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Hazard Evaluation of Soil Contaminants with Aquatic Animals and Plant Toxicity Tests

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Deleterious effects upon the biota should be one of the principal characteristics used to perform the initial assessment of contamination and the acceptable level of clean-up at hazardous waste sites. Acute toxicity tests are probably the best means for conducting rapid preliminary assessment of distribution and extent of toxic conditions at a site. On the other hand acute toxicity tests may not be adequate indicators of potential effects at critical life stages or responses to longer term exposure to contaminants. Chronic toxicity tests are generally more sensitive than acute tests, and can be used to predict "no effect" or "safe" levels of contamination. In addition, chronic tests provide a better index of field population responses and more closely mimic actual exposure in the field. Partial chronic tests such as the 7 d *Ceriodaphnia* sp. survival and reproduction test and 7 d fathead minnow survival and growth test are widely used to predict effects upon critical stages in the life cycle of chemical and mixtures (Mount and Norberg 1984; Rand and Petrocelli 1985). The overall objective of this project was to evaluate the potential hazard of contaminants at an abandoned oil refinery upon aquatic ecosystems within the vicinity. A battery of acute and partial chronic toxicity tests were used to evaluate potential effects of contaminated soil and leachates of soil upon rice seed germination and root growth, *Ceriodaphnia* acute survival, fathead minnow acute survival, Microtox acute response, 7 d *Ceriodaphnia* survival and reproduction, and 7 d fathead minnow survival and growth. The specific tests used to accomplish the overall objective included; 1) To estimate phytotoxicity of the soil at the selected contaminated areas within the refinery, 2) to determine potential for leaching at the selected contaminated areas within the refinery, and 3) to assess the relative toxicity of each of the six contaminated areas in the refinery.

MATERIALS AND METHODS

The study site was an old abandoned oil refinery located in Cyril, in Caddo County, Oklahoma. The refinery site covered approximately 63 ha, which included the main processing plant facility in addition to an array of unlined earthen ponds, storage pits, and 3.4 ha soil farm facility used to treat oily sludges. Crude oil had been refined into petroleum products for distribution

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since early 1920's. This practice had produced considerable quantities of waste materials requiring treatment, storage, and disposal. From 1920 to 1984 process waste were placed in over 50 impoundments, many unlined. Also, some process waste was applied to the soil and treated in a land-farming operation. Soil samples were collected from predefined grids covering known areas of contamination (Figure 1 and Table 1). The trap grids were used by other co-investigators for collecting small feral rodents from the site and the enclosures were used for confining populations of laboratory-raised white-footed deer mice on site for controlled mesocosm exposures (McMurry 1993). Random surface soil samples were collected during April 1992 from the grids, during May 1992 from the enclosures, and during January 1993 from both the grids and enclosures. A composite consisting of > 6 random soil samples was mixed on site and subsampled to obtain aliquots for laboratory analyses. Soil samples from each area were homogenized, air dried and ground in a blender in preparation for seed germination tests. Aqueous leachates of soil samples were prepared in accordance with Dredged Material and Testing Manual US-ACE method (US-ACE 1991). Rice (*Oryza saliva*) seeds were selected for the phytoassays because published effects data indicated sensitivity to toxic effluents (Wang 1990; Misra and Behera 1990), and air pollutants (Anbhazhagan and Bhagwat 1991; Kats et al. 1985). Rice seeds were also recommended by the Organization for Economic Cooperation and

Table 1. Cyril Refinery, Oklahoma, sampling locations, and site description.

Site	Grids	Enclosures
1	North side and upstream upslope of the Refinery. Unused land.	A reference site and placed in Grid 1. Unused land.
2	Within the Refinery, Asphalt Drum Storage Area with pits filled with asphalt wastes.	Within the Refinery. Placed in the leaded gasoline tank area and the soil was soaked with gasoline
3	Within the Refinery, the area surrounding the oil/sludge traps	Within the Refinery placed near oil/sludge traps.
4	Within the Refinery, Land Farming Area, used for processing sediments from oil/sludge traps.	Within the refinery, placed in the Land Farming area.
5	A reference site, 1 Mile southwest of the Refinery A cultivated farm land.	A reference site, placed at the southern border of the Refinery. Unused land.
6	A reference site, 4 Miles east of the Refinery. Unused land.	A reference site adjacent to Enclosure 5. Unused land.

Development (OECD 1984) for terrestrial plant growth tests. Generally seed germination tests have been used as a measure of phytotoxicity in hazardous waste site evaluation (Green et al. 1988). However, some of the toxicants in the hazardous wastes may have no effect on seed germination but still may interfere with growth. Therefore, we included rice shoot growth as a additional measure to identify phytotoxic chemicals that could suppress growth or interfere with nutrient availability. Shoot growth was determined after 10 d seed germination in the contaminated soil.

