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Acute Toxicity Screening of Hanford Site Waste Grouts Using Aquatic Invertebrates

Prepared for the U.S. Department of Energy
Environmental Restoration and
Waste Management

Westinghouse Hanford Company Richland, Washington

Hanford Operations and Engineering Contractor for the
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ABSTRACT

Waste grouts prepared by mixing a simulated nonradioactive liquid waste with a dry solids blend consisting of cement, fly ash, and clay were screened for their acute toxicity using aquatic invertebrates (D. magna, D. pulex, and C. dubia) as test organisms and a fluorogenic substrate (4-methylumbelliferyl b-d galactoside) as the toxic stress indicator. After one hour of exposing juvenile daphnids to grout extracts of varying concentrations, followed by a 15-minute reaction with the fluorogenic substrate, the degree of in vivo enzymatic inhibition was measured by the number of resulting fluorescent daphnids. The effective concentration at which 50% of the daphnids were adversely affected (EC50) values calculated by probit analysis were 2,877 mg/L, 2,983 mg/L, and 3,174 mg/L for D. pulex, D. magna, and C. dubia, respectively. The results indicated that the grout extracts studied are nonhazardous and not dangerous to daphnids.

INTRODUCTION

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the Hanford Site's 50 years of operation. The Hanford Site, which is owned by the U.S. Department of Energy, is located in south-eastern Washington State, near Richland, Washington (refer to Figure 1). These wastes are stored temporarily in single-shell (refer to Figure 2) and double-shell carbon steel tanks (refer to Figure 3) at the Hanford Site. One of the strategies for the disposal of the low-level radioactive portion of these wastes involves immobilizing the waste in the form of grout. Because the potential risk of animal and plant exposure to grouts is unknown, grout toxicity and dangerous environmental exposure concentrations must be measured. The utility of aquatic invertebrates (D. magna, D. pulex, and C. dubia) to assess and detect the toxicity of Hanford Site grouts was investigated.

METHOD

A nonradioactive waste grout was prepared by mixing a simulated waste (refer to Table 1) with a dry blend consisting of Portland cement, fly ash,

Figure 1. Location and Regional Map of the Hanford Site.

