USE OF DAPHNIA MAGNA 
TO ASSESS POTENTIALLY CONTAMINATED BUILDINGS

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C.W. Kurnas

RESEARCH DIRECTORATE

June 1992

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Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.
Random concrete core samples taken from a loading dock were used in determining the toxicity of concrete to Daphnia magna. The cores were ground to powder and analyzed for volatiles and chemical agents before being subjected to aquatic toxicology studies using Daphnia magna. Particle size, pH, and ion exchange mechanisms were investigated as part of methodology development. No effects were observed in daphnia exposed to concrete concentrations up to 400 mg/L after the pH was adjusted. It was determined that an ion exchange reaction between sodium bicarbonate and concrete caused the water hardness to drop. Concrete spiked with sodium lauryl sulfate, copper sulfate, and β-aminoethylarylisothio sulfate were investigated to determine if concrete would alter the toxicity. The resulting EC50s were 17.2, 1.42, and 17.3 mg/L, respectively. The copper sulfate toxicity was reduced by two orders of magnitude. The changes in sodium lauryl sulfate and β-aminoethylarylisothio sulfate were not significant.
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PREFACE

The work described in this report was authorized under Project No. 1NGK-X6-XXA, Clean Pilot Plant. This work was started in April 1990 and completed in March 1991.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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This report has been approved for release to the public.

Acknowledgments

The authors express their appreciation to Dennis Beattie and his crew at the pilot plant for their support in equipment set up, sampling, and meeting all time deadlines required for TCLP and U.S. Environmental Protection Agency requirements. The authors also thank Dr. Dupont Durst for providing us with computer drawings of the compounds listed in Appendix A.
QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 89). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

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<td>22 May 1991</td>
</tr>
<tr>
<td>Final report</td>
<td>14 May 1991</td>
<td>22 May 1991</td>
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To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

DENNIS W. JOHNSON
Quality Assurance Coordinator, Rshf
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<th>Section</th>
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USE OF DAPHNIA MAGNA
TO ASSESS POTENTIALLY CONTAMINATED BUILDINGS

1. INTRODUCTION

The U.S. Army is conducting a program at the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) using various areas of expertise in environmental toxicology and chemistry to assess a contaminated building being prepared for demolition due to deterioration. In 1941, a four-story building (Pilot Plant, Building E-5625) was erected at the Center and used to manufacture CC2 impregnate. Shortly after World War II, the building was converted into laboratories operating with blister, blood, simulant, riot control, and incapacitating chemical agents. In 1986, all operations in this building were stopped. The laboratory equipment was removed in the years to follow. The interior of the building (heating, air-conditioning, piping, etc.) was stripped, decontaminated, and removed. The walls and floors of the entire building were washed with caustic materials used in the decontaminating operations of chemical agents.

The objective of this study was to develop methods using Daphnia Magna to identify areas of contamination on the floors of Building E-5625 using concrete core samples. The contaminated portions of the building would be dismantled and incinerated before disposal. The uncontaminated portions would be taken directly to a landfill. Incinerating only the contaminated portions of a building can potentially save millions of dollars in disposal fees.

A detailed evaluation plan, that will identify contaminated areas using historical records, personnel interviews, mobile mass spectrometry, and core sampling for chemical analysis and environmental studies, has been implemented.

This report will focus on the methodology development for using daphnia to assess the toxicity of concrete core samples. Subsequent reports will address the analytical methodology development and analysis and other environmental screening assays.

2. METHODS AND MATERIALS

2.1 Concrete Preparation.

The back loading dock of the Pilot Plant was determined to be the least likely contaminated concrete floor and was used as a negative control for determining base line toxicities. A flow chart summary of the concrete sample processing is presented...
in Figure 1. The loading dock floor was divided into a 1-m by
1-m grid. Each square was assigned a number in increasing order.
A random number table was used in selecting the squares to be
sampled. Several 5-1/2 in. diameter concrete cores were taken
with a DCM II Diamond Coring System by Hilti, Incorporated.
Cores were sealed separately in plastic bags and allowed to stand
for a minimum of 4 hr at 70 °F. The bags were sampled for
volatiles that may have leached from the core. Any steel rein-
forcing bars running through the cores were removed by breaking
the cores into large chunks. The concrete chunks were passed
through a Brinkmann Instruments' BB2 Jaw Crusher several times to
produce a fine powder. The powder was collected and analyzed for
chemical agents (refer to Appendix A for a listing of the chemi-
cal agents for which the powder was analyzed). If a sample was
contaminated, that particular sampling area would be designated
hot and would merit detailed sampling (none of the loading dock
samples contained agent contamination). If it was determined to
be below detectable limits for chemical agent contamination, a
sample would be released for environmental studies.

