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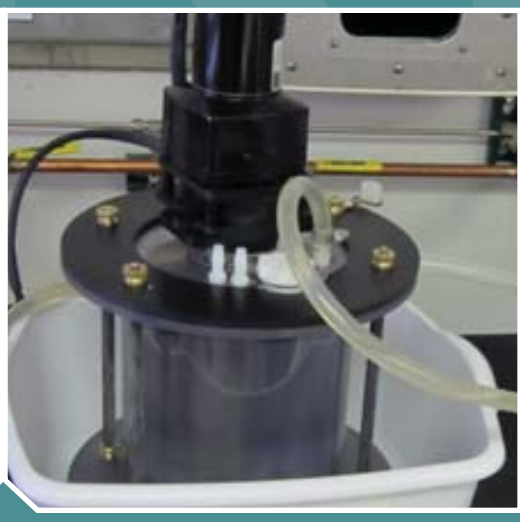
Chemical Contaminant Persistence and Decontamination in Drinking Water Pipes

Results using the EPA Standardized Persistence and Decontamination Experimental Design Protocol



Office of Research and Development
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LIST OF ABBREVIATIONS

AR	annular reactor
ASTM	ASTM International
AWWA	American Water Works Association
cm	centimeters
cfu	colony forming units
EPA	U.S. Environmental Protection Agency
°C	degrees Celsius
F	flushing
ft/s	feet per second
h	hour
HC	hyperchlorination
HPC	heterotrophic plate counts
IC	ion chromatography
IS	internal standard
in.	inch
g	gram
GC-MS	gas chromatographic mass spectrometry
K_{ow}	octanol-water partitioning coefficient
KOH	potassium hydroxide
LOQ	limit of quantitation
LFM	laboratory fortified matrix
L	liter
Lpm	liter per minute
µL	microliter
µg	microgram
mA	milliamp
mM	millimolar
mg	milligrams
mm	millimeters
mL	milliliters
min	minute
ng	nanogram
NHSRC	National Homeland Security Research Center
QAPP	Quality Assurance Project Plan
QC	quality control
%R	percent recovery
%P	percent persistence
PE	persistence evaluation
PDEDP	Persistence and Decontamination Experimental Design Protocol
PVC	polyvinyl chloride
rpm	revolutions per minute
s	second
SIM	selected ion monitoring
SPME	solid phase micro extraction

EXECUTIVE SUMMARY

The objective of this project was to develop and test a standardized Persistence and Decontamination Experimental Design Protocol (PDEDP) that could be used across laboratories to perform pipe decontamination research. To test the protocol for chemical contaminants, data were collected pertaining to the adsorption, persistence, and possible decontamination approaches for chlordane and sodium fluoroacetate (SFA) on cement-lined and polyvinyl chloride (PVC) pipe material.

Experimental Design Protocol. Implementation of the PDEDP simulated conditions within operational drinking water pipes using annular reactors (AR). The ARs consist of a glass outer cylinder and a rotating polycarbonate inner cylinder with 20 flush mounted rectangular coupons that are made of materials that simulate drinking water pipe materials. The annular reactor was selected because it is relatively inexpensive, permits the protocol to be easily reproduced across different laboratories, and eliminates potential variability among various studies.

For this work, cement-lined and PVC coupons were used. Shear stress was applied to the coupon surfaces by setting the AR inner cylinder rotation to 100 revolutions per minute (rpm), which produces flow similar to 1 foot per second (ft/s) (30.5 centimeters (cm)/s) in a 6 inch (15.2 cm) diameter pipe[*]. For the flushing evaluation, the AR inner cylinder rotation was set to 200 rpm (1.64 ft/s) (50.3 cm/s) and subsequently 250 rpm (1.91 ft/s) (58.2 cm/s) to simulate increased flow[*]. During normal operation, the flow of drinking water through the AR (connected directly to the tap) was maintained at a mean velocity of 200 milliliters (mL) per minute so that the mean residence time of the water in the AR was 5 minutes. Prior to use of any pipe material coupons, a biofilm was grown on all of the coupons.

The PDEDP includes five components:

- Surface extraction method verification - determines if a contaminant could be extracted from a pipe material surface
- Surface contamination method verification - determines if the pipe material coupon would be contaminated when exposed to bulk solution of contaminated water
- Persistence evaluation – pipe material coupons contaminated and then exposed to fresh tap water in ARs operating at 100 rpm (1 ft/s)
- Flushing evaluation – pipe material coupons contaminated and then exposed to fresh tap water in ARs operating at 200 rpm (1.64 ft/s) or 250 rpm (1.91 ft/s)
- Hyperchlorination evaluation – pipe material coupons contaminated and then exposed to solutions of 25 mg/L and 50 mg/L of free chlorine in ARs with no rotation

Chlordane on Cement Results. The surface extraction method confirmed that chlordane can be extracted from the cement after direct

[*] Based on calculations provided in the User Manual of the BioSurface Technologies (421 Griffin Drive #2, Bozeman, MT 58715) Model 1120/1320 LS Biofilm Annular Reactor. Assumes a Hazen-Williams coefficient of 120. Corresponding Reynolds Numbers calculated for velocities of 1.0, 1.64, and 1.91 ft/s are 53800, 88771, and 102759 respectively (all Re are turbulent flow).

contamination of the coupon. The surface contamination method verification confirmed that a cement coupon can be contaminated with chlordane by exposing it to a solution of contaminated water. The results from the persistence and flushing evaluations exhibited results that were very similar to one another. The percent persistence (%P) after 24 h for the persistence evaluation (AR operated at 100 rpm) was 9% ± 3% and the %P after 24 h during the flushing evaluation (AR operated at 200 rpm) was 6% ± 1%. Results from the hyperchlorination evaluation showed that hyperchlorination without increased flow is not an effective means of decontaminating chlordane from cement.

Chlordane on Polyvinyl Chloride Results. The surface extraction method verification confirmed that chlordane can be extracted from the PVC surface after direct contamination of the PVC coupon. The surface contamination method verification confirmed that a PVC coupon can be contaminated with chlordane by exposing it to a solution of contaminated water. The results from the persistence and flushing evaluations for the PVC exhibited very similar results. The %P after 24 h for the persistence evaluation (AR operated at 100 rpm (1 ft/s)) was 14% ± 4% and the %P after 24 h during the flushing evaluation (AR operated at 200 rpm (1.64 ft/s)) was 14% ± 6%. Again, as for the chlordane on cement testing, results from the hyperchlorination evaluation showed that hyperchlorination without flow is not an effective means of decontaminating chlordane from PVC.

Sodium Fluoroacetate on Cement Results. The surface extraction method confirmed that SFA can be extracted from the cement after direct contamination of the coupon. The surface contamination method verification confirmed that a cement coupon can be contaminated with SFA by exposing it to a solution of contaminated water. The results from the persistence, evaluation, and hyperchlorination evaluations showed that SFA was persistent in each of these experimental scenarios.

Future Research Needs. This work has laid the foundation for a PDEDP that can be adapted to accommodate additional research priorities. Below are a few possible areas for further study:

- Importance of biofilm to pipe decontamination research – During the SFA surface contamination method verification step, two cement coupons without biofilm (only two because of the limited capacity of the AR and the fact that this impromptu experiment was outside the context of the PDEDP) were contaminated with SFA along with the coupons covered with biofilm. For these two coupons, five times as much SFA was adsorbed as the coupons with biofilm. This very limited data set suggested that the presence or absence of biofilm could significantly impact the results of pipe adsorption/decontamination research. More rigorous experimentation would need to be performed to better characterize the role of biofilm, which is typically expected in actual field studies.
- Broadening of adsorption/decontamination data set by expanding on list of chemical contaminants tested using the PDEDP (e.g., organophosphates as available toxic chemicals and simulated chemical agents, metals to simulate heavy metal, or radiological contamination).
- Study of adsorption/decontamination of

biological organisms using the PDEDP.

- Use of additional pipe materials with additional chemicals and biological organisms as well as additional chemical pipe cleaning materials as possible decontamination agents.
- Research on shearing stress, dynamic pressure, and the effects that laminar, transient, and turbulent flow has on biofilm removal as part of the PDEDP on different diameter pipes.
- Scaling up of AR experiments into experiments with real pipe using a pipe loop in order to study how well the AR experiments translate into scenarios with real pipe.
- Study of risk assessment questions addressing how much persistence of various chemicals is acceptable.

