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TOXICITY ASSESSMENTS OF ANTIMONY, BARIUM, BERYLLIUM, AND MANGANESE FOR DEVELOPMENT OF ECOLOGICAL SOIL SCREENING LEVELS (ECO-SSL) USING ENCHYTRAEID REPRODUCTION BENCHMARK VALUES

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14. ABSTRACT
The U.S. Environmental Protection Agency is developing Ecological Soil Screening Level (Eco-SSL) benchmarks for ecological risk assessment of contaminants at Superfund sites. Benchmarks for invertebrates were developed from existing literature. Insufficient information for Ba, Be, Mn, and Sb to generate Eco-SSLs necessitated standardized toxicity testing to fill the data gaps. We used the Enchytraeid Reproduction Test (ERT) with *Enchytraeus crypticus* in this study. This test was selected on the bases of its ability to measure chemical toxicity to ecologically relevant test species during chronic assay, and its inclusion of at least one reproductive component among the measurement endpoints. Tests were conducted in Sassafras sandy loam soil, which supports relatively high bioavailability of metals. Aging/weathering procedures for amended treatment soil were incorporated into the study to better reflect the "real world" exposure conditions. The toxicity order based on juvenile production was Be > Mn > Sb > Ba with EC_{20} values of 45, 116, 194, and 585 mg kg^{-1}, respectively. These results show that ERT is a robust and sensitive assay for toxicity assessments and is appropriate for the Eco-SSL development.

15. SUBJECT TERMS

Barium	Antimony	Bioavailability	Ecological Soil Screening Level
Beryllium	Toxicity Assessment	Natural soil	
Manganese	Aging/weathering	*Enchytraeus crypticus*	

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PREFACE

The work described in this report was authorized under Sales Order No. 9KNM22. The work was started in February 2000 and completed in September 2002.

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TOXICITY ASSESSMENTS OF ANTIMONY, BARIUM, BERYLLIUM, AND MANGANESE FOR DEVELOPMENT OF ECOLOGICAL SOIL SCREENING LEVELS (ECO-SSL) USING ENCHYTRAEID REPRODUCTION BENCHMARK VALUES

1. INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) is developing Ecological Soil Screening Levels (Eco-SSLs) for ecological risk assessment of contaminants at Superfund sites. Eco-SSLs are soil concentrations of chemicals which, when not exceeded, will theoretically protect terrestrial ecosystems from unacceptable harmful effects. They are derived using data generated from laboratory toxicity tests with different test organisms, which represent the vast array of ecological receptors. Whenever sufficient quantity and quality of information existed, Eco-SSLs for soil invertebrates were developed from studies reported in literature. However, insufficient information to generate Eco-SSLs for barium (Ba), beryllium (Be), Manganese, (Mn), and antimony (Sb) necessitated standardized toxicity testing to fill the data gaps.

This study was designed to produce benchmark data for the development of an Eco-SSL for Ba, Be, Mn and Sb for soil invertebrates, and meet specific criteria (USEPA, 2000), including: (1) tests were conducted in soil having physico-chemical characteristics that support relatively high bioavailability of metals; (2) experimental designs for laboratory studies were documented and appropriate; (3) both nominal and analytically determined concentrations of chemicals of interest were reported; (4) tests included both negative and positive controls; (5) chronic or life cycle tests were used; (6) appropriate chemical dosing procedures were reported; (7) concentration-response relationships were reported; (8) statistical tests used to calculate the benchmark and level of significance were described; and (9) the origin of test species were specified and appropriate.

Several soil invertebrate toxicity tests, for which standardized protocols have been developed, can effectively be used to assess the toxicity and to derive protective benchmark values for metals (Stephenson *et al.*, 2002; Løkke and Van Gestel, 1998). We used the Enchytraeid Reproduction Test in this study. This test was selected on the bases of its ability to measure chemical toxicity to ecologically relevant test species during chronic assays, and its inclusion of at least one reproductive component among the measurement endpoints.

Special consideration in assessing chemical toxicity for Eco-SSL development was given to the effects of aging/weathering of soil contaminants on the exposure of relevant ecological receptors, as commonly occurs at Superfund sites. During chemical aging/weathering in soil, reduction in the exposure to the chemical may occur due to volatilization, microbial degradation and immobilization, or other fate processes (e.g., photodecomposition, hydrolysis, and hysteresis, etc.). This can result in a dramatic reduction in the amount of chemical that is bioavailable, compared to tests conducted with freshly-amended chemicals or those tested following a short equilibration period (e.g., 24 h). Standardized methods for aging/weathering of chemicals in soil are not available. We used the approach developed to simulate at least partially, the aging and weathering process that included exposing soils amended with chemicals to periodic alternating wetting and air-drying cycles for three weeks, in a green house.

2. MATERIAL AND METHODS

2.1 Test Soil.

A natural soil, Sassafras sandy loam [Fine-loamy, siliceous, mesic Typic Hapludult] (SSL) was used in this study to assess the toxicity of test chemicals to *E. crypticus*. This soil was selected for developing ecotoxicological values protective of soil biota because it has physical and chemical characteristics supporting relatively high bioavailability of the test chemicals (low pH, organic matter and clay contents). The SSL soil was collected from an open grassland field on the property of the U.S. Army Aberdeen Proving Ground (APG; Edgewood, MD). Vegetation and the organic matter horizon were removed to just below the root zone and the top six inches of the A horizon were then collected. The soil was sieved through a $5mm^2$ mesh screen, air-dried for at least 72h and mixed periodically to ensure uniform drying, passed through a 2-mm sieve, then stored at room temperature before use in testing. Soil was then analyzed for physical and chemical characteristics by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory, College Park, MD. Results of these analyses are presented in Table 1.

Table 1. Physical and chemical characteristics of Sassafras sandy loam soil analyzed by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory, College Park, MD.

Soil Parameter	Sassafras Sandy Loam
Sand %	71
Silt %	18
Clay %	11
Texture %	Sandy loam
CEC cmol kg^{-1}	4.27
Organic matter %	1.2
pH	5.0

2.2 Test Chemicals.

The goal of this study was to determine the toxicity of Ba, Be, Mn, and Sb to *E. crypticus*. The assessment was done using sulfate salts, including $BaSO_4$ (CAS #7727-43-7, 97%; stock #13989; lot #I10J20, Alfa Aesar), $BeSO_4*4H_2O$ (CAS #7787-56-6, 99.99%; stock #16104; lot #H09J07, Alfa Aesar), $MnSO_4*H_2O$ (CAS #10034-96-5, ACS, 98.0-101.0%, stock

#33341; lot #I18I29, Alfa Aesar), and $Sb_2(SO_4)_3$ (CAS #7446-32-4, 97%, stock #33492; lot #L21I28, Alfa Aesar). Additional tests were done for Ba and Sb to determine how carrier salts and their relative solubilities affect the toxicity to *E. crypticus*. For Ba, these compounds including BaO (CAS #1304-28-5, 97%, lot #12101BI, Aldrich Chemical Company), $Ba(NO_3)_2$ (CAS #10022-31-8, ACS, lot #000420, Fisher Scientific Co.), and $Ba(C_2H_3O_2)_2$ (CAS #543-80-6, ACS, lot #995963, Fisher Scientific Co.). For Sb, we used antimony D-tartrate $Sb_2(C_4H_4O_6)_3*6H_2O$ (CAS # 126506-93-2, lot #111004-2, Pfaltz & Bauer). The positive control used in this study was 4-Nitrophenol (CAS #100-02-7, 98%, lot #6623HE, Aldrich). The main carrier salt control was sulfate as $CaSO_4*2H_2O$ (CAS #10101-41-4, ACS, Reagent grade 100%, lot #C07704, J.T. Baker). ASTM type I water (American Society of Testing and Materials, http://www.astm.org) obtained using Milli-RO® 10 Plus followed by Milli-Q® PF Plus systems (Millipore®, Bedford, MA) was used throughout the studies.

2.3 Soil Amendment Procedures.

Treatment concentrations for toxicity tests with all sulfate salts and barium oxide were prepared by adding test chemicals to SSL soil in appropriate proportions to achieve nominal target concentrations. Soil was mixed for three hours on a three dimensional rotary mixer. After mixing, soil was hydrated with ASTM type I water to 100% of the soil water holding capacity (WHC; 18% water, on a the basis of the dry soil mass) for toxicity testing, or 60% of the WHC for the aging/weathering procedure. Soil prepared for range finding toxicity tests was allowed to equilibrate for 24 hours before exposing potworms. The exception was soil amended with barium acetate, which was incubated for 5 days before exposing potworms to allow acetate degradation by soil microbes. Treatment concentrations of $Ba(C_2H_3O_2)_2$, $Ba(NO_3)_2$ and $Sb_2(C_4H_4O_6)_3$ were prepared by dissolving appropriate amounts of each chemical in ASTM type I water, then hydrating pre-weighed amounts of SSL soil to achieve target treatment concentrations in soil for each chemical, respectively, at the required moisture level.

2.4 Treatment Concentrations.

2.4.1 Range Finding Tests

Range finding tests for Ba, Be, Mn, and Sb were initially conducted using $BaSO_4$, $BeSO_4$, $MnSO_4$, and $Sb_2(SO_4)_3$. Concentrations of Ba and Mn were 100, 500, 1000, 5000 and 10000 mg kg^{-1}. Concentrations of Be and Sb were 1, 10, 100, 500 and 1000 mg kg^{-1}. Additional range finding testing for Ba using BaO, $Ba(NO_3)_2$ and $Ba(C_2H_3O_2)_2$, and for Sb using $Sb_2(C_4H_4O_6)_3$, were done using the same concentrations as for the sulfate salts.

2.4.2 Definitive Tests

Data from the range finding tests were used to determine the respective chemical form with higher toxicity values for *E. crypticus*, and to determine treatment concentrations for definitive tests. Additional considerations in the selection of the chemical form for definitive toxicity testing was given to chemical solubility in water and the effect each chemical form had on soil pH level. Concentrations selected for definitive tests are shown in Table 2.

Table 2. Nominal barium, beryllium, manganese, and antimony concentrations selected for definitive toxicity studies with *E. crypticus*, as determined from range finding tests.

Chemical	Ba	Be	Mn	Sb
First positive concentration tested:				
1	451	10	10	100
2	597	14	18	140
3	686	20	31	196
4	789	27	54	274
5	907	38	94	384
6	1043	54	164	538
7	1200	75	287	753
8	1314	105	503	1054
9	1551			
10	1830			

Controls included positive (30 mg kg^{-1} 4-Nitrophenol), negative (no chemical added) and sulfate ($CaSO_4$). Sulfate controls were based on estimated sulfate amounts in highest treatment concentrations, and were 7,000 and 35,000 mg kg^{-1} SO_4^{2-}, respectively. Four replicates were used for each treatment concentration and controls.

2.5 Aging/Weathering of Amended Soil.

All soil treatment concentrations and negative controls were subjected to simulated aging/weathering procedure, which included alternating wetting/air-drying cycles for three weeks prior to commencement of definitive tests. Aging/weathering of test soils was conducted in open plastic bags in the green house. Soil treatments were initially hydrated to 60% of water holding capacity (WHC), and then allowed to begin drying. All soil treatments were weighed and adjusted to 60% of WHC twice each week, and afterward brought to 100% of WHC (18% water, on the basis of the dry soil mass) for initiation of bioassays. A separate study was conducted using Mn as a model chemical to determine if the three-week duration of aging/weathering procedure was adequate. The duration of this study was 18 weeks. Nominal Mn treatment concentrations included 0, 10, 18, 31, 54, 94, 164, 287, and 503 mg kg^{-1}. Samples from each treatment concentration were analyzed for exchangeable Mn concentrations at three-week intervals to determine if increase in duration of aging/weathering procedure beyond three weeks affects exchangeable Mn concentrations (directly related to bioavailable Mn).

2.6 Chemical Extraction and Analyses.

Soil was analyzed for total metal concentrations following USEPA Method 200.8 (USEPA, 1994) using inductively coupled plasma mass spectrometry (ICP-MS). Additional analysis was done to determine exchangeable Mn fraction. Exchangeable Mn was extracted from soil using $0.05M\ CaCl_2$ with agitation on a reciprocating shaker for 24h. All reagents used in extraction of chemicals from soils were either reagent or trace metal grade, and ASTM type I water was used throughout the analytical studies. Glassware was washed with phosphate-free detergent followed by rinses with tap water, ASTM type I water, nitric acid 1% (v/v) and finally again with ASTM type I water. Analyses of exchangeable Mn concentrations were conducted using a Perkin-Elmer 5100 PC Atomic Absorption Spectrophotometer equipped with an AS-90 autosampler.

2.7 Toxicity Assessment.

The Enchytraeid Reproduction Test (ERT) was used to assess the effects of Ba, Be, Mn and Sb on the reproduction of the enchytraeid worm *Enchytraeus crypticus*. The test is an application of the ISO/CD 16387 (Draft). *Soil quality — Effects of pollutants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction and survival (January 2001).* The ERT is a Chronic/Life-Cycle Assay. The ISO Guideline for this assay was originally developed for use with Artificial Soil (USEPA Standard Artificial Soil), however our research showed that this test could also be conducted using natural soils (Kuperman and Simini, 1999). The ISO ERT was designed using the enchytraeid worm species *Enchytraeus albidus*. Results of our previous studies using *E. albidus* showed that this species requires soils containing high organic matter content with a soil pH 6 (∀0.5) for optimal test conditions. This species performed poorly in natural soils with physical and chemical characteristics that support a higher level of metal bioavailability (Kuperman and Simini, 1999). The species of Enchytraeidae, *E. crypticus*, listed in the ISO protocol as an acceptable alternative to *E. albidus,* was selected for toxicity testing.

2.7.1 Principle of the Test

Adult *E. crypticus* are exposed to a range of concentrations of the test chemical added to soil. The test consists of two steps. They are a range finding test in which adult survival and total number of juveniles produced are assessed using few treatment concentrations (five) and reduced number of replicates (two), and a definitive test in which the same endpoints are assessed using greater number of concentrations and replicates. The duration of each test is four weeks. After the first two weeks, the adult worms are removed, counted, and any morphological changes are recorded. After an additional two-week incubation, the number of juveniles produced is counted. The number of adults and juveniles in treatment concentrations are compared to numbers in the control(s) to quantify ecotoxicological parameters. These parameters include the bounded No Observed Effect Concentration (NOEC), the bounded Lowest Observed Effect Concentration (LOEC) and the effective concentration that causes a p percent reduction in juvenile numbers, i.e. ECp (e.g. EC_{20}, EC_{50}).

2.7.2 Validity of the Test

The validity criteria are included in the test as part of the Quality Control procedures. They include the following performance parameters for the negative controls:

(1) The adult mortality does not exceed 20% after 14 days, in the range finding and definitive tests
(2) The average number of juveniles is higher than 25 per test container at the end of the test assuming that 10 adult worms per test container were used
(3) The coefficient of variation for the mean number of juveniles is ≤50% at the end of the test

2.7.3 Culturing Conditions

Enchytraeids were bred in 4.3-L clear plastic boxes (34 x 20 x 10 cm) filled with 2 kg (dry mass) SSL soil. The culture was kept in an incubator at 22±1°C with continuous light. Soil moisture level was adjusted to 100% of WHC, and was maintained by periodic (once per week) mass checks and water adjustments. Soil in the breeding culture was aerated by carefully mixing it once per week.

