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EFFECTS OF SPECIFIC DOSAGES OF MAGNESIUM AND ZINC ON THE TERATOGENICITY OF CADMIUM, NICKEL, AND COBALT IN *XENOPUS* EMBRYOS, AS ASSESSED BY THE FETAX TEST

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□ The objective of this study was to determine if exposure to divalent cations, Cd^{2+}, Ni^{2+}, and Co^{2+} would lead to malformations in *Xenopus laevis* embryos, and whether addition of $Mg^{2+}$and Zn^{2+}; separately and in combination, would reduce their toxicity and teratogenicity on the embryos of *Xenopus laevis* as assessed by 96-h FETAX tests. Results indicate that exposure to Cd^{2+}, Ni^{2+} or Co^{2+} lead to an increase in toxicity and teratogenicity in embryos, whereas Mg^{2+}, Zn^{2+}, or a combination of them reduced the toxic and teratogenic effects of these divalent cations. Modulation of Cd^{2+}, Ni^{2+} or Co^{2+} toxicity and teratogenicity by Mg^{2+} and Zn^{2+}, varied with the metal. Zn^{2+} was observed to be a better suppressor of Co^{2+} toxicity and teratogenicity than Mg^{2+}. In contrast, Ni^{2+}, and Cd^{2+} teratogenicity was reduced more prominently by Mg^{2+}. On the other hand, combination of Mg^{2+} and Zn^{2+} showed potentialization effect on all divalent cation toxicity and teratogenicity. We concluded that Mg^{2+} and Zn^{2+} reduced the toxicity and teratogenicity of Cd^{2+}, Ni^{2+}, Co^{2+}.

Keywords: FETAX, Magnesium (Mg^{2+}), Metal Interaction, Zinc (Zn^{2+}), Xenopus

INTRODUCTION

Hill and Matrone (1970) first suggested that the biologically essential 'bioelements' interact with toxic metals due to their similar physical and chemical properties (Brzoska, and Jakonuik 2001a). In recent years, such interactions have been determined to exist among bioelements like Zn^{2+}, Cu^{2+}, Fe^{2+}, Se^{2+}, and Ca^{2+}, and the toxic metals such as Cd^{2+}, Ni^{2+}, Co^{2+} (Brzoska and Jakonuik, 2001b). The antagonistic and synergistic interactions were evaluated with regard to their competition for metal-specific target molecules or the metalloproteins, metalloenzymes, and the binding sites on membranes (Güven, 1999).

Ni^{2+}, Co^{2+} and Cd^{2+} have been demonstrated to be potentially teratogenic as shown on *Xenopus laevis* by the FETAX test (Plowman et al., 1991, TOX Probe, 1993, Plowman et al.,1994, Costa et al., 2001, Kasprzak, 2002).

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It has been found that addition of Mg^{2+} at various concentrations to Ni^{2+}, Co^{2+}, Cd^{2+} and Zn^{2+} decreased the embryotoxic and teratogenic effects of these cations in proportion with the Mg^{2+} concentration on *Xenopus laevis* (Luo et al.,1993). The same investigators speculated that this effect was the result of the competition between Mg^{2+} and these divalent cations for physiological process such as binding and intake into cells.

The interaction of Zn^{2+} with Cd^{2+} and Ni^{2+} have also been investigated on *Bufo arenarum* embryos (Herkovits et al.,1998, Herkovits et al., 2000, Waalkes, 2000, Fort et al., 2001). In these studies, Zn^{2+} was found to have a protective agent against retarded growth and lethal effects of these heavy metals on the embryos. However, in biological systems, the interactions between the heavy metals depend on the amount of material present, the tested species, concentration and length of exposure. In this manner, different results have been obtained from the exposure of fish and plants to different heavy metals (Herkovits et al., 1998).

In biological systems, Cd^{2+} and Zn^{2+} macromolecules bind to sulphur (S), oxygen (O) and nitrogen (N) and reacts avidly with the S^{-}, O^{-}, and N^{-} donors. Cadmium and Zn^{2+} are bound preferentially to the same proteins in plasma, and proteins and metallothionein (MT) in tissues. Various studies have shown Cd^{2+} and Zn^{2+} to have high affinities for metallothionein and that their interactions lead to the induction of the synthesis of MT. MT has been shown to be synthesized in various tissues especially from the intestines, liver and kidneys (Pasky et al.,1996, Brzoska and Jakonuik, 1998). On the other hand, the binding of heavy metals by homeostatically-protective sulfur, oxygen, or nitrogen-based ligands were highly variable (Herkovits et al., 2000). In each case the intake of the metals into cells and their roles in other processes depend on their concentrations (Rainbow, 2002, Brzoska and Jakoniuk, 2001b, Falchuk, 1998a). The result of this is the tendency for Cd^{2+} to competitively bind intercellular sites resulting in the exclusion of Zn^{2+} from critical processes (Rink and Gabriel, 2001, Sanstead, 2000, Falchuk, 1998b, Pasky et al., 1996).