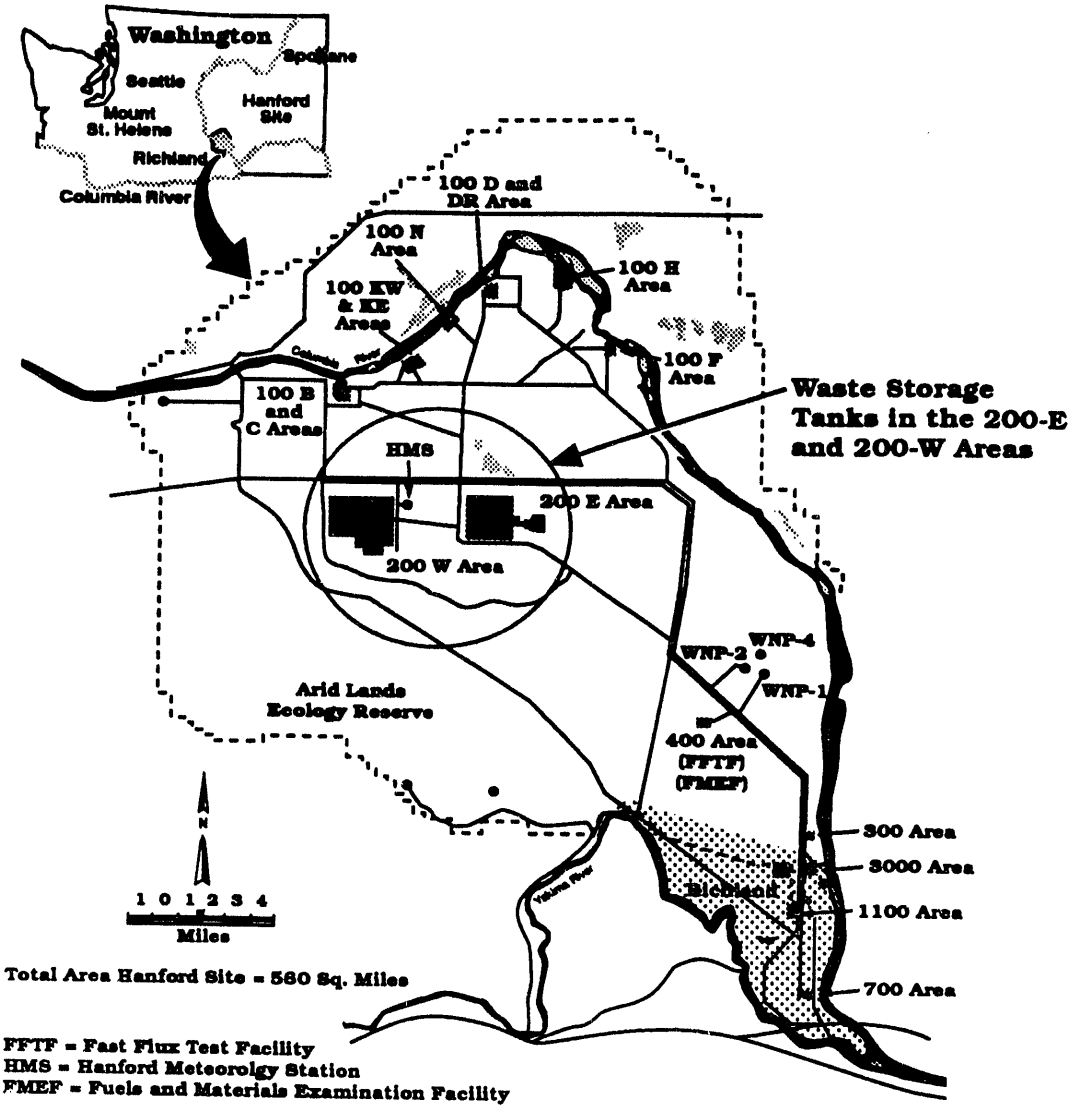
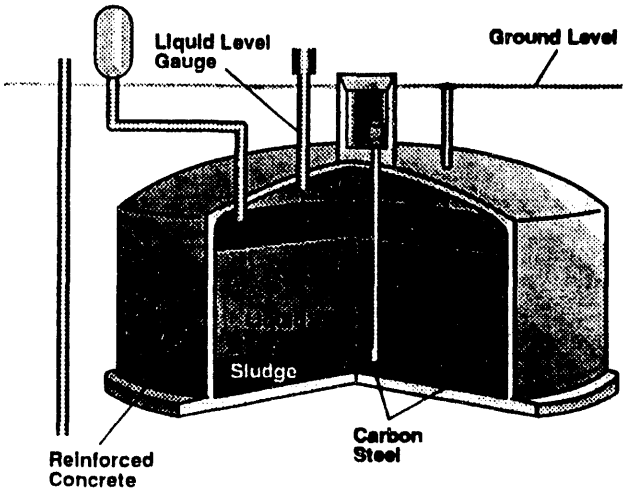


Figure 2. Single-Shell Tank.



- 149 Tanks Constructed 1943-64
- ~210 m^3 to 3,800 m^3 Capacity (55 kgal to 1 Mgal)
- Bottom of Tanks at Least 50 m (150 Feet) Above Groundwater
- No Waste Added to Tanks Since 1980
- Tanks Currently Contain:

  ~136,800 m^3 (36 Mgal) of Salt Cake, Sludge, and Liquid

  ~555 x 10^{16} Bq (150 MCi)
- 67 Are Assumed to Have Leaked ~ 3,800 m^3 (~1 Mgal)

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Figure 3. Double-Shell Tank.