The potworms were fed approximately twice a week with a proper amount of ground oats spread on the soil surface. If food from the previous feeding date remained on the soil surface, the amount of food given was adjusted. Every 2-3 months, the worms were transferred into a freshly prepared culture substrate.

Culturing conditions were regarded satisfactory if:

(1) Worms did not try to leave soil
(2) They moved quickly through the soil
(3) They exhibited a shiny outer surface without soil particles clinging to it
(4) They were whitish in color
(5) Worms of different ages were present

The worm culture was considered healthy if worms reproduced continuously.

2.7.4 Test Performance

Glass test containers (42 mm ID; 45 mm deep) were rinsed with acetone, tap water, and ASTM type I water before the test. Twenty grams of prepared soil hydrated to 100% of WHC were added to each test container and 0.05 g of grounded oats were mixed with soil. The mass of each container (without lid) with soil was recorded. Each treatment and controls were replicated four times for definitive tests (two for range finding tests). Soil was allowed to equilibrate 24 hours in the range finding test. Definitive tests were conducted using soil subjected to simulated aging/weathering procedure for three weeks.

Enchytraeid adults with eggs in the clitellum region were collected from culture established in the same soil type (SSL) as soil used in the test. The selected worms were placed in a petri dish filled with a small amount of ASTM type I water for examination using a stereomicroscope. Worms with no eggs were discarded. Any invertebrates living in the cultures such as mites were also removed. Ten enchytraeid worms selected for uniformity (approximately 1 cm) were placed on top of prepared soil in each test container. Test containers were placed randomly on trays and incubated at 21±1°C with continuous light cycle. The containers were weighed once a week and the mass loss was replenished with the appropriate amount of ASTM type I water. Ground oats (0.05 g) were added to each test container at that time.

After two weeks, soil in each test container was carefully searched and adult worms were removed and counted. Worms were examined for any morphological or behavioral changes. The remaining test substrate, including any cocoons laid during the first two weeks of the test, was incubated for additional two weeks. After four weeks from the start of the test, soil in the test containers was fixed with 70% ethanol, and seven drops of Rosebengal biological stain (1% solution in ethanol) was added. Staining continued for minimum of 24 hours. The content of each test container was wet-sieved on No. 100 (150 *u*m) mesh and transferred to a counting tray and worms were counted. Measurement endpoints included number of surviving adults after 14 days and number of juveniles produced after 28 days.

2.8 Data Analysis.

Adult survival and reproduction data were analyzed using nonlinear regression models, described in Stephenson *et al.* (2000). Histograms of the residuals and stem-and-leaf graphs were examined to ensure that normality assumptions were met. Variances of the residuals were examined to decide whether or not to weight the data, and to select potential models. The logistic (Gompertz) model had the best fit for data in all toxicity tests. The fit of the line was closest to the data points, the variances were the smallest, and the residuals had the best appearance (i.e., most random scattering). There were the megaphone-shaped patterns in the "residual vs. concentration" graphs for Mn and Sb, suggesting potential heteroscedasticity (Appendix D). Additional analyses were done with data weighted with the inverse of the variances of each concentration. These produced no appreciable difference in the confidence intervals and only minor differences in the endpoint estimates. Based on these results the Gompertz-modeled analyses were left unweighted. The model is:

$$Y = a \times e^{([\log(1-p)] \times [C/\mathrm{ECp}] \wedge b)}$$

where Y is the number of adults or juveniles produced, a is the control response, e is the base of the natural logarithm, p is the percent inhibition/100 (e.g., 0.5 for EC_{50}), C is the exposure concentration in test soil, ECp is the estimate of effect concentration for a specified percent effect, and b is the scale parameter. The ECp parameters used in this study included the metal concentration producing a 20% (EC_{20}) or 50% (EC_{50}) reduction in the measurement endpoint. The EC_{20} parameter based on a reproduction endpoint is the preferred parameter for deriving soil invertebrate Eco-SSL benchmarks. The EC_{50}, more commonly used in the past, and survival data were included to enable comparisons of the results produced in this study with results reported by other researchers. The asymptotic standard error (a.s.e.) and 95% confidence intervals (CI) associated with the point estimates were determined.

Analysis of Variance (ANOVA) was used to determine the bounded No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for adult survival or juvenile production data (Appendix D). Mean separations were done using Fisher's Least Significant Difference (LSD) pairwise comparison tests. A significance level of $P < 0.05$ was accepted for determining the NOEC and LOEC values. When NOAEC (bounded no observed adverse effect concentration) or LOAEC (bounded lowest observed adverse effect concentration) values were determined, the same statistical methods were used. All analyses were done using measured metal concentrations. Statistical analyses were performed using SYSTAT 7.0.1 (SPSS, 1997).

3. RESULTS

3.1 Soil Analyses.

Analysis of negative control soil showed that beryllium concentration in natural SSL soil used in this study was below method detection limit (MDL) of 2.5 mg kg^{-1}. Total beryllium concentrations in the experimental treatments ranged from 95 to 124% and averaged 107% of nominal (Table 3).

The natural background manganese concentration determined in the negative control treatment was 94 mg kg^{-1}. Total extractable manganese concentrations (in excess of background) in the experimental treatments ranged from 50 to 117% and averaged 94% of nominal (Table 3). Exchangeable Mn fraction expressed as percent of total concentration increased with increasing soil Mn loads (Table 3). There were no trends within any treatment concentration in the amount of exchangeable Mn fraction beyond three weeks during the 18-week aging/weathering study (Table 4). These results confirmed that the three-week duration for simulated aging/weathering procedure used in to the definitive study design was adequate for the Eco-SSL benchmark development.

Analytical procedures for antimony determination did not confirm agreement with the nominal treatment concentrations. Total antimony treatment concentrations determined using USEPA Method 200.8 ranged from 4 to 21% and averaged 8% of nominal concentration. These results showed that this standard method was not sufficient for total Sb analysis in SSL soil. Additional effort was made in the attempt to improve the analytical procedure. Soils were digested using procedures described in SW-846 Method 3050B (USEPA, 1996). This improved the efficiency of antimony extraction, however it remained relatively low and averaged 58% of nominal concentration added to the soil. For this reason, nominal Sb concentrations were used in determining ecotoxicological parameters for antimony; however because ERA relies on the determination of chemical concentrations extracted from soil, toxicity parameters determined from nominal concentrations may have to be adjusted to 58% of their values before determining an Sb Eco-SSL to best conservatively-correspond to the level of Sb extracted from soil at specific levels of Sb toxicity in soil.

The natural background barium concentration determined in the negative control treatment was 34 mg kg^{-1}. Total barium concentrations (in excess of background) in the experimental treatments ranged from 88 to 134% and averaged 110% of nominal (Table 3).

The SSL soil pH value of 5.29 was within the range of Eco-SSL's soil matrix of properties that support high bioavailability of cationic metals in natural soils. Soil pH generally decreased with increasing chemical loads but the decrease did not exceed one pH unit for Be, Mn, and Ba treatments (Table 5). The decrease in the highest Sb treatment was 1.2 pH unit compared with untreated SSL soil (negative control). In the sulfate control, soil pH decreased by less than 1.0 pH unit in both 7000 and 35000 mg kg^{-1} SO_4^{2-} treatments compared with negative control.

Table 3. Nominal and measured concentrations of metals in soil following a three-week aging/weathering procedure for total beryllium, manganese, barium, and antimony amended individually in SSL soil. Measured concentrations were determined using USEPA Method 200.8 and inductively coupled plasma mass spectrometry (ICP-MS).

Beryllium			Manganese			Barium			Antimony		
Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery %	Nominal mg kg^{-1}	Measured mg kg^{-1}	Recovery§ %
0	2.5*		0	94		0	34		0	2.5*	
10	12	95**	10	99	50**	451	433	88**	100	6.4	4
14	18	111	18	110	89	597	744	119	140	12	7
20	24	108	31	119	80	686	689	95	196	17	7
27	36	124	54	157	117	789	791	96	274	9.6	3
38	43	107	94	191	103	907	843	89	384	27	6
54	57	101	164	267	105	1043	1429	134	538	37	6
75	83	107	287	386	102	1200	1333	108	753	157	21
105	110	102	503	644	109	1314	1798	134	1054	135	13
						1551	2000	127			
						1830	2111	113			

* Method Detection Limit is reported when no metal amount could be determined in negative control soil.

** Percent recovery was determined after correcting metal concentration in treatment soils for the amount present in negative control soil.

§ Using USEPA Method 3050B on selected samples yielded 58% recovery, on average.

Table 4. Exchangeable manganese fractions during 18-week aging/weathering study using SSL soil amended with manganese sulfate.

Nominal Mn treatment (mg kg^{-1})	Exchangeable Mn fraction (% of total)						Treatment mean (% of total)
	Week 3	Week 6	Week 9	Week 12	Week 15	Week 18	
0	5.4	4.9	7.3	6.6	6.2	7.7	6.4
10	18.0	16.3	19.9	20.1	16.3	17.8	18.1
18	27.1	25.6	28.7	30.1	23.5	27.9	27.2
31	42.3	37.3	39.1	44.2	38.8	40.5	40.4
54	60.1	52.4	54.9	60.4	48.5	54.5	55.1
94	85.8	75.9	76.0	82.4	65.3	76.7	77.0
164	75.2	63.9	66.7	70.7	56.3	68.9	66.9
287	106.3	93.8	94.3	98.5	82.2	95.8	95.2
503	127.3	99.8	104.7	110.4	101.7	90.3	105.7

Table 5. Summary of soil pH data following a three-week aging/weathering procedure determined in studies of beryllium, manganese, antimony, and barium amended individually in SSL soil.

Be treatment	pH	Mn treatment	pH	Sb treatment	pH	Ba treatment	pH
0	5.29	0	5.29	0	5.29	0	5.29
10	5.01	10	5.39	100	5.11	451	4.72
14	4.95	18	5.35	140	4.99	597	4.63
20	4.89	31	5.30	196	4.85	686	4.63
27	4.76	54	5.22	274	4.76	789	4.54
38	4.63	94	5.14	384	4.68	907	4.50
54	4.51	164	5.06	538	4.56	1043	4.48
75	4.45	287	4.96	753	4.35	1200	4.45
105	4.29	503	4.86	1054	4.08	1314	4.44
						1551	4.38
						1830	4.36

3.2 Range Finding Tests.

Range finding test for beryllium was conducted using $BeSO_4 * 4H_2O$ (cold water solubility 42.5 g per 100 cc). Adult survival decreased by 58% at 100 mg kg^{-1} and juveniles production decreased by 18% at 10 mg kg^{-1}. There was a 99.9 % reduction in juvenile numbers at the 100 mg kg^{-1} treatment concentration. No surviving adults or juveniles were recovered in 500 and 1000 mg kg^{-1} treatment concentrations (Appendix A). Beryllium sulfate hydrate was retained for the definitive test, using Be concentrations shown in Table 3.

Range finding test for manganese was conducted using $MnSO_4*H_2O$. Adult survival decreased by 10% at 500 mg kg^{-1} and by 95% at 1,000 mg kg^{-1}. Juvenile production decreased by 33% at 100 mg kg^{-1}. There were no surviving adults above 1,000 mg kg^{-1} or juveniles above the 100 mg kg^{-1} treatment concentrations (Appendix A). Manganese sulfate monohydrate ($MnSO_4*H_2O$) was retained for the definitive test, using Mn concentrations shown in Table 3.

Range finding test for antimony was conducted using $Sb_2(SO_4)_3$ and antimony D-tartrate [$Sb_2(C_4H_4O_6)_3*6H_2O$] to determine if a carrier salt form affects Sb toxicity to *E. crypticus*. Significant ($P = 0.001$) reduction in juvenile production at 538 mg kg^{-1} antimony D-tartrate resulted only in 18% decrease compared with negative control. Numbers of juveniles in the preceding treatment concentration of 384 mg kg^{-1} were actually 13% higher compared with negative control (Appendix A). Toxicity of antimony sulfate to *E. crypticus* juvenile production was higher compared with antimony D-tartrate (Appendix A). The reduction in juvenile production at 500 mg kg^{-1} Sb as $Sb_2(SO_4)_3$ was 62% ($P < 0.0001$). Antimony sulfate was chosen for the definitive test, using Sb concentrations shown in Table 3.

Range finding test for barium was initially conducted using $BaSO_4$ salt. This test showed that even at the highest concentration tested (10,000 mg kg^{-1}), this form of barium is essentially insoluble in water, and did not affect adult survival after 14 days. Toxicity to juveniles after 28 days was low at the same concentration resulting only in a 36% reduction in juvenile numbers compared with control (Appendix A). Such low percent reduction in the reproductive endpoint would not have allowed ECp determination at the 50% level in the definitive test. This necessitated additional range finding tests to determine Ba toxicity to *E. crypticus* with alternative Ba forms. These tests were done using Ba forms soluble in water, including BaO, $Ba(NO_3)_2$, and $Ba(C_2H_3O_2)_2$. All three barium forms produced $LOEC_{juveniles}$ at 1,000 mg kg^{-1} and 100% mortality at 5,000 mg kg^{-1} (Appendix A). Both BaO and $Ba(C_2H_3O_2)_2$ amendments increased soil pH levels beyond boundaries required by the Eco-SSL guidance for soil parameters supporting high cationic metal bioavailability. Barium oxide increased soil pH to 8.69 and barium acetate increased soil pH to 8.61 at 5,000 mg kg^{-1}, respectively. Soil pH in the barium nitrate test decreased to 4.12 in the 5,000 mg kg^{-1} treatment. Additionally, reproductive toxicity of Ba added as $Ba(NO_3)_2$ was higher compared with the other two Ba forms. Percent reductions from control at the 1,000 mg kg^{-1} treatment were 83.4, 50.6, and 29.6 in $Ba(NO_3)_2$, BaO and $Ba(C_2H_3O_2)_2$ tests, respectively. Based on the results of these range finding tests barium nitrate was selected for the definitive test using Ba concentrations shown in Table 3.

3.3 Definitive Tests.

Test results complied with the validity criteria defined in the test guideline. Mean adult survival in negative controls ranged from 97.5 to 100%. The mean juvenile production in negative controls ranged from 735 to 1104 juveniles, and the coefficient of variation ranged from 6 to 18%. Sulfate control treatments showed no statistically significant ($P > 0.05$) effect on adult survival and reproductive measurement endpoints compared with negative controls. Soil pH decreased by less than 1.0 pH unit in both 7000 and 35000 mg kg^{-1} SO_4^{2-} treatments compared with negative control. These results confirmed that the toxicological effects determined in the definitive tests were most likely due to test metal concentrations. Direct comparisons of the results of positive control are not possible because ERT is a new test and no reference values are available from the literature. Limited information available from our earlier studies of 4-nitrophenol with different enchytraeid species, *E. albidus*, in USEPA/OECD standard artificial soil was used as a reference for comparisons. Juvenile production in positive control was within the range of previous study resulting in 66% reduction from negative control.

Definitive tests with aged/weathered SSL soil using the Enchytraeid Reproduction Tests were conducted to assess the effects of Ba, Be, Mn, or Sb on the reproduction of the enchytraeid worm *E. crypticus*. Adult *E. crypticus* were exposed in SSL soil to a range of concentrations for each metal, in independent investigations. Measurement endpoints were assessed using 8-10 treatment concentrations determined from the range-finding studies and included number of surviving adults after 14 days and number of juveniles after 28 days. All ecotoxicological parameters for Ba, Be, and Mn were estimated using measured chemical concentrations for each treatment level. Ecotoxicological parameters for Sb were estimated using nominal concentrations.