The influence of Zn^{2+} on to Ni^{2+} toxicity was suggested to be due to various factors such as metal intake, which leads to a fall in tissue concentration. This finding indicated the requirement of one metal for the intake of the other. Zinc applied at concentrations of 0.5 mg /l led to little or no effect which can be attributed to the presence of Ni^{2+}. At higher concentrations of Zn^{2+} (2-30 mg/l) however, the intake of Ni^{2+} increased as a result of synergistic toxic effect. At much higher concentrations 60-100 mg/l Zn^{2+}, by competing with Ni^{2+} for uptake into cells, may increase its intake. This result resembles a U-shaped dose—response curve. A typical U-shaped dose-response curve is a relation in which concentrations below a certain level for a particular chemical shows benefits and an opposite effect at higher doses *(Arndt-Schulz law* (Herkovits et al., 2000*)*.

The beneficial effect of Zn^{2+} on the toxicity of Ni^{2+} is most likely due to its induction of the metabolic enzymes, GSH (reduced GSH) and GST (Glutathion—S-Transferase), and is likely to be similarly affected by Cd^{2+}, Ag^{2+}, Hg^{2+}, Pb^{2+}, and Se^{2+} which all are considered to be xenobiotics (foreign to the organism) (Herkovits et al.,1998). Glutathion conjugation has an important toxicological function especially in the excretion of reactive intermediate products (OH^-, H_2O_2). Glutathion, is a tripeptide (glutamic acid, glycine, and cysteine) compound found in several mammalian tissues (particularly in the liver). Normally, the sulphydryl groups attack exposed reactive or electrophilic metabolites. These reactions are catalyzed by the glutathion transferases. The importance of this class of enzymes lies in their role in the metabolism of electrophilic groups like alkyl, aldehydes and ketones (Herkovits et al.,1998).

The frog, particularly the South African Claved frog *Xenopus laevis*, has been used as a model of embryonic development for more than 100 years. Alternative bioassys such as Frog Embryo Teratogenesis Assay: Xenopus (FETAX) provide a rapid, simple, and cost effective method for evaluating mechanisms of developmental toxicity on a preliminary basis. FETAX is a 96-h whole embryo, static renewal assay using *Xenopus laevis* embryos (Fort et al., 2001). It is essentially an organogenesis test, and organogenesis is highly conserved across amphibians and mammals. The first 96 hours of embryonic development in Xenopus parellels many major processes of human organogenesis. Thus, FETAX should be useful in predicting potential human developmental toxicants and teratogens (American Society for Testing and Materials (ASTM), 1998). The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) assessed the validation status of FETAX as a screening assay for detecting potential human teratogens, and for its use in the ecotoxicological assessment of water /soil/sediment/samples (NICEATM, 2004).

The aim of this study was to evaluate embryotoxic and teratogenic effects of the xenobiotic divalent metals Cd^{2+}, Ni^{2+} and Co^{2+}, and the interactions of the presence of Mg^{2+}, Zn^{2+} and their combination using the FETAX test.

MATERIALS AND METHODS

Xenopus laevis embryos (N: 13, n: 6660) were used in this study and were obtained by in vitro fertilization. According to ASTM FETAX Guideline (1998), in practice, 95% normal, live embryos should be obtained routinely. In the present study, at every phase approximately 700 embryos were squeezed, and the ratio of normal embryos were appropriate to ASTM guidelines.

Cl^- salts of Cd^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+}, Zn^{2+} divalent cations obtained from Sigma chemical Co. (St.Louis, Missouri, USA) were used to make stock solutions. Stock solutions were prepared and the concentrations were confirmed using a Parkin-Elmer 3100 model Atomic Absorbtion spectrophotometer (AAS). To prevent the precipitation of metallic hydroxides, the solutions were adjusted to a pH of 6.8. Stock solutions were stored at +4°C.

The tested concentrations of Cd^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+}, Zn^{2} were prepared in FETAX solution and pipetted into a petri dish. After a period of five hours, embryos reached phases 7-8 of development; were placed in the test solutions, and were incubated at 23± 1°C. Solutions were changed every 24 hours. The number of normal, abnormal and dead embryos were evaluated at the 96^{th} hour under a stereo microscope (Kyowa). Evaluations were conducted according to the Nieuwkoop's normal table atlas (Nieuwkoop, and Faber, 1994). Live, but not considered fit normal embryos were accepted as abnormal. Mortality at 96^{th} (stage 45) hour was confirmed by an arrested heart in the transparent embryo. Dead malformed embryos were not included in the malformed count (ASTM, 1998, Boga et al., 2000).

The experiment was conducted in four stages. In the first stage, the xenobiotic cation solutions including Cd^{2+} (5, 7.2, 8.5,10 µmol/l), Ni^{2+} (10, 80, 100, 120 µmol/l), Co^{2+} (20, 56, 112, 120 µmol/l) were evaluated alone. A total of 1800 Xenopus embryos (from 5 females) were used in 5 replicated experiments. Five petri dishes each containing 20 embryos were used for each replicate.