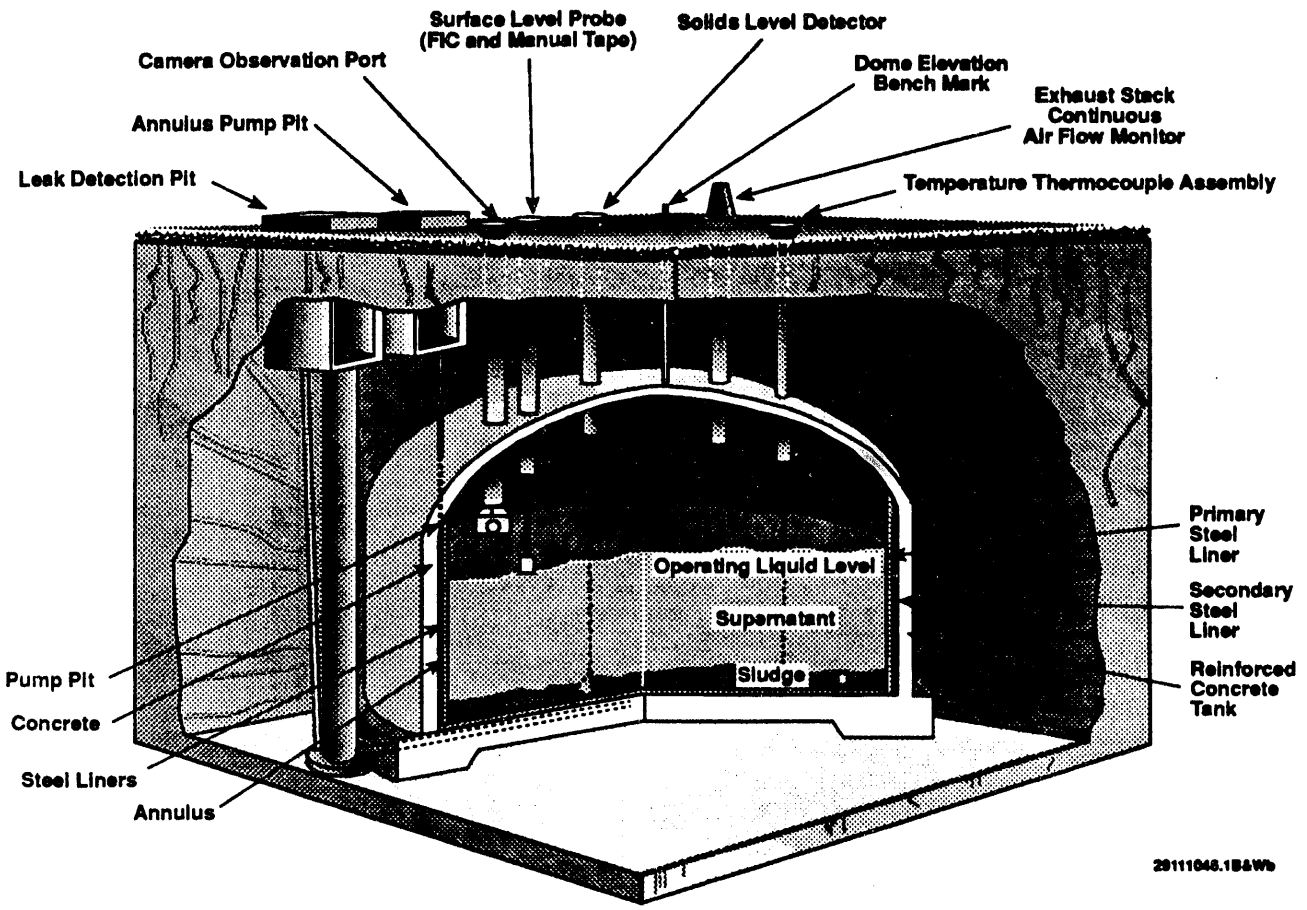

Table 1. Simulated Waste Composition Major Component.