Stock solutions of 4000 mg/L of concrete were prepared
using well water and left to stand for 24 hr to allow for any
leaching to occur. After 24 hr, the pH of the concrete solution
was adjusted to within a range of 7.5-8.3 using 10% HCl. The
samples were not filtered so the daphnia would have the opportu-
nity to ingest the particles to provide an added pathway of
toxicity.

All aquatic testing followed current American Society
for Testing and Materials (ASTM) and U.S. Environmental Protec-
tion Agency (EPA) guidelines.1,2

2.2 Concrete Water Chemistry.

A concrete core sample was run through the jaw crusher
only once to provide an assortment of particle sizes. The
concrete was then placed into a shaker sieve equipped with 20-,
10-, 2.8-, 0.8-, 0.7-, and 0.2-mm sieves for 5 min. Stock solu-
tions of 2000 mg/L of the various concrete particle sizes were
prepared, and 
 determinations were made at 0, 4, 6, 24, and
72 hr.

Overall particle size distribution was determined on a
concrete core sample that was processed through the jaw crusher
to its final powdered form. This was done using a Brinkmann
Instruments' Optical Particle Size Analyzer.

Hardness studies were conducted in distilled and well
waters using concrete concentrations up to 4000 mg/L. Studies
were also conducted in combination with suggested EPA additives
for reconstituted water2 to determine how these additives affect
water hardness when concrete is added. The additives were placed
separately into distilled water at EPA-suggested concentrations
SAMPLE AREA GRIDED RANDOM SAMPLES DETERMINED

CORE SAMPLE TAKEN

SEALED IN PLASTIC

ANALYZED FOR VOLATILES

GRINDING
Jaw Crusher, BB2

ANALYZED FOR AGENTS

IF CONTAMINATED
INCINERATION NECESSARY

IF CLEAN

SAMPLE DISTRIBUTED FOR TOXICITY STUDIES

Figure 1. Flow Chart Summarizing the Methods Involved in Concrete Core Sample Preparation
and above, while the concrete concentration remained at 4000 mg/L. Total and calcium hardnesses were determined using titration methods described by ASTM, and hardness kits made by Ecologic Instruments.

2.3 Daphnia Assays.

*Daphnia magna* were obtained from Dr. Freida Taub at the University of Washington (Seattle, WA) and reared for the past 8 years in this laboratory using methods described by Goulden and co-workers. Daphnia stock cultures were fed a mixture of vitamin enriched *Ankistrodesmus falcatus*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardtii*. Daphnia culture media was derived from well water, which was passed through a treatment system containing limestone pH adjustment, Zeata Sol iron removal, carbon filtration, and UV sterilization. The well water is monitored for 92 commonly found ground water pollutants every 4 months by Watercheck National Testing Laboratories, Incorporated (Ypsilanti, MI). Appendix B lists the compounds and parameters measured.

Serial dilutions, which ranged from 500 to 4000 mg/L of concrete, were prepared. The test beakers were placed into a temperature-controlled room of 20 °C with a light-dark cycle of 16:8 hr at 315 ft-c of light. Two replicates per each concentration contained 10 daphnia, less than 24 hr old, in a total of 100 mL of solution. The pH, conductivity, and hardness measurements were taken at the start of each test. Daphnia were gently touched with a pasteur pipet at 24 and 48 hr. If the daphnia could not swim actively for 15 s, immobilization was recorded. The EC₅₀ (effective concentration at which 50% of the organisms are immobilized) values were computed using the probit analysis as prepared by Stephan (Personal communication). The EC₅₀'s were also tabulated graphically using a least square regression analysis and were used to verify all probit analyses.