Results showed that Be did not affect ($P = 0.174$) adult *E. crypticus* survival up to 83 mg kg^{-1} treatment concentration (Table 6). Adult survival in this definitive test was significantly ($P < 0.0001$) reduced at 110 mg kg^{-1} (LOEC). The bounded NOEC for juvenile production was 43 mg kg^{-1} ($P = 0.06$). The bounded LOEC for juvenile production was 57 mg kg^{-1} ($P < 0.0001$). The juvenile production EC_{20} and EC_{50} values for Be were, respectively 45 and 52 mg kg^{-1} (Table 7; Figure C 1, Appendixes C, D).

Table 6. Ecotoxicological parameters (mg kg^{-1}) for adult *E. crypticus* survival determined in aged/weathered SSL soil independently amended with beryllium, manganese, antimony, and barium using Enchytraeid Reproduction Test.

Endpoint	Beryllium	Manganese	Antimony*	Barium
NOEC	83	191	384	1798
LOEC	110	267	538	2000

* Parameters determined using nominal concentrations of Sb in soil.

Manganese did not affect ($P = 0.721$) adult *E. crypticus* survival up to 191 mg kg^{-1} concentration. Adult survival was reduced by 5% ($P = 0.48$) at 267 mg kg^{-1} (Table 6). Juvenile numbers were significantly ($P < 0.0001$) higher in the 99 mg kg^{-1} treatment compared to negative control suggesting the hormetic effect of Mn on reproduction at this exposure level. The bounded No Observed Adverse Effect Concentration (NOAEC) for juvenile production was 157 mg kg^{-1} ($P = 0.52$). The bounded Lowest Observed Adverse Effect Concentration (LOAEC) for juvenile production was 191 mg kg^{-1} ($P < 0.0001$) (Table 7). No juveniles were produced in 644 mg kg^{-1} treatment (Appendix B). The hormetic effect at the 99 mg kg^{-1} concentration level suggested the use of the hormetic model to estimate ECp parameters for Mn data. This model produced EC_{50} and EC_{20} estimates, but the fit was not good and the value for the hormetic component of the model seemed extreme. In addition, the variance was very large and the residuals distinctly displayed a pattern. Varying the parameters in the hormetic model did not improve the fit. Based on these results the Gompertz-modeled analysis was accepted for Mn data (Figure C 2, Appendixes C, D). The juvenile production EC_{20} and EC_{50} values for Mn were 116 and 192 mg kg^{-1}, respectively (Table 7).

Antimony did not affect ($P = 0.407$) adult *E. crypticus* survival up to 384 mg kg^{-1} concentration. Adult survival was reduced by 50% ($P < 0.0001$) at 538 mg kg^{-1} (Table 6). The bounded NOEC for juvenile production was 100 mg kg^{-1} ($P = 0.69$). The bounded LOEC for juvenile production was 140 mg kg^{-1} ($P = 0.027$). The juvenile production EC_{20} and EC_{50} values for Sb were 194 and 316 mg kg^{-1}, respectively (Table 7; Figure C 3, Appendixes C, D).

Barium did not significantly affect ($P = 0.467$) adult *E. crypticus* survival up to 1798 mg kg^{-1} concentration. Adult survival was reduced by 20% ($P = 0.006$) at 2000 mg kg^{-1} (Table 6). The bounded NOEC for juvenile production was 433 mg kg^{-1} ($P = 0.597$). The bounded LOEC for juvenile production was 689 mg kg^{-1} ($P = 0.031$). The juvenile production EC_{20} and EC_{50} values for Ba were, respectively 585 and 947 mg kg^{-1} (Table 7; Figure C 4, Appendixes C, D).

Table 7. Ecotoxicological parameters (mg kg^{-1}) for juvenile production determined in aged/weathered SSL soil independently amended with beryllium, manganese, antimony, and barium using Enchytraeid Reproduction Test; parenthetical values are 95% confidence intervals.

Endpoint	Beryllium	Manganese	Antimony*	Barium
NOAEC	43	157	100	433
LOAEC	57	191	140	689
EC_{20}	45 (42-49)	116 (56-176)	194 (155-234)	585 (447-722)
EC_{50}	52 (50-54)	192 (147-238)	316 (285-347)	947 (830-1064)

* Parameters determined using nominal concentrations of Sb in soil.

4. DISCUSSION

Development of screening level benchmarks for Ecological Risk Assessment (ERA) of contaminated soils has become a critical need in recent years (USEPA, 2000). To address this problem, the USEPA in conjunction with stakeholders is developing Eco-SSLs to identify concentrations of chemicals in soil that, when not exceeded, theoretically protective of terrestrial ecosystems within specific soil boundary conditions from unacceptable harmful effects. An extensive review of literature (USEPA, 2000) determined that there was insufficient information for beryllium, manganese, antimony, and barium to generate Eco-SSL benchmarks for soil invertebrates. Our toxicity studies were designed to specifically fill this knowledge gap.

The majority of soil toxicity tests that were reported in literature used standard artificial soil with high organic matter content (10%) and near neutral pH. In contrast, we selected SSL soil to meet the criteria for Eco-SSL development, in large part because it has characteristics supporting relatively high bioavailability of cationic metals. In addition, our aging/weathering procedure of the soils loaded with the range of metal concentrations allowed us to more realistically assess the toxicity under conditions more closely resembling the potential toxic effects of beryllium, manganese, antimony, and barium in the field.

Definitive toxicity tests conducted with aged/weathered soils amended with test chemicals showed that chemical toxicity order based on EC_{20} for juveniles production in tests with *E. crypticus* was Be > Mn > Sb > Ba (Table 7). However because ERA relies on the determination of soil concentrations extracted from soil, Sb toxicity parameters determined from nominal concentrations may have to be adjusted to 58% of their values before determining an Sb Eco-SSL to best conservatively-correspond to the level of Sb extracted from soil at specific levels of Sb toxicity in soil. If the EC_{20} for juveniles production is adjusted by 58% to account for reduced extractability, the toxicity order for *E. crypticus* becomes Be > Mn = Sb > Ba. Reproductive endpoints in all tests were more sensitive compared with adult survival (Tables 6, 7). This supports the Eco-SSL requirement of the use of reproductive endpoints for benchmark development. Because this study was designed to produce benchmark data to be used in the development of Eco-SSLs for beryllium, manganese, antimony, and barium for soil invertebrates, the test conditions and the resulting data had to meet specific criteria (USEPA, 2000). Thus results from these studies may not directly compare to those of other studies in the literature, since none of them were designed to specifically quantify metal toxicity to soil invertebrates under Eco-SSL conditions of testing using soils that support relatively high bioavailability of cationic metals.

Beryllium is one of the least studied metals regarding its effects on soil invertebrates, although it is considered one of the problem metals of the future (Newland, 1982). It is a component of various fossil fuel types and is increasingly used in aircraft industry, space research, nuclear energy development (Ireland, 1986), X-ray tube, windows manufacturing, and in production of non-sparking tools composed of copper-beryllium alloy (Thorat *et. al.*, 2001). Be concentrations in Aberdeen Proving Ground (APG) soil (including contaminated sites) in the areas adjacent to soil collection ranged from 0.3 to 1.4 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Extensive toxicological studies of Be exposure effects in humans and experimental animals have established that it can cause pulmonary and systemic granulomatous disease known as chronic beryllium disease (Sprince and Kazami, 1980), necrosis and tumors in animals (Witschi, 1971), can inhibit certain enzymes, including alkaline phosphatase (Reiner, 1971), and can inhibit plant and animal growth (Newland, 1982). Ireland (1986) reported increased mortality and growth suppression in a terrestrial snail *Achatina fulica* (Pulmonata) fed 10 $\mu g\ ml^{-1}$ Be in the diet containing the sub-optimal calcium concentrations. Beryllium was the most toxic metal among the four chemicals tested in our study, and the estimated ecotoxicological parameters for *E. crypticus* are the first in the available literature for a soil invertebrate species.

Natural manganese concentration in SSL soil of 94 mg kg^{-1} was within the range of Mn concentrations reported for soils (including contaminated sites) at the Aberdeen Proving Ground, which ranged from 4.9 to 1140 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Manganese is a required nutrient essential for plants and animals. Manganese was the most previously investigated of the four metals in this study, however none of the previous studies involved invertebrate exposures in natural soils. Reinecke and Reinecke (1996) reported reduction in growth and development (measured as time needed for clitellum development) of *E. fetida* fed with cattle manure spiked with Mn at 151.7 mg kg^{-1}. This value falls within the effect concentrations range of 20-50% reduction in reproductive endpoint determined in our study. In a later study, Reinecke and Reinecke (1997) reported damage to spermatozoan structure from treatments containing food spiked with Mn at 61.57 mg kg^{-1}. Nottrot *et al.* (1987) reported no effect on feeding activity and growth of collembolan *Orchesella cincta* fed with green algae spiked with up to 25 μmol Mn g^{-1} dry mass, however that study was conducted on dental plaster. Joosse *et al.* (1983) reported no effect on respiration of woodlice fed with litter containing Mn at 1000 mg kg^{-1} on a porous tile. There was no soil exposure incorporated in that study.

Few studies have investigated antimony concentrations in soil (Cal-Prieto *et al.*, 2001; Crecelius *et al.*, 1974; Kabata-Pendias and Pendias, 1992; van der Voet and de Wolff, 1996). Reported concentrations ranged from 0.17 mg kg^{-1} in organic soils in Norway to 1489 mg kg^{-1} in vicinity of an Sb smelter in northeast England (Ainsworth and Cooke, 1991), and these corresponded with treatment concentrations used in our study. Antimony concentrations in soil (including contaminated sites) at the Aberdeen Proving Ground in the areas adjacent to the location where the SSL soil was collected ranged from 0.1 to 501 mg kg^{-1} (Hlohowskyj *et al.*, 1999). No information could be found in the available literature on ecotoxicological effects of antimony to soil invertebrates. Developing such information is especially important since input to the soil ecosystems was estimated at 26000 t y^{-1} of Sb (Cal-Prieto *et al.*, 2001). This anthropogenic contribution of antimony is 10-fold higher compared with the Sb emissions from natural sources (ca. 2600 t y^{-1}) reported by Nriagu (1990). Limited data for soil biota was reported by Rafel and Popov (1988) as part of a validation effort for developing the USSR maximum allowable concentrations of antimony in soil. These authors reported 23-52% reduction in seed germination and 26-62% reduction in root growth at 1002 mg kg^{-1} Sb in tests with barley, wheat, radish, pees, and onion. Decrease in ammonia mineralization and nitrate accumulation was observed at Sb concentrations of 52 and 102 mg kg^{-1} in their study. Other measures of soil biological activity were also affected, including decrease in soil enzyme catalase activity and stimulation of soil respiration at 102 mg Sb kg^{-1} (Rafel and Popov, 1988).

Difficulties encountered with the efficiency of extraction of Sb that is aged/weathered in soil prior to analytical determination, using natural SSL amended with antimony, may be symptomatic of a larger problem regarding chemical characterization data during ERA activities at contaminated sites. Low Sb recovery rates using standard USEPA methods suggest that true concentrations of this metal will be underestimated during site characterization efforts. The recovery rates of 8 and 58 percent determined for Sb aged/weathered in soil in our study, using USEPA methods 200.8 and 3050B, respectively were below recovery rates of 70 and 88 percent previously reported for freshly amended soils. This clearly indicates that USEPA method 3050B appears better suited to extract aged/weathered Sb from soil at Superfund and other contaminated sites, and this potential discrepancy in extractability should be corrected for at the time of compilation of a list of contaminants of potential ecological concern (COPEC) in the screening phase of ERA. To use the ecotoxicological parameters from this study, which are based on nominal Sb values, it is recommended that these nominal Sb values be adjusted to 58% of nominal to account for the aging/weathering of Sb in soil (i.e., adjusted to 58% of nominal prior to determining the Eco-SSL). Aging/weathering of Sb in soils typically occurs even more extensively in the field, but simulated aging/weathering provides a conservative estimate of what might otherwise be extractable from field soils. This is especially important given a steep slope of the concentration-response curve for reproductive endpoint determined from the Enchytraeid Reproduction Test in our study (Figure C 3, Appendix C), which establishes a narrow toxicity threshold range from 194 to 316 mg kg^{-1} (nominal) based on EC_{20} and EC_{50} estimates (Table 6). The 39 percent difference between these two estimates is within the potential recovery error rate of analytical methods used. Disregarding this potential error, especially without adjustment of the Eco-SSL for aging/weathering, can otherwise lead to a removal of antimony from the COPEC list while its extracted concentrations represent field concentrations toxic to relevant ecological receptors. Adjustment of the values of the ecotoxicological parameters determined from nominal concentrations, prior to determination of the Eco-SSL, is properly left to those evaluating benchmarks for Eco-SSL development; however, in these studies an adjustment to 58% of nominal corresponds to the mean recovery rate following three weeks of aging/weathering of Sb in soil.

Natural barium concentration in SSL soil of 34 mg kg^{-1} was within the Ba concentrations found in soils (including contaminated sites) at the Aberdeen Proving Ground, which ranged from 9.8 to 1580 mg kg^{-1} (Hlohowskyj *et al.*, 1999). Limited barium ecotoxicological information for soil invertebrates is available from literature. Grace (1990) investigated oral toxicity of barium metaborate to the Eastern Subterranean Termite *Reticulitermes flavipes* (Kollar) in no-choice assays by feeding termite workers for 15 days on filter papers treated with concentrations of 500-40,00 mg kg^{-1} (356-28,472 mg Ba kg^{-1}, recalculated by Kuperman). Results of this study comport with result of the adult survival (14 days) portion of our definitive test. Grace (1990) reported 19% mortality in 1780 mg Ba kg^{-1} treatment, which was comparable with 20% adult mortality at 2000 mg Ba kg^{-1} treatment observed in our investigation. However, direct comparisons of feeding assays results with soil exposure studies using different species should be treated with caution.

5. CONCLUSIONS

This study has produced ecotoxicological data for beryllium, manganese, antimony, and barium using ecologically relevant soil invertebrate species *E. crypticus*. Relative toxicity of the four metals tested in this study was Be > Mn > Sb > Ba. When the EC_{20} for juveniles production is adjusted by 58% to account for reduced extractability of Sb after three weeks of aging/weathering in soil, the toxicity order for *E. crypticus* becomes Be > Mn = Sb > Ba. It is strongly recommended that the nominal Sb benchmark values from this study be adjusted to 58% of nominal. To account for the aging/weathering of Sb in soil (i.e., adjusted to 58% of nominal prior to determining the Eco-SSL). Study results showed that tests based on reproductive endpoint provide a more sensitive evaluation of effect than adult survival and therefore should be used to set screening criteria. These tests were performed using a natural soil, Sassafras sandy loam. Sassafras sandy loam has relatively low pH, low organic matter, low cation exchange capacity, and high sand content. Such characteristics support relatively high bioavailability of cationic metals in soil. Furthermore, aging and weathering of the amended soil produced a soil microenvironment more similar to field conditions than previous studies where soil invertebrates were exposed immediately following amendment of soil. These study results will be provided to the Ecological Soil Screening Level (Eco-SSL) workgroup for review. Results will undergo quality control review by the Eco-SSL task group before inclusion in the Eco-SSL database, and before being used for developing Ecological Soil Screening Levels (Eco-SSLs) for Be, Mn, Sb, and Ba.

