AQUATIC TOXICITY TESTS ON INORGANIC ELEMENTS OCCURRING IN OIL SHALE

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ABSTRACT

Using the rainbow trout (Salmo gairdneri), embryo-larval toxicity tests were performed on 33 elements which occur in oil shale and other fossil fuels. Continuous exposure was maintained from fertilization through 4 days posthatching, employing static renewal procedures and test responses were based on lethality and teratogenesis. The LC_{50}s were under 1.0 mg/l for 19 of the 33 elements, indicating high sensitivity of developmental stages of the rainbow trout to a wide range of elements which occur in oil shale, spent shale, and process waters. Elements which proved most toxic to trout eggs and larvae were Hg, Ag, La, Ge, Ni, Cu, and Cd, with probit-derived LC_{50}s of 0.005, 0.01, 0.02, 0.05, 0.05, 0.11, and 0.14 mg/l, respectively.

Exposure levels which produced 1% control-adjusted impairment of test populations (LC_1) were also determined by log probit analysis, to provide a basis for estimating threshold concentrations. The LC_1 values were at or under 10 µg/l for 12 elements, including Ag, Be, Cd, Cu, Ge, Hg, La, Ni, Pb, Tl, V, and Zr. To determine reliability of the LC_1 values, they were compared with maximum acceptable toxicant concentrations developed in continuous flow embryo-larval and chronic reproductive studies and with current freshwater criteria. Good correlations generally were obtained where data were adequate to permit comparisons. Results showed that static renewal tests with trout embryo-larval stages afforded a reliable and economical means of screening oil shale contaminants for toxic properties, identifying those of greatest concern to aquatic ecosystems, and estimating concentrations which may produce hazardous effects. To assist further in prioritizing elements for studies on environmental monitoring and biological effects, oil shale, spent shale, and retort waters were compared for elemental composition.

Trout embryo-larval tests also were conducted on simple metal mixtures, to evaluate possible antagonistic, additive, or synergistic interactions. Mercury was mixed in equal proportions with each of three other metals, including cadmium, copper, and selenium. Analysis of dose-response data clearly indicated that the type of interaction varied with concentration. At lower exposure levels, copper-mercury was antagonistic, and the other

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mixtures were additive to antagonistic. All mixtures became synergistic at or above median lethal concentrations. As synergism was dependent on high exposure levels, this interaction appeared less likely to be significant under ambient conditions.

INTRODUCTION

Upwards of 65 elements reportedly occur in oil shale, and spent shale and retort waters contain significant concentrations of many elements considered hazardous to aquatic biota.[1,2,3] Environmental outfall of inorganic contaminants may approach or equal that observed for coal. Using data from recent investigations,[1-18] the composition of oil shale, coal and their waste products was compared for 33 of the elements which may prove detrimental to aquatic life (Table 1). Utilization of oil shale will yield a high ratio of waste products, averaging about 91 tons of spent shale and 400 to 3,000 liters of retort water for every 100 tons of shale processed.[4] Aqueous leaching of solid wastes may constitute the principal threat to surface and groundwaters, particularly as retort and other wastewaters may be used in wetting down spent shale.[4] Fallout from atmospheric discharges also may reach aquatic ecosystems, including waters affected by local deposition flux, atmospheric scavenging, and terrestrial runoff.[5,19,20] The Green River oil shale formation covers approximately 17,000 square miles in Colorado, Utah, and Wyoming, and reserves have been calculated at 600 billion barrels of oil, considering only that shale estimated to yield a minimum of 25 gallons of oil per ton.[6] Due to the potential magnitude of this new energy technology and the expansive geographic regions which will be affected, it is essential to establish waste disposal guidelines which will ensure environmental acceptability.

However, definitive freshwater criteria have been slow to develop and currently exist for only a small fraction of the elements found in oil shale. If an adequate data base for hazard assessment is to be achieved within the time frame contemplated for the implementation of oil shale technology, it is essential to develop a more rapid and economical means of delineating guidelines for waste disposal. McKim[21] recently reviewed data for a wide range of aquatic toxicants and concluded that continuous flow fish embryo-larval tests which extended beyond hatching by 30 days or more yielded responses comparable to those produced in chronic life-cycle studies. As suggested by McKim, this affords a somewhat more economical procedure for estimating maximum acceptable toxicant concentrations (MATC). In addition, continuous flow embryo-larval tests of even shorter duration have produced sensitivity equal to that observed with chronic testing procedures.[22-24] However, considering the many aquatic contaminants which may result from new energy technologies, still simpler and more rapid screening procedures are required.[25] In this investigation, static renewal toxicity tests with embryos and larvae of the rainbow trout were used (1) to compare toxicity of 33 elements which occur in oil shale, (2) to identify particularly hazardous elements for more comprehensive study, and (3) to provide a basis for estimating initial freshwater guidelines in instances where established criteria are lacking.

