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Correlation Between the *In Vitro* Cytotoxicity of Inorganic Metal Compounds to Cultured Fathead Minnow Fish Cells and the Toxicity to *Daphnia magna*

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Much toxicological research has been performed during the last years on the replacement of animals by cultured cells. The prediction of the human toxicity is the final goal in this case. The development of appropriate ecotoxicity assays is another important task. Indeed, the continuously increasing production and use of chemicals cause heavily contaminated fresh-, coastal- and seawater in most of the industrialized regions of the world. Different cultured fish cell lines were have thus far been used for measuring the cytotoxicity of several groups of chemicals (for a review, see Babich and Borenfreund 1991).

We have previously shown that a good correlation exists between *in vitro* cytotoxicity to cultured fathead minnow fish cells and the lethality data for a series of 50 chemicals belonging to very different groups (Brandao et al. 1992). A comparable correlation was observed for the *in vitro* toxicity of 45 pesticides to goldfish GF-scale (GFS) cells (Saito et al. 1991). However, the aquatic ecosytem is very complex. Fish and water fleas are the most commonly used model organisms in short-term lethality testing. Since these organisms need special equipment, it would be useful if these ecotoxicological models could be replaced by cultured cells. Here we report on the cytotoxicity of 19 metals to cultured fathead minnow (FHM) fish cells, as measured by the neutral red uptake inhibition assay. Inorganic salts of the following metals were tested : Hg, Ag, Cu, Zn, Co, Cd, As, Fe, Ni, Mn, Sn, Ba, Al, Sr, K, Mg, Ca, Na and Sb. The cytotoxicity results are compared with those obtained after 24hr and 48hr for the classical test organism *Duphnia magna* (Khangorot and Ray 1989), expressed by the EC50, representing the effective concentration at which 50% immobilization response was recorded.

MATERIALS AND METHODS

FHM cells are an established fish cell line (American Type Culture Collection no. CCL42) derived from tissue posterior to the anus from fathead minnow *(Pimephales promelas)*. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 1% non-essential amino acids, 100 units/ml penicillin, and 0.1 mg/mL streptomycin (complete medium) and incubated in a humidified atmosphere of 5% CO_2 at 34°C.

The cytotoxicity was measured by the neutral red uptake inhibition assay of Borenfreund and Shopsis (1985), slightly adapted as described (Dierickx and Van

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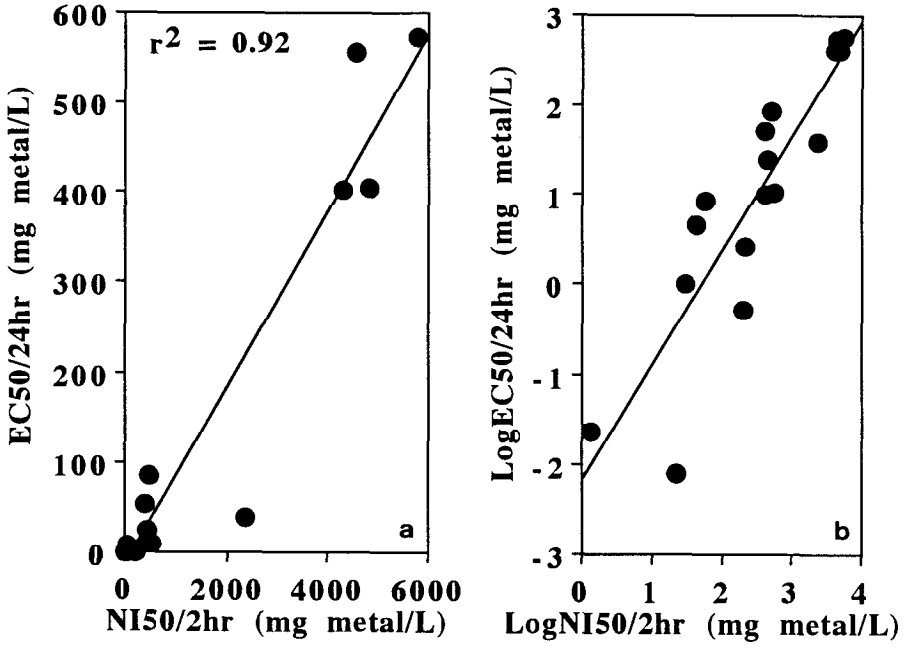

Figure 1. Linear regression analysis of the relationship between the neutral red uptake inhibition in FHM cells and the toxicity in *Daphnia magna,* expressed on a linear (a) or logarithmic (b) scale.

de Vyver 1991). The cells were seeded into 64 wells of a titer plate (Nunc) at $6x10^4$ cells in 0.2 mL complete medium per well. After incubation for 24hr the cultures were treated with 0.2 mL aliquots of different concentrations of the freshly prepared test compounds in complete medium (8 wells/concentration). The highest concentration was chosen according to results of preliminary range-finding experiments. The cells were treated for 2hr or 24hr and further analyzed as previously described (Borenfreund and Shopsis 1985; Dierickx and Van de Vyver 1991). The results were calculated in percentages compared to control cultures. The relative toxicity of the test compounds is established by the determination of the NI50; this is the concentration of test compound required to induce a 50% inhibition in neutral red uptake. Standard deviations of less than 5% were found in completely independent assays.

RESULTS AND DISCUSSION

The cytotoxicity results of the 19 metal compounds are summarized in Table 1. The NI50 values are expressed both on a molar basis, allowing the comparison of the relative metal toxicity, and on a weight/volume basis, allowing the comparison with the *Dahnia magna* toxicity data as measured by Khangorot and Ray (1989). *Daphia magna* was more sensitive to the test chemicals than the FHM cells. This difference was very pronounced for the very toxic heavy metals as Hg and Ag, and was relatively small for some less toxic metals as Ca and Mg. A comparable

Table 1. Neutral red uptake inhibition induced by the 19 metals in cultured fathead minnow cells. The results are compared with the toxicity of the same metals in *Daphnia magna*

Test Compound[b]	NI_{50} (mM) in FHM		NI_{50} (mg metal/L) in FHM		EC_{50} (mg metal/L) in *D. magna*[a]	
	2hr	24hr	2hr	24hr	24hr	48hr
$HgCl_2$	0.11	0.031	22	6.2	0.0081	0.0052
$AgNO_3$	0.012	0.014	1.3	1.5	0.023	0.010
$CuSO_4.5H_2O$	3.2	0.58	206	37	0.536	0.093
$ZnSO_4.7H_2O$	0.48	0.43	31	28	1.00	0.56
$CoCl_2.6H_2O$	3.6	0.56	214	33	2.61	1.49
$CdCl_2.H_2O$ [$CdSO_4.8H_2O$]	0.38	0.030	43	3.4	4.66	1.88
As_2O_3 [Na_3AsO_3]	0.38	0.13	58	19	8.45	6.23
$FeSO_4.7H_2O$	7.9	4.3	440	242	24.50	7.20
$NiCl_2.6H_2O$	9.2	1.8	540	104	10.90	7.59
$MnCl_2$ [$MnSO_4.2H_2O$]	7.7	4.3	426	237	10.00	8.28
$SnCl_2.2H_2O$	20	4.8	2362	564	38.00	21.56
$BaCl_2$ [$BaSO_4$]	2.9	4.5	398	551	52.82	32.00
$AlNH_4(SO_4)_2.12H_2O$	18	11	498	283	85.92	59.60
$SrCl_2.6H_2O$	116	37	10188	3242	162.93	94.00
KCl	199	70	7800	2742	327.94	141.46
$MgSO_4.7H_2O$	198	52	4804	1262	405.98	343.56
$CaCl_2.2H_2O$	144	34	5782	1363	573.06	383.60
NaCl	186	112	4278	2585	402.60	402.60
$SbCl_3$ [Sb_2O_3]	37	33	4534	4003	555.26	423.45

[a]Results from Khangorot and Ray (1989).

[b]The compounds tested in this study. If different, the salts tested by Khangorot and Ray are given within brackets.

discrepancy was previously observed by Lilius et al. (1994) for freshly isolated rainbow trout hepatocytes.

Relatively poor correlations existed between the NI50/2hr or NI50/24hr and the EC50/24hr or EC50/48hr (r^2=0.37 - 0.61. However, when Sr and K were omitted, good correlations were observed for the remaining 17 metals (Table 2). This is illustrated for the NI50/2hr versus EC50/24hr in Fig. 1, the log presentation showing a rather homogeneous distribution of the correlation points arround the regression line. Compared to the other chemicals, Sr and K are less toxic to FHM cells than to water fleas. A correlation coefficient r^2=0.50 was observed between the cytotoxicity, measured by Rb leakage, of 38 chemicals in freshly isolated rainbow trout hepatocytes and the toxicity in *Daphnia magna* (Lilius et al. 1994).

Table 2. Linear correlation values between the NI50 in FHM cells and the EC50 in *Daphnia magna* for the investigated metals, without strontium and potassium.

	EC50/24hr	EC50/48hr
NI50/2hr	r^2 = 0.92	r^2 = 0.90
NI50/24hr	r^2 = 0.76	r^2 = 0.79

Thus, for 17 of the 19 investigated metals a good correlation was observed between the cytotoxicity to cultured FHM cells and the toxicity to *Daphnia magna*, meaning that the neutral red uptake inhibition assay in cultured cells can be considered as a valuable tool for *in vitro* ecotoxicity testing.

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