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APPENDIX A

RANGE FINDING TESTS DATA

Range-finding invertebrate assays

Fresh SSL soil

Compound: Be [$BeSO_4$]

Start Date: 21-Apr-00

Invertebrate: *E. crypticus*

Nominal Be (mg/kg)	Rep	Adults 5/5/00	MEAN S.E.	Juveniles 5/19/00	MEAN S.E.	Reduction %
0	1	10	9.5	721	722.5	0
0	2	9	0.3	704	10.4	
0	3	9		752		
0	4	10		713		
1	1	10	10	845	705	2.4
1	2	10	0	565	140	
10	1	9	9.5	596	590.5	18.3
10	2	10	0.5	585	5.5	
100	1	8	4	2	1	99.9
100	2	0	4	0	1	
500	1	0		0		100
500	2	0		0		
1000	1	0		0		100
1000	2	0		0		

Range-finding invertebrate assays

Fresh SSL soil

Compound: Mn [$MnSO_4$]

Start Date: 21-Apr-00

Invertebrate: *E. crypticus*

Nominal Mn (mg/kg)	Rep	Adults 5/5/00	MEAN S.E.	Juveniles 5/19/00	MEAN S.E.	Reduction %
0	1	10	9.5	721	722.5	0
0	2	9	0.29	704	10.43	
0	3	9		752		
0	4	10		713		
100	1	10	9.5	503	486	32.73
100	2	9	0.50	469	17.00	
500	1	7	8.5	0		100
500	2	10	1.50	0		
1000	1	0	0.5	0		100
1000	2	1	0.50	0		
5000	1	0		0		100
5000	2	0		0		
10000	1	0		0		100
10000	2	0		0		

Range-finding invertebrate assays

Fresh SSL soil

Compound: Sb [Sb-d-tartrate]

Start Date: 18-Jan-01

Invertebrate: *E. crypticus*

Nominal Sb (mg/kg)	Rep	Initial container mass (g)	Adults 2/1/01	Juveniles 2/15/01	MEAN S.E.
0	1	96.4	10	962	976.75
0	2	95.1	10	950	13.59
0	3	100.5	10	1012	
0	4	97.8	9	983	
274	1	95.7	10	1109	1084.25
274	2	95.8	10	1095	39.63
274	3	95.8	10	973	
274	4	95.2	10	1160	
384	1	97.8	10	1112	1105.25
384	2	100.2	10	1103	7.30
384	3	98	10	1086	
384	4	97.9	10	1120	
538	1	101.6	10	884	801.75
538	2	97.3	10	771	28.31
538	3	94.9	10	759	
538	4	95.8	10	793	

Range-finding invertebrate assays

Fresh SSL soil

Compound: Sb [$Sb_2(SO_4)_3$]

Start Date: 20-Apr-00

Invertebrate: *E. crypticus*

Nominal Sb (mg/kg)	Rep	Adults 5/4/00	MEAN S.E.	Juveniles 5/18/00	MEAN S.E.	Reduction %
0	1	10	9.5	721	722.5	0
0	2	9	0.29	704	10.43	
0	3	9		752		
0	4	10		713		
1	1	7	8.5	642	728	-0.76
1	2	10	1.50	814	86.00	
10	1	10	10	604	649	10.17
10	2	10	0	694	45.00	
100	1	10	10	615	667	7.68
100	2	10	0	719	52.00	
500	1	9	8.5	341	274	62.08
500	2	8	0.50	207	67.00	
1000	1	4	4	0	0	100
1000	2	4	0	0	0	

Range-finding invertebrate assays

Fresh SSL soil

Compound: Ba [$BaSO_4$]

Start Date: 20-Apr-00

Invertebrate: *E. crypticus*

Nominal Ba (mg/kg)	Rep	Adults 5/4/00	MEAN S.E.	Juveniles 5/18/00	MEAN S.E.	Reduction %
0	1	10	9.5	721	722.5	0
0	2	9	0.3	704	10.4	
0	3	9		752		
0	4	10		713		
100	1	9	9.5	705	690	4.5
100	2	10	0.5	675	15	
500	1	8	8.5	435	513.5	28.9
500	2	9	0.5	592	78.5	
1000	1	9	8.5	360	443	38.7
1000	2	8	0.5	526	83	
5000	1	9	9.5	515	563.5	22.0
5000	2	10	0.5	612	48.5	
10000	1	10	9	484	462.5	36.0
10000	2	8	1	441	21.5	

Range-finding invertebrate assays

Fresh SSL soil

Compound: Ba [BaO]

Start Date: 27-Jun-00

Invertebrate: *E. crypticus*

Nominal Ba (mg/kg)	Rep	Adults 7-11-00	MEAN S.E.	Juveniles 7-25-00	MEAN S.E.	Reduction %
0	1	10	9.75	679	702.25	0
0	2	10	0.25	712	30.01	
0	3	9		638		
0	4	10		780		
100	1	10	10	612	621.5	11.50
100	2	10	0	631	9.5	
500	1	10	9	887	730.5	-4.02
500	2	8	1	574	156.5	
1000	1	1	1	385	347	50.59
1000	2	1	0	309	38	
5000	1	0	0	0	0	100
5000	2	0	0	0	0	
10000	1	0	0	0	0	100
10000	2	0	0	0	0	

Range-finding invertebrate assays

Fresh SSL soil

Compound: Ba [$Ba(NO_3)_2$]

Start Date: 27-Sep-00

Invertebrate: *E. crypticus*

Nominal Ba (mg/kg)	Rep	Initial container mass (g)	10/11/00 Adults	Mean S.E.	10/25/00 Juveniles	Mean S.E.
0	1	91.22	10	10	1275	1261.5
0	2	91.82	10	0	1248	13.5
100	1	96.7	10	10	1530	1598.5
100	2	90.5	10	0	1667	68.5
500	1	90.31	10	9.5	1310	1159.5
500	2	95.47	9	0.5	1009	150.5
1000	1	90.63	10	10	276	209
1000	2	96.48	10	0	142	67
5000	1	91.12	0		0	
5000	2	90.3	0		0	
10000	1	90.83	0		0	
10000	2	92.76	0		0	

Range-finding invertebrate assays

Fresh SSL soil

Compound: Ba [$Ba(C_2H_3O_2)_2$]

Start Date: 2-Oct-00

Invertebrate: *E. crypticus*

Nominal Ba (mg/kg)	Rep	Initial container mass (g)	10-16-00 Adults	10-30-00 Juveniles	Mean S.E.	Reduction %
0	1	91.75	10	859	893	0
0	2	91.04	10	927	34	
100	1	91	10	1026	948.5	-6.22
100	2	91.12	9	871	77.5	
500	1	96.48	10	922	990	-10.86
500	2	90.62	9	1058	68	
1000	1	90.75	10	545	629	29.56
1000	2	96.42	8	713	84	
5000	1	95.52	0	0	0	100
5000	2	90.97	0	0	0	
10000	1	96.02	0	0	0	100
10000	2	93.25	0	0	0	

APPENDIX B

DEFINITIVE TESTS DATA

Definitive invertebrate assays

Aged SSL soil

Compound: Be [$BeSO_4$]

Start Date: 20-Oct-00

Invertebrate: *E. crypticus*

Nominal Be (mg/kg)	Rep	Initial container mass (g)	11/3 Adults	Mean S.E.	11/17 Juveniles	Mean S.E.
0	1	93.01	10	10	1112	1104.3
0	2	96.36	10	0	1149	43.069
0	3	91.6	10		1175	
0	4	92.78	10		981	
10	1	90.83	10	10	1166	1051.5
10	2	92.27	10	0	906	54.216
10	3	90.97	10		1083	
10	4	91.53	10		1051	
14	1	95.91	10	9.75	1164	1117.3
14	2	96.84	10	0.25	1339	90.133
14	3	96.05	10		1054	
14	4	90.99	9		912	
20	1	90.22	10	10	1208	1102.5
20	2	90.85	10	0	979	64.888
20	3	90.9	10		1002	
20	4	91.47	10		1221	
27	1	90.83	10	10	1095	1077.8
27	2	92.7	10	0	1174	37.172
27	3	96.59	10		1038	
27	4	96.06	10		1004	
38	1	90.78	10	10	1018	946.5
38	2	90.63	10	0	1129	77.039
38	3	91.19	10		803	
38	4	91.47	10		836	
54	1	92.87	10	10	233	244.5
54	2	90.28	10	0	146	68.27
54	3	90.67	10		441	
54	4	92.47	10		158	
75	1	90.74	10	9	12	9
75	2	92.85	9	0.71	9	2.12
75	3	91.18	10		12	
75	4	92.51	7		3	
105	1	97.05	8	4.5	1	1.25
105	2	91.1	5	1.32	0	0.95
105	3	92.92	2		4	
105	4	91.59	3		0	

Definitive invertebrate assays

Aged SSL soil

Compound: Mn [$MnSO_4$]

Start Date: 5-Jul-00

Invertebrate: *E. crypticus*

Nominal Mn (mg/kg)	Rep	Initial container mass (g)	Adults 7-19-00	MEAN S.E.	Juveniles 8-2-00	MEAN S.E.
0	1	96.7	9	9.75	759	735.3
0	2	93	10	0.25	672	21.11
0	3	90.8	10		754	
0	4	92.8	10		756	
10	1	90.3	10	9.5	1097	965
10	2	90.3	10	0.5	1021	75.82
10	3	96.4	10		995	
10	4	95.2	8		747	
18	1	95	9	9.5	684	727.3
18	2	90.3	9	0.29	662	41.17
18	3	95.3	10		717	
18	4	89.9	10		846	
31	1	92.5	10	9.75	649	669.3
31	2	90.3	10	0.25	627	22.38
31	3	90.1	9		670	
31	4	95.5	10		731	
54	1	95.8	10	10	638	700.3
54	2	90.6	10	0	693	24.02
54	3	90.8	10		752	
54	4	90.7	10		718	
94	1	95.1	10	9.5	481	417.5
94	2	91.1	9	0.29	397	47.81
94	3	96.8	10		501	
94	4	90.7	9		291	
164	1	90.2	9	9.25	136	236.5
164	2	90.6	10	0.25	313	41.03
164	3	90.7	9		293	
164	4	90.1	9		204	
287	1	90.7	7	5	33	32.5
287	2	96.9	2	1.22	14.00	8.25
287	3	90.8	4		29	
287	4	92.5	7		54	
503	1	96.5	1	0.25	0	
503	2	90.8	0	0.25	0	
503	3	90.7	0		0	
503	4	90.6	0		0	

Definitive invertebrate assays

Aged SSL soil

Compound: Sb [$Sb_2(SO_4)_3$]

Start Date: 6-Jul-00

Invertebrate: *E. crypticus*

Nominal Sb (mg/kg)	Rep	Initial container mass (g)	Adults 7-20-00	MEAN S.E.	Juveniles 8-3-00	MEAN S.E.
0	1	96.7	9	9.75	759	735.3
0	2	93	10	0.25	672	21.11
0	3	90.8	10		754	
0	4	92.8	10		756	
100	1	92.8	9	9.5	675	755
100	2	96.44	10	0.29	753	29.09
100	3	97.15	9		782	
100	4	90.62	10		810	
140	1	96.31	10	9.25	634	621
140	2	90.21	9	0.25	629	31.43
140	3	90.92	9		535	
140	4	92.84	9		686	
196	1	95.46	9	9.5	561	579
196	2	95.59	9	0.29	593	15.38
196	3	97.05	10		547	
196	4	90.89	10		615	
274	1	96.03	10	9.5	586	470
274	2	92.84	10	0.50	399	74.52
274	3	92.26	8		294	
274	4	92.66	10		601	
384	1	91.16	10	8.75	362	254
384	2	92.54	8	0.48	147	47.04
384	3	90.67	8		295	
384	4	97.14	9		212	
538	1	93.18	6	5	22	52.75
538	2	93.17	7	1.35	85	20.79
538	3	90.81	1		12	
538	4	97.16	6		92	
753	1	90.49	0	4.75	1	15.25
753	2	92.9	7	1.65	9	7.98
753	3	90.65	5		13	
753	4	93.27	7		38	
1054	1	96.89	0	1	4	1.5
1054	2	91.26	0	1.00	1	0.87
1054	3	91.15	4		1	
1054	4	92.72	0		0	

Definitive invertebrate assays

Aged SSL soil

Compound: Ba [$Ba(NO_3)_2$]

Start Date: 21-Nov-00

Invertebrate: *E. crypticus*

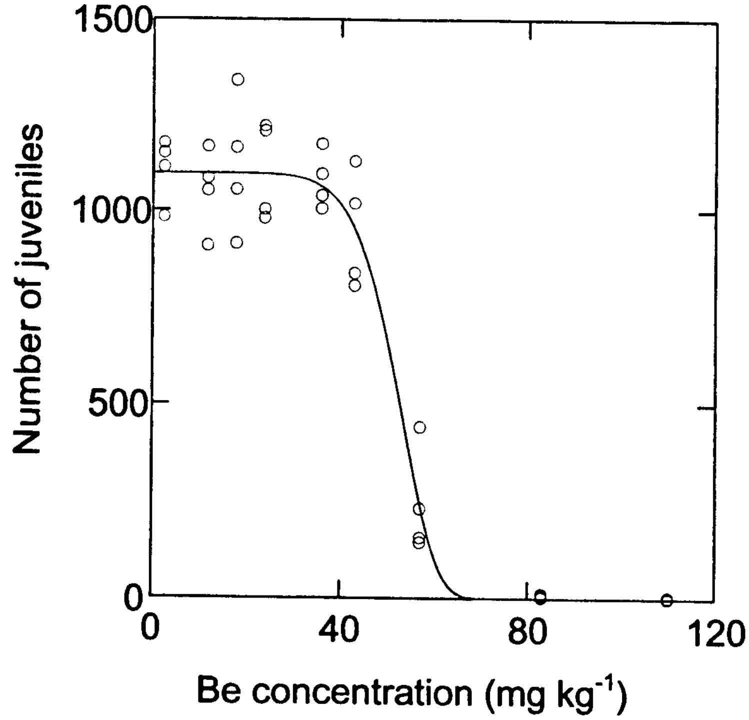
Nominal Ba (mg/kg)	Rep	Initial container mass (g)	12/5 Adults	Mean S.E.	12/19 Juveniles	Mean S.E.
0	1	94.22	10	10	1166	951.5
0	2	94.92	10	0	752	87.44
0	3	94.11	10		998	
0	4	93.01	10		890	
451	1	94.35	10	10	958	913.5
451	2	92.17	10	0	912	32.9
451	3	96.89	10		963	
451	4	98.42	10		821	
597	1	92.31	10	10	1020	843
597	2	91.79	10	0	925	78.05
597	3	98.51	10		741	
597	4	97.43	10		686	
686	1	98.12	10	10	896	790.8
686	2	92.08	10	0	681	60.23
686	3	97.31	10		894	
686	4	92.31	10		692	
789	1	94.67	10	9.5	795	560.5
789	2	92.71	9	0.29	512	80.42
789	3	97.39	9		506	
789	4	92.45	10		429	
907	1	91.77	10	9.75	470	392.8
907	2	92.59	10	0.25	301	36.98
907	3	98.26	10		369	
907	4	92.25	9		431	
1043	1	96.66	10	10	238	244
1043	2	91.75	10	0	261	9.772
1043	3	94.29	10		258	
1043	4	92.73	10		219	
1200	1	91.74	9	9.25	88	159.3
1200	2	92.17	9	0.25	182	26.63
1200	3	92.51	10		213	
1200	4	92.57	9		154	
1314	1	96.51	10	9.5	157	86.25
1314	2	102.37	9	0.29	35	27.57
1314	3	95.65	10		51	
1314	4	96.95	9		102	
1551	1	101.29	9	8	27	17
1551	2	95.5	7	0.91	21	4.397