Both *Ceriodaphnia dubias* and fathead minnows cultured in hard water were obtained from the Water Quality Research Laboratory, Oklahoma State University, Oklahoma. Rice seeds were obtained from Rice Research of USDA in Beaumont, Texas. Aqueous soil extracts were made from hard synthetic water for toxicity assays with *Ceriodaphnia dubia*, and fathead minnow. Soil extracts were made with deionized tap water for rice root growth test and for Microtox soil extracts were made with Millipore Milli-Q^{R} water. The reference Grids 1 and 6, and Enclosures 1, 5, and 6 soil were hard to very hard (EPA 1989) having values range from 140 -230 mg $CaCO_3$/L. The Grid 5 reference soil samples collected from farmland had a hardness value of 900 mg $CaCO_3$/L and the soil extract significantly reduced *Ceriodaphnia* reproduction (Table 1) when compared with the laboratory control, the hard synthetic water. The Grid 5 reference soil samples were not used to compare with the treatments. Reference soils Grid 1 and 6 and Enclosure 1 were not significantly different from the laboratory control in the toxicity tests and they were used to compare with the treatments. The Enclosure 3 soil extract was turbid and dark brown in color and it was difficult to measure hardness in the 100% soil extract and the hardness of 1% soil extract was 174 mg $CaCO_3$/L.

The 7 d *Ceriodaphnia* survival and reproduction effects test and 7 d fathead minnow survival and growth test were performed according to the effluent toxicity test methods described by Weber et al. (US EPA 1989). *Ceriodaphnia* neonates and fathead minnow larvae were exposed to 100% aqueous soil extracts and the toxic soil extracts were diluted with hard synthetic water. Microtox was performed with a Microtox Model 500 (Microbics Corporation, Carlsbad, California), according to the instructions described in the Microtox manual. Rice seed germination tests were performed according to modified Neubauer seed germination test described by Thomas and Cline (1985). Rice seeds were exposed directly to the contaminated soils and for toxic soil dilutions were made by mixing with sterile sand. Rice seeds were exposed to soil for 10 d and after 10 d exposure the germinated seeds were counted and shoot dry wt was estimated. Rice root elongation tests were performed by combining two methods, the root elongation test described by Green et al. (1988) and the seed rack method described by Myhill and Konzak (1967). Root elomgation test was performed in 100% aqueous soil extracts and for toxic soil dilution were made with deionized tap water. The seeds were

exposed for 5 d and root length was estimated after 5 d exposure.

LC50's were calculated by Probit analysis for *Ceriodaphnia* survival, fathead minnow larval survival and lethal responses of the rice seed germination toxicity tests. The Probit analysis was also used to calculate EC50's for rice shoot growth and root growth inhibition proportion data. Data from partial chronic toxicity test with fish and *Ceriodaphnia* was subjected to the decision process described by Weber et al. (EPA 1989). The t-test was used to compare the reference sites (uncontaminated) with the blank. If the reference site was not significantly different from the blank, the reference site was used to compare the treatments. In the Microtox assay the reference soil extracts were colored so the blank, the Microtox diluent was used to compare the treatments. The reference soil extracts were unavailable for fathead minnow assays so the the laboratory control, the hard synthetic water was used to compare with the treatments.

RESULTS AND DISCUSSION

The three contaminated enclosures showed differential responses among the organisms tested (Table 2 and 3). Enclosure 3 aqueous soil leachates were highly lethal to *Ceriodaphnia* and fathead minnows. Also rice seeds did not germinate when tested in Enclosure 3 soil. The soil had a noxious odor and appeared to be saturated with oily wastes. Enclosure 3 soil was difficult to extract because it was sticky and clumped together. The aqueous extract was dark brown in color, turbid and had an oily appearance and odor. Aqueous leachates of Enclosure 3 soil collected in 1992 caused 100% mortality of *Ceriodaphnia* exposed to 0.01% soil extract (Table 2). The threshold of lethal effects was very sharp since at 0.005% leachate there was 100% survival (Table 3). The LC50 value was 0.0071% in the 48 h acute survial test and 7 d chronic survival and reproduction test. Trimmed Spearman-Karber method was used to calculate LC50 instead of probit analysis, since there were no intermediate responses between 100% and 0% mortality. The 1993 Enclosure 3 soil was comparatively less toxic than 1992 samples and *Ceriodaphnia* survival LC50 value had increased to 12.85%. But sublethal effects still persisted at low concentration as seen in partial chronic test and a significant effect upon *Ceriodaphnia* neonate production was observed in 0.01% extract.