2.4 Concrete Spiking.

In an effort to determine the effects of concrete on the overall toxicity of chemicals to daphnia, separate studies using concrete spiked with copper sulfate, sodium lauryl sulfate, and β-aminooethylaryltiosulfonate were conducted. The toxicants were added separately to solutions of concrete and allowed to stand for 24 hr. The pH was adjusted to within a range of 7.5-8.3 using 10% HCl before the daphnia were exposed to the test solutions. The concrete concentrations remained constant, (4000 mg/L) while the toxicant levels were varied. Studies were also run using the toxicants without concrete additions for toxicity comparison.
3. RESULTS

Concrete particle size has an affect on the overall pH change in water (Figure 2). Particles of 10.0-0.2 mm raised the pH of the solution to above tolerable limits for daphnia.* Particles of 20.0 mm in size caused the smallest change in pH. The size of the particle did not affect the time it took for the pH to stabilize. The pH of the solution stabilized 6 hr into the leachate process. Particle sizes used in the toxicity studies ranged from 3-55 μm.

Daphnia exposed to concrete concentrations of 500-4000 mg/L without adjusting the pH resulted in an EC$_{50} = 2288.4$ mg/L. At a concentration of 1000 mg/L, no mortality was observed (Figure 3). After the pH was adjusted, there was no mortality recorded in concentrations up to 4000 mg/L of concrete.

As the concrete concentration increased, the water hardness was reduced by approximately 60% (Figure 4). However, the hardness increased when concrete was added to distilled water (Figure 4). The investigation of various salts, both those present in the well water and those required in suggested EPA hardening formulations, resulted in the discovery that sodium bicarbonate causes a reduction in water hardness when concrete powder is added (Figure 5). When the other EPA-recommended additions of salts were added, the hardness increased as expected.

The table lists the EC$_{50}$s of compounds before and after being added to solutions of 4000 mg/L of concrete. The EC$_{50}$ of copper sulfate was drastically reduced by two orders of magnitude. Toxicity changes of the other compounds were only slight.

4. DISCUSSION

The choice of negative control concrete was not an easy task. There are no historic records that can prove what vintage concrete was used in 1941 to construct this building. Over the years, the source of aggregates and the composition of the cement may have changed considerably. The only concrete available that was guaranteed to have never been exposed to chemical contamination was of newer vintage, and using that for toxicity comparison would be erroneous. The most likely choice was a loading dock located on the outside of the building. Due to low traffic, the southern-most end of the loading dock was the suggested sampling area for negative controls.

Figure 2. Effects of Concrete Particle Size on the Overall Water pH

Figure 3. Mortality of Daphnia Due to Increased pH Caused by Concrete Additions
ADDITION OF CONCRETE TO DISTILLED WATER

![Graph showing the effect of concrete addition to distilled water on pH and total hardness.](image)

ADDITION OF CONCRETE ADDED TO WELL WATER

![Graph showing the effect of concrete addition to well water on pH and total hardness.](image)

Figure 4. Concrete Additions to Distilled and Well Water Types
Figure 5. Change in Water Hardness with Salt Additions

Table. Toxicity of Compounds Before and After Being Added to 4000 mg/L of Concrete

<table>
<thead>
<tr>
<th>Compound</th>
<th>48 Hr EC_{50} (mg/L)*</th>
<th>48 Hr EC_{50} (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Concrete Added</td>
<td></td>
<td>With 4000 mg/L Concrete</td>
</tr>
<tr>
<td>Sodium Lauryl Sulfate</td>
<td>23.3</td>
<td>17.2</td>
</tr>
<tr>
<td>Copper Sulfate</td>
<td>0.04</td>
<td>1.42</td>
</tr>
<tr>
<td>β-aminoethylarylthio-sulfonate (VX-Simulant)</td>
<td>12.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Concrete (pH Adjusted)</td>
<td></td>
<td>&gt;4000.0</td>
</tr>
<tr>
<td>Concrete (no pH Adjustment)</td>
<td></td>
<td>2288.4</td>
</tr>
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</table>