1551	3	96.33	6		7	
1551	4	97.94	10		13	
1830	1	97.44	5	6.5	11	8.75
1830	2	101.56	5	1.19	17	3.276
1830	3	97.11	6		3	
1830	4	96.44	10		4	

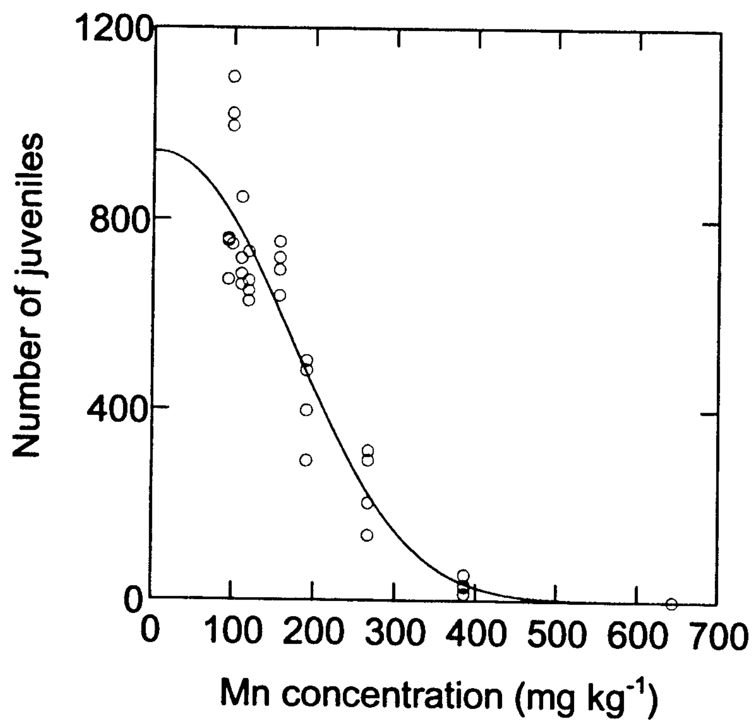
APPENDIX C

CONCENTRATION-RESPONSE CURVES FOR REPRODUCTION ENDPOINT DETERMINED FROM ERT USING JUVENILE PRODUCTION DATA IN AGED AMENDED SSL SOIL

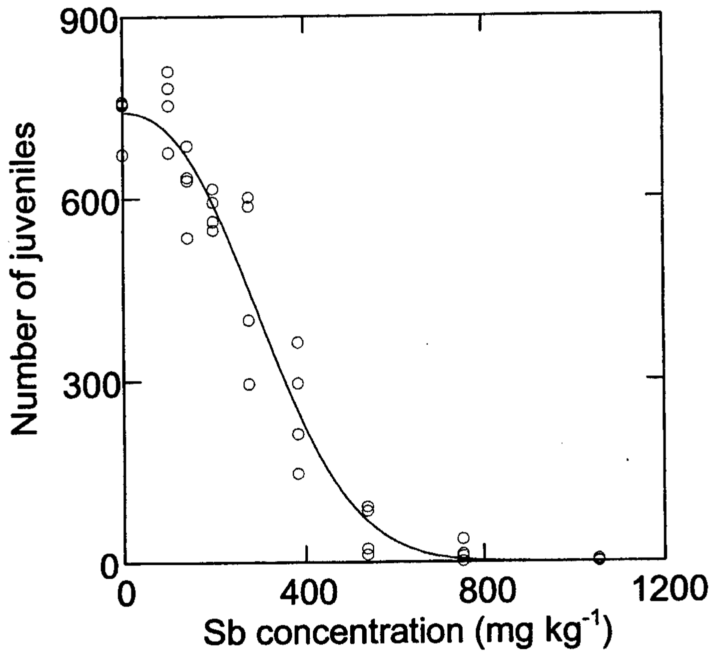
C 1. Effect of beryllium on *E. crypticus* juvenile production.



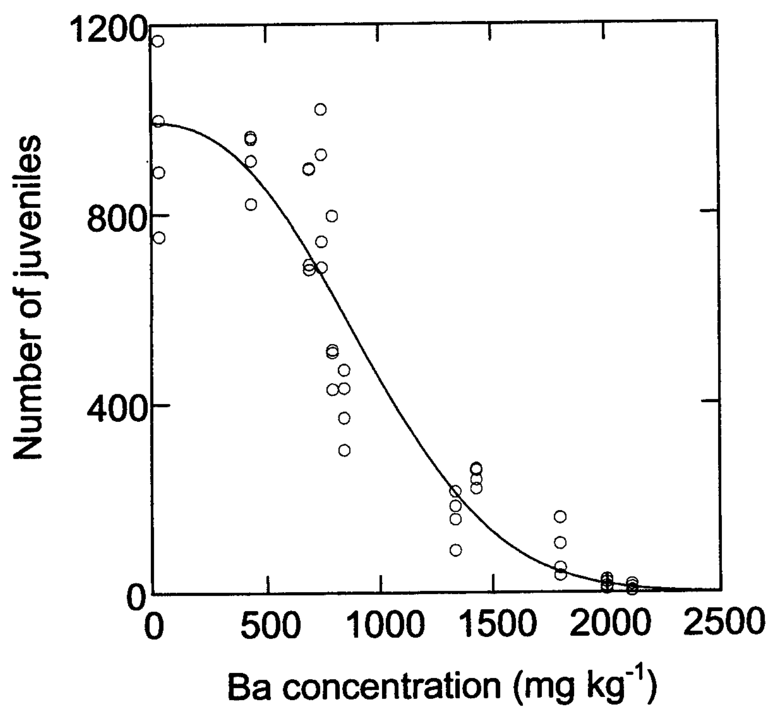
C 2. Effect of manganese on *E. crypticus* juvenile production.



C 3. Effect of antimony on *E. crypticus* juvenile production.



C 4. Effect of barium on *E. crypticus* juvenile production.



Blank

APPENDIX D

STATISTICAL ANALYSES OF THE DEFINITIVE TESTS DATA

SYSTAT VERSION 7.0.1
COPYRIGHT (C) 1997, SPSS INC.

Welcome to SYSTAT!

EC_{50} determination for Be effect on *E. crypticus* using Gompertz model.

```
MODEL:
nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBE50 /
resid
estimate/ start = 1100, 40, 2 iter=200
```

36 cases have been saved into a SYSTAT file

Iteration No.	Loss	G	X	B
0	.233815D+07	.110000D+04	.400000D+02	.200000D+01
1	.125614D+07	.983256D+03	.607419D+02	.373687D+01
2	.707919D+06	.113273D+04	.476012D+02	.407977D+01
3	.441396D+06	.107003D+04	.531308D+02	.664166D+01
4	.362585D+06	.109485D+04	.516959D+02	.841714D+01
5	.361045D+06	.109652D+04	.519856D+02	.841232D+01
6	.361045D+06	.109652D+04	.519858D+02	.841631D+01
7	.361045D+06	.109652D+04	.519857D+02	.841614D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	2.76125E+07	3	9204157.081
Residual	361044.758	33	10940.750
Total	2.79735E+07	36	
Mean corrected	8292462.556	35	

Raw R-square (1-Residual/Total) = 0.987
Mean corrected R-square (1-Residual/Corrected) = 0.956
R(observed vs predicted) square = 0.956

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	1096.517	25.298	43.344	1045.048	1147.987
X	51.986	1.064	48.855	49.821	54.151
B	8.416	1.539	5.467	5.284	11.548

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	1112.000	1096.517	15.483
2	1149.000	1096.517	52.483
3	1175.000	1096.517	78.483
4	981.000	1096.517	-115.517
5	1166.000	1096.514	69.486
6	906.000	1096.514	-190.514
7	1083.000	1096.514	-13.514
8	1051.000	1096.514	-45.514
9	1164.000	1096.416	67.584

10	1339.000	1096.416	242.584
11	1054.000	1096.416	-42.416
12	912.000	1096.416	-184.416
13	1208.000	1095.381	112.619
14	979.000	1095.381	-116.381
15	1002.000	1095.381	-93.381
16	1221.000	1095.381	125.619
17	1095.000	1062.557	32.443
18	1174.000	1062.557	111.443
19	1038.000	1062.557	-24.557
20	1004.000	1062.557	-58.557
21	1018.000	952.935	65.065
22	1129.000	952.935	176.065
23	803.000	952.935	-149.935
24	836.000	952.935	-116.935
25	233.000	243.565	-10.565
26	146.000	243.565	-97.565
27	441.000	243.565	197.435
28	158.000	243.565	-85.565
29	12.000	0.000	12.000
30	9.000	0.000	9.000
31	12.000	0.000	12.000
32	3.000	0.000	3.000
33	1.000	0.000	1.000
34	0.0	0.000	0.000
35	4.000	0.000	4.000
36	0.0	0.000	0.000

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.449	1.000	
B	-0.455	0.600	1.000

Residuals have been saved.
Residuals have been saved.

EC_{20} determination for Be effect on *E. crypticus* using Gompertz model.

MODEL:

```
nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBE20n /
resid
estimate/ start = 1100, 40, 2 iter=200
```

Iteration

No.	Loss	G	X	B
0	.220852D+07	.110000D+04	.400000D+02	.200000D+01
1	.100630D+07	.115691D+04	.311839D+02	.261898D+01
2	.968446D+06	.104284D+04	.472301D+02	.478036D+01
3	.591771D+06	.112268D+04	.382929D+02	.509404D+01
4	.411737D+06	.108543D+04	.453549D+02	.711621D+01
5	.361131D+06	.109566D+04	.455155D+02	.842984D+01
6	.361045D+06	.109652D+04	.454342D+02	.841464D+01
7	.361045D+06	.109652D+04	.454357D+02	.841621D+01

8 .361045D+06 .109652D+04 .454356D+02 .841614D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	2.76125E+07	3	9204157.081
Residual	361044.758	33	10940.750
Total	2.79735E+07	36	
Mean corrected	8292462.556	35	

Raw R-square (1-Residual/Total) = 0.987
Mean corrected R-square (1-Residual/Corrected) = 0.956
R(observed vs predicted) square = 0.956

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	1096.517	25.298	43.344	1045.048	1147.987
X	45.436	1.835	24.765	41.703	49.168
B	8.416	1.539	5.467	5.284	11.548

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	1112.000	1096.517	15.483
2	1149.000	1096.517	52.483
3	1175.000	1096.517	78.483
4	981.000	1096.517	-115.517
5	1166.000	1096.514	69.486
6	906.000	1096.514	-190.514
7	1083.000	1096.514	-13.514
8	1051.000	1096.514	-45.514
9	1164.000	1096.416	67.584
10	1339.000	1096.416	242.584
11	1054.000	1096.416	-42.416
12	912.000	1096.416	-184.416
13	1208.000	1095.381	112.619
14	979.000	1095.381	-116.381
15	1002.000	1095.381	-93.381
16	1221.000	1095.381	125.619
17	1095.000	1062.557	32.443
18	1174.000	1062.557	111.443
19	1038.000	1062.557	-24.557
20	1004.000	1062.557	-58.557
21	1018.000	952.935	65.065
22	1129.000	952.935	176.065
23	803.000	952.935	-149.935
24	836.000	952.935	-116.935
25	233.000	243.565	-10.565
26	146.000	243.565	-97.565
27	441.000	243.565	197.435
28	158.000	243.565	-85.565
29	12.000	0.000	12.000
30	9.000	0.000	9.000
31	12.000	0.000	12.000
32	3.000	0.000	3.000
33	1.000	0.000	1.000
34	0.0	0.000	0.000
35	4.000	0.000	4.000
36	0.0	0.000	0.000

```
Asymptotic Correlation Matrix of Parameters
                        G            X            B
 G                    1.000
 X                   -0.505        1.000
 B                   -0.455        0.914        1.000

Residuals have been saved.

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\ert\models\reBE20n
.SYD,
contains variables:
 JUVENILES    CONCENTR     ESTIMATE     RESIDUAL

RESIDUALS MODEL:

graph
use
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBE20n
plot residual*concentr
plot residual*estimate


Stem and Leaf Plot of variable:      RESIDUAL, N = 36
         Minimum:      -190.514
         Lower hinge:      -72.061
         Median:         2.000
         Upper hinge:       66.324
         Maximum:       242.584

              -1    98
              -1    4111
              -0 H 9985
              -0    4421100
               0 M 00001113
               0 H 56667
               1    112
               1    79
               2    4


                       RESIDUAL
  N of cases                 36
  Minimum              -190.514
  Maximum               242.584
  Mean                    1.179
  Std. Error             16.926
  Standard Dev          101.559
  Variance            10314.134
```

ANOVA for juveniles

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
2.5, 12, 18, 24, 36, 43, 57, 83, 110

Dep Var: JUVENILES N: 36 Multiple R: 0.979 Squared multiple R: 0.958

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	7943123.556	8	992890.444	76.739	0.000
Error	349339.000	27	12938.481		

Durbin-Watson D Statistic 2.569
First Order Autocorrelation -0.285

COL/ ROW	CONCENTR
1	2.5
2	12
3	18
4	24
5	36
6	43
7	57
8	83
9	110

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 12938.481 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-52.750	0.0			
3	13.000	65.750	0.0		
4	-1.750	51.000	-14.750	0.0	
5	-26.500	26.250	-39.500	-24.750	0.0
6	-157.750	-105.000	-170.750	-156.000	-131.250
7	-859.750	-807.000	-872.750	-858.000	-833.250
8	-1095.250	-1042.500	-1108.250	-1093.500	-1068.750
9	-1103.000	-1050.250	-1116.000	-1101.250	-1076.500

	6	7	8	9
6	0.0			
7	-702.000	0.0		
8	-937.500	-235.500	0.0	
9	-945.250	-243.250	-7.750	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.517	1.000			
3	0.873	0.421	1.000		
4	0.983	0.531	0.856	1.000	
5	0.744	0.747	0.627	0.761	1.000
6	0.060	0.203	0.043	0.063	0.114
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.000	1.000		
8	0.000	0.007	1.000	
9	0.000	0.005	0.924	1.000

ANOVA for adults.