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Table 1. CONCENTRATIONS (ppm) OF TOXIC ELEMENTS OCCURRING IN OIL SHALE, COAL, AND THEIR WASTE PRODUCTS

Element	Oil Shale	Spent Oil Shale	Oil Shale Retort Water	Coal	Ash Pond Effluent	Coal Conversion Process Waters
Aluminum	5000 - >10000	-	0.11 - 0.66	4300 - 30400	1.4 - 7.2	0.007 - 3
Antimony	0.20 - 11	1	0.004 - 0.036	0.2 - 8.9	-	0.007 - 0.5
Arsenic	2.6 - 108	15	4.6 - 10	0.5 - 106	0.005 - 0.038	0.001 - 1
Barium	32 - 750	180	0.002 - 0.22	33 - 750	0.1 - 0.3	0.02 - 0.1
Beryllium	0.26 - 35	0.3	-	0.2 - 31	<0.01 - 0.01	<0.02 - 0.02
Boron	12 - 140	53	4.4 - 8.8	1.2 - 356	-	0.4 - 82
Cadmium	0.02 - 1.4	≤0.8	-	0.1 - 65	0.001 - 0.037	0.01
Cesium	0.06 - 11	7	0.002 - 0.007	0.49 - 1.5	-	<0.005 - 0.01
Chromium	21 - 1000	125	0.011 - 0.037	0.3 - 610	0.004 - 0.067	0.004 - 0.6
Cobalt	0.78 - 39	11	0.002 - 0.65	0.5 - 43	-	0.002 - 0.5
Copper	15 - 120	48	0.007 - 0.16	1.8 - 185	0.01 - 0.31	0.002 - 5.0
Gallium	1.1 - 18	14	-	1.1 - 61	-	<0.005
Germanium	0.37 - 2.9	0.65	0.001 - 0.007	1.0 - 819	-	0.001 - 0.01
Lanthanum	1.1 - 50	20	0.002 - 0.010	3.3 - 98	-	0.05 - 0.46
Lead	1.0 - 70	19	0.062 - 0.37	4 - 218	0.01 - 0.06	0.002 - 1.0
Lithium	1.9 - 850	160	0.004 - 0.75	3.1 - 25	-	0.001 - 0.020
Magnesium	5000 - >10000	-	5.3 - 8.7	100 - 2500	0.4 - 14	0.32 - 50.0
Manganese	9 - 390	405	0.042 - 0.14	6 - 181	0.01 - 0.58	0.01 - 15.0
Mercury	0.2 - 1.4	-	<0.1 - 0.1	0.01 - 1.6	0.0002 - 0.038	0.007 - 0.030
Molybdenum	4.9 - 87	. 6	0.056 - 0.340	1.0 - 73	-	0.001 - 0.5
Nickel	28 - 760	15	0.37 - 2.6	0.4 - 104	0.05 - 1.1	0.001 - 10.0
Selenium	0.08 - 5.2	1	0.003 - 0.98	0.4 - 7.7	0.002 - 0.065	0.002 - 0.3
Silver	0.04 - 1.7	1.7	0.002 - 0.230	0.03 - 0.19	<0.01 - 0.01	<0.02
Strontium	59 - 2700	460	0.003 - 0.48	10.0 - 37	-	0.015 - 0.120
Tantalum	0.04 - 4.8	<0.2	-	0.25	-	≤0.1 - 0.3
Tellurium	0.11 - 0.35	<0.2	0.001	0.5	-	<0.003
Thallium	0.3 - 1.4	<0.2	-	0.29 - 2.0	-	<0.003
Tin	0.11 - 11	1.5	8.9 - 100	1.0 - 51	-	<0.02 - 0.1
Titanium	150 - 2600	-	0.64 - 21	20 - 3200	-	0.003 - 0.3
Tungsten	0.03 - 2.9	1.25	0.003 - 0.024	0.04 - 3.0	-	0.03 - 0.07
Vanadium	10 - 280	135	0.004 - 11	10 - 1281	-	0.001 - 0.033
Zinc	12 - 136	35	0.26 - 0.47	15 - 5600	0.03 - 1.51	0.007 - 5.0
Zirconium	3.0 - 60	110	0.008 - 0.390	8 - 133	-	0.02 - 0.1