Enclosure 3 soil collected in 1992 also caused significant toxic effect to fathead minnow larval survival and 100% mortality was observed in 0.1% soil extract which was 10 fold higher value than *Ceriodaphnia* survival (Table 2). The NOEC value for fish survival was 0.01%. But no significant effect upon fish larval growth was observed (Table 3). The 1993 Enclosure 3 soil was less toxic than 1992 soil and fish survival was reduced to 25% in 20% soil extract. Toxicity tests with daphnids and fish larvae could not be conducted above 20% soil extract, since the dark color of the extract prevented visual observation of daphnid neonates and fish larvae and thus determination of toxic

effects. The Enclosure 2 soil though not extremely toxic like Enclosure 3 soil still had significant effects on aquatic organisms tested. The soil collected in 1992 had no toxic effect but suppressed 78% *Ceriodaphnia* reproduction (Table 2), whereas soil collected in 1993 caused 100% mortality in 100% soil extract and 0% *Ceriodaphnia* reproduction in 50% soil extract. The abnormality such as shedding the eggs by the adult was observed in 50% soil extract. The Enclosure 2 soil had no significant effect on fathead minnow larval survival and growth.

The soil samples collected in 1992 and 1993 from the contaminated Grids 3 and 4 never showed any toxic effects to rice seed germination, but significant effects were observed on rice shoot growth (Table 2). Initially the seed germination test was conducted for 5 d and then the test was extended up to 10 d to determine the maximum germination rate and also to determine toxic effects on plant growth. Seed germination was 0% in 100% Enclosure 3 soil on d 5, but it increased > 50% on d 10. In the next dilution namely 50% there was 100% germination. Shoot growth was suppressed to 81% in Enclosure 3 soil collected in 1992 and shoot growth was suppressed to 71% in soil collected in 1993. Though shoot dry weight decreased to 71% in Enclosure 3 soil collected in 1993, it did not increase at low dilutions (0.5) due to nutrient deficiency in sand and an EC50 could not be calculated. Enclosure 3 soil collected in 1992 also caused significant effect on rice root growth, i.e., rice root length EC50 value was 14% (Table 3). In addition, morphological changes produced shorter and thicker roots. Enclosure 3 soil collected in 1993 was less toxic to rice root growth than the previous year similar to aquatic animals tested. In this study both root length and root dry weight seemed to be useful measures to determine toxic effects of the oil refinery wastes. Sometimes soil contaminants had more significant effect on root dry weight than on root length. Enclosure 2 soil collected in 1992 had more effect on root dry weight than on root length. Enclosure 2 soil collected in 1993 had no significant effect on root length but still suppressed root dry weight.

When compared with *Ceriodaphnia* and fish bioassays, Microtox was found to be the least sensitive to leachates of the oil refinery soil (Table 2 and 3). Though soil collected from Grids 3 and 4 (1992) caused considerable light diminution when compared to Microtox diluent controls, the reduction was not statistically significant. Microtox was not a sensitive indicator of toxicity in this study. The presence of dark colored dissolved substances in both the reference soil and Enclosure 3 soil caused light diminution. One percent solutions of soil leachates from Enclosure 3 soil collected in 1992 and 1993 caused a 9 % and 25 % light diminution. There was 100% diminution at solutions greater than 5% prohibiting calculation of EC50. However, the light diminution was not judged to be a toxic effect but due to a decrease in transparency of the test solution. In contrast the contaminated soil extracts were colorless which may be due to wastes complexing with soil particles and

settling by centrifugation. The Microtox diluent blank was used to compare the contaminated soil extracts.

The bioassay results indicated that selected sites at the refinery contain levels of water soluble contaminants that pose a toxic threat to both aquatic and terrestrial communities. Enclosure 3 was the most toxic site among the selected sites. *Ceriodaphnia* was the most sensitive species of all the bioassay organisms tested in this study. Enclosure 2 soil collected in 1993 contained visibly greater quantities of gasoline and was more toxic to *Ceriodaphnia* than

Table 2. Toxicity test results from exposure to either soil extracts or soil samples collected from grids (G1-G6)and enclosures (E1-E6) at the Oklahoma Oil Co. Refinery Superfund waste site. Values expressed as percent inhibition relative to controls.