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
2.5, 12, 18, 24, 36, 43, 57, 83, 110

Dep Var: ADULTS   N: 36   Multiple R: 0.889   Squared multiple R: 0.791

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	105.000	8	13.125	12.770	0.000
Error	27.750	27	1.028		

Durbin-Watson D Statistic 2.266
First Order Autocorrelation -0.173

COL/ ROW	CONCENTR
1	2.5
2	12
3	18
4	24
5	36
6	43
7	57
8	83
9	110

Using least squares means.
Post Hoc test of ADULTS

Using model MSE of 1.028 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	0.000	0.0			
3	-0.250	-0.250	0.0		
4	0.000	0.0	0.250	0.0	
5	0.000	0.0	0.250	0.0	0.0
6	0.000	0.0	0.250	0.0	0.0
7	0.000	0.0	0.250	0.0	0.0
8	-1.000	-1.000	-0.750	-1.000	-1.000
9	-5.500	-5.500	-5.250	-5.500	-5.500

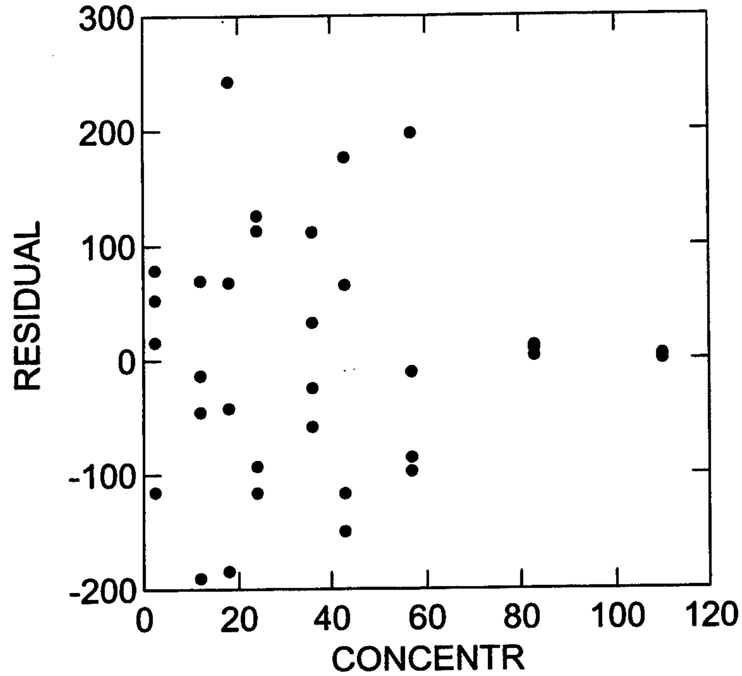
	6	7	8	9
6	0.0			
7	0.0	0.0		
8	-1.000	-1.000	0.0	
9	-5.500	-5.500	-4.500	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

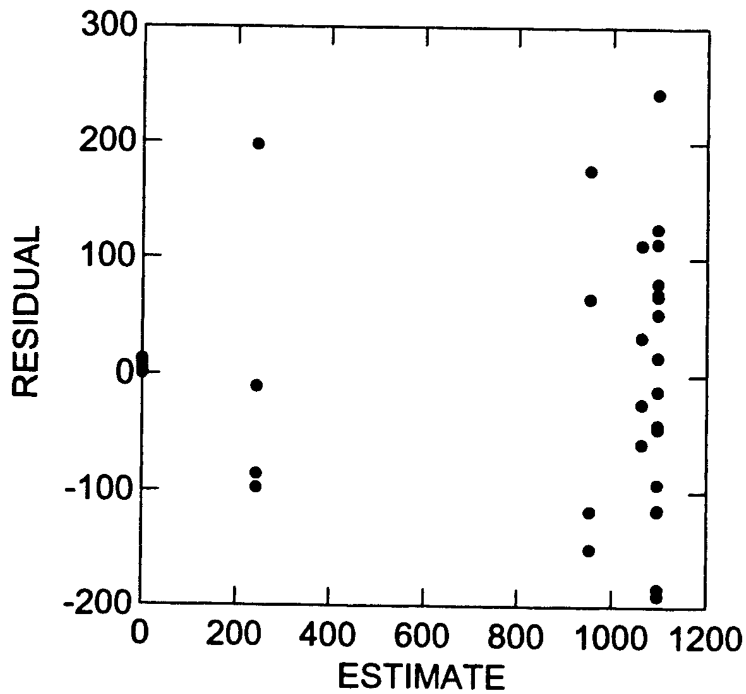
	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	0.730	0.730	1.000		
4	1.000	1.000	0.730	1.000	
5	1.000	1.000	0.730	1.000	1.000
6	1.000	1.000	0.730	1.000	1.000
7	1.000	1.000	0.730	1.000	1.000
8	0.174	0.174	0.305	0.174	0.174
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	1.000	1.000		
8	0.174	0.174	1.000	
9	0.000	0.000	0.000	1.000

Residuals for beryllium.



Residuals for beryllium.



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EC_{50} determination for Mn effect on *E. crypticus* using Gompertz model.

```
SYSTAT Rectangular file
C:\DOCUME~1\RGKUPERM\MYDOCU~1\SYSTAT\ROMAN3\\NONLINRE\\NAVY\ERT\DATA\MNSDANEW.
SYD,
contains variables:

JUVENILES    CONCENTR

 Iteration

 No.      Loss        G            X            B

   0 .559258D+07 .700000D+03 .100000D+03 .200000D+01
   1 .305280D+07 .103502D+04 .809053D+02 .901236D+00
   2 .301590D+07 .714606D+03 .134369D+03 .122146D+01
   3 .112391D+07 .619380D+03 .302422D+03 .275802D+01
   4 .102054D+07 .766991D+03 .218266D+03 .147993D+01
   5 .638838D+06 .930454D+03 .171675D+03 .154953D+01
   6 .381747D+06 .896718D+03 .205888D+03 .222469D+01
   7 .366625D+06 .946253D+03 .190418D+03 .219337D+01
   8 .366075D+06 .941904D+03 .192547D+03 .223653D+01
   9 .366075D+06 .942280D+03 .192479D+03 .223580D+01
  10 .366075D+06 .942276D+03 .192480D+03 .223583D+01
  11 .366075D+06 .942276D+03 .192480D+03 .223583D+01

Dependent variable is JUVENILES

    Source   Sum-of-Squares    df  Mean-Square
 Regression      1.24710E+07    3  4156989.043
   Residual       366074.870   33    11093.178

      Total      1.28370E+07   36
Mean corrected   3902921.000   35

       Raw  R-square (1-Residual/Total)           =        0.971
Mean corrected R-square (1-Residual/Corrected) =        0.906
          R(observed vs predicted) square         =        0.906

                                                          Wald Confidence Interval
Parameter          Estimate        A.S.E.     Param/ASE        Lower < 95%> Upper

 G                  942.276       117.981         7.987      702.241      1182.310
 X                  192.480        22.479         8.563      146.746       238.214
 B                    2.236         0.643         3.480        0.929         3.543

            JUVENILES    JUVENILES
  Case      Observed     Predicted     Residual
     1       759.000      819.497       -60.497
     2       672.000      819.497      -147.497
     3       754.000      819.497       -65.497
     4       756.000      819.497       -63.497
     5      1097.000      805.562       291.438
```

6	1021.000	805.562	215.438
7	995.000	805.562	189.438
8	747.000	805.562	-58.562
9	684.000	772.707	-88.707
10	662.000	772.707	-110.707
11	717.000	772.707	-55.707
12	846.000	772.707	73.293
13	649.000	743.792	-94.792
14	627.000	743.792	-116.792
15	670.000	743.792	-73.792
16	731.000	743.792	-12.792
17	638.000	607.146	30.854
18	693.000	607.146	85.854
19	752.000	607.146	144.854
20	718.000	607.146	110.854
21	481.000	476.758	4.242
22	397.000	476.758	-79.758
23	501.000	476.758	24.242
24	291.000	476.758	-185.758
25	136.000	223.079	-87.079
26	313.000	223.079	89.921
27	293.000	223.079	69.921
28	204.000	223.079	-19.079
29	33.000	35.290	-2.290
30	14.000	35.290	-21.290
31	29.000	35.290	-6.290
32	54.000	35.290	18.710
33	0.0	0.031	-0.031
34	0.0	0.031	-0.031
35	0.0	0.031	-0.031
36	0.0	0.031	-0.031

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.943	1.000	
B	-0.927	0.856	1.000

Residuals have been saved.

SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\ert\models\resMn50
.SYD,

contains variables:

JUVENILES CONCENTR ESTIMATE RESIDUAL

```
Stem and Leaf Plot of variable:     RESIDUAL, N = 36
        Minimum:       -185.758
        Lower hinge:       -69.644
        Median:          -4.290
        Upper hinge:        50.387
        Maximum:        291.438
```

```
                -1   8
                -1   411
                -0 H 9887766655
                -0 M 211000000
                 0 M 0123
                 0 H 6788
                 1   14
                 1   8
                 2   1
        * * * Outside Values * * *
                 2   9
```

	RESIDUAL
N of cases	36
Minimum	-185.758
Maximum	291.438
Mean	-0.040
Std. Error	17.045
Standard Dev	102.271
Variance	10459.280

EC_{20} determination for Mn effect on *E. crypticus* using Gompertz model.

Iteration

No.	Loss	G	X	B
0	.711361D+07	.700000D+03	.490000D+02	.200000D+01
1	.618281D+07	.902002D+03	.335404D+02	.143387D+01
2	.381836D+07	.141525D+04	.662866D+01	.642051D+00
3	.380141D+07	.135601D+04	.758002D+01	.661399D+00
4	.377875D+07	.130350D+04	.858887D+01	.680595D+00
5	.375140D+07	.125682D+04	.965010D+01	.699617D+00
6	.372022D+07	.121519D+04	.107584D+02	.718451D+00
7	.368593D+07	.117794D+04	.119086D+02	.737083D+00
8	.364915D+07	.114451D+04	.130953D+02	.755505D+00
9	.361038D+07	.111444D+04	.143135D+02	.773709D+00
10	.357006D+07	.108729D+04	.155585D+02	.791692D+00
11	.354357D+07	.964517D+03	.218936D+02	.880484D+00
12	.341518D+07	.885479D+03	.287133D+02	.966831D+00
13	.327682D+07	.682300D+03	.560622D+02	.129431D+01
14	.106814D+07	.616562D+03	.185842D+03	.287100D+01
15	.768567D+06	.760705D+03	.120754D+03	.188895D+01
16	.432874D+06	.986246D+03	.951375D+02	.197507D+01
17	.370064D+06	.926436D+03	.115542D+03	.220970D+01
18	.366075D+06	.942343D+03	.115927D+03	.223563D+01
19	.366075D+06	.942275D+03	.115938D+03	.223583D+01
20	.366075D+06	.942276D+03	.115938D+03	.223583D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	1.24710E+07	3	4156989.043
Residual	366074.870	33	11093.178
Total	1.28370E+07	36	
Mean corrected	3902921.000	35	

Raw R-square (1-Residual/Total) = 0.971
Mean corrected R-square (1-Residual/Corrected) = 0.906
R(observed vs predicted) square = 0.906

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	942.276	117.981	7.987	702.241	1182.310
X	115.938	29.326	3.953	56.273	175.602
B	2.236	0.643	3.480	0.929	3.543

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	759.000	819.497	-60.497
2	672.000	819.497	-147.497
3	754.000	819.497	-65.497
4	756.000	819.497	-63.497
5	1097.000	805.562	291.438
6	1021.000	805.562	215.438
7	995.000	805.562	189.438
8	747.000	805.562	-58.562
9	684.000	772.707	-88.707
10	662.000	772.707	-110.707
11	717.000	772.707	-55.707
12	846.000	772.707	73.293
13	649.000	743.792	-94.792
14	627.000	743.792	-116.792
15	670.000	743.792	-73.792
16	731.000	743.792	-12.792
17	638.000	607.146	30.854
18	693.000	607.146	85.854
19	752.000	607.146	144.854
20	718.000	607.146	110.854
21	481.000	476.758	4.242
22	397.000	476.758	-79.758
23	501.000	476.758	24.242
24	291.000	476.758	-185.758
25	136.000	223.079	-87.079
26	313.000	223.079	89.921
27	293.000	223.079	69.921
28	204.000	223.079	-19.079
29	33.000	35.290	-2.290
30	14.000	35.290	-21.290
31	29.000	35.290	-6.290
32	54.000	35.290	18.710
33	0.0	0.031	-0.031
34	0.0	0.031	-0.031
35	0.0	0.031	-0.031
36	0.0	0.031	-0.031

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.969	1.000	
B	-0.927	0.971	1.000

Residuals have been saved.

ANOVA for Juveniles

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
94, 99, 110, 119, 157, 191, 267, 386, 644

Dep Var: JUVENILES N: 36 Multiple R: 0.980 Squared multiple R: 0.960

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	3746881.000	8	468360.125	81.042	0.000
Error	156040.000	27	5779.259		

Durbin-Watson D Statistic 1.928
First Order Autocorrelation 0.034

COL/ ROW	CONCENTR
1	94
2	99
3	110
4	119
5	157
6	191
7	267
8	386
9	644

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 5779.259 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	229.750	0.0			
3	-8.000	-237.750	0.0		
4	-66.000	-295.750	-58.000	0.0	
5	-35.000	-264.750	-27.000	31.000	0.0
6	-317.750	-547.500	-309.750	-251.750	-282.750
7	-498.750	-728.500	-490.750	-432.750	-463.750
8	-702.750	-932.500	-694.750	-636.750	-667.750
9	-735.250	-965.000	-727.250	-669.250	-700.250

	6	7	8	9
6	0.0			
7	-181.000	0.0		
8	-385.000	-204.000	0.0	
9	-417.500	-236.500	-32.500	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.000	1.000			
3	0.883	0.000	1.000		
4	0.230	0.000	0.290	1.000	
5	0.520	0.000	0.620	0.569	1.000
6	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.002	1.000		
8	0.000	0.001	1.000	
9	0.000	0.000	0.550	1.000

ANOVA for Adults

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
94, 99, 110, 119, 157, 191, 267, 386, 644

Dep Var: ADULTS N: 36 Multiple R: 0.965 Squared multiple R: 0.931

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	349.889	8	43.736	45.418	0.000
Error	26.000	27	0.963		

Durbin-Watson D Statistic 2.313
First Order Autocorrelation -0.168

COL/ ROW	CONCENTR
1	94
2	99
3	110
4	119
5	157
6	191
7	267
8	386
9	644

Using least squares means.
Post Hoc test of ADULTS

Using model MSE of 0.963 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-0.250	0.0			
3	-0.250	0.000	0.0		
4	0.0	0.250	0.250	0.0	
5	0.250	0.500	0.500	0.250	0.0
6	-0.250	0.000	0.000	-0.250	-0.500
7	-0.500	-0.250	-0.250	-0.500	-0.750
8	-4.750	-4.500	-4.500	-4.750	-5.000
9	-9.500	-9.250	-9.250	-9.500	-9.750