Species	Concentration (M)
AlO_2^-	0.383
NO_2^-	0.536
NO_3^-	1.279
SO_4^{2-}	0.029
PO_4^{3-}	0.173
OH^-	1.880
CO_3^{2-}*	0.359
TOC**	0.248

*Calculated from total carbon (inorganic and organic)
**Calculated from ethylenediamine tetraacetic acid (EDTA), n-hydroxyethyl ethylenediamine triacetic acid (HEDTA), glycolate, and citrate additions.

Table 2. Composition of Dry Blend.

Composition	Weight %
Attapulgite clay	11.0
Fly ash	20.7
Portland cement	68.3

and attapulgite clay (refer to Table 2). The simulated waste was designed to mimic the expected compositional range of low-level radioactive liquid wastes stored in the waste tanks at the Hanford Site. Aqueous extracts of the cured grout were then screened for acute toxicity using daphnids as test organisms and a fluorogenic substrate (4-methylumbelliferyl b-d, galactoside) as the toxic stress indicator. The method is based on the ability of healthy daphnids to ingest and metabolize the fluorogenic substrate that is added to the grout extract. Daphnids that are able to cleave the fluorescent marker from the substrate fluoresce brightly under long wave ultraviolet light while those that have impaired enzymatic systems caused by their exposure to toxic levels of the grout extract do not fluoresce. The mechanism of the reaction is illustrated in Figure 4.

The steps to prepare the grout extract are listed in Table 3. The cured grout was dried at 110 °C, pulverized, and passed through a 75-um sieve. A 1-g aliquot of the dried pulverized grout was dispersed in 100 mL of distilled deionized water. The resulting dispersion was shaken for 30 minutes, allowed to stand for 30 minutes, and then filtered using a 0.45 um filter.

The procedure for conducting the acute toxicity test closely follows the Daphnia Magna IQ Toxicity Test* of Aqua Survey Inc. (Aqua Survey 1992). The protocol is summarized in Table 4. Briefly, the test involves exposing juvenile daphnids to a series of aqueous grout extracts with varying concentrations for a period of one hour. The fluorogenic substrate was then added to the extracts and after an incubation period of 15 minutes, the effect of the exposure of the daphnids to the grout extracts was assessed by illuminating the exposure chamber/container with long wave ultraviolet light. A parallel control test using distilled deionized water in place of the grout extract was also conducted. The degree of enzymatic inhibition was measured by the number of resulting fluorescent daphnids. The test was scored by comparing the response of the daphnids to each grout concentration against that of the control daphnids. If the light emission of a daphnid was less than that of the control daphnids, it was scored as affected. The EC50 was then calculated using probit analysis.

RESULTS

The EC50 values of the three different species of daphnids are tabulated in Table 5. The EC50 values are 2,877 mg grout/L, 2,983 mg grout/L, and 3,174 mg grout/L for D. pulex, D. magna, and C. dubia, respectively. The slight difference in the responses may be attributed to the subjective pass-fail scoring of the fluorescence criterion. Based on the acute toxicity rating scales of the Washington State Department of Ecology (Ecology 1981),

*The Daphnia Magna IQ Toxicity Test is a trademark of Aqua Survey, Inc.

Figure 4. Enzymatic Reaction of Daphnids with a Fluorogenic Substrate.

