni	N.D.
Co	N.D.	Pb	N.D.
Cd	N.D.	As	N.D.
Se	N.D.	Hg	N.D.
V	N.D.	Ag	N.D.

N.D.: less than 0.002 ppm

RESULTS

The results of the present study are shown in Table 1. No fry died in replicate blank tests. In each 1 ppm solution of Hg, Cu, Cd, or Ag, all of the 10 fry died while 8-10 fry survived in the corresponding solutions of all the other metals. In each 10 ppm solution of Mn, Fe, As, Se, Co., or V, 1-9 fry died and 7-9 fry died in each 100 ppm solution of Mn, Fe, or Co. In the 1000 ppm solution of Mn, 9 fry died but one survived. At the end of the experiment, this single fry had sunk to the bottom of the beaker, and when stimulated by gentle prodding it showed no response. Its heart, however, was still beating rhythmically when it was observed under a stereoscopic microscope. In the 0.1 ppm solution of Ag, all of the 10 fry died but 5-9 fry died in each 0.1 ppm solution of Hg, Cu, or Cd. In each 0.01 ppm solution of Hg, Cu, Cd, or Ag, none of the fry died.

DISCUSSION

The intensity level of acute toxicity of the 14 different kinds of metals affecting medaka fry was examined by comparing the number of deaths at each concentration and by using Fisher's exact test. In each 1 ppm solution of Ag, Hg, Cu, or Cd all of the fry died and 2 fry died in the corresponding solution of Zn, but none of the fry died in each 1 ppm solution of the other metals. The number of dead fry in the 1 ppm solution of Ag, Hg, Cu, or Cd was significantly higher than that for Zn ($p<0.01$:Table 3). It was therefore inferred that the most toxic metals tested were Ag, Hg, Cu, and Cd. Next, the numbers of deaths in the 1 and 10 ppm solutions of Zn, As, Cr, Ni, Pb, Se, or V were compared because 1-3 fry survived even in each 100 ppm solution of Mn, Fe, or Co. The numbers of dead fry in each 10 ppm solution of Zn, As, Cr, Ni, or Pb were significantly higher compared with those in corresponding solutions of Se or V ($p<0.01$: Table 3) and there was little difference between the numbers of dead fry in the 10 ppm solutions of Se or V (Table 1). Nine fry died in the 100 ppm solution of Fe or Co and 7-8 fry died in each 10 ppm solution of these two metals. The number of dead fry in the 100 ppm solution of Mn was lower than that in the corresponding solution of Fe or Co and one fry survived even in the 1000 ppm solution of Mn. Accordingly it is inferred that, of the metals

Table 3. Verification of the significant difference between two metals using Fisher's exact test

	Cr	Zn	As	Se	Ni	Pb	V
Cr							
Zn	—						
As	5.00×10^{-1}	5.00×10^{-1}					
Se	1.55×10^{-3}**	1.55×10^{-3}**	9.88×10^{-3}**				
Ni	—	—	5.00×10^{-1}	1.55×10^{-3}**			
Pb	—	—	5.00×10^{-1}	1.55×10^{-3}**	—		
V	5.95×10^{-5}**	5.95×10^{-5}**	5.47×10^{-4}**	2.91×10^{-1}	5.95×10^{-5}**	5.95×10^{-5}**	

Figures are probabilities.
**: $p < 0.01$
—:The number of dead fry is almost the same for the two metals.

tested, those with secondary toxicity were Zn, As, Cr, Ni, and Pb, and those with tertiary were Se, V, Fe, and Co, the least toxic metal tested being Mn (Table 4).

Zn, Fe, and Mn were detected in the tap water which was used as the diluent, their levels being 0.22, 0.60, and 0.006 ppm respectively. As the tap water was mixed with the same volume of deionized water including one concentration of one of the test metals when test solutions were prepared, the levels of these metals were reduced by half. The comparative ratios of these metals included in tap water to the amounts of metals which were added were great when exposure experiments were carried out using concentration of less than 1 ppm. However, in the case of Zn exposure experiments, no fry died in at a concentration of 0.1 ppm and only two fry died at 1 ppm. Also, in the case of Fe or Mn no fry died in at 1 ppm concentration. In the experiments using 10 ppm, ratios of the metal content in tap water to the amounts of metals added were 1% for Zn, 3% for Fe, and 0.03% for Mn. Based on the above-mentioned reasons, it is considered that Zn, Fe, and Mn in tap water did not affect the conclusions of the present experiments at all.

There was 6.5 mg/liter Cl^- in the test solution used in the present study, but there was no problem in conducting the exposure experiments except for the case of Ag, because no fry died in the replicate blank test. In the case of the Ag exposure experiments, added Ag(I) ions acted with the Cl ions, forming AgCl, which was precipitated. The Ag toxic level of the present study might therefore not be exact.

Table 4. Orders of relative toxicity levels for medaka fry

1.	Ag, Hg, Cu, Cd
2.	Zn, As, Cr, Ni, Pb
3.	Se, V, Fe, Co
4.	Mn

Also, from our observation that when a high concentration of Fe(II) ion was added iron(III) hydroxide was formed, the toxic level of the Fe(II) ion concentration in the present study may have been lower than the true toxic level.

It is known that the toxicity of Zn and Cu is not so intense in humans[3] but in the results the present study these metals showed much higher toxicity to the medaka fry. It is not clear whether this difference depends on the method of administration and/or the kind of animals used in the experiments. Certainly, it seemed that the toxicity of As and Cr to medaka fry was as intense as in humans[1,7]. The orders of toxic level were roughly classified because these experiments were not carried out with replication and reproducibility, which are generally speaking, not so good in experiments using animals. As there is, however, no report comparing the toxicity levels of 14 different kinds of metals under the same conditions and at the same time, it was considered that this conclusion is very valuable.

REFERENCES

1. **Gleason, M.N.** 1969. Clinical toxicology of commercial products. Acute poisoning. 3rd Ed., The Williams and Willkins Co., Baltimore.

2. **Hiraoka, Y.** 1980. Studies on the vertebral abnormalities of medaka as the biological index of water pollution. III. Experimental investigation concerning cadmium exposure. Hiroshima M.J. **28**: 625—632.
3. **Koda, Y. and Hayashi, H.** 1982. The order of potential toxicity of heavy metal elements in natural environment. *Kogai to Taisaku*. **18**: 1057—1062.
4. **Ohmori, K.** 1965. Studies on absorption and excretion of organic lead compounds. 1. The absorption and excretion of tetraethyllead. Jpn. J. Hyg. **20**: 340-345.
5. **Pickering, Q.H. and Gast, M.H.** 1972. Acute and chronic toxicity of cadmium to the fathead minnow *(Pimephales promelas)*. J. Fish. Res. Board Can. **29**: 1099—1106.
6. **Underwood, E.J.** 1971. Trace elements in human and animal nutrition. 3rd. Ed. Academic Press Inc. p.207—243. New York.
7. **Yamane, Y., Takahata, E. and Uchiyama, M.** 1980. Toxicological aspects of environmental pollution. Inorganic chemicals. p. 137. Nanko-do, Tokyo.