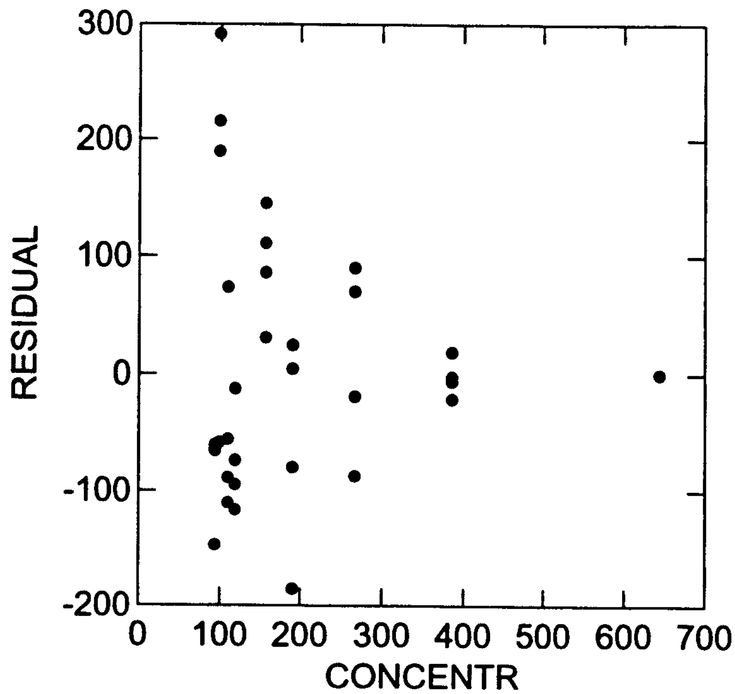
	6	7	8	9
6	0.0			
7	-0.250	0.0		
8	-4.500	-4.250	0.0	
9	-9.250	-9.000	-4.750	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

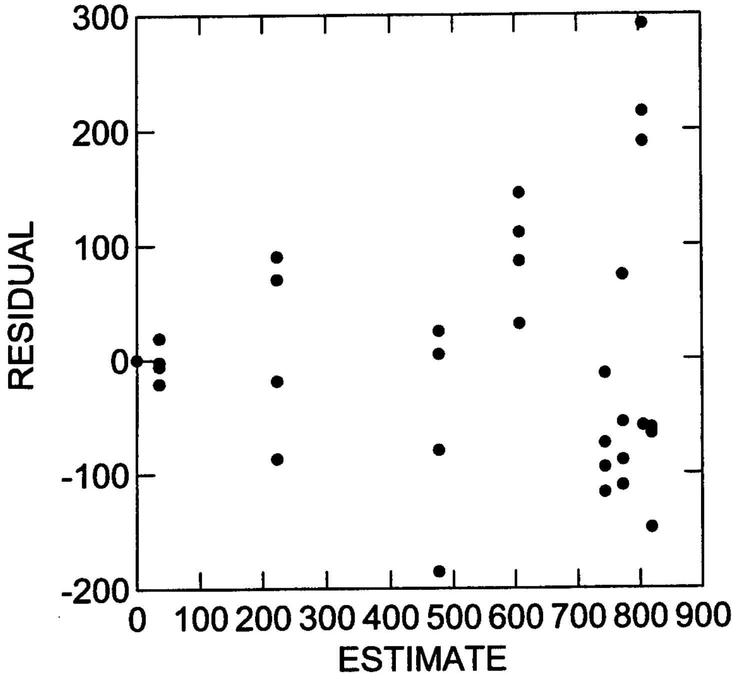
	1	2	3	4	5
1	1.000				
2	0.721	1.000			
3	0.721	1.000	1.000		
4	1.000	0.721	0.721	1.000	
5	0.721	0.477	0.477	0.721	1.000
6	0.721	1.000	1.000	0.721	0.477
7	0.477	0.721	0.721	0.477	0.289
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.721	1.000		
8	0.000	0.000	1.000	
9	0.000	0.000	0.000	1.000

Residuals for manganese.



Residuals for manganese.



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EC_{50} determination for Sb effect on *E. crypticus* using Gompertz model.

```
Model:
nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\resSb50j
/ resid
estimate/ start = 700, 316, 2 iter=200
```

Iteration

No.	Loss	G	X	B
0	.202239D+06	.700000D+03	.316000D+03	.200000D+01
1	.151117D+06	.742440D+03	.315357D+03	.229931D+01
2	.151086D+06	.741629D+03	.316066D+03	.232190D+01
3	.151085D+06	.741545D+03	.316123D+03	.232313D+01
4	.151085D+06	.741539D+03	.316127D+03	.232320D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	8457997.541	3	2819332.514
Residual	151085.459	33	4578.347
Total	8609083.000	36	
Mean corrected	3215076.750	35	

Raw R-square (1-Residual/Total)	=	0.982
Mean corrected R-square (1-Residual/Corrected)	=	0.953
R(observed vs predicted) square	=	0.953

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	741.539	27.090	27.373	686.425	796.654
X	316.127	15.332	20.619	284.934	347.319
B	2.323	0.314	7.402	1.685	2.962

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	759.000	741.539	17.461
2	672.000	741.539	-69.539
3	754.000	741.539	12.461
4	756.000	741.539	14.461
5	675.000	706.918	-31.918
6	753.000	706.918	46.082
7	782.000	706.918	75.082
8	810.000	706.918	103.082
9	634.000	667.974	-33.974
10	629.000	667.974	-38.974
11	535.000	667.974	-132.974
12	686.000	667.974	18.026
13	561.000	590.177	-29.177
14	593.000	590.177	2.823
15	547.000	590.177	-43.177

16	615.000	590.177	24.823
17	586.000	451.028	134.972
18	399.000	451.028	-52.028
19	294.000	451.028	-157.028
20	601.000	451.028	149.972
21	362.000	249.543	112.457
22	147.000	249.543	-102.543
23	295.000	249.543	45.457
24	212.000	249.543	-37.543
25	22.000	68.357	-46.357
26	85.000	68.357	16.643
27	12.000	68.357	-56.357
28	92.000	68.357	23.643
29	1.000	4.065	-3.065
30	9.000	4.065	4.935
31	13.000	4.065	8.935
32	38.000	4.065	33.935
33	4.000	0.009	3.991
34	1.000	0.009	0.991
35	1.000	0.009	0.991
36	0.0	0.009	-0.009

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.691	1.000	
B	-0.652	0.521	1.000

Residuals have been saved.

EC_{20} determination for Sb effect on *E. crypticus* using Gompertz model.

Model:

```
nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\resSb20j
/ resid
estimate/ start = 700, 190, 2 iter=200
```

Iteration

No.	Loss	G	X	B
0	.198039D+06	.700000D+03	.190000D+03	.200000D+01
1	.151252D+06	.744755D+03	.190552D+03	.226355D+01
2	.151086D+06	.741733D+03	.193877D+03	.231952D+01
3	.151085D+06	.741553D+03	.194068D+03	.232305D+01
4	.151085D+06	.741540D+03	.194080D+03	.232320D+01
5	.151085D+06	.741539D+03	.194080D+03	.232321D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	8457997.541	3	2819332.514
Residual	151085.459	33	4578.347
Total	8609083.000	36	
Mean corrected	3215076.750	35	

Raw R-square (1-Residual/Total) = 0.982
Mean corrected R-square (1-Residual/Corrected) = 0.953

R(observed vs predicted) square = 0.953

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	741.539	27.090	27.373	686.424	796.654
X	194.080	19.434	9.987	154.541	233.619
B	2.323	0.314	7.402	1.685	2.962

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	759.000	741.539	17.461
2	672.000	741.539	-69.539
3	754.000	741.539	12.461
4	756.000	741.539	14.461
5	675.000	706.918	-31.918
6	753.000	706.918	46.082
7	782.000	706.918	75.082
8	810.000	706.918	103.082
9	634.000	667.974	-33.974
10	629.000	667.974	-38.974
11	535.000	667.974	-132.974
12	686.000	667.974	18.026
13	561.000	590.177	-29.177
14	593.000	590.177	2.823
15	547.000	590.177	-43.177
16	615.000	590.177	24.823
17	586.000	451.028	134.972
18	399.000	451.028	-52.028
19	294.000	451.028	-157.028
20	601.000	451.028	149.972
21	362.000	249.543	112.457
22	147.000	249.543	-102.543
23	295.000	249.543	45.457
24	212.000	249.543	-37.543
25	22.000	68.357	-46.357
26	85.000	68.357	16.643
27	12.000	68.357	-56.357
28	92.000	68.357	23.643
29	1.000	4.065	-3.065
30	9.000	4.065	4.935
31	13.000	4.065	8.935
32	38.000	4.065	33.935
33	4.000	0.009	3.991
34	1.000	0.009	0.991
35	1.000	0.009	0.991
36	0.0	0.009	-0.009

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.764	1.000	
B	-0.652	0.911	1.000

Residuals have been saved.

RESIDUALS MODEL:

```
graph
use
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\resSb20j
plot residual*concentr
plot residual*estimate
```

```
SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\ert\models\resSb20
j.SYD,
contains variables:

 JUVENILES     CONCENTR      ESTIMATE      RESIDUAL

Stem and Leaf Plot of variable:      RESIDUAL, N = 36

         Minimum:      -157.028
         Lower hinge:      -38.258
         Median:         3.407
         Upper hinge:       24.233
         Maximum:       149.972



              -1    53
      * * * Outside Values * * *
              -1    0
              -0
              -0    6
              -0    5544
              -0 H  33332
              -0    00
               0 M  00000011111
               0 H  223
               0    44
               0    7
               0
               1    01
      * * * Outside Values * * *
               1    34


                          RESIDUAL
  N of cases                   36
  Minimum                -157.028
  Maximum                 149.972
  Mean                      0.460
  Std. Error               10.950
  Standard Dev             65.700
  Variance               4316.510

Graph Model:

graph
begin
plot juveniles*concentr / title='', xlab='Sb concentration (mg kg-1)',
ylab='Number of juveniles',
     xmax=1200, xmin=0, ymax=900, ymin=0

fplot y=741.539*exp((log(.5))*(concentr/316.127)^2.323); xmin=0, xmax=1200,
xlab='' ymin=0, ylab='',
     ymax=900
end
```

ANOVA for Juveniles

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
0, 100, 140, 196, 274, 384, 538, 753, 1054

Dep Var: JUVENILES   N: 36   Multiple R: 0.980   Squared multiple R: 0.960

Estimates of effects $B = (X'X)^{-1} X'Y$

		JUVENILES
CONSTANT		387.083
CONCENTR	0	348.167
CONCENTR	100	367.917
CONCENTR	140	233.917
CONCENTR	196	191.917
CONCENTR	274	82.917
CONCENTR	384	-133.083
CONCENTR	538	-334.333
CONCENTR	753	-371.833

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	3085725.500	8	385715.688	80.512	0.000
Error	129351.250	27	4790.787		

Durbin-Watson D Statistic   2.491
First Order Autocorrelation   -0.248

COL/ ROW	CONCENTR
1	0
2	100
3	140
4	196
5	274
6	384
7	538
8	753
9	1054

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 4790.787 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	19.750	0.0			
3	-114.250	-134.000	0.0		
4	-156.250	-176.000	-42.000	0.0	

5	-265.250	-285.000	-151.000	-109.000	0.0
6	-481.250	-501.000	-367.000	-325.000	-216.000
7	-682.500	-702.250	-568.250	-526.250	-417.250
8	-720.000	-739.750	-605.750	-563.750	-454.750
9	-733.750	-753.500	-619.500	-577.500	-468.500
	6	7	8	9	
6	0.0				
7	-201.250	0.0			
8	-238.750	-37.500	0.0		
9	-252.500	-51.250	-13.750	0.0	

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.690	1.000			
3	0.027	0.011	1.000		
4	0.004	0.001	0.398	1.000	
5	0.000	0.000	0.005	0.034	1.000
6	0.000	0.000	0.000	0.000	0.000
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000
	6	7	8	9	
6	1.000				
7	0.000	1.000			
8	0.000	0.450	1.000		
9	0.000	0.304	0.781	1.000	

ANOVA for Adults

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (9 levels)
0, 100, 140, 196, 274, 384, 538, 753, 1054

Dep Var: ADULTS   N: 36   Multiple R: 0.896   Squared multiple R: 0.804

Estimates of effects $B = (X'X)^{-1} X'Y$

		ADULTS
CONSTANT		7.444
CONCENTR	0	2.306
CONCENTR	100	2.056
CONCENTR	140	1.806
CONCENTR	196	2.056
CONCENTR	274	2.056
CONCENTR	384	1.306
CONCENTR	538	-2.444
CONCENTR	753	-2.694

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P

CONCENTR	310.889	8	38.861	13.806	0.000
Error	76.000	27	2.815		

Durbin-Watson D Statistic 2.835
First Order Autocorrelation -0.428

COL/ ROW	CONCENTR
1	0
2	100
3	140
4	196
5	274
6	384
7	538
8	753
9	1054

Using least squares means.
Post Hoc test of ADULTS

Using model MSE of 2.815 with 27 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-0.250	0.0			
3	-0.500	-0.250	0.0		
4	-0.250	0.0	0.250	0.0	
5	-0.250	0.000	0.250	0.000	0.0
6	-1.000	-0.750	-0.500	-0.750	-0.750
7	-4.750	-4.500	-4.250	-4.500	-4.500
8	-5.000	-4.750	-4.500	-4.750	-4.750
9	-8.750	-8.500	-8.250	-8.500	-8.500

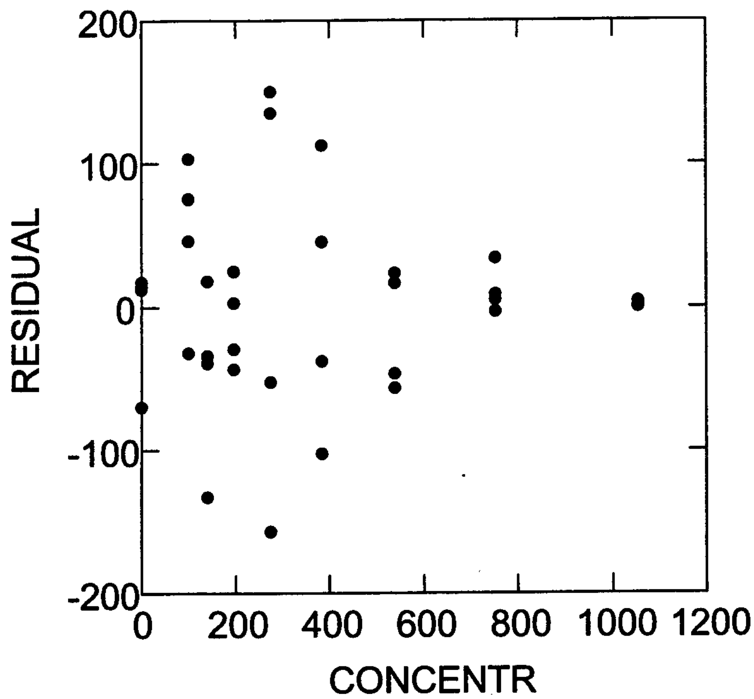
	6	7	8	9
6	0.0			
7	-3.750	0.0		
8	-4.000	-0.250	0.0	
9	-7.750	-4.000	-3.750	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

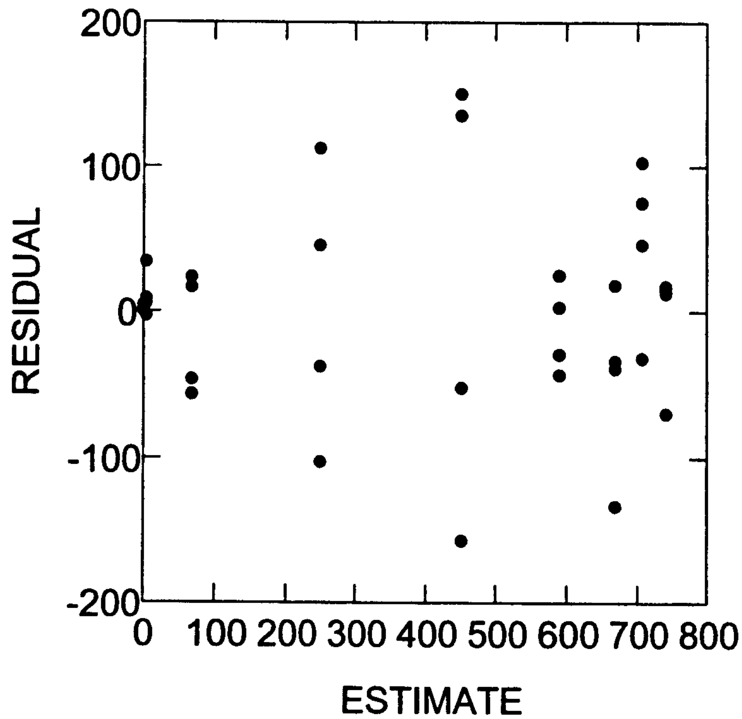
	1	2	3	4	5
1	1.000				
2	0.835	1.000			
3	0.677	0.835	1.000		
4	0.835	1.000	0.835	1.000	
5	0.835	1.000	0.835	1.000	1.000
6	0.407	0.533	0.677	0.533	0.533
7	0.000	0.001	0.001	0.001	0.001
8	0.000	0.000	0.001	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000

	6	7	8	9
6	1.000			
7	0.004	1.000		
8	0.002	0.835	1.000	
9	0.000	0.002	0.004	1.000

Residuals for antimony



Residuals for antimony



SYSTAT VERSION 7.0.1
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Welcome to SYSTAT!