INTRODUCTION

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) conducts research to protect, detect, respond to, and recover from terrorist attacks on the nation's water and wastewater infrastructure. The objective of this project was the development and testing of a standardized Persistence and Decontamination Experimental Design Protocol (PDEDP) to quantitatively determine the persistence of individual priority contaminants to various drinking water pipe materials as well as the testing of techniques for decontaminating affected pipe surfaces if the contaminant persists. This report provides a summary of the results from the testing that was performed following the development of the experimental design protocol, which is included in Appendix A. As thoroughly described in the PDEDP, testing included use of an annular reactor (AR) as the device used to simulate flow past materials from which drinking water pipe is made. The annular reactor was selected because it is relatively inexpensive, permits the protocol to be easily reproduced across different laboratories, and eliminates potential variability associated with laboratories constructing their own apparatus, which would likely occur even with detailed instructions. Annular reactors have been used for several previous EPA persistence and decontamination studies[4,5].

The drinking water pipe materials used for the study included cement–lined (with contaminants chlordane and sodium fluoroacetate [SFA]) and polyvinyl chloride (PVC) with only chlordane. These two contaminants were selected based in part on their absorption properties; chlordane is a low solubility organic while SFA is ionic. Specifically, the absorption characteristics of a chemical can be described by its octanol-water partitioning coefficient (K_{ow}). Chemicals with high K_{ow} values are more likely to partition out of the water and onto the pipe surface and chemicals with low K_{ow} values are more likely to remain in the water than absorb onto the pipe. Of the two contaminants, chlordane is the high K_{ow} value contaminant (log K_{ow} of 6.2) and sodium fluoroacetate is a chlorine resistant contaminant with ion-exchange (log K_{ow} of -0.061) sorption characteristics. The following report includes a summary of the experimental design as well as study results, with one section dedicated for each pipe material and contaminant combination that was tested.

1. SUMMARY OF EXPERIMENTAL DESIGN PROTOCOL

This project included five components of testing that were completed for each combination of pipe material and contaminant. They included 1) the surface extraction method verification, 2) the surface contamination method verification, 3) the persistence evaluation, 4) the flushing evaluation, and 5) the hyperchlorination evaluation. Summaries of the experimental set up, each component of the experimental design, and details of the analytical methods are provided below.

1.1. Experimental Reactor System

For the persistence and decontamination experiments described in this experimental design, the conditions within operational drinking water pipes were simulated in annular reactors (AR) (BioSurface Technologies Corporation, Bozeman, MT). The ARs consist of a glass outer cylinder and a rotating polycarbonate inner cylinder with 20 flush mounted rectangular coupons that are made of materials that simulate drinking water pipe materials. For this testing, cement-lined and PVC coupons (BioSurface Technologies Corporation, Bozeman, MT) were used. For the cement-lined coupons, the cement used for the coupons met the requirements of the C150-07 American Society for Testing and Materials (ASTM) Standard Specification for Portland Cement[2] and the thickness of the cement was approximately 1.3 mm, slightly less than as specified in American Water Works Association (AWWA) C104-03 Standard for Cement-Mortar Lining for Ductile-Iron Pipe and Fittings for Water[3]. The cement coupons were made from a polycarbonate backing with the cement applied at the above thickness. Because of the porosity of the cement, some of the contaminants passed through the cement and adsorbed to the polycarbonate backing. Therefore, the cement was separated from the polycarbonate and the two components were analyzed separately. The PVC coupons were made entirely of PVC so no separation was required. In this manner, the adsorption to the infrastructure material could be investigated independent of other adsorption processes occurring in the AR set-up.

The coupons had surfaces that were 0.55 inch (in.) (14 millimeters (mm)) × 5.8 in. (148 mm). Shear stress was applied to the coupon surfaces by setting the inner AR cylinder rotation to 100 revolutions per minute (rpm), which produces shear similar to 1 foot (ft)/second (s) (30.5 centimeter (cm)/s) flow in a 6 inch (in.) (15.2 cm) pipe[4]. For the flushing evaluation, the AR inner cylinder rotation was set to 200 rpm (1.64 ft/s) (50.3 cm/s) and subsequently 250 rpm (1.91 ft/s) (58.2 cm/s) to simulate increased flow. During normal operation, the flow of drinking water through the AR (connected directly to the tap) was maintained at a mean velocity of 200 milliliters (mL) per minute, so the mean residence time of the water in the AR was five minutes. This flow velocity prevented the depletion of chlorine level over the course of the experiments. The short residence time decreased the chance that desorbing contaminant could re-contaminate a surface.

Columbus, Ohio tap water from the laboratory faucet was used for the study and no range of water quality parameters was specified. However, experience in the same laboratory has shown that the free chlorine level is typically between 1.0 mg/L and 2.0

mg/L, the pH between 7.5 and 8.0, and the temperature between 22 and 25 degrees Celsius (°C). The pH, temperature, and free chlorine concentration of the drinking water was measured daily using a multi-parameter water monitor (Rosemount Analytical Model WQS, Rosemount Analytical, Irvine, CA). The ARs were always operated in the dark by covering them completely with aluminum foil. Because some contaminant was likely to adsorb onto the non-coupon components of the AR and affect the amount of contaminant that was available for coupon contamination, the concentration of the bulk contamination solutions was measured to ensure that an adequate concentration of contaminant was maintained to achieve coupon contamination.

1.2 Coupon Biofilm Growth

Prior to performing each component of the PDEDP, a biofilm was grown on all of the coupons by submerging the required number of coupons into a container (an 8L plastic tub) that allowed recirculation of dechlorinated tap water (outlet near the top of the container and inlet near the bottom of the container) fortified with 1 gram (g) of yeast extract as a nutrient to stimulate more rapid biofilm growth. This container was filled with water and kept in the dark (to better simulate biofilm growth in an enclosed pipe) and recirculated using a pump for at least four days with an additional 1 g of yeast added after every two days. The biofilm growth was measured, using heterotrophic plate counts (HPC), from one of the coupons in the biofilm growth container. However, there was not a strict biofilm density required for use in experiments. Following the detailed procedure included in the PDEDP, coupons to be measured for HPC were centrifuged in a Triton X solution, mixed using a vortex mixer, and then decanted. Two tenfold dilutions of that decanted solution were prepared and plated in triplicate on tryptic soy agar plates (Rainin L200, L19304, Rainin Instrument LLC, Oakland, CA). After incubation for 48 hours at 35-37 °C, the distinguishable colonies on each plate were counted and surface density of HPC was calculated by dividing the number of colonies by the surface area of the coupons.

Throughout the cement and PVC experiments, seven sets of coupons were used and the HPC densities were determined for six of the seven sets. On average, the HPC densities were 1.6×10^6 colony forming units (cfu)/cm^2. The standard deviation of the HPC densities was 1.3×10^6 cfu/cm^2. The HPC concentration in the biofilm growth water was determined for all seven sets of coupons. The average HPC concentration was 6.3×10^5 cfu/mL with a standard deviation of 1.1×10^6 cfu/mL. While there was not a target HPC density to be grown on the pipe material coupons, the consistent growth of biofilm (densities within one log of one another) provided a means to simulate pipe conditions on pipe material coupons.

The one set of coupons for which no HPC measurement was made was used for the cement-chlordane persistence evaluation. The HPC measurement was not made because the colonies on the enumeration plates of the dilution level used were too few to count. A more concentrated dilution (that had been refrigerated for two days) was then plated and incubated, but there were again too few colonies to count. The concentration of HPC in the water used for biofilm growth on that set of coupons was 9.0×10^4 cfu/mL, which was similar to the water HPC concentrations measured in the biofilm growth water for the rest of the coupons. Because none of the other biofilm

growth conditions had been altered and there were similar levels of HPC in the biofilm growth water, it was determined that colony growth from the more concentrated dilution was apparently inhibited by storage during the incubation of the original plate and it was likely that there had been biofilm on that set of coupons.

1.3 Pipe Coupon Contamination Method Verification Experiments

The generation of persistence and decontamination data from this experimental design included contamination of coupons by exposing them to bulk solutions of chlordane and SFA. Thereafter, the persistence of each contaminant on the coupons and/or the application of a decontamination approach were investigated to determine both the propensity of each contaminant to persist on the coupons and the effectiveness of decontamination approaches in removing the applicable contaminant from the coupon surface. The usefulness of results from such experiments relies on the accuracy of the required contaminant measurements. In order to be confident in these measurements, two important questions needed to be answered about the approach to contaminant measurement.

- When adsorbed to the coupon surface, how well can a contaminant be extracted from that surface?
- When a coupon has been exposed to a bulk solution at a given concentration, how much of the contaminant is adsorbed to the coupon surface?

To answer these two questions, two method verification steps were conducted as the first two steps of the experimental design. First, the surface contamination extraction method was validated. Second, the coupon surface contamination method was validated.