*The effective concentration at which 50% of the organisms are immobilized. The lower the EC_{50}, the more toxic the material.
When concrete is added to water, the pH rises drastically. Concrete is primarily composed of several forms of silicate (CaSiO$_3$, CaSiO$_4$, and CaSiO$_5$). The following chemical reaction (Equation 1), with tricalcium silicate and water, creates calcium hydroxide, which causes the pH to rise. Similar reactions occur with all forms of calcium silicate cited above.

$$2\text{Ca}_3\text{SiO}_4 + 6\text{H}_2\text{O} \rightarrow \text{Ca}_3(\text{SiO}_4)\text{2(OH)}_3 + 3\text{Ca(OH)}_2$$ (1)

When conducting the daphnia assays, pH adjustments were necessary. At pH = 10 and above, daphnia mortality rises above 10%.* Even though no mortality was observed at 1000 mg/L of concrete before pH adjustments, investigators decided to conduct the assays up to 4000 mg/L and follow with pH adjustments. Adjusting the pH would allow four times the concrete load in water without having harmful pH effects and also provide higher contaminate concentrations.

The amount of time the concrete dust should stand in water to ensure total dissolution of possible contaminates was addressed. The pH of concrete dust stabilized after 6 hr. The pH stabilization trend followed the same pattern with the graded sizes of concrete particulate. Therefore, it was assumed that 24 hr was more than enough time to ensure that soluble contaminates would be dissolved. Concrete particle sizes of 20.0 mm did not increase the water pH to above tolerable limits for daphnia. However, 20.0-mm particles were not small enough to facilitate a suspension and were not available for the daphnia to ingest. Using the smallest particle sizes provided another pathway of possible toxicity through the ingestion of alkaline materials. Therefore, the concrete used in the assays was crushed to the finest powder the jaw crusher could provide (within the range of 3-55 μm).

During the initial phases of this study, assays were conducted using well water hardened with salts suggested by EPA. The investigators discovered that when concrete was added to water with these salts, the hardness would decrease dramatically. Knowing that concrete consists of mostly calcium silicates, the

water hardness was expected to increase, not decrease. The same phenomenon occurred in well water without the salts added. Studies using distilled water and concrete showed the hardness to increase as expected (Figure 4), proving that the constituents in the water were causing the reduction in hardness. It was determined that sodium bicarbonate in water caused a decrease in hardness as concrete concentration increased (Figure 5). The following equation (Equation 2) theorizes the possible chemical reaction that may cause an ion exchange phenomenon to occur with the calcium in concrete:

\[
\text{sodium bicarbonate} \quad \text{NaHCO}_3
\]

\[
\rightarrow \text{Na}^+ + \text{HCO}_3^- \rightarrow \text{H}^+ + \text{CO}_3^{2-} \quad (2)
\]

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3
\]

Sodium bicarbonate is introduced into the well water due to the materials used for pH adjustment needed for iron removal purposes. When calcium binds to the carbonate ion to form a precipitate, free calcium is removed from the water column. Calcium carbonate is reported to be practically insoluble in water. This phenomenon needs to be investigated further to determine the exact chemical reaction causing the hardness to decrease.

Three compounds of known toxicities were used to spike concrete solutions of 4000 mg/L (table). It was hoped that when toxicants were added to concrete, a synergistic effect would occur, making the daphnia test a more sensitive model. However, this was not the case. There was no significant change in the toxicity of the two organic compounds. The toxicity of copper sulfate was decreased two orders of magnitude. It has been shown in numerous publications that pH and water hardness have significant affects on metal toxicity. The higher the hardness, the less toxic metals are to aquatic organisms. Since the water hardness decreased below control levels with the concrete additions, it was expected that the toxicity of copper sulfate would increase; however, this was not the case. Even though CuSO₄ toxicity was reduced when mixed in concrete solutions, it was still highly toxic to daphnia. Further research is needed using other metal compounds mixed with concrete to determine if similar results will occur.

The question of daphnia response to agent or other contamination below analytical detection limits still remains. Studies using low level agent contamination in concrete is needed.
to fully test daphnia response to these types of materials. Future experiments will look at concrete contaminated with mustard used as a positive control to fully test the validity of this model.