EC_{50} determination for Ba effect on *E. crypticus* using Gompertz model.

MODEL:

```
nonlin
print=long
model juveniles=g*exp((log(1-.5))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBA50 /
resid
estimate/ start = 1000, 800, 2 iter=200
```

44 cases have been saved into a SYSTAT file

Iteration

No.	Loss	G	X	B
0	.992081D+06	.100000D+04	.800000D+03	.200000D+01
1	.703536D+06	.972099D+03	.961583D+03	.239042D+01
2	.701177D+06	.993959D+03	.944922D+03	.233348D+01
3	.701119D+06	.991798D+03	.947307D+03	.235458D+01
4	.701117D+06	.992360D+03	.946634D+03	.235188D+01
5	.701117D+06	.992264D+03	.946729D+03	.235264D+01
6	.701117D+06	.992284D+03	.946705D+03	.235253D+01
7	.701117D+06	.992281D+03	.946709D+03	.235255D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	1.41810E+07	3	4726985.947
Residual	701117.160	41	17100.419
Total	1.48821E+07	44	
Mean corrected	5909866.795	43	

Raw R-square (1-Residual/Total)	=	0.953
Mean corrected R-square (1-Residual/Corrected)	=	0.881
R(observed vs predicted) square	=	0.882

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	992.281	59.002	16.818	873.124	1111.437
X	946.709	57.875	16.358	829.828	1063.590
B	2.353	0.348	6.759	1.650	3.055

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	1166.000	992.007	173.993
2	752.000	992.007	-240.007
3	998.000	992.007	5.993
4	890.000	992.007	-102.007
5	958.000	888.873	69.127
6	912.000	888.873	23.127
7	963.000	888.873	74.127
8	821.000	888.873	-67.873
9	1020.000	669.664	350.336
10	925.000	669.664	255.336
11	741.000	669.664	71.336
12	686.000	669.664	16.336
13	896.000	714.637	181.363
14	681.000	714.637	-33.637
15	894.000	714.637	179.363
16	692.000	714.637	-22.637
17	795.000	630.064	164.936
18	512.000	630.064	-118.064
19	506.000	630.064	-124.064
20	429.000	630.064	-201.064
21	470.000	585.480	-115.480
22	301.000	585.480	-284.480
23	369.000	585.480	-216.480
24	431.000	585.480	-154.480
25	238.000	159.814	78.186
26	261.000	159.814	101.186
27	258.000	159.814	98.186
28	219.000	159.814	59.186
29	88.000	210.523	-122.523
30	182.000	210.523	-28.523
31	213.000	210.523	2.477
32	154.000	210.523	-56.523
33	157.000	43.181	113.819
34	35.000	43.181	-8.181
35	51.000	43.181	7.819
36	102.000	43.181	58.819
37	27.000	17.693	9.307
38	21.000	17.693	3.307
39	7.000	17.693	-10.693
40	13.000	17.693	-4.693
41	11.000	10.252	0.748
42	17.000	10.252	6.748
43	3.000	10.252	-7.252
44	4.000	10.252	-6.252

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.777	1.000	
B	-0.619	0.541	1.000

Residuals have been saved.

Residuals MODEL:

```
graph
use
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBA50
plot residual*concentr
plot residual*estimate
```

```
SYSTAT Rectangular file
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\\nonlinre\\navy\ert\models\reBA50.
SYD,

Stem and Leaf Plot of variable:     RESIDUAL, N = 44
          Minimum:      -284.480
          Lower hinge:       -62.198
          Median:         2.892
          Upper hinge:        72.731
          Maximum:       350.336

               -2   8
      * * * Outside Values * * *
               -2   410
               -1   5
               -1   22110
               -0 H 65
               -0   32210000
                0 M 000000012
                0 H 5567779
                1   01
                1   6778
                2
                2   5
      * * * Outside Values * * *
                3   5
```

	RESIDUAL
N of cases	44
Minimum	-284.480
Maximum	350.336
Mean	4.097
Std. Error	19.240
Standard Dev	127.624
Variance	16287.878

EC_{20} determination for Ba effect on *E. crypticus* using Gompertz model.

MODEL:

```
nonlin
print=long
model juveniles=g*exp((log(1-.2))*(concentr/x)^b)
save
c:\Docume~1\rgkuperm\MyDocu~1\systat\roman3\nonlinre\navy\ert\models\reBA20 /
resid
estimate/ start = 1000, 600, 2 iter=200
```

Iteration No.	Loss	G	X	B
0	.897903D+06	.100000D+04	.600000D+03	.200000D+01
1	.707471D+06	.995592D+03	.562714D+03	.218228D+01
2	.701491D+06	.988928D+03	.588580D+03	.234812D+01
3	.701127D+06	.992679D+03	.583758D+03	.234486D+01
4	.701117D+06	.992128D+03	.584929D+03	.235278D+01
5	.701117D+06	.992301D+03	.584724D+03	.235230D+01
6	.701117D+06	.992276D+03	.584768D+03	.235257D+01
7	.701117D+06	.992282D+03	.584760D+03	.235254D+01

Dependent variable is JUVENILES

Source	Sum-of-Squares	df	Mean-Square
Regression	1.41810E+07	3	4726985.947
Residual	701117.160	41	17100.419
Total	1.48821E+07	44	
Mean corrected	5909866.795	43	

Raw R-square (1-Residual/Total) = 0.953
Mean corrected R-square (1-Residual/Corrected) = 0.881
R(observed vs predicted) square = 0.882

Parameter	Estimate	A.S.E.	Param/ASE	Wald Confidence Interval Lower < 95%>	Upper
G	992.282	59.002	16.818	873.126	1111.438
X	584.760	68.027	8.596	447.376	722.144
B	2.353	0.348	6.759	1.650	3.055

Case	JUVENILES Observed	JUVENILES Predicted	Residual
1	1166.000	992.007	173.993
2	752.000	992.007	-240.007
3	998.000	992.007	5.993
4	890.000	992.007	-102.007
5	958.000	888.873	69.127
6	912.000	888.873	23.127
7	963.000	888.873	74.127
8	821.000	888.873	-67.873
9	1020.000	669.664	350.336
10	925.000	669.664	255.336
11	741.000	669.664	71.336
12	686.000	669.664	16.336
13	896.000	714.637	181.363
14	681.000	714.637	-33.637
15	894.000	714.637	179.363
16	692.000	714.637	-22.637
17	795.000	630.064	164.936
18	512.000	630.064	-118.064
19	506.000	630.064	-124.064
20	429.000	630.064	-201.064
21	470.000	585.480	-115.480
22	301.000	585.480	-284.480
23	369.000	585.480	-216.480
24	431.000	585.480	-154.480
25	238.000	159.814	78.186
26	261.000	159.814	101.186
27	258.000	159.814	98.186
28	219.000	159.814	59.186

29	88.000	210.523	-122.523
30	182.000	210.523	-28.523
31	213.000	210.523	2.477
32	154.000	210.523	-56.523
33	157.000	43.181	113.819
34	35.000	43.181	-8.181
35	51.000	43.181	7.819
36	102.000	43.181	58.819
37	27.000	17.693	9.307
38	21.000	17.693	3.307
39	7.000	17.693	-10.693
40	13.000	17.693	-4.693
41	11.000	10.252	0.748
42	17.000	10.252	6.748
43	3.000	10.252	-7.252
44	4.000	10.252	-6.252

Asymptotic Correlation Matrix of Parameters

	G	X	B
G	1.000		
X	-0.787	1.000	
B	-0.619	0.897	1.000

Residuals have been saved.

ANOVA for Juveniles

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (11 levels)
34, 433, 689, 744, 791, 843, 1333, 1429, 1798, 2000, 2111

Dep Var: JUVENILES   N: 44   Multiple R: 0.971   Squared multiple R: 0.943

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	5575324.045	10	557532.405	54.996	0.000
Error	334542.750	33	10137.659		

Durbin-Watson D Statistic 2.752
First Order Autocorrelation -0.445

COL/ ROW	CONCENTR
1	34
2	433
3	689
4	744
5	791
6	843
7	1333
8	1429

9	1798
10	2000
11	2111

Using least squares means.
Post Hoc test of JUVENILES

Using model MSE of 10137.659 with 33 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	-38.000	0.0			
3	-160.750	-122.750	0.0		
4	-108.500	-70.500	52.250	0.0	
5	-391.000	-353.000	-230.250	-282.500	0.0
6	-558.750	-520.750	-398.000	-450.250	-167.750
7	-792.250	-754.250	-631.500	-683.750	-401.250
8	-707.500	-669.500	-546.750	-599.000	-316.500
9	-865.250	-827.250	-704.500	-756.750	-474.250
10	-934.500	-896.500	-773.750	-826.000	-543.500
11	-942.750	-904.750	-782.000	-834.250	-551.750

	6	7	8	9	10
6	0.0				
7	-233.500	0.0			
8	-148.750	84.750	0.0		
9	-306.500	-73.000	-157.750	0.0	
10	-375.750	-142.250	-227.000	-69.250	0.0
11	-384.000	-150.500	-235.250	-77.500	-8.250

	11
11	0.0

Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.597	1.000			
3	0.031	0.094	1.000		
4	0.137	0.329	0.468	1.000	
5	0.000	0.000	0.003	0.000	1.000
6	0.000	0.000	0.000	0.000	0.025
7	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000
9	0.000	0.000	0.000	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000
11	0.000	0.000	0.000	0.000	0.000

	6	7	8	9	10
6	1.000				
7	0.002	1.000			
8	0.044	0.242	1.000		
9	0.000	0.313	0.034	1.000	
10	0.000	0.054	0.003	0.338	1.000
11	0.000	0.042	0.002	0.284	0.908

	11
11	1.000

ANOVA for Adults

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CONCENTR (11 levels)
34, 433, 689, 744, 791, 843, 1333, 1429, 1798, 2000, 2111

Dep Var: ADULT   N: 44   Multiple R: 0.785   Squared multiple R: 0.617

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONCENTR	49.045	10	4.905	5.307	0.000
Error	30.500	33	0.924		

Durbin-Watson D Statistic   2.033
First Order Autocorrelation   -0.217

COL/ ROW	CONCENTR
1	34
2	433
3	689
4	744
5	791
6	843
7	1333
8	1429
9	1798
10	2000
11	2111

Using least squares means.
Post Hoc test of ADULT

Using model MSE of 0.924 with 33 df.
Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.0				
2	0.0	0.0			
3	0.0	0.0	0.0		
4	0.0	0.0	0.0	0.0	
5	-0.500	-0.500	-0.500	-0.500	0.0
6	-0.250	-0.250	-0.250	-0.250	0.250
7	-0.750	-0.750	-0.750	-0.750	-0.250
8	0.000	0.000	0.000	0.000	0.500
9	-0.500	-0.500	-0.500	-0.500	0.000
10	-2.000	-2.000	-2.000	-2.000	-1.500
11	-3.500	-3.500	-3.500	-3.500	-3.000

	6	7	8	9	10
6	0.0				
7	-0.500	0.0			
8	0.250	0.750	0.0		
9	-0.250	0.250	-0.500	0.0	
10	-1.750	-1.250	-2.000	-1.500	0.0
11	-3.250	-2.750	-3.500	-3.000	-1.500

	11
11	0.0

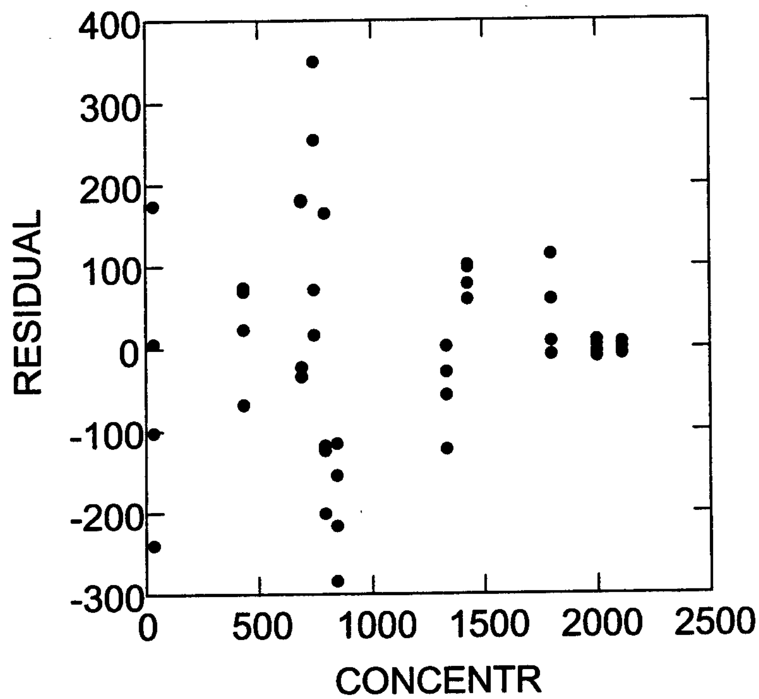
Fisher's Least-Significant-Difference Test.
Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	1.000	1.000	1.000		
4	1.000	1.000	1.000	1.000	
5	0.467	0.467	0.467	0.467	1.000
6	0.715	0.715	0.715	0.715	0.715
7	0.278	0.278	0.278	0.278	0.715
8	1.000	1.000	1.000	1.000	0.467
9	0.467	0.467	0.467	0.467	1.000
10	0.006	0.006	0.006	0.006	0.034
11	0.000	0.000	0.000	0.000	0.000

	6	7	8	9	10
6	1.000				
7	0.467	1.000			
8	0.715	0.278	1.000		
9	0.715	0.715	0.467	1.000	
10	0.015	0.075	0.006	0.034	1.000
11	0.000	0.000	0.000	0.000	0.034

	11
11	1.000

Residuals for barium.



Residuals for barium.