Toxicity test	Year	G1	G2	G3	G4	G5	G6	E1	E2	E3	E4	E5	E6
Ceriodaphnia	1992	NE	NE	NE	NE	NE	NE	NE	NE	*	NE	NE	NE
7 d survival[e]	1993	NE	NE	NE	NE	NE	NE	NE	100	**	NE	NE	NE
Ceriodaphnia	1992	NE	NE	NE	NE	22	NE	NE	78	*	NE	NE	NE
7 d reproductio[e]	1993	NE	NE	NE	NE	42	47	NE	100	**	NE	29	17
Fathead minnow	1992	+	+	+	+	+	+	+	+	β	+	+	+
7 d survival[e]	1993	+	+	+	+	+	+	+	+	γ	+	+	+
Rice seed	1992	NE	NE	NE	NE	20	14	NE	NE	44	NE	20	14
10 d germinatio[s]	1993	NE	NE	10	8	24	NE	NE	NE	46	NE	NE	NE
Rice shoot	1992	NE	NE	22	25	NE	NE	NE	31	81	13	NE	NE
10 d dry wt[s]	1993	NE	NE	21	30	24	NE	NE	21	71	NE	NE	NE
Rice root	1992	NE	NE	NE	NE	NE	NE	NE	24	91	NE	NE	NE
5 d length[e]	1993	NE	NE	NE	NE	14	NE	NE	NE	19	NE	NE	18
Rice Root	1992	NE	NE	27	NE	26	NE	NE	44	54	NE	NE	NE
5 d dry wt[e]	1993	NE	NE	NE	NE	NE	NE	NE	27	25	NE	NE	NE
Microtox[e]	1992	19	NE	24	20	58	29	25	NE	ℰ	NE	37	29
5 min test	1993	24	NE	NE	NE	23	23	23	NE	∝	NE	18	29

[e] aqueous soil extract,
[s] soil,
NE = no biologically significant effect in highest concentrations tested
* = *Ceriodaphnia* 0% survival in 0.01% Enclosure 3 soil extract (1992)
** = *Ceriodaphnia* 0% survival in 20% Enclosure 3 soil extract (1993)
ℰ = Microtox 9% light inhibition in 1% Enclosure 3 soil extract (1992)
∝ = Microtox 26% light inhibition in 1% Enclosure 3 soil extract (1993)
β = Fathead minnow 0% survival in 0.1% Enclosure 3 soil extract (1992)
γ = Fathead minnow 25 % survival in 20% Enclosure 3 soil extract (1993)
+ = not tested

the soil collected in 1992. In contrast the 1993 Enclosure 3 soil was less toxic to aquatic organisms than the previous year. These temporal variations were probably due to climatic variability. In 1993 soil sampling in January was followed by heavy rains in the area which could have shifted the contaminants. The contaminants in the soil samples also possibly differed due to random sampling method. In this study three reference sites were used to compare three contaminated sites. Sometimes the reference (uncontaminated) sites exhibited physical and chemical parameters that caused adverse effects to the test battery. A laboratory control can be used for preliminary screening of the reference sites. In this study rice had shown sensitivity to the refinery site soil and therefore rice seeds could be used in the test battery employed for a hazardous waste site evaluation. As this study provides information on short-term acute effects as well as partial chronic effects, both lethal and "no effect" concentrations of the wastes could be used for site characterization.

Table 3. LC50 (confidence interval), EC50, and NOEC responses caused by soil contaminants in Enclosure 2 (E2) and Enclosure 3 (E3), at the Oklahoma Oil Co. Refinery Superfund waste site.

	Year	LC50*		EC50*		NOEC*	
		E2	E3	E2	E3	E2	E3
Ceriodaphnia	1992	NE	0.0071+			NE	0.005
7 d survival[e]	1993	70.71+	12.8 (7.5-17.7)			50	5
Ceriodaphnia	1992						0.001
7 d reproduc[e]	1993					12.5	0.005
Fathead minno	1992	NE	0.06 (.05-.08)			NE	0.01
7 d survival[e]	1993	NE	>20			NE	5
Fathead minno	1992					NE	NE
7 d growth[e]	1993					NE	NE
Rice seed	1992	NE					
10 d germinat[s]	1993	NE					
Rice shoot	1992						
10 d dry wt.[s]	1993				∝		
Rice 5 d	1992				14 (6.7-25.4)		
root length[e]	1993			NE			
Rice 5 d root	1992				∝		
dry wt[e]	1993						
Microtox[e]	1992			NE	>5	NE	0.5
5 min test	1993			NE	>5	NE	0.5

* % soil elutriate or soil; "soil elutriate; "soil; NE no biological effect
+ = all or none response in 48 acute survival test and 7 d chronic survival test. LC50 value was calculated by Trimmed Spearman-Karber method
∝ = shoot dry wt never increased at lowest concentration of E3 soil tested

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