In the second stage, a single concentration of magnesium (40 mmol/l) was added to solutions of different concentrations of the xenobiotic divalent cations including, Cd^{2+} (5, 7.2, 8.5, 10 µmol/l); Ni^{2+} (10, 80,100,120 µmol/l); Co^{2+} (20, 56, 112, 120 µmol/l). A total of 1800 Xenopus embryos (from 5 females) were used in 5 replicated experiments. Five petri dishes each containing 20 embryos were used for each replicate.

In the third stage, a single concentration of zinc (40 µg/l) was added to the solutions of different concentrations of the xenobiotic divalent cations including Cd^{2+} (5, 7.2, 8.5, 10 µmol/l), Ni^{2+} (10, 80,100,120 µmol/l), Co^{2+} (20, 56, 112, 120 µmol/l). A total of 1260 Xenopus embryos (from 3 females) were used in 3 replicated experiments. Three petri dishes each containing 20 embryos were used for each replicate.

In the fourth stage, a combination of magnesium (40 mmol/l) + zinc (40 µg/l) was added to solutions of xenobiotic cations including Cd^{2+} (5, 7.2, 8.5,10 µmol/l), Ni^{2+} (10, 80, 100, 120 µmol/l), Co^{2+} (20, 56, 112, 120 µmol/l). A total of 1800 Xenopus embryos (from 5 females) were used in 5 replicated experiments. Five petri dishes each containing 20 embryos were used for each replicate.

DATA ANALYSIS

The percentages of normal, malformed, and dead embryos were analyzed using the multivariated Logistic regression. SPSS 14.0 was used for data analysis. A multivariate logistic regression analysis was applied to assess the Odds Ratio (OR) for anomaly, death and anomaly or death. The OR's given for group and dose were adjusted for the effect of each other. Significance level was accepted as $p \leq 0.05$.

RESULTS

The level of risk for each teratogen dose and combination group was evaluated statistically. The risk of anomaly , death, anomaly/death of Cd^{2+} treatment are presented in Table 1. As can be seen from Table 1 anomaly, death, anomaly or death risk rose parallel to the increase in doses.

According to $ref_1(Cd^{2+}$-5µmol/l), the risk of anomaly of the treatments of 7.2, 8.5, 10 µmol/l Cd^{2+} was 1.47 (1.04-2.07) p=0.029, 1.80 (1.26-2.55) p=0.001, 2.47 (1.68-3.63) p<0.001 respectively; the death risk was 2.42 (1.58-3.71) p<0.001, 1.87 (1.21-2.9) p=0.004, 3.77 (2.44-5.70) p<0.001 respectively, and the anomaly/death risk was 1.63 (1.20-2.21) p=0.001, 1.62 (1.19-2.220) p=0.002, 2.48 (1.81-3.4) p<0.001, respectively. Figure 1 shows the risk of anomaly, death, and anomaly or death together due to the Cd^{2+} doses on *Xenopus laevis* embryos. As seen in this figure; the highest death risk was with 10 µmol/l Cd^{2+}.

The risk of anomaly for groups T (Teratogen) + Mg^{2+}, T + Zn^{2+}, T + Mg^{2+} + Zn^{2+} compared to $ref_1(Cd^{2+})$ was 0.067 (0.045-.099) p<0.001, 0.163 (.116-

TABLE 1. Odds Ratio (OR) (95%C.I) of Cd, Cd + Mg; Cd + Zn and Mg + Zn + Cd on Xenopus embryos, by using FETAX.

	Anomaly OR (95 %C.I)	p	Death OR (95 %C.I)	p	Abnormal or Death OR (95 %C.I)	p
Dose						
5	ref1	—	ref1	—	ref1	—
.2	1.47(1.04-2.08)	.029	2.42(1.58-3.71)	<0.001	1.63(1.20-2.21)	.001
8.5	1.80(1.26-2.55)	.001	1.87(1.22-2.9)	.004	1.62(1.19-2.200)	.002
10	2.47(1.68-3.63)	<0.001	2.77(2.145-5.70)	<0.001	2.48(1.82-3.4)	<0.001
10	ref2	—	ref2	—	ref2	—
8.5	.728(.501-1.059)	.097	.497(.331-.748)	.001	.651(.476-.890)	.007
7.2	.595(.406-.873)	.008	.644(.431-.961)	.031	.658(.482-.898)	.008
Group						
Cd	ref1	—	ref1	—	ref1	—
Cd+Mg	0.067(0.045-.099)	<0.001	.337(.226-.501)	<0.001	.143(.103-.199)	<0.001
Cd+Zn	0.163(.116-.229	<0.001	.021(.009-.049)	<0.001	.128(.092-.178)	<0.001
Cd+Mg+Zn	0.302(.203-.443)	<0.001	1.320(.883-1.97)	<0.001	.556(.396-.781)	.001
Cd+Mg+Zn	ref2	—	ref2	—	ref2	—
Cd+Mg	.221(.146-.334)	<0.001	.255(.182-.358)	<0.001	.258(.191-.348)	<0.001
Cd+Zn	.539(.377-.771)	.001	.016(.007-.036)	<0.001	.230(.170-.311)	<0.001