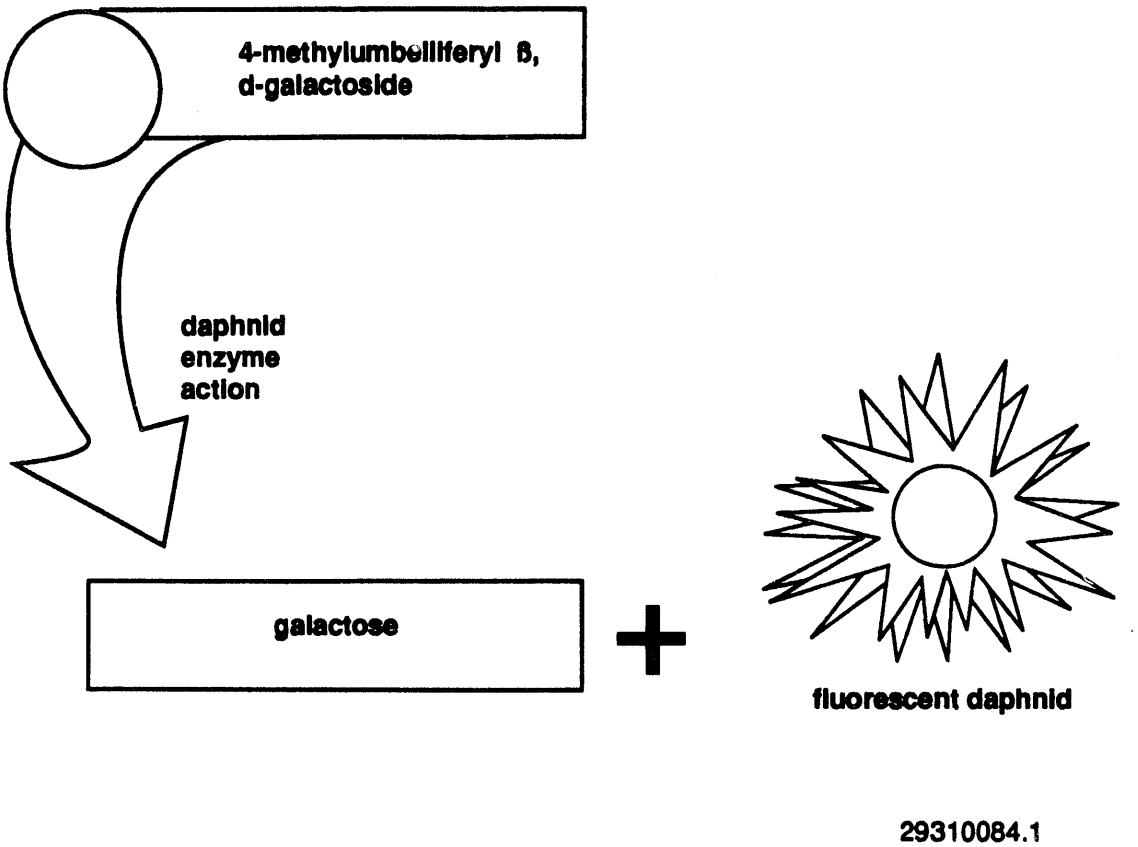

Table 3. Grout Extract Preparation.

Dry cured grout to constant weight at 110 °C.
Pulverize dried grout and pass through 75-um sieve.
Weigh 1.0 gram of sieved pulverized grout.
Disperse grout in 100 mL distilled water
Shake for 30 minutes.
Allow suspension to stand for 30 minutes.
Filter suspension using 0.45-um filter.

Table 4. Acute Toxicity Procedure.

Expose daphnids to a series of grout extracts for one hour. Note: 6 or more daphnids per extract concentration.
Add fluorogenic substrate to extract containing the daphnids.
Incubate fluorogenic-containing extract for 15 minute at 20 °C.
Illuminate incubated extract with longwave UV light.
Score light emission of daphnids against control.
Count daphnids with light emission greater or equal to those of control daphnids.
Calculate EC50 of grout extract by probit analysis.

Table 5. EC50 of Daphnids.

Species	EC50, mg/L
D. pulex	2,877
D. magna	2,983
C. dubia	3,174

the grout extracts are nontoxic and not dangerous to the daphnids. A substance that has an EC50 value of less than 1,000 mg/L is classified as dangerous and hazardous. These EC50 values compare very well with those obtained using bacteria as the test organism (Rebagay et al. 1992).

CONCLUSION

The test using daphnids is rapid (1.5 hour duration) and may be used as a screening test to assess the acute toxicity of grouts.

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