1.3.1 Method Verification Step 1: Surface Contamination Extraction

The purpose of this step is to determine whether it is possible to extract the contaminant if adsorbed to a pipe material surface. The extraction must be statistically quantifiable in order to make valid conclusions about contaminant removal; otherwise the extraction procedure must be further developed. The verification required 20 half coupons of the applicable material type with a biofilm developed as described in Section 1.1. These coupons were removed from the biofilm growth container and allowed to air dry until water droplets were not visible on the surface, but the surface was still damp (mean time of seven minutes). This drying step ensured that the contaminant was added to the coupon surface and not to the water remaining on the coupon surface following the time period that the coupon was immersed in water during biofilm growth.

Each coupon, including blanks, was cut in half with scissors and five drops of contaminant solution were applied directly to each half coupon using a micropipette (Eppendorf Research Plus, Eppendorf International, Hauppauge, NY) approximately 10 mm apart. For chlordane, the volume of each drop was 5 µL and for sodium fluoroacetate, the drop volume was 15 µL. This verification included low, medium, and high spike levels to determine the effectiveness of the extraction at various contamination levels. Table 1 gives the concentration of the three chlordane and SFA spiking solutions.

Table 1. Contaminant Analytical Techniques, Limit of Quantitation, and Stock Solution Concentrations

Contaminant	Analytical Technique	Approx. Limit of Quantitation	Concentration of Spike Solutions
Chlordane	Gas Chromatographic Mass Spectrometer	0.002 mg/L	0.8, 4, and 40 mg/L
Sodium Fluoroacetate	Ion Chromatography	0.1 mg/L	133, 667, 6,667 mg/L

Each coupon received drops of a different contaminant concentration and each concentration was applied to five coupons (for a total of 15 coupons per contaminant). The drops were allowed to air dry until they were not visible on the surface (mean of seven minutes) to ensure that the contaminant was being extracted from the surface of the coupon (and not from a droplet of spiking solution). Five non-contaminated coupons were also extracted as blanks.

The surface contamination extraction method included the extraction of the entire coupon, both the cement surface and the polycarbonate backing supporting the cement. The cement coupons were extracted (for separate analysis) by removing the cement from the polycarbonate backing and placing the cement and polycarbonate backing into separate test tubes (Kimble #73785-50, VWR, West Chester, PA or Fisherbrand #03-337-14, Fisherbrand, Pittsburgh, PA) filled with an appropriate extraction solvent. The extraction solvent for chlordane was 9:1 hexane:acetone and for SFA, ASTM International (ASTM) Type I water. For the chlordane extractions, after inserting both components of the coupons into separate test tubes, the test tubes were sealed with a cap and sonicated for 10 minutes, solvent decanted and replaced with fresh solvent, and then sonicated for another 10 minutes. The decanted solvent was combined. The resulting solution was centrifuged and supernatant solution collected for analysis.

The SFA coupons were extracted in a similar manner but only one sonication step was performed. The PVC coupons required no separation and were extracted following the same method as for the polycarbonate backing of the cement coupons. For chlordane, the extraction solution was concentrated using nitrogen evaporation prior to analysis using a gas chromatograph-mass spectrometer (GC-MS). For SFA, ion chromatography (IC) was used as the measurement technique without sample concentration.

The percent recovery (%R) was calculated using the following equation

$$\%R = \frac{C_R}{C_o} \times 100$$

where C_R is the mass of contaminant recovered from the coupon surface (area 22.5 cm^2) and C_o is the mass of contaminant originally dispensed onto the coupon surface.

1.3.2 Method Verification Step 2: Surface Contamination

Step 2 involved validating a method to contaminate the surface of the coupons in a way that simulates an actual intentional contamination of a water distribution system. The surface contamination method to be validated incorporated:

- Preparing coupons with biofilm
- Exposing the coupons to contaminated water (1 mg/ liter [L] -

chlordane and 500 mg/L – SFA) in the AR without flow (batch mode)
- Extraction of the contaminant from the coupon using the method validated in Step 1.

To begin the verification, 10 coupons were prepared with a biofilm. Then, the AR was filled with contaminated water at the above concentration levels and five of the coupons were added to the AR and five were collected as blank samples. Then, the AR was operated at 100 rpm (1 ft/s), but the flow of tap water through the AR was stopped to increase the contact time between the contaminated water and the coupons. Two hours following the contamination of the water, the coupons were removed, rinsed twice with 25 mL of ASTM Type I water (which was then discarded), and then extracted and analyzed following the surface contamination extraction and measurement method described in Section 1.3.1. This rinse step was to ensure that the contaminant is extracted from the surface of the coupon and not just an artifact of residual contamination solution on the surface of the coupon. The bulk solution was sampled at the start of the contamination time period, at the half-way point, and at the end and the concentration of contaminant was measured via the applicable measurement technique to confirm the availability of the contaminant for adsorption.

1.4 Evaluation of Contaminant Persistence

This section describes the approach to evaluating the persistence of a contaminant on various pipe coupon materials. Table 2 provides an overview of the persistence evaluation (PE). For each combination of coupon material and contaminant, biofilm was grown on 20 coupons as described in Section 1.2. Two coupons with biofilm were the non-contaminated blank coupons and the rest of the coupons were contaminated with a bulk solution following the surface contamination method. Immediately following the coupon contamination step, three coupons were removed to serve as control coupons. The amount of contaminant on the surface of these control coupons were compared with the amount remaining on the coupons that were left in the AR for various lengths of time following the removal of the control coupons.

Thereafter, a stopped flow scenario was evaluated by stopping the rotation of the AR and stopping the flow of water through the AR (after the contaminant water is replaced by uncontaminated drinking water). This stopped flow scenario was held for 24 hours after which three PE coupons were removed. After that 24 hour period, the flow of drinking water and AR rotation was resumed to normal operating conditions (AR rotating at 100 rpm (1 ft/s) and tap water flow through the AR at 200 mL/min, with a mean hydraulic retention time of 5 minutes.

Table 2. Persistence Evaluation

PE Step	Description	Coupons removed (20 total)
PE 1	Developed biofilm (confirmed with heterotrophic plate count) on 20 coupons; remove two coupons as blank control coupons	2
PE 2	Stopped flow through AR, filled AR with contaminated bulk solution concentration, inserted 18 coupons into AR, operated AR at 100 rpm, waited 2 hours	0
PE 3	Sampled bulk contamination solution at start, half-way point, and end of contamination period	0
PE 4	Following 2 hour contamination period, removed three coupons as contaminated control coupons	3
PE 5	Stopped AR rotation to simulate stopped flow. Replaced bulk contamination solution with uncontaminated water and remained at stopped flow for 24 hours; collected three coupons	3
PE 6	Restarted the AR rotation and flow through the AR. Removed three coupons at 4 hours, 1 day, 3 days, and 7 days after restart of AR rotation and flow	12
PE 7	Measured amount of contaminant remaining on coupons and compared to amount remaining on contaminated control coupons	0

Following the stopped flow scenario, sets of three PE coupons were collected from the AR at four different time increments (4 hours, 1 day, 3 days, and 7 days) following the resumption of flow. Following the removal of each of these sets of PE coupons, they were extracted and the amount of contaminant on the coupon surfaces compared with the amount on the control coupons collected just after the coupon contamination step.

This comparison was made by calculating the percent persistence (%P) of the contaminant on the coupons as described by the following equation.

$$\%P = \frac{C_{PE}}{C_C} \times 100$$

where C_{PE} is the mass of contaminant recovered from the coupon surface and C_C is the average mass of contaminant originally measured from the surfaces of the control coupon surfaces.

1.5 Evaluation of Decontamination Approaches

This section describes the evaluation of two approaches to decontaminating pipe, flushing (F) and hyperchlorination (HC). Table 3 provides an overview of the flushing evaluation and Table 4 provides an overview of the HC evaluation. As was the case for the persistence evaluation, a biofilm was grown on 20 coupons of the desired material and 18 were loaded in the AR and contaminated using the validated surface contamination method. Then three contaminated coupons were removed to serve as the control coupons. The amount of contaminant on the surface of these control coupons were compared with the amount remaining on the coupons that were left in the AR (operated under increased flow conditions to simulate flushing).

For the flushing evaluation, following coupon contamination, the AR inner cylinder rotation was raised from 100 rpm (1 ft/s) to 200 rpm (1.64 ft/s), which

corresponded to a water velocity of 0.5 ms^{-1} in a 15.2 cm (6 in.) pipe[3]. This increased rotational speed was held for one day. Sets of three coupons were collected from the AR at three different time increments (1 hour, 4 hours, and 1 day) following the coupon contamination. Then, the rotational speed was increased again to 250 rpm (1.91 ft/s) and held for another day, with the collection of three coupons after 4 hours and after 1 day of 250 rpm (1.91 ft/s) conditions. Following the removal of each set of three coupons, the coupons were extracted and the amount of contaminant on the coupon was compared with the amount on the control coupons collected just after the surface contamination step. Comparisons were made using a recognized statistical approach, as illustrated in the study results.