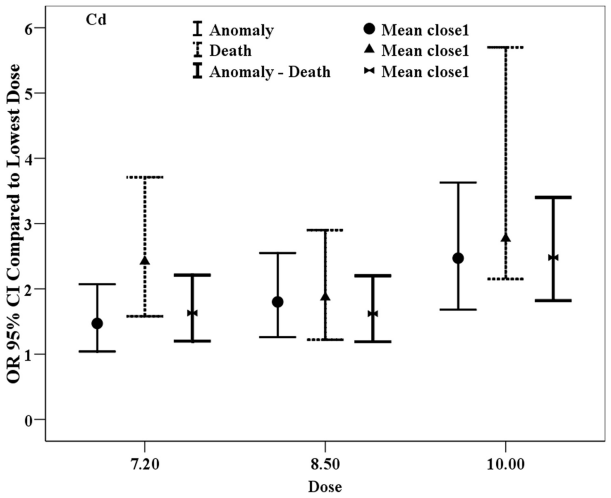


FIGURE 1. Odds Ratio (OR) (95%C.I) of Cd^{2+} doses adjusted for group.

.229) p<0.001, 0.302 (.203-.443) p<0.001, the risk of death was 0.337 (.226-.501) p<0.001, 0.021 (.009-.049) p<0.001, 1.320 (.883-1.97) p=0.175, and the risk of anomaly/death was 0.143 (.103-.199) p<0.001, 0.128 (.092-.178) p<0.001, .556 (.396-.781) p=0.001, respectively (Table 1, Figure 2, 3, 4).

It appears that among all combinations, Cd^{2+} + Mg^{2+} 0.0067 (0.045-.099) p<0.001) induced the lowest risk of anomaly, and also appeared to

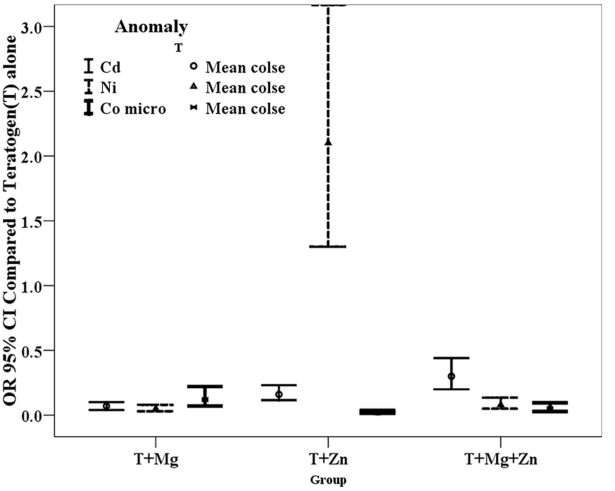


FIGURE 2. Odds Ratio (OR) (95%C.I) of Cd^{2+}, Ni^{2+}, Co^{2+} anomaly group adjusted for doses.

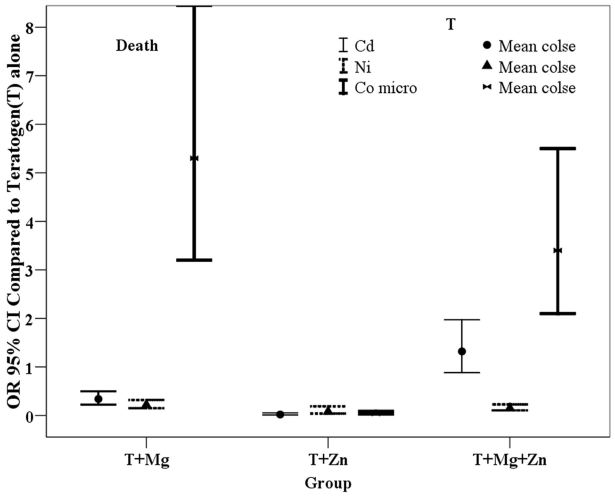


FIGURE 3. Odds Ratio (OR) (95%C.I) of Cd^{2+}, Ni^{2+}, Co^{2+} death group adjusted for doses.

decrease the risk of death 0.337 (.226-.501) p<0.001. The Cd^{2+} + Mg^{2+} + Zn^{2+} combinations induced significantly lower risk of anomaly 0.302 (0.203-0.443) p<0.001 but induced a higher risk of death 1.320 (.883-1.97) p=0.175 whereas this increase was not signifcantly higher than for