5. CONCLUSIONS

As a result of the study conducted, the following conclusions are provided:

- The pH of 4000 mg/L of concrete stabilized after 6 hr; therefore, it was determined that 24 hr was enough time for possible contaminates to leach out of the concrete powder.

- Calcium hydroxide formation caused the pH to increase dramatically, making it necessary to run daphnia assays under pH adjusted conditions.

- Sodium bicarbonate caused the hardness of concrete solution to drop considerably.

- Conducting daphnia assays using 4000 mg/L of concrete, under pH adjusted conditions, allows a higher concrete load in solution; therefore, increasing the concentration of possible low level contaminates.

- Copper sulfate mixed with 4000 mg/L of concrete showed a reduction in toxicity by two orders of magnitude.
LITERATURE CITED


4. Hardness Kits, Ecological Instruments, Bohemia, NY.


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<tr>
<td>291-64-5</td>
<td>Cycloheptane</td>
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<tr>
<td>106-44-5</td>
<td>4-methylphenol</td>
</tr>
<tr>
<td>3913-71-1</td>
<td>2-decanol</td>
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<tr>
<td>620-17-7</td>
<td>3-ethylphenol</td>
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<td>84-66-2</td>
<td>Diethyl phthalate</td>
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<td>544-63-8</td>
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<td>123-29-5</td>
<td>Nonanoic acid, ester</td>
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<td>57-10-3</td>
<td>Hexadecanoic acid, ester</td>
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<td>111-48-8</td>
<td>Thiodiglycol, (HD)</td>
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<td>2-chlorovinylarsenious acid, (CVAA)</td>
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<td>Bis(2-chloroethyl)ethylamine, (Mustard, HN1)</td>
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<td>Pinacolylmethylphosphonofluoridate,</td>
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* - CAS Number not available
APPENDIX B
WATER QUALITY MEASUREMENTS USED IN MONITORING WELL WATER

MICROBIOLOGICALS
Total Coliform

METALS
Arsenic
Barium
Cadmium
Chromium
Copper
Iron
Lead
Manganese
Mercury
Selenium
Silver
Sodium
Zinc

INORGANICS AND PHYSICAL PARAMETERS
Alkalinity
Chloride
Fluoride
Nitrite
Nitrate
Hardness
pH
Total Dissolved Solid
Turbidity

ORGANICS
Bromoform
Bromodichloromethane
Chloroform
Dibromochloromethane
Benzene
Vinylchloride
Carbon tetrachloride
1,2-Dichloroethene
Trichloroethylene
1,4-Dichlorobenzene
1,1-Dichloroethylene
1,1,1-Trichloroethane
Bromobenzene
Bromoethane
Chlorobenzene
Chloroethane

ORGANICS CONT
Chloroethylvinyl ether
Chloromethane
0-Chlorotoluene
P-Chlorotoluene
Dibromochloropropane
Dibromomethane
1,2-Dichlorobenzene
1,3-Dichlorobenzene
Dichlorodifluoromethane
1,1-Dichloroethane
Trans-1,2-Dichloroethylene
Cis-1,2-Dichloroethylene
Dichloromethane
1,2-Dichloropropane
Trans-1,3-Dichloropropene
Cis-1,3-Dichloropropene
2,2-Dichloropropane
1,1-Dichloropropene
1,3-Dichloropropene
Ethylbenzene
Ethylene dibromide
Styrene
1,1,1,2-Tetrachloroethane
1,1,2,2-Tetrachloroethane
Tetrachloroethylene
Trichlorobenzene
1,1,2-Trichloroethane
Trichlorofluoromethane
1,2,3-Trichloropropane
Toluene
Xylene

ORGANICS (Pesticides)
Alachlor
Atrazine
Chlordane
Aldrin
Dichloran
Dieldrin
Endrin
Heptachlor
Heptachlor Epoxide
Hexachlorobenzene
Hexachloropentadiene
Lindane
Methoxychlor
PCBs
Pentachloronitrobenzene
Silvex 2,4,5-TP
Simazine
Toxaphene
Trifluralin
2,4-D