The evaluation of hyperchlorination as a decontamination approach was performed as shown in Table 4. The evaluation was started in a similar way as for the flushing evaluation. However, instead of increasing the rotational velocity of the AR, the rotation of the AR was stopped and the drinking water flow through the AR was also stopped to simulate a stopped flow scenario. The free chlorine concentration was then increased first to 25 mg/L and then to 50 mg/L after several increments of time after which coupons were collected from the AR. This comparison was made by calculating the %P as described in the previous section.

Table 3. Evaluation of Flushing as Decontamination Approach

Step	Description	Coupons removed (20 total)
F 1	Developed biofilm (confirm with heterotrophic plate count) on 20 coupons of same material; removed two coupons as blanks	2
F 2	Injected enough contaminant into AR to make desired bulk concentration within AR; inserted 18 coupons and operated AR at 100 rpm, waited 2 hours	0
F 3	Sampled bulk contaminant solution at start, half-way point, and end of contamination time and sample bulk contamination solution	0
F 4	Following 2 hour contamination period, replaced bulk contamination solution with uncontaminated water and removed three coupons as contaminated control coupons	3
F 5	Increased AR rotational velocity to 200 rpm (1.64 ft/s) from original velocity of 100 rpm (1 ft/s)	0
F 6	Removed three coupons at 2 hours, 4 hours, and 1 day following increase in rotational velocity	9
F 7	Increased AR rotational velocity to 250 rpm (1.91 ft/s) from 200 rpm	0
F 8	Removed three coupons at 4 hours and 1 day following increase in rotational velocity to 250 rpm	6
F 9	Measured amount of contaminant remaining on coupons and compared to amount remaining on contaminated control coupons	0

Table 4. Evaluation of Hyperchlorination as Decontamination Approach

Step	Description	Coupons removed (20 total)
HC 1	Developed biofilm (confirm with heterotrophic plate count) on 20 coupons of same material; removed two coupons as blanks	2
HC 2	Injected enough contaminant into AR to make desired bulk concentration within AR; inserted 18 coupons and operate AR at 100 rpm, waited 2 hours	0
HC 3	Sampled bulk contaminant solution at start, half-way point, and end of contamination time and sampled bulk contamination solution	0
HC 4	Following the 2 hour contamination period, replaced bulk contamination solution with uncontaminated water and removed three coupons as contaminated control coupons	3
HC 5	Stopped flow through AR and stopped rotation of AR; increased free chlorine concentration to 25 mg/L	0
HC 6	Removed three coupons at 2 hours, 4 hours, and 1 day following increase in free chlorine concentration	9
HC 7	Increased free chlorine concentration to 50 mg/L	0
HC8	Removed three coupons at 4 hours and 1 day following increase in free chlorine concentration to 50 mg/L	6
HC 9	Calculated percent persistence for all coupons by comparing residual contaminant on the surface with contaminated control coupons	0

1.6 Analytical Methods

1.6.1 Chlordane

The analytical standard for chlordane (Chem Service, West Chester, PA) was a mixture of the isomers alpha-chlordane (30%), beta-chlordane (37%), gamma-chlordane (10%), and trans nonachlor (23%). The relative abundances were determined through evaluation of peak areas during repeated analysis of a 100 nanogram (ng)/mL calibration standard. The standard solutions for chlordane were made in hexane. Trichlornate (ChemService, West Chester, PA) was used as the internal standard (IS). The samples were analyzed by GC-MS (Agilent 5973, Agilent, Santa Clara, CA) operating in the selected ion monitoring (SIM) mode. Table 5 gives details pertaining to the GC-MS:

Table 5. Information about the GC-MS

Component	Description
Analytical column	Rtx-5MS (Restek, Bellefonte, PA), 30m x 0.25 mm x 0.25 micrometer (μm) film or equivalent
Helium flow rate	1 mL/min
Injection volume	1-2 μL
Injection port	300°C, splitless for 0.75 min
Oven temperature program	120°C for 1 min, 120-300°C at 9°/min, hold 300 ºC for hold for 10 min
Transfer line temperature	300°C
Quantitation Ions	Chlordane 373/375/377 Trichlornate 297/299/269

Calibration standards were prepared at total chlordane concentrations from 2-1000 ng/mL. Each calibration standard contained the IS at a constant level. The calibration curve was analyzed followed by a blank and then the samples. The limit of quantitation (LOQ) for this method was 2 ng/mL. If the concentration of a sample exceeded the highest calibration point, that sample was diluted into the calibration range and re-analyzed.

Two continuing calibration check solutions (lowest and middle calibration levels, respectively) were analyzed after every 10 samples and at the end of the sequence in order to verify instrument sensitivity and calibration throughout the analysis. The results of these samples were targeted to be between 70 -130% of the known concentration. A laboratory reagent blank consisting of hexane was analyzed at the beginning of the sequence and bracketed all calibration and check standards in order to verify system cleanliness and prevent carryover. In addition, 200 ng/mL chlordane was added to a split sample of 10% of the total samples analyzed to create laboratory fortified matrix (LFM) samples. Target recoveries for the LFM samples were from 70-130%.

The coupon extracts for chlordane were concentrated to 1 mL and transferred to a GC-MS analysis vial for direct analysis. Sample concentration was performed using a TurboVap LV (Biotage, Charlotte, NC). In summary, the sample was transferred to the TurboVap LV tubes by rinsing the original extraction test tube. The nitrogen was turned on to 4 pounds per square inch and then the solution was checked periodically to determine remaining volume, taking care to avoid concentrating the sample below the target volume. Each sample was removed from the concentrator as the sample reached a final 1 mL volume. The bulk contamination solution samples were analyzed using solid phase micro extraction (SPME, Supelco 57341-U, 3-pack) to extract the contaminants out of the aqueous solution. A 3 mL volume of the bulk contamination solution was extracted by placing the samples in SPME vials (ChromSys, 18 03 1309-10mL) and analyzed directly by GC-MS. A relative determination of peak areas was used to evaluate if there was chlordane available for binding throughout the time period of contamination.

1.6.2 Sodium Fluoroacetate

SFA calibration standards were prepared in ASTM Type I water from a high purity (>99%) standard from Riedel-de Haën PESTANAL® Analytical Standard Catalog #36755 (Sigma-Aldrich, St. Louis, MO). The IC system consisted of a Dionex LC 20 with EG40 Eluent Generator, AS3500 Autosampler, GP40 Gradient Pump, and Dionex Ionpac® AS11 analytical column (4 x 250 mm) (Dionex, Bannockburn, IL). Table 6 gives a few details pertaining to the IC method. QC criteria are described subsequently.

Table 6. Information about the Ion Chromatograph

Component	Description
Detector	ED40 Electrochemical Detector working in conductivity mode with 5 milliamp (mA) suppression current
Mobile phase	0.5 millimolar (mM) potassium hydroxide (KOH) in ASTM Type I water at a 2.00 mL/minute (min) flow rate
Elution	Gradient starting at 0.5 mM KOH for 1.5 min followed by linear ramp from 0.5 mM to 10.5 mM KOH over next five minutes (2 mM/min). Next three minutes consist of cleanout step where mobile phase increases to 40 mM KOH. System re-equilibration then achieved by decreasing eluent concentration to 0.5 mM KOH for 14.5 min resulting in 24 minute run time

Quantitative analysis was performed using external standards. A five-point calibration curve was generated at the beginning of the sequence. The calibration levels ranged from 0.1 mg/ liter (L) to 2.5 mg/L. The LOQ for this method was 0.1 mg/L. If the concentration of a sample exceeded the highest calibration point, that sample was diluted into the calibration range and re-analyzed.

One continuing calibration check solution (0.5 mg/L) was analyzed after every 10 samples and at the end of the sequence in order to verify instrument sensitivity and calibration throughout the analysis. The acceptable recovery these samples was for their concentration to be between 90 -110% of the known concentration. A laboratory reagent blank consisting of ASTM Type I water was analyzed at the beginning of each sequence to verify system cleanliness. In addition, 0.5 mg/L of sodium fluoroacetate was added to a split sample of 10% of the total samples analyzed to create LFM samples. Acceptable recoveries for the LFM samples ranged from 75-125%. The calibration standards, water samples, and sample extracts were directly injected onto the IC at a volume of 100 µL.

1.7 Quality Control

Quality control samples for the contaminant reference methods including continuing calibration checks, laboratory blanks, and laboratory fortified matrix samples are described in Section 2. The data quality objectives for each of these samples are provided in Table 7. The acceptable ranges were intended to limit the error introduced into the experimental work.

Table 7. Data Quality Objectives for Contaminant Reference Methods

Method	Sample Type	QC Target
GC-MS analysis of chlordane	Continuing calibration check at lowest and middle calibration levels	70-130% of known concentration, include with each batch of 10 samples
	Laboratory reagent blank	<LOQ for analyte; include with each batch of 10 samples
	Laboratory fortified matrix samples	70-130% of known concentration; 10% of all samples
IC analysis of sodium fluoroacetate (similar to EPA Method 300.0)	Continuing calibration check at middle calibration level	90-110% of known concentration, include with each batch of 10 samples
	Laboratory reagent blank	<LOQ for analyte; include with each batch of 10 samples
	Laboratory fortified matrix samples	75-125% of known concentration; 10% of all samples

2. RESULTS REPORT

Testing of the PDEDP included use of chlordane and SFA with cement-lined AR coupons as well as chlordane with PVC AR coupons. The results are divided into separate sections for each combination of contaminant and coupon type.

2.1 Results from Testing with Chlordane on Cement Pipe Coupons

The following sections describe results from performing quality control, verification, and evaluation experimental design procedures for chlordane on cement pipe coupons.

2.1.1 Chlordane on Cement Quality Control Results

Continuing calibration verification (CCV) samples were analyzed on the GC-MS throughout each analysis set. After every 10 samples analyzed, a low concentration calibration solution (2 ng/mL or 5 ng/mL) and a middle concentration calibration solution (100 ng/mL) were reanalyzed. In addition, 10% of the samples were split and 200 ng of chlordane was spiked into the sample extract to create a laboratory fortified matrix (LFM) samples. Target recoveries for each of these QC samples were between 70% and 130%. Tables 8 and 9 show the results obtained during testing.

For the CCV samples, the recoveries of the low concentration samples ranged from 84% to 246%. These low concentration samples were very close to the LOQ so small changes in peak area greatly impacted the percent recoveries of the CCV samples. Specifically all four Step 1 surface extraction method verification low concentration CCV samples (recovery range:185%-198%) and two out of the four low concentration CCV samples exceeded the acceptable range of recoveries during the analyses applicable to both the flushing (210% and 246%) and hyperchlorination (132% and 149%) evaluations. However, no corrective action was taken with these results (i.e. results were used) because the peak areas measured during these components of the evaluation were closer to the middle and higher parts of the calibration curve. The recoveries of the middle concentration (100 mg/mL) CCV samples ranged from 64% to 118% with an average recovery of 79% with a standard deviation of 16%. The middle concentration CCV was never more than 6% outside the targeted acceptable range and within each sample set there was at least one CCV sample that was within the targeted range.

For the LFM samples, the recoveries ranged from 86% to 209%. All but two of the LFM samples that were outside of the targeted range of recovered occurred during the Step 1 and Step 2 method verification experiments, which were used to qualitatively determine the feasibility of extracting chlordane from the surface of the coupon as well as contaminating the surface from a bulk solution. One LFM each from the persistence and hyperchlorination evaluations were the only other LFM samples to be outside of the target range. No corrective action was made because the LFM samples that were outside of the targeted recovery range were paired with several other LFM samples that were recovered within the targeted range.

Table 8. Chlordane on Cement GC-MS Continuing Calibration Verification Results

Component of Testing	Low Calibration Standard (%R of Expected)	Mid Calibration Standard (%R of Expected)
Step 1 - Surface Extraction Method Verification	198%	70%
	196%	67%
	185%	68%
	199%	70%
	186%	66%
Step 2 - Surface Contamination Method Verification	121%	79%
	94%	75%
Persistence Evaluation	87%	73%
	85%	70%
	84%	69%
Flushing Evaluation	210%	97%
	246%	68%
	121%	64%
	100%	75%
Hyperchlorination Evaluation	103%	95%
	121%	92%
	132%	118%
	149%	110%
Average	**145%**	**79%**
Standard Deviation	**51%**	**16%**

Table 9. Chlordane on Cement Laboratory Fortification Matrix Sample Results

Component of Testing	Laboratory Fortified Matrix (%R)
Step 1 - Surface Extraction Method Verification	161%
	142%
	171%
	131%
Step 2 - Surface Contamination Method Verification	113%
	201%
	145%
Persistence Evaluation	150%
	110%
	129%
	86%
	119%
Flushing Evaluation	121%
	106%
	86%
Hyperchlorination Evaluation	128%
	116%
	209%
Average	**135%**
Standard Deviation	**34%**

The bulk contamination solution was sampled at the beginning, middle, and end of the 2 h contamination time during the Step 2 method verification, the persistence evaluation, the flushing evaluation, and the hyperchlorination evaluation. These samples were analyzed by direct SPME injection and a relative comparison of chlordane isomer peak areas was used to evaluate if there was chlordane available for binding throughout the time period of contamination. Across those four experiments, the peak areas of the initial 1 milligram (mg)/L contamination solution were considered the 100% chlordane levels. The samples collected at the 1 h point of the contamination step retained a 22%±2% of the peak area and the sample collected at the end of the contamination period retained 17%±2% of the peak area. Therefore, while there was a considerable loss of chlordane during the first hour, chlordane was available throughout the entire 2 h contamination period.

2.1.2 Method Verification Step 1: Chlordane on Cement Surface Extraction

The objective of this component of testing was to determine if chlordane could be extracted from the surface of the coupon. Cement coupons were spiked with 20 ng, 100 ng, and 1,000 ng of chlordane. When the chlordane was spiked onto the cement coupon, some of the chlordane adsorbed to the cement surface and some flowed through the cement and adsorbed to the polycarbonate backing on which the cement was mounted. The cement and backing were extracted separately using the method

described in Section 1.3.1 and the results were reported for both the cement and the backing for all five components of the testing. Table 10 gives the results including the amount of chlordane spiked onto the coupons, the amount extracted from the backing and cement, the total recovery, and the standard deviation. Overall, the total recovery ranged from 44% to 68% with standard deviations of the total percent recovered across the five replicates of less than 5%. This indicates that chlordane could be reproducibly extracted and measured from both the cement surface and the polycarbonate backing of the coupons. The amounts recovered from the backing and cement show that considerably more chlordane adsorbed to the cement surface than passing through the cement and adsorbing to the polycarbonate backing. The concentration of each spiking solution was confirmed using GC-MS. The low, middle, and high spiking solutions had percent recoveries of 76%, 83%, and 87% of the target concentration levels.

Table 10. Chlordane on Cement Surface Contamination Extraction

	Amount spiked (ng)	Avg. amount recovered from cement (ng)	Avg. amount recovered from backing (ng)	Avg. total recovered (ng)	Total % Recovery	SD
Low level	20	8.8	4.8	14	68%	2%
Mid level	100	34	13	47	47%	2%
High level	1000	340	100	440	44%	3%

Five replicates were spiked and extracted at each concentration level.

2.1.3 Method Verification Step 2: Chlordane on Cement Surface Contamination

This verification indicates if a contaminant will adsorb to the cement surface containing biofilm in the event that it is exposed to a bulk solution. Table 11 gives the results from the surface contamination method verification for chlordane on cement including the amount of chlordane extracted from each part of the coupon after a two hour exposure to 1 mg/L chlordane. Overall, an average of 3.1 µg ± 0.4 µg was adsorbed to the coupon surfaces (cement and backing combined) out of a total of 1,000 µg of chlordane (0.31%) that was available in the bulk contamination solution. Albeit to a small percentage, the results show that chlordane reproducibly adsorbed to the surface of the cement coupons as well as the polycarbonate backing.

During this experiment, very similar amounts of chlordane adsorbed to the cement surface and the polycarbonate backing. This was compared to the previous experiment during which the chlordane was spiked directly onto the coupons and more chlordane ended up adsorbing to the cement surface. It is not entirely clear what caused this, but it likely has something to do with the duration of contaminant exposure. In the first experiment, only five drops of contaminated solution were added to the coupon while in the second experiment, the coupon was equilibrated with the contaminated solution for two hours, providing more opportunity for the chlordane to come to equilibrium between the two components of the coupon.

Table 11. Chlordane on Cement Surface Contamination

Contaminated Coupon	Amount Recovered from Cement (µg)	Amount Recovered from Backing (µg)	Total Amount Recovered from Coupon (µg)
#1	1.0	2.3	3.3
#2	1.4	1.1	2.5
#3	1.3	2.1	3.4
#4	1.7	1.4	3.1
#5	1.3	1.4	2.8
Avg.	1.4	1.7	3.1
St. Dev.	0.3	0.5	0.4
%RSD	19%	30%	12%

2.1.4 Chlordane on Cement Persistence Evaluation

Figure 1 shows the results from the persistence evaluation for chlordane on the cement coupon surfaces as well as the polycarbonate backing. The vertical axes show the amount of chlordane remaining on the coupons after each time period (shown across the horizontal axis) during which fresh tap water is flowing through the AR and the AR is rotating at 100 rpm (1 ft/s). The average free chlorine concentration in the tap water during this evaluation was 1.54 mg/L ±0.17 mg/L, the average pH was 7.6 ± 0.1, and average temperature was 24.5°C ±0.7 °C. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The error bars on the graphs are the standard deviations of the remaining chlordane on the three coupons. The %P that corresponds with each time period is given across the top of each graph.

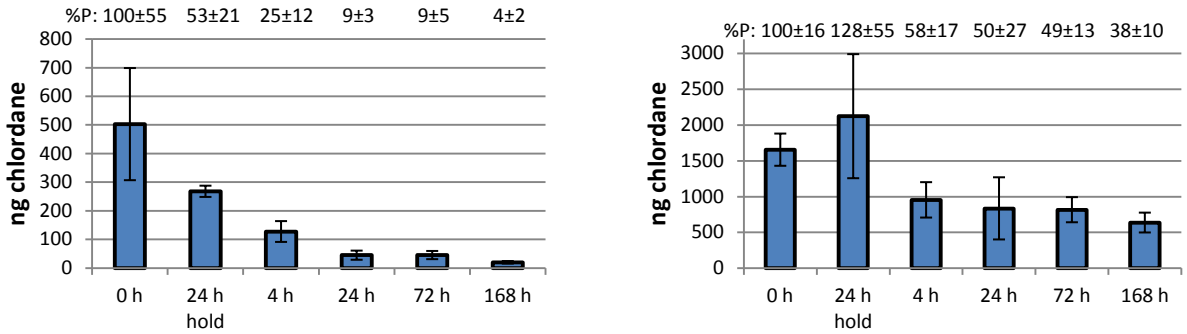

Figure 1. Persistence evaluation - percent persistence and chlordane remaining on cement (left) and backing (right)

In order to further clarify the data, t-tests were performed to determine what time periods exhibited significant differences from one another at the 95% confidence interval. The null hypotheses of the t-tests were that the difference in amount of chlordane remaining on the coupons across the various time periods was zero. The

probabilities (p) generated by the t-test were the probabilities of the null hypothesis being confirmed. Therefore, p-values less than 0.05 indicated a small likelihood that the difference between the two data sets was zero, and thus, are considered to be significantly different from one another.

Table 12 gives the p-values for comparisons of each possible set of coupons collected at the various time periods. The data that exhibited significant differences are highlighted in gray. For the cement, the initial contamination level was not significantly different from the 24 h hold level (largely due to the rather high variability in the initial concentration chlordane level), but the chlordane levels at the initial contamination, after the 24 h hold, and 4 h after resumption of flow were all significantly different from the chlordane levels collected 24 h, 72 h, and 168 h after the resumption of flow. Therefore, after the initial 24 h hold, the residual chlordane decreased until 24 h after the resumption of flow and then the chlordane residuals became steady. The cement was initially contaminated with 500 ng ± 200 ng of chlordane and 24 h after the resumption of flow, the chlordane levels had decreased to 45 ng ± 16 ng which was not significantly different from the levels at 72 h (46ng ± 14 ng) or 168 h (20ng ± 4 ng). The %P after 24 h of flow (after which there was no additional decrease) was 9% ± 3%.

For the backing, the chlordane residual decreased through 4 h after the flow was resumed and then there was no further decrease until 168 h. The 24 h, 72 h, and 168 h samples were not different from one another, indicating the steady residual after the 4 h sample. The backing was initially contaminated with 1,700 ng ± 230 ng of chlordane and 4 h after the resumption of flow, the chlordane levels had decreased to 950 ng ± 250 ng and no further significant decrease was noted until 168 h when the chlordane levels were 640 ng ± 140 ng. The %P after 168 h of flow was 38% ± 10%. The increased %P for the backing with respect to the cement was likely due to the fact that the shear of the flowing water more directly impacted the cement surface which served to shield the backing.

Table 12. Chlordane on Cement – Probability Value Matrix for Persistence Evaluation

		probability (p) values (< 0.05 - significant difference)				
	Persistence Evaluation Times	**24 h hold**	**4 h**	**24 h**	**72 h**	**168 h**
Cement	**0 h**	0.086	0.028	0.024	0.029	0.026
	24 h hold		0.010	0.002	0.004	0.001
	4 h			0.011	0.039	0.020
	24 h				0.486	0.078
	72 h					0.066
Backing	**0 h**	0.167	0.002	0.011	0.030	0.005
	24 h hold		0.043	0.018	0.077	0.041
	4 h			0.199	0.311	0.011
	24 h				0.481	0.213
	72 h					0.213

Read as matrix, for times at left, read right for p-value to determine possible differences.
Light shading – significant differences

2.1.5 Chlordane on Cement Flushing Evaluation

Figure 2 shows the results from the flushing evaluation for chlordane on the cement coupon surfaces as well as the polycarbonate backing. As was the case for the persistence evaluation, the vertical axes show the amount of chlordane remaining on the coupons after each time period and flushing condition that is shown across the horizontal axes. The average free chlorine concentration in the tap water during this evaluation was 1.45 mg/L ± 0.17 mg/L, the average pH was 7.6 ± 0.1, and average temperature was 25.4°C ± 0.3 °C. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The error bars on the graphs are the standard deviations of the remaining chlordane on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of each graph.

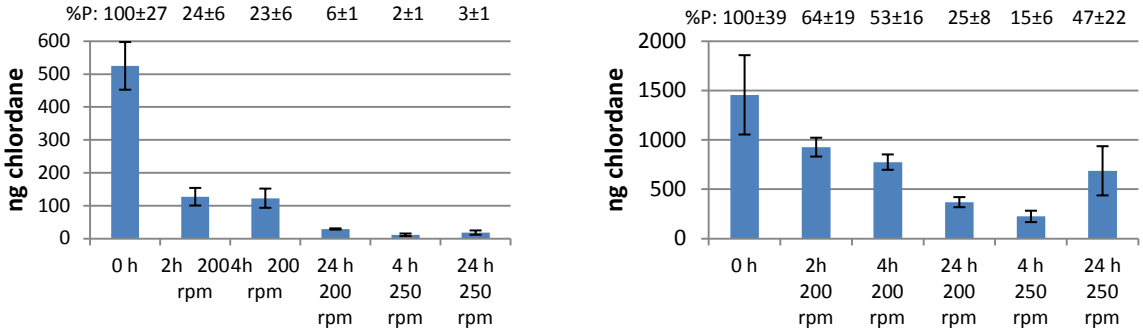

Figure 2. Flushing evaluation - percent persistence and chlordane remaining on cement (left) and backing (right)

Similar to the persistence evaluation, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 13 gives the p-values for comparisons of each possible set of coupons collected at the various flushing conditions. The significant differences are highlighted in gray. For the cement, the initial contamination level was significantly different from all of the other scenarios. In addition, while the residual chlordane after 2 h and 4 h of 200 rpm (1.64 ft/s) was not different, the residual chlordane decreased with each scenario until there was no change between the 4h and 24 h 250 rpm (1.91 ft/s) samples. The cement was initially contaminated with 530 ng ± 70 ng of chlordane and it decreased to 130 ng ± 27 ng after 2 h at 200 rpm where it held steady for the next 2 h and decreased to 29 ng ± 2 ng after 24 h at 200 rpm. Another significant decrease took place after the rotation of the AR was increased to 250 rpm for 4 h (11 ng ± 4 ng) which was not significantly different that the chlordane levels after 24 h at 250 rpm (18 ng ± 7 ng). The %P after the time period including 24 h of 200 rpm and 4 h of 250 rpm (after which there was no additional decrease) was 2%± 1%.

Table 13. Chlordane on Cement – Probability Value Matrix for Flushing Evaluation

	Flushing Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
		2hr - 200 rpm	4hr - 200 rpm	24 hr - 200 rpm	4 hr - 250 rpm	24 hr - 250 rpm
Cement	0 h	0.010	0.008	0.003	0.003	0.003
	2hr - 200 rpm		0.415	0.014	0.010	0.015
	4hr - 200 rpm			0.017	0.014	0.015
	24 hr - 200 rpm				0.005	0.029
	4 hr - 250 rpm					0.100
Backing	0 h	0.101	0.054	0.018	0.014	0.080
	2hr - 200 rpm		0.101	0.007	0.005	0.060
	4hr - 200 rpm			0.015	0.009	0.329
	24 hr - 200 rpm				0.0003	0.081
	4 hr - 250 rpm					0.044

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences

For the backing, the chlordane residual decreased from the initial contamination to the 2 h and 4 h 200 rpm (1.64 ft/s) samples (that were not different from one another) and then the chlordane residual decreased after 24 h at 200 rpm and then again after 4 h at 250 rpm. However, then the chlordane residual increased in the 24 h 250 rpm samples. The chlordane level on the backing decreased from an initial concentration of 1,500 ng ± 400 ng to 930 ng ± 95 ng after 2 h at 200 rpm where it held steady for the next 2 h and decreased to 370 ng ± 51 ng after 24 h at 200 rpm. Another significant decrease took place after the rotation of the AR was increased to 250 rpm for 4 h (220 ng ± 58 ng), but then the observed chlordane level unexpectedly increased after 24 h at 250 rpm. There was no apparent reason for this increase. The %P after 24 h of 200 rpm and 4 h of 250 rpm was 15% ± 6%. The increased %P for the backing with respect to the cement was likely for the same reasons as the similar observation during the persistence evaluation.

2.1.6 Chlordane on Cement Hyperchlorination Evaluation

Figure 3 shows the results from the hyperchlorination evaluation for chlordane on the cement coupon surfaces as well as the polycarbonate backing in a similar way as was done for the persistence and flushing evaluations. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP, specifically, the amount of time that the coupons were exposed to either 25 mg/L or 50 mg/L free chlorine. The error bars on the graphs are the standard deviations of the remaining

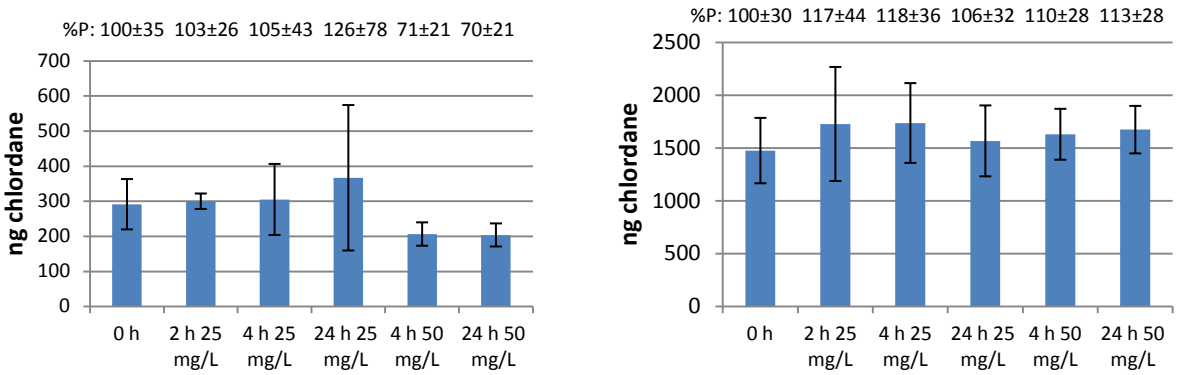

Figure 3. Hyperchlorination evaluation - percent persistence and chlordane remaining on cement (left) and backing (right)

chlordane on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of each graph.

As for the persistence and flushing evaluations, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 14 gives the p-values for comparisons of each possible set of coupons collected at the various

Table 14. Chlordane on Cement – Probability Value Matrix for Hyperchlorination Evaluation

	Hyperchlorination Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
		2 h 25 mg/L FC	4 h 25 mg/L FC	24 h 25 mg/L FC	4 h 50 mg/L FC	24 h 50 mg/L FC
Cement	0 h	0.442	0.439	0.322	0.080	0.140
	2 h 25 mg/L FC		0.464	0.300	0.047	0.014
	4 h 25 mg/L FC			0.215	0.166	0.125
	24 h 25 mg/L FC				0.184	0.153
	4 h 50 mg/L FC					0.468
Backing	0 h	0.120	0.282	0.279	0.324	0.284
	2 h 25 mg/L FC		0.493	0.203	0.412	0.453
	4 h 25 mg/L FC			0.330	0.188	0.279
	24 h 25 mg/L FC				0.409	0.357
	4 h 50 mg/L FC					0.121

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences
FC-free chlorine

hyperchlorination conditions. The significant differences are highlighted in gray. For the cement, the only significant differences occurred between the residual chlordane concentration after 2 h exposure to 25 mg/L free chlorine and the residual chlordane present after exposure to both 4 h and 24 h of 50 mg/L free chorine. These data suggested that hyperchlorination with no flow is not an effective decontamination

approach for chlordane on cement. Similarly, for the backing, there were no differences in residual chlordane concentration through the duration of the hyperchlorination experiment.

2.2 Results from Testing with Chlordane on PVC Pipe Coupons

The following sections describe results from performing quality control, verification, and evaluation experimental design procedures for chlordane on PVC coupons.

2.2.1 Chlordane on PVC Quality Control Results

The same QC procedures were followed for these measurements as in the previous section. Tables 15 and 16 show the results obtained during testing.

Table 15. Chlordane on PVC GC-MS Continuing Calibration Verification Results

Component of Testing	Low Calibration Standard (%R of Expected)	Mid Calibration Standard (%R of Expected)
Step 1 - Surface Extraction Method Verification	80%	45%
	72%	55%
Step 2 - Surface Contamination Method Verification	141%	95%
	113%	97%
Persistence Evaluation	42%	81%
	0%	61%
Flushing Evaluation	0%	69%
	0%	79%
	32%	77%
Hyperchlorination Evaluation	174%	92%
	137%	83%
	140%	84%
	130%	91%
	124%	90%
Average	85%	78%
Standard Deviation	61%	16%

For the CCV samples, the recoveries of the low concentration samples ranged from 0% to 174%. These low concentration samples were very close to the LOQ so small changes in peak area greatly impacted the %Rs. During the persistence and flushing measurement, the 5 ng/mL standard was not detectable during the analysis set. However, the low end of the concentration range was not applicable to these samples. Throughout testing, the peak areas that most of the samples were measured at were in the middle and higher parts of the calibration curve and often the samples had to be diluted to bring the peak areas into the linear range of the calibration curve. Therefore, no corrective action was taken in response to these CCV results. The recoveries of the middle concentration CCV samples (100 ng/mL) ranged from 45% to 97% with an average recovery of 78% with a standard deviation of 16%. The two lowest recoveries (45% and 55%) were during the Step 1 surface extraction method verification which was meant to determine if the chlordane could be extracted from the

surface of the cement coupons. No corrective action was taken because of the qualitative nature of the question being explored in Step 1. For the rest of the 100 ng/mL CCV samples, only two were outside of the acceptable range (61% and 69%) and those were both in the same sample sets with another 100 ng/mL CCV sample that was within the acceptable range of recoveries. Therefore, no corrective action was taken.

Table 16. Chlordane on PVC Laboratory Fortification Matrix Sample Results

Component of Testing	Laboratory Fortified Matrix (%R)
Step 2 - Surface Contamination Method Verification	98%
Persistence Evaluation	98%
Flushing Evaluation	151%
	105%
	237%
Hyperchlorination Evaluation	106%
	118%
Average	**130%**
Standard Deviation	**51%**

For the LFM samples, with the exception of two samples with recoveries of 151% and 237%, the recoveries ranged from 98% to 118%. The two outlying samples occurred during analysis of the flushing evaluation. These samples were in an analysis set with one other LFM samples recovered at 105% and two CCV samples that were within the acceptable range. There was no clear reason why these two samples were over recovered. Because of the reasons stated, and because the flushing data is interpreted based on the relative change in concentration over time, no corrective action was made.

As for the chlordane testing on the cement coupons, the bulk contamination solution was sampled at the beginning, middle, and end of the 2 h contamination time during the Step 2 method verification, the persistence evaluation, the flushing evaluation, and the hyperchlorination evaluation and analyzed as described above. Across those four experiments, the peak areas of the initial 1 milligram (mg)/L contamination solution were considered the 100% chlordane levels. The samples collected at the 1 h point of the contamination step retained a 25%±1% of the peak area and the sample collected at the end of the contamination period retained 20%±2% of the peak area. Therefore, as in the previous example using the cement coupons, while there was a considerable loss of chlordane during the first hour, chlordane was available throughout the entire 2 h contamination period.

2.2.2 Method Verification Step 1: Chlordane on PVC Surface Extraction

Table 17 gives the results from the surface contamination extraction method verification for chlordane on PVC. Overall, the total recovery ranged from 35% to 62% with standard deviations across the five replicates of less than 14%, indicating that chlordane could be reproducibly extracted and measured from the PVC coupons. The concentration of each spiking solution was confirmed using GC-MS. The low, middle,

and high spiking solutions had percent recoveries of 61%, 53%, and 68% of the target concentration levels.

Table 17. Chlordane on PVC Surface Contamination Extraction

	Amount spiked (ng)	Avg. amount recovered from PVC (ng)	Total % Recovery	SD
Low level	20	9.4	47%	14%
Mid level	100	35	35%	5%
High level	1000	620	62%	7%

Five replicates were spiked and extracted at each concentration level.

2.2.3 Method Verification Step 2: Chlordane on PVC Surface Contamination

This verification indicated if a contaminant would adsorb to the PVC surface containing biofilm in the event that it is exposed to a bulk solution. Table 18 gives the results from the surface contamination method verification for chlordane on PVC including the amount of chlordane extracted from each coupon after a two hour exposure to 1 mg/L chlordane. Overall, an average of 3.6 µg ± 0.6 µg was adsorbed to the coupon surfaces out of a total of 1,000 µg of chlordane that was available in the bulk contamination solution (0.36%). As for the cement, albeit a small percentage, these results indicate that chlordane did adsorb to the PVC coupon following exposure to the bulk contamination solution.

Table 18. Chlordane on PVC Surface Contamination

Contaminated Coupon	Amount Recovered from PVC (µg)
#1	3.4
#2	2.8
#3	4.4
#4	3.4
#5	3.8
Avg.	3.6
St. Dev.	0.6
%RSD	17%

2.2.4 Chlordane on PVC Persistence Evaluation

Figure 4 shows the results from the persistence evaluation for chlordane on the PVC coupon. The vertical axes show the amount of chlordane remaining on the coupons after each time period (shown across the horizontal axis) during which fresh tap water is flowing through the AR and the AR is rotating at 100 rpm (1 ft/s). The average free chlorine concentration in the tap water during this evaluation was 1.34 mg/L ± 0.11 mg/L, the average pH was 7.8 ± 0.1, and average temperature was 25.5°C ± 0.0 °C. The columns at the far left side of the graphs represent the initial contamination level (as measured on the

contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The error bars on the graphs are the standard deviations of the remaining chlordane on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graph.

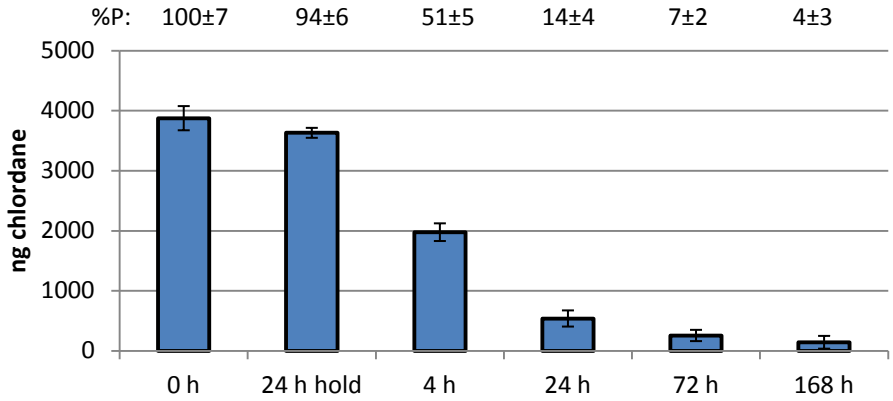

Figure 4. Persistence evaluation - percent persistence and chlordane remaining on PVC

As for the chlordane cement evaluations described above, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 19 gives the p-values for comparisons of each possible set of coupons collected at the various flushing conditions. The data that exhibit significant differences are highlighted in gray. The initial contamination level was not significantly different from the 24 h hold level, but the chlordane levels at the initial contamination and 24 h hold were significantly different from the chlordane levels on the rest of the coupons. The chlordane levels dropped significantly from the initial and 24 h hold levels after 4 h and again after 24 h of resumed flow. Thereafter, the chlordane concentration steadied with only another significant difference between the 24 h and 168 h chlordane levels. The PVC was initially contaminated with 3,900 ng ± 200 ng of chlordane and 4 h after the resumption of AR rotation at 100 rpm (1 ft/s), the chlordane levels had decreased to 2,000 ng ± 150 ng and after 24 h the levels decreased to 540 ng ± 130 ng which was not significantly different from the levels at 72 h (260 ng ± 94 ng) and the 72 h chlordane levels were not different from the 168 h chlordane levels (180 ng ± 110 ng), but the 168 h levels had decreased in comparison to the 24 h levels. The %P after 24 h of flow was 14% ± 4% and after 168 h, 5% ± 3%.

Table 19. Chlordane on PVC – Probability Value Matrix for Persistence Evaluation

Persistence Evaluation Times	probability (p) values (< 0.05 - significant difference)				
	24 h hold	4 h	24 h	72 h	168 h
0 h	0.063	4.17E-03	4.37E-04	1.10E-03	2.21E-04
24 h hold		1.11E-03	3.41E-05	3.81E-04	1.66E-05
4 h			1.71E-03	8.88E-04	1.35E-03
24 h				0.066	0.025
72 h					0.27

Read as matrix, for times at left, read right for p-value to determine possible differences.
Light shading – significant differences

2.2.5 Chlordane on PVC Flushing Evaluation

Figure 5 shows the results from the flushing evaluation for chlordane on the PVC coupons. As was the case for the persistence evaluation, the vertical axes show the amount of chlordane remaining on the coupons after each time period and flushing condition that is shown across the horizontal axes. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The average free chlorine concentration in the tap water during this evaluation was 1.34 mg/L ± 0.11 mg/L, the average pH was 7.8 ± 0.1, and average temperature was 25.5°C ± 0.0 °C. The error bars on the graphs are the standard deviations of the remaining chlordane on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graph.

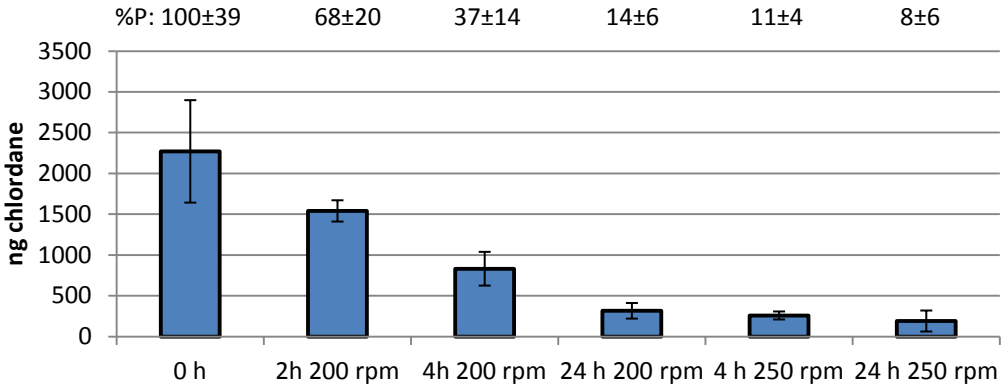

Figure 5. Flushing evaluation - percent persistence and chlordane remaining on PVC

Statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 20 gives the p-values for comparisons of each possible set of coupons collected at the various flushing conditions. The data that exhibit significant differences are highlighted in gray. The initial contamination level (2,300 ng ± 630 ng) was significantly different from all of the other scenarios and the residual chlordane levels decreased significantly until the significant decreases in residual chlordane stopped after the 24 h of the AR rotating at

200 rpm (1.64 ft/s) (320 ng ± 96 ng). Increasing the AR rotation to 250 rpm (1.91 ft/s) did not cause additional decreases in the residual chlordane levels. The %P after 24 h of 200 rpm (1.64 ft/s) rotation (after which there was no additional decrease) was 14% ± 6%.

Table 20. Chlordane on PVC – Probability Value Matrix for Flushing Evaluation

Flushing Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
	2hr - 200 rpm	4hr - 200 rpm	24 hr - 200 rpm	4 hr - 250 rpm	24 hr - 250 rpm
0 h	0.063	0.047	0.012	0.017	0.011
2hr - 200 rpm		0.033	0.00020	0.003	0.011
4hr - 200 rpm			0.049	0.014	0.030
24 hr - 200 rpm				0.270	0.087
4 hr - 250 rpm					0.233

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences

2.2.6 Chlordane on PVC Hyperchlorination Evaluation

Figure 6 shows the results from the hyperchlorination evaluation for chlordane on PVC coupons. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP, specifically, the amount of time that the coupons were exposed to either 25 mg/L or 50 mg/L free chlorine. The error bars on the graphs are the standard deviations of the remaining chlordane on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graph.