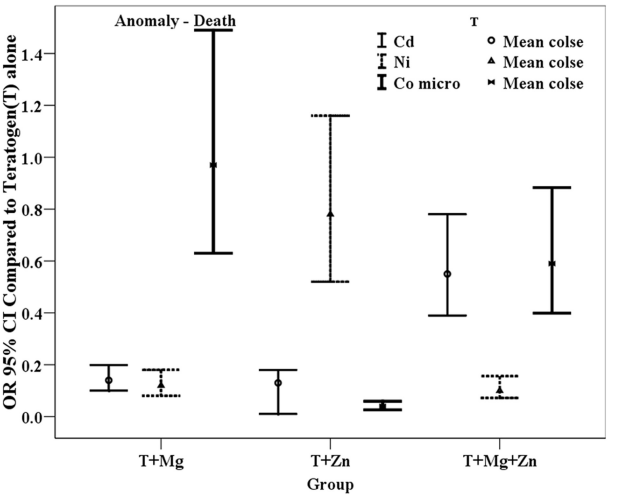


FIGURE 4. Odds Ratio (OR) (95%C.I) of Cd^{2+}, Ni^{2+}, Co^{2+} anomaly/death group adjusted for doses.

TABLE 2. Odds Ratio (OR) (95%C.I) of Ni, Ni + Mg, Ni + Zn, and Mg + Zn + Ni on Xenopus embryos, by using FETAX

	Anomaly OR (95 %C.I)	p	Death OR (95 %C.I)	p	Anomaly or Death OR (95 %C.I)	p
Dose						
10	ref1	—	ref1	—	ref1	—
80	10.6(6.61-17.0)	<0.001	3.31(2.21-4.96)	<0.001	6.45(4.57-9.12)	<0.001
100	14.5(8.95-23.6)	<0.001	3.74(2.48-5.64)	<0.001	7.83(5.50-11.1)	<0.001
120	12.9(7.64-21.9)	<0.001	6.92(4.58-10.45)	<0.001	11.55(7.97-16.7)	<0.001
120	ref2	—	ref2	—	ref2	—
100	1.124(.687-1.84)	.643	.541(.365-.801)	.002	.678(.474-.969)	.033
80	.820(.500-1.34)	.430	.479(.325-.705)	<0.001	.559(.392-.796)	.001
Group						
Ni	ref1	—	ref1	—	ref1	—
Ni+Mg	.051(.030-.088)	<0.001	.225(.154-.327)	<0.001	.124(.085-.181)	<0.001
Ni+Zn	2.12(1.3-3.4)	.002	.087(.039-.196)	<0.001	.782(.523-1.16)	.231
Ni+Mg+Zn	.082(.050-.135)	<0.001	.155(.105-.228)	<0.001	.106(.072-.156)	<0.001
Ni+Mg+Zn	ref2	—	ref2	—	ref2	—
Ni+Mg	.625(.387-1.011)	0.056	1.45(1.05-1.99)	.022	1.16(.060-1.57)	.317
Ni+Zn	25.9(16.4-40.9)	<0.001	.561(.249-1.264)	.163	7.3(5.12-10.5)	<0.001

Cd^{2+} alone. Zinc appeared to induce the lowest mortality 0,021 (.009-.049) p<0,001, and also decrease the risk of anomaly 0.163 (.116-.229) p<0.001 which may indicate that Zn^{2+} was found to be the most protective 0.128 (.092-.178) p<0.001, (Table 1).

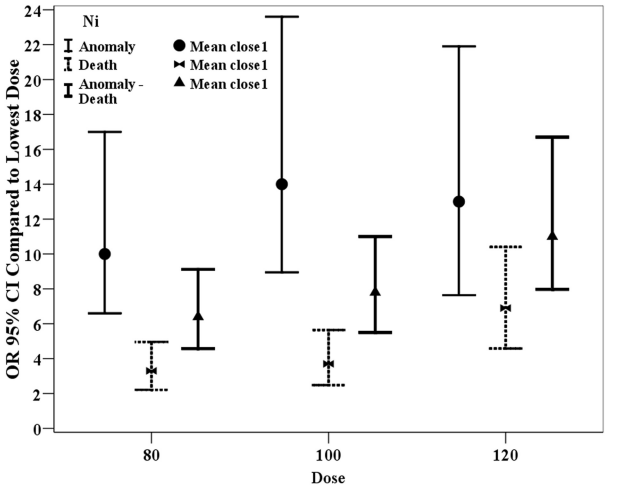


FIGURE 5. Odds Ratio (OR) (95%C.I) of Ni^{2+} doses adjusted for group.

The anomaly, death, anomaly/death risk of the Ni^{2+} treatment are presented in Table 2. Similar to the results illustrated in Table 1, we can see that the increase/decrease in anomally, death, anomaly/death risk also dependant on the doses. According to ref_1(Ni^{2+}-10µmol/l), the risk of anomaly of the treatments of 80, 100, 120 µmol/l Ni^{2+}, was 10.6 (6.61-17.0) p<0.001, 14.5 (8.95-23.6) p<0.001, 12.9 (7.64-21.9) p<0.001 respectively; the risk of death was 3.31 (2.21-4.96) p<0.001, 3.74 (2.48-5.64) p<0.001, 6.92 (4.58-10.45) p<0.001 respectively and the risk of, anomaly/death was 6.45 (4.57-9.12) p<0.001, 7.83 (5.50-11.1) p<0.001, 11.55 (7.97-16.7) p<0.001, respectively. Figure 5 shows the risk of anomaly, death, and anomaly or death together due to the Ni^{2+} treatments on *Xenopus laevis* embryos. As seen in Figure 5, higher anomaly risk was found in all doses when compared to ref_1 (Ni^{2+}).