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MATERIALS AND METHODS

Embryo-larval toxicity tests were performed with the rainbow trout (Salmo gairdneri), using static renewal procedures previously described by Birge et al.[26] Test water and toxicant were changed at regular 12-hour intervals. Treatment was maintained continuously from fertilization through 4 days posthatching, giving an exposure period of 28 days. Water hardness ranged from 92 to 110 mg/l $CaCO_3$, and pH varied from 6.9 to 7.8. Moderate aeration was used to maintain dissolved oxygen within a range of 9.3 to 10.1 mg/l. A minimum of 7 mg/l has been recommended for trout and salmon spawning waters.[27] Other physicochemical characteristics of the test water have been described by Birge et al.[22] All tests were conducted in environmental rooms and temperature was maintained at 12° to 13°C. Test populations were examined each day to tabulate frequencies of lethality and teratogenesis. Log probit analysis was used to determine control-adjusted LC_1, LC_{10}, and LC_{50} values with 95% confidence limits. The lethal concentrations were determined with the method of Finney,[28] rather than the procedure of Daum[29] used in earlier investigations.[3,26] Teratic survivors, as described by Birge and Black,[30] were counted as lethals in probit calculations. Minimum sample size was set at 100 eggs, using 500-ml exposure chambers.

Elements and compounds selected for testing are given in Table 2. Hydrated salts were used for Al, Ba, Cd, Co, Cu, Fe, Mg, Mn, Mo, Ni, Sr, and Te. Concentrations of elements contained in prepared test solutions added to exposure chambers were confirmed with a Perkin-Elmer atomic absorption spectrophotometer (Model 503), equipped with an HGA 2100 graphite furnace and a mercury analyzer.[3,31] Test water was monitored for temperature, dissolved oxygen, water hardness, and pH, using a YSI telethermometer with thermocouple, YSI oxygen meter (Model 51A), Orion divalent cation electrode, and a Corning digital pH meter (Model 110).

RESULTS AND CONCLUSIONS

Tests conducted on embryonic and larval stages of the rainbow trout are summarized in Table 2. Median lethal concentrations (LC_{50}) and other values (LC_{10}, LC_1) were based on control-adjusted responses (lethality, teratogenesis) incurred during the 28-day expsoure period. Control populations, maintained simultaneously with experimentals, survived at frequencies ranging from 83% to 96%. The LC_{50}s were under 1.0 mg/l for 19 of the 33 elements, indicating high sensitivity of developmental stages of the rainbow trout to a wide range of elements which occur in oil shale and coal. Mercury, silver, and lanthanum were the most toxic. Of the remaining 14 elements, LC_{50}s ranged from 1.1 to 7.3 mg/l for Zr, Zn, Mn, Ga, Ta, Se, and Ti, and those elements which exhibited the lowest toxicity, based on median lethal concentrations, included B, Ba, Cs, Li, Mg, Te, and W. The high sensitivity of rainbow trout embryos and alevins has been noted in numerous previous investigations.[26,32-34] In particular, McKim et al.[34] observed life-cycle stages of the rainbow trout to be more susceptible to copper than were those of seven other fish species. Considering the LC_{50}s given in Table 2, a number of toxic elements occur in oil shale, retort waters, and

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Table 2. TROUT EMBRYO-LARVAL BIOASSAYS ON ELEMENTS OCCURRING IN OIL SHALE