The risk of anomaly for groups T (teratogen) + Mg^{2+}, T + Zn^{2+}, T + Mg^{2+} + Zn^{2+} compared to ref1 (Ni^{2+}) was 0.051 (.030-.088) p<0.001, 2.12 (1.3-3.4) p:0.002, 0,082 (.050-.135) p<0.001, respectively, the risk of death was 0.225 (.154-.327) p<0.001, 0.087 (.039-.196) p<0.001, 0.155 (.105-.228) p<0.001 respectively, and the risk of anomaly/death was 0.124 (.085-.181) p<0.001, 0.782 (.523-1.16) p:0.231) and 0.106 (.072-.156) p<0.001, respectively (Table 2, Figures 2, 3, 4).

It appears that among all combinations, Ni^{2+} + Mg^{2+} 0.051 (.030-.088) p<0.001 induced the lowest risk of anomaly and the Ni^{2+} + Mg^{2+} + Zn^{2+} combination 0.106 (.072-.156) p<0.001 induced the lowest risk of anomaly/death. The difference in this of risk of anomaly/death was not statistically significant from that of the Ni^{2+} + Mg^{2+} treatment. Zinc appeared to induce the lowest mortality, due to the Ni^{2+} treatments 0.087 (.039-.196) p<0.001, but increased the risk of anomaly of the Ni^{2+}2.12 (1.3-3.4), p= 0.002 (Table 2).

The anomaly, death, anomaly/death risk of Co^{2+} treatment are presented in Table 3. Here, again as in Table 1 and 2, increase in anomally, death, anomaly/death risk was also found to be closely related to the rise in doses. According to ref_1(Co^{2+}-20µmol/l), the risk of anomaly of the treatment of 56, 112, 120 µmol/l Co^{2+} was 3.14 (1.88-5.2) p<0.001, 8.9 (5.1-15.5) p<0.001, 23.9 (13.5-39.8) p<0.001 respectively; the risk of death was 1.56 (1.04-2.33) p=0.029, 4.5 (2.8-7.2) p<0.001, 6.4 (3.5-11.6) p<0.001 respectively, and the risk of anomaly/death was 2.0 (1.4-2.8) p< 0.001, 4.7 (3.2-7.0) p<0.001, 7.3 (4.8-11.1) p<0.001, respectively. Figure 6 shows the risk of anomaly, death, and anomaly/death of the Co^{2+} doses. The OR for anomaly was highest at 120µmol/l.

As risk of anomaly compared to ref_1 (Co^{2+}) T (teratogen) + Mg^{2+}, T + Zn^{2+}, T+ Mg^{2+}+ Zn^{2+} was 0.126 (.072-.221) p<0.001, 0.022 (.014-.036) p<0.001, 0.052 (.029-.095) p<0.001 respectively, the risk of death was 5.3 (3.21-8.9) p<0.001, 0.047 (.025-.088) p<0.001, 3.45 (2.1-5.5) p<0.001 respectively, and the risk of anomaly/death was 0.977 (.63-1.49) p=0,914,

TABLE 3. Odds Ratio (OR) (95%C.I) of Co, Co + Mg, Co + Zn, and Mg + Zn + Cd on Xenopus embryos, by using FETAX.

	Anomaly OR (95 %C.I)	p	Death OR (95 %C.I)	p	Anomaly or Death OR (95 %C.I)	p
Dose						
20	ref1	—	ref1	—	ref1	—
56	3.14(1.88-5.2)	<0.001	1.56(1.04-2.33)	.029	2.0(1.4-2.8)	<0.001
112	8.9(5.1-15.5)	<0.001	4.5(2.8-7.2)	<0.001	4.7(3.2-7.0)	<0.001
120	23.2(13.5-39.8)	<0.001	6.4(3.5-11.6)	<0.001	7.3(4.8-11.1)	<0.001
120	ref2	—	ref2	—	ref2	—
112	.383(.236-.623)	<0.001	.700(.379-1.295)	.256	.642(.419-.982)	.041
56	.135(.083-.221)	<0.001	.243(.134-.440)	<0.001	.272(.179-.413)	<0.001
Group						
Co	ref1	—	ref1	—	ref1	—
Co + Mg	.126(.072-.221)	<0.001	5.3(3.21-8.9)	<0.001	.977(.63-1.49)	.914
Co + Zn	.022(.014-.036)	<0.001	.047(.025-.088)	<0.001	.039(.026-.059)	<0.001
Co+Mg+Zn	.052(.029-.095)	<0.001	3.45(2.1-5.5)	<0.001	.594(.399-.883)	.010
Co+Mg+Zn	ref2	—	ref2	—	ref2	—
Co + Mg	2.4(1.26-4.56)	.008	1.55(1.03-2.32)	.034	1.64(1.1-2.4)	.014
Co + Zn	.421(.243-.731)	.002	.014(.008-.024)	<0.001	.066(.046-.096)	<0.001

0.039 (.026-.059) p<0.001, 0.594 (.399-.883) p=0.010), respectively (Table 3, Figure 2, 3, 4).

It appeared that among all exposure combinations, the combination of Co^{2+} and Zn^{2+} induced the lowest risk of mortality 0.047 (.025-.088) p<0.001, the lowest risk of anomaly 0.022 (.014-.036) p<0.001, and the lowest risk of anomaly/death 0.039 (.026-.059) p<0.001. The Mg^{2+} treat-

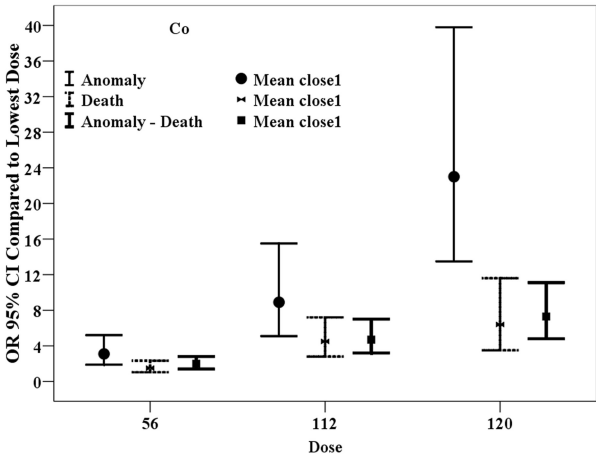


FIGURE 6. Odds Ratio (OR) (95%C.I) of Co^{2+} doses adjusted for group.

ment appeared to increase the toxicity of Co^{2+} exposure. The OR value for Co^{2+} + Mg^{2+} + Zn^{2+} combination was lower than that for the Co^{2+} + Mg^{2+} combination, but higher than that for the Co^{2+} + Zn^{2+} combination, and it was significantly lower compared to the $ref_1(Co^{2+})$.

It also appeared that there may have been some competetive interaction between Mg^{2+} and Zn^{2+} since the protective effects of Zn^{2+} were not clearly evident when Mg^{2+} was included.

DISCUSSION

Since the 1990s, various investigators have studied the effect of metallic ions on teratogenicity indices and mechanisms. Interactions that exist between the bioelements and the toxic metals have been discovered (Brzoska, and Jakonuik, 2001b, Kasprzak, 2002, Plowman et al.,1994, Costa et al., 2001, TOX Probe, 1993, Plowman et al.,1991, Miller and Landesman, 1978).

The protective effect of Mg^{2+} on all the divalent cations (Luo et al., 1993) and that of Zn^{2+} on Ni^{2+} (Herkovits et al., 2000) and Cd^{2+} (Waalkes, 2000, Brzoska and Jakoniuk, 2001b) have been investigated. In addition, Kasprzak (2002) and Costa et al.(2001) demonstrated the protective effect of Mg^{2+} against the the toxic and teratogenic effects of Ni^{2+} compounds. We evaluated not only the effect of Mg^{2+} against toxic effect of Cd^{2+} and Ni^{2+} but also the effect of Zn^{2+} alone and in combination with Mg^{2+} on the toxic effect of Cd^{2+}, Ni^{2+} and Co^{2+}. We found that these combinations reduced toxicity and teratogenicity of these xenobiotic metals.

According to Luo et al. (1993), a possible mechanism for the effect of magnesium on the other divalent cations is the competition between these ions for transport mechanisms; including absorption, intake into cells, or binding to critical molecules (e.g. DNA polimerase) (Luo et al.,1993). This finding may explain our results of Zn^{2+} supression of the teratogenic effect of Co^{2+}, and Mg^{2+} supression of the teratogenicity of Cd^{2+} and Ni^{2}.

The FETAX test seems to be, useful for rapid screening of samples as the method provide adequate information on the teratogenicity of various agents using a standardized procedure (Dawson and Bantle, 1987). Although, the FETAX test meets most of the foreseen criteria for testing in vitro teratogenesis, combination with Mixed Function Oxidase System (MFO) proves to be a more effective method in transforming proteratogenic compounds into teratogenic metabolites. By making use of the data from Kitchin and Woods (1979) who utilized whole rat embryo cultures, Dawson and Bantle (1987), employed recently induced rat liver microsomes as the exogenous metabolic activator system (MAS) for the FETAX test. Potentially teratogenic substances that tested negative in these tests may become transformed into teratogenic forms causing congenital defects.

Zinc is a component of the metalloenzymes which plays a role in the metabolism of animals at all stages of their development. At all cell development stages, it plays regulatory and protective part in the process of protein synthesis. It also stabilizes biological membranes by competing with the redox active divalent metals such as copper, mercury and cadmium for binding onto cell membrane sites (sulfhydryl groups). It synthesizes metallothionein which also binds to the heavy metals to eradicate OH^- radicals, which can be formed by cobalt as well as Ni^{2+}, Cu^{2+}, Cr^{2+}, Fe^{2+} (Plowman et al.,1994, Tox Probe,1993, Plowman et al.,1991). Zinc was also found to decrease the teratogenic effect of Co^{2+} thus, in turn, leading to the decrease of level of OH^- radicals.

Deficiency of zinc has a negative impact on development and reproduction, causing development of abnormal eggs, changes in meiotic and ovulatory processes, abnormal spermatozoa and a high incidence of congenital anomalies. On the other hand, high zinc concentrations affect reproduction and viability. Zinc enables absortion of food, provides wellbeing, reproduction and longevity in invertebrates. No adverse effect was encountered in *Bufo arenarum* embryos exposed to a concentration of up to 130 mg/l Zn^{2+}. In studies conducted, beneficial effects of Zn^{2+} on amphibian embryos were found to be a reduction in the rate of spontaneous anomalies and malformations, and protection against the retarded growth and development and death due to metals like Cd^{2+}, Pb^{2+}, Al^{2+}, Hg^{2+}, and Cu^{2+} (Rink and Gabriel, 2001, Sandstead, 2000, Falchuk, 1998a, Pasky et al., 1996).

The effect of cadmium is neutralized by the addition of zinc, which decreases absorption of Cd^{2+}. Zinc deficiency leads to accumulation and increases toxicity of Cd^{2+}. In the biological system, Cd^{2+} and Zn^{2+} bind specifically to macromolecules, specifically through sulphur (S), oxygen (O) and Nitrogen (N), and interact with the S^-, O^- and N donors. The ions of Cd^{2+} and Zn^{2+} compete for entry into various cells and in many biological processes Zn^{2+} may take the place of Cd^{2+}. Such interactions can be either competitive or non competitive and largely occur in the intestines where MT and Cd^{2+} absorption occurs (Pasky et al., 1996, Brzoska and Jakonuik, 2001a). Zinc was also found to be more effective than magnesium reducing the toxic effect of Cd^{2+} in our study.

An earlier study, demonstrated that 0.5 mg/l of Zn^{2+} did not affect the toxicity of cadmium in zebra fish *(Brachydanio rerio)* (Küçükoglu, 1996). Contrary to this finding, we observed that zinc, even at µg/l concentrations, was enough to decrease the teratogenicity on *Xenopus laevis* embryos.

The toxicity of Ni^{2+} on embryos of *Bufo arenarum* was found to be lower compared to other metals (Cu^{2+}> Cd^{2+}> Hg^{2+}> Al^{2+}> Pb^{2+}> Ni^{2+}> Zn^{2+}) whereas Zn^{2+} sulphate concentrations under 130 mg/l were found to have no lethal effect on the embryo. Embryos of amphibians, com-

pared to other species such as daphnia, algae, and fish, are more tolerant to Zn^{2+} at least in their last developmental stage (Herkovits et al., 2000).

In the same study, the Ni^{2+}-Zn^{2+} interaction forms a U shaped dose-response curve. While Zn^{2+} at 0.5 mg/l failed to suppress the toxicity of Ni^{2+}, concentrations of this metal at 2-30 mg/l increased the death rate but desired beneficial effects were observed at higher concentrations of 60-100 mg/l. A synergistic effect of combined Zn^{2+} and Ni^{2+} was observed at concentrations of 2-30mg/l.

The mechanism of Ni^{2+} toxicity, similar to other metals (Cd^{2+}, Co^{2+}), increases lipid peroxidation, cellular injury and death. Inhibition of cellular defence against peroxidative injury is through the inhibition of protective enzymes against free radicals and/or through free oxygen radical production. On the other hand, Ni^{2+} under in vitro conditions binds to proteins and DNA while, under in vivo conditions, it binds to chromatins. By binding to macromolecules, Ni^{2+} compounds interfere with DNA synthesis, and hence lead to increased chromosomal abnormalities (Herkovits et al.,2000). In our study, Mg^{2+} at mmol/l concentrations significantly decreased the teratogenic effect of Ni^{2+}. Thus, we suggest Mg^{2+} may have competed with Ni^{2+} for Ni^{2+}'s target.

In conclusion, bioelements such as Zn^{2+}, and Mg^{2+} can suppress teratogenic and toxic effects of xenobiotic cations such as Cd^{2+}, Ni^{2+},Co^{2+}. These interactions can be evaluated by FETAX, and therefore *Xenopus laevis* is an effective model for this evaluation. In the light of such studies, metal interaction research provides a scientific basis for establishment of water quality criteria for protection of humans from wildlife.

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