Test Element	LC_{50} (mg/l)	95% Confidence Limits	LC_{10} (µg/l)	95% Confidence Limits	LC_1 (µg/l)	95% Confidence Limits
Mercury ($HgCl_2$)	0.005	0.004 - 0.005	0.9	0.7 - 1.2	0.2	0.1 - 0.3
Silver ($AgNO_3$)	0.010	0.008 - 0.011	0.9	0.7 - 1.2	0.1	0.1 - 0.2
Lanthanum ($LaCl_3$)	0.02	0.01 - 0.03	3.7	2.3 - 5.2	0.9	0.4 - 1.5
Germanium (GeO_2)	0.05	0.04 - 0.05	2.8	1.9 - 3.8	0.3	0.2 - 0.5
Nickel ($NiCl_2$)	0.05	0.04 - 0.06	10.6	7.4 - 13.9	3.0	1.7 - 4.5
Copper ($CuSO_4$)	0.11	0.09 - 0.14	16.5	10.1 - 23.7	3.4	1.6 - 5.9
Cadmium ($CdCl_2$)	0.14	0.13 - 0.16	29.2	22.8 - 36.1	8.0	5.4 - 10.9
Vanadium (V_2O_5)	0.17	0.14 - 0.21	33.8	22.0 - 46.8	9.0	4.7 - 14.6
Thallium ($TlCl_3$)	0.18	0.14 - 0.22	36.3	24.0 - 50.0	9.9	5.2 - 15.8
Chromium (CrO_3)	0.19	0.15 - 0.23	56.9	34.6 - 80.2	21.5	10.3 - 35.2
Lead ($PbCl_2$)	0.22	0.19 - 0.25	40.9	31.1 - 51.4	10.3	6.9 - 14.6
Strontium ($SrCl_2$)	0.25	0.20 - 0.30	49.0	32.0 - 67.7	13.0	6.7 - 21.2
Beryllium ($BeCl_2$)	0.38	0.26 - 0.53	42.0	19.8 - 72.1	7.0	2.2 - 15.5
Tin ($SnCl_2$)	0.42	0.35 - 0.50	75.5	53.4 - 99.9	18.6	10.9 - 28.3
Cobalt ($Co(NO_3)_2$)	0.49	0.38 - 0.59	120	64.4 - 176	38.2	14.1 - 69.6
Arsenic ($NaAsO_2$)	0.55	0.49 - 0.61	134	104 - 164	42.1	28.6 - 57.4
Aluminum ($AlCl_3$)	0.56	0.51 - 0.61	369	301 - 420	260	190 - 315
Antimony ($SbCl_3$)	0.66	0.53 - 0.79	157	101 - 216	48.9	24.8 - 79.2
Molybdenum (Na_2MoO_4)	0.79	0.61 - 0.99	125	76.5 - 183	27.8	13.5 - 48.3
Zirconium ($ZrCl_4$)	1.08	0.70 - 1.57	79.0	32.2 - 150	10.3	2.4 - 24.1
Zinc ($ZnCl_2$)	1.12	1.00 - 1.24	451	366 - 533	216	157 - 275
Manganese ($MnCl_2$)	2.91	2.60 - 3.23	958	779 - 1134	388	280 - 501
Gallium ($GaCl_3$)	3.51	2.47 - 4.73	316	156 - 540	44.5	15.6 - 96.7
Tantalum ($KTaO_3$)	4.33	3.08 - 5.84	525	260 - 869	94.0	32.0 - 198
Selenium (Na_2SeO_4)	5.17	4.15 - 6.26	786	483 - 1137	169	79.9 - 296
Titanium ($TiCl_4$)	7.31	5.33 - 9.51	981	513 - 1578	191	72.7 - 381
Lithium (LiCl)	9.28	6.68 - 12.3	1783	901 - 2826	464	163 - 916
Tungsten (Na_2WO_4)	16.5	14.0 - 19.4	3651	2609 - 4748	1066	629 - 1591
Tellurium (K_2TeO_3)	21.6	14.5 - 30.6	1263	523 - 2377	125	31.5 - 327
Barium ($BaCl_2$)	42.7	32.2 - 54.2	9543	5566 - 14097	2813	1267 - 4924
Boron (H_3BO_3)	70.1	37.0 - 184	1016	156 - 2067	31.6	0.8 - 191
Cesium (CsCl)	181	133 - 235	21826	9807 - 37054	3887	1092 - 8842
Magnesium ($MgCl_2$)	1355	1199 - 1507	660500	517600 - 788000	367600	254800 - 475800

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solid wastes at concentrations sufficient to pose appreciable risk to trout and other aquatic biota (Table 1).

Particular attention was given to exposure levels which produced 10% (LC_{10}) and 1% (LC_1) impairment of test populations, to evaluate use of such probit-derived values for (1) approximating threshold concentrations, and (2) application in initial hazard assessment programs. To determine reliability of the LC_1 values, they were compared with MATCs or no effect concentrations developed in continuous flow embryo-larval and chronic life-cycle tests, as well as with current freshwater criteria.[35] The LC_1 of 0.2 µg/l mercury was in close agreement with MATCs determined in chronic studies with the fathead minnow[21] (0.07-0.13 µg/l), flagfish[21] (0.17-0.33 µg/l), and brook trout[36] (0.29-0.93 µg/l), and with the freshwater criterion of 0.05 µg/l.[35] However, in continuous flow embryo-larval and chronic reproductive tests with the rainbow trout, developmental stages suffered lethality at 0.1 µg/l mercury.[22,32] Though data on silver were limited, the LC_1 of 0.1 µg/l agreed closely with a long term no effect concentration set between 0.09 and 0.17 µg/l in an 18-month study with the rainbow trout.[37] The copper LC_1 of 3.4 µg/l was close to estimated MATC ranges of 3.0 to 5.0 and 5.0 to 8.0 µg/l determined for the brook trout by Sauter et al.,[38] and just below the no effect concentration of 9.4 µg/l given by McKim and Benoit.[39]

The cadmium LC_1 of 8.0 µg/l was in agreement with estimated MATCs for eight species of fish,[21] including the range of 3.8 to 11.7 µg/l determined for brown trout.[40] An MATC of 1.7 to 3.4 µg/l was established for chronically exposed brook trout,[41] and present EPA criteria for salmonids were set at 0.4 and 1.2 µg/l for cadmium in soft and hard water, respectively.[35] The chromium LC_1 was 21.5 µg/l, compared to an estimated MATC of 51 to 105 µg/l established in a 60-day test with embryonic, larval, and juvenile stages of the rainbow trout.[38] In a complete life-cycle study with the brook trout, the MATC range for chromium was 200 to 350 µg/l,[42] and the EPA criterion for aquatic life was set at 100 µg/l.[35] In life-cycle studies with Daphnie magna, Biesinger and Christensen[43] reported 16% reproductive impairment at a chromium concentration of 330 µg/l, while Trabalka and Gehrs[44] observed significant effects on survival and reproduction at exposure levels as low as 10 µg/l.

In studies with lead, MATC ranges of 31.3 to 62.5 and 58 to 119 µg/l were determined in chronic reproductive tests on the flagfish[21] and brook trout,[45] respectively. An MATC for rainbow trout was estimated to fall between 71 and 146 µg/l in 60-day tests on developmental and juvenile stages.[38] However, the toxicity of lead may vary substantially depending on water hardness and other test conditions.[35,46] In chronic studies with the rainbow trout,[46] MATCs for total lead administered in soft water were within ranges of 4.1 to 7.6 µg/l and 7.2 to 14.6 µg/l, depending on whether exposure was initiated at the eyed stage or after hatching. The most sensitive test responses included discoloration of the tail and abnormalities of the spinal column (i.e., lordosis, scoliosis). These MATCs closely approximated the LC_1 of 10.3 µg/l given in Table 2. It is important to note that the latter value was determined by combining frequencies for embryo-larval lethality and teratogenesis, basing exposure on total lead administered in moderately hard water.

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No chronic data were available for beryllium, but the LC_1 did not differ significantly from the EPA criterion of 11 µg/l established for aquatic life exposed in soft water.[35] The LC_1 for cobalt was 38.2 µg/l, and this was in reasonable agreement with an MATC of 48.7 to 112.5 µg/l, which we estimated from results on growth and survival obtained in 30-day tests with embryos and larvae of the fathead minnow.[47] In the latter investigation, the bioconcentration of cobalt was significant at 48.7 µg/l.

Though a final criterion for arsenic has not been developed, the EPA recommendation for domestic water supplies (50 µg/l) was considered adequate to protect aquatic life.[35] The arsenic LC_1 was 42.1 µg/l. Zinc gave an LC_1 of 216 µg/l, compared to MATCs of 30 to 180 and 532 to 1368 µg/l determined in chronic reproductive studies with the fathead minnow[48] and brook trout,[21] respectively. In addition, the LC_1 was in close agreement with the estimated MATC of 139 to 267 µg/l obtained in 30-day tests with the flagfish.[49] The boron LC_1 of 31.6 µg/l was obtained in tests conducted in moderately hard water (100 mg/l $CaCO_3$) and was approximately midrange between values reported in continuous flow tests in which trout embryos and larvae were exposed in soft and hard water.[30] Compared with a boron LC_{50} of 70.1 mg/l, the LC_1 was unusually low. However, this was due in large part to teratogenic effects of boron observed at low concentrations.[30] The LC_{10} of 1016 µg/l further characterized the gradual slope of the dose-response curve obtained for boron. Though chronic data were not available for magnesium, the LC_{50} of 1355 mg/l appeared reasonable in view of 96-hour LC_{50}s which ranged up to 4200 mg/l for adult fish.[50] In tests with magnesium, water hardness was substantially increased at the higher exposure levels.

A poor correlation between LC_1 and MATC values was observed for nickel. Despite the importance of nickel in hazard assessment programs for oil shale and coal, chronic toxicity tests with this element have been limited to very few aquatic species. The most comprehensive investigation was conducted on the fathead minnow by Pickering.[51] Nickel concentrations up to 1.6 mg/l did not affect survival or growth of the first generation of fish, which were 6 weeks of age at the onset of exposure. Spawning began after approximately 5 months, and both fecundity and egg hatchability were sharply reduced at a mean nickel concentration of 730 µg/l. The average number of eggs per spawning was 66 and hatchability was 42%, compared to control values of 188 and 94%, respectively. Though egg production appeared repressed at lower exposure levels, results could not be verified statistically. For example, when nickel was administered at 380, 180, and 82 µg/l, mean egg production per female for all spawnings was 13% to 31% less than observed for controls. The maximum acceptable toxicant concentration for nickel in hard water was judged to fall between 380 and 730 µg/l, and Pickering[51] predicted an MATC of 68 to 132 µg/l for fathead minnows exposed in soft water.

In other investigations, Biesinger and Christensen[43] reported 50% and 16% reproductive impairment in Daphnia at nickel concentrations of 95 and 30 µg/l, respectively. While Daphnia and the fathead minnow may differ in their tolerances to nickel,[35] the wide variation between results of Biesinger and Christensen[43] and Pickering[51] probably resulted in part from the different statistical procedures applied to their data. Biesinger and

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Christensen obtained concentrations for reproductive impairment using the method of Litchfield and Wilcoxon,[52] which involved fitting a regression line to dose-response data plotted on logarithmic-probability paper.[53] On the other hand, Pickering applied analysis of variance to his results. Even though he used four replicates per treatment level and obtained good precision in regulating exposure concentrations of nickel, it was not possible to show significance for the consistent reductions in fecundity observed at all exposure levels below 730 µg/l. Time and cost limitations involved in long term investigations frequently curtail use of sufficient replicate exposures to provide adequate differentiation of low-level test responses using the more traditional statistical procedures (e.g., analysis of variance). Therefore, when the dose-response is adequately characterized, regression analysis generally provides a more effective means of approximating threshold concentrations for toxic effects.[24] When data obtained with trout embryo-larval stages were analyzed using log probit regression, sensitivity to nickel equalled or exceeded that observed for Daphnia (Table 2). The LC_{10} and LC_1 values were 10.6 and 3.0 µg/l. In other static renewal tests with embryos and larvae, nickel LC_1s of 3.6, 10.6, and 97.7 µg/l were obtained for the channel catfish, largemouth bass, and goldfish.[54] It should be noted that these values, as well as those presneted in Table 2, were determined with the probit method of Finney,[28] rather than by Daum's procedure[29] which was used in previous investigations. This, together with inclusion of some additional data from replicate experiments, gave lethal concentrations which differed slightly from preliminary findings.[3,26,32]

The data correlations reviewed above were complicated somewhat by differences in test procedures, water conditions, and animal test species. However, where data were sufficient to permit comparisons, LC_1s obtained in static renewal embryo-larval tests with trout were in reasonable agreement with no effect concentrations and MATCs determined in continuous flow embryo-larval and chronic life-cycle studies and with most existing EPA criteria for freshwater biota.[35] Differences between LC_1s and MATCs for specific elements generally were no greater than variations among MATCs reported in different investigations (Table 3). Also as shown in Table 3, an interesting relationship existed between LC_{10} values and metal concentrations which produced 16% reproductive impairment in Daphnia magna.[43] Compared with Daphnia on this basis, trout embryo-larval stages were more sensitive to As, Hg, Mn, Ni, Sn, and Sr, about equally affected by Al, Ba, Cu, and Pb, and more tolerant to Cd, Co, Mg, and Zn. When the different elements were compared for relative toxicity, the order varied somewhat depending on whether LC_{50}, LC_{10}, or LC_1 values were used (Table 2). The order of toxicity of metals to chronically exposed Daphnia also varied to some extent when determined by LC_{50}s. Maximum acceptable toxicant concentrations given in Table 3 were estimated from 30- to 90-day continuous flow embryo-larval tests or determined in partial and complete life-cycle studies, and the values for Daphnia were taken from Biesinger and Christensen.[43] The EPA Red Book[35] was the source for criteria for freshwater aquatic life, as revisions currently in progress were not available for inclusion in this study.

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Table 3. MATC'S COMPARED WITH LC_1 AND LC_{10} VALUES DETERMINED IN STATIC RENEWAL TESTS WITH TROUT EMBRYO-LARVAL STAGES

Element[1]	LC_{10}[2] (µg/l)	LC_1[2] (µg/l)	MATC[3] (µg/l)	Species	Test[4]	Daphnia[5] (µg/l)
Aluminum	369	260	-	-	-	320
Arsenic	134	42.1	-	-	-	520
Barium	9543	2813	-	-	-	5800
Cadmium	29.2	8.0	1.7 - 3.4	brook trout[41]	clc	0.17
			3.0 - 6.5	flagfish[21]	el	
			3.8 - 11.7	brown trout[40]	el	
			4.1 - 12.5	coho salmon[40]	el	
			7.4 - 16.9	flagfish[21]	clc	
			8.1 - 16.0	flagfish[49]	el	
Chromium	56.9	21.5	51 - 105	rainbow trout[38]	el	330
			200 - 350	brook trout[42]	clc	
Cobalt	120	38.2	-	-	-	10
Copper	16.5	3.4	3.0 - 5.0	brook trout[38]	el	22
			5.0 - 8.0	brook trout[38]	el	
			9.4 - 17.4	brook trout[39]	clc	
Lead	40.9	10.3	4.1 - 7.6	rainbow trout[46]	plc	30
			7.2 - 14.6	rainbow trout[46]	plc	
			31.3 - 62.5	flagfish[21]	clc	
			58 - 119	brook trout[45]	clc	
			71 - 146	rainbow trout[38]	el	
Magnesium	660500	367600	-	-	-	82000
Manganese	958	388	-	-	-	4100
Mercury	0.9	0.2	0.07 - 0.13	fathead minnow[21]	clc	3.4
			0.17 - 0.33	flagfish[21]	plc	
			0.29 - 0.93	brook trout[36]	clc	
Nickel	10.6	3.0	380 - 730	fathead minnow[51]	plc	30
Silver	0.9	0.1	0.09 - 0.17	rainbow trout[37]	plc	-
Strontium	49.0	13.0	-	-	-	42000
Tin	75.5	18.6	-	-	-	350
Zinc	451	216	30 - 180	fathead minnow[48]	plc	70
			139 - 267	flagfish[49]	el	
			532 - 1368	brook trout[21]	plc	

[1]Administered in static renewal tests from fertilization through 4 days post-hatching.
[2]Determined with the probit method of Finney[28], rather than the procedure of Daum[29] used in earlier investigations[3,26,32].
[3]Additional values were presented by McKim[21].
[4]MATC's were estimated from 30 to 90-day embryo-larval tests (el) or determined in partial (plc) and complete (clc) life-cycle studies.
[5]Chronic values for 16% reproductive impairment given by Biesinger and Christensen[43].

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Firm criteria for aquatic biota have been developed for only a small fraction of the elements which occur in process waters and solid wastes associated with oil shale and coal (Table 1), and energy engineers are faced with an uncertain future concerning regulatory guidelines for waste disposal. Consistent with recommendations of the Interagency Workshops on Oil Shale[55] and Coal Conversion,[25] early identification of potential hazards is essential to assure environmental acceptability of new and rapidly emerging energy technologies. The promulgation of freshwater criteria has progressed slowly since implementation of the Water Quality Act of 1965, due in substantial measure to the stringent requirements of the present testing program. Static renewal bioassays evaluated in the present investigation can be conducted at a small fraction of the time and cost involved in partial and complete chronic life-cycle tests generally used to establish MATCs for aquatic life. As rainbow trout are endemic to many waters which potentially may be affected by the processing of oil shale, the LC_1s and LC_{10}s given in Table 2 should be useful in estimating impact of contaminants on aquatic biota, pending development of regulatory criteria by State and Federal agencies. It should be noted, however, that toxicity of trace elements in antural waters may be affected by various transport-fate phenomena, water characteristics (e.g., pH, hardness, suspended solids), or chemical form and solubility of the contaminant.[35,56,57] The comparative toxicological ranking given in Table 2 should also be useful in prioritizing trace elements for more comprehensive studies on environmental monitoring and biological effects. Particular attention should be given to the more toxic elements which appear at appreciable concentrations in oil shale waste products (Table 1).

Trout embryo-larval tests also were conducted on simple metal mixtures, to evaluate possible antagonistic, additive, or synergistic interactions. Mercury was mixed in equal proportions with each of three other metals, and the resulting LC_{50}s (µg/l) with 95% confidence limits given parenthetically were 10 (6-18), 10 (9-12), and 18 (12-25) for mercury-cadmium, mercury-selenium, and mercury-copper, respectively. Given in the same order, LC_{50}s (µg/l) calculated for additive effects were 25 (19-32), 90 (64-131), and 15 (12-20). Except for mercury-copper, the actual LC_{50}s reflected net synergism. However, as noted in earlier studies,[22,26] analysis of dose-response data clearly indicated that the type of interaction varied with exposure concentration. The results for mercury-copper are shown in Figure 1. Antagonism was observed at 1 to 10 µg/l ($P < 0.005$). Throughout this exposure range, the hatchability of trout eggs consistently exceeded frequencies calculated for additive effects, but synergism became significantly at 50 µg/l ($P < 0.001$). Based on LC_{50} values given in Table 2, mercury was more than 20 times as toxic to trout eggs as copper. However, the mercury-copper mixture was less toxic than copper at lower exposure levels, but equally as toxic as mercury at high concentrations. Below median lethal concentrations, mercury-selenium and mercury-cadmium were moderately antagonistic to additive, and synergism was observed only at higher exposure levels. On the basis of these initial results, it appears that synergism usually is dependent on high exposure concentrations and, therefore, less likely to be a significant factor in most natural trout waters. This is consistent with

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earlier results of *in situ* embryo-larval tests conducted on coal ash effluents which contained complex metal mixtures.[3]

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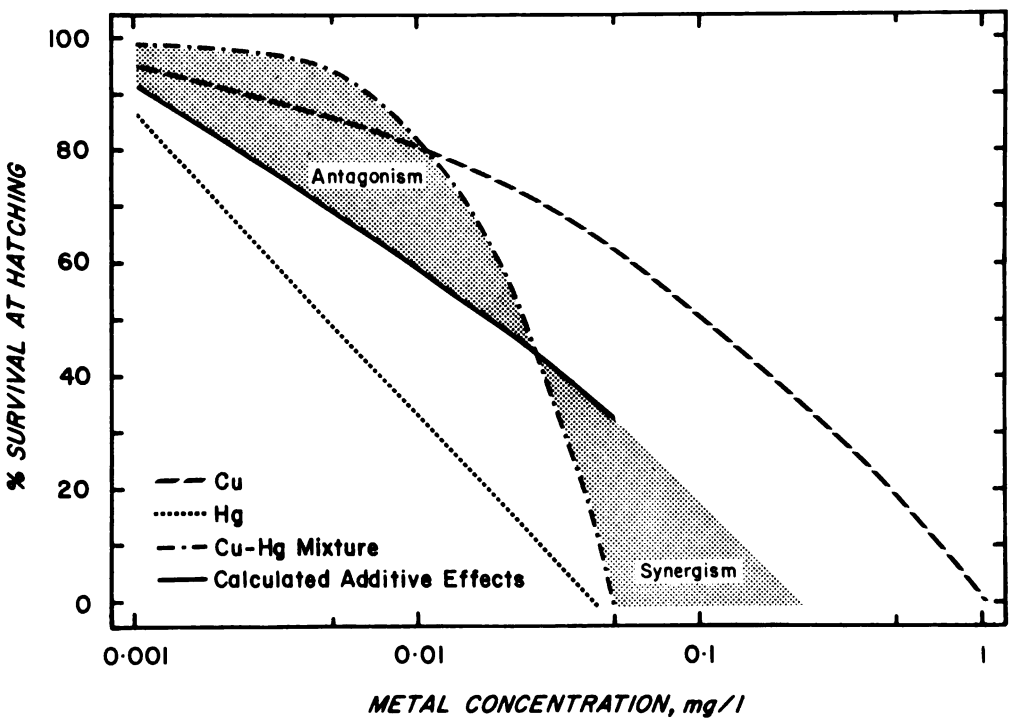

Figure 1. Effects of mercury-copper mixture on rainbow trout embryos. Mercury and copper were mixed in equal proportions and administered from fertilization through hatching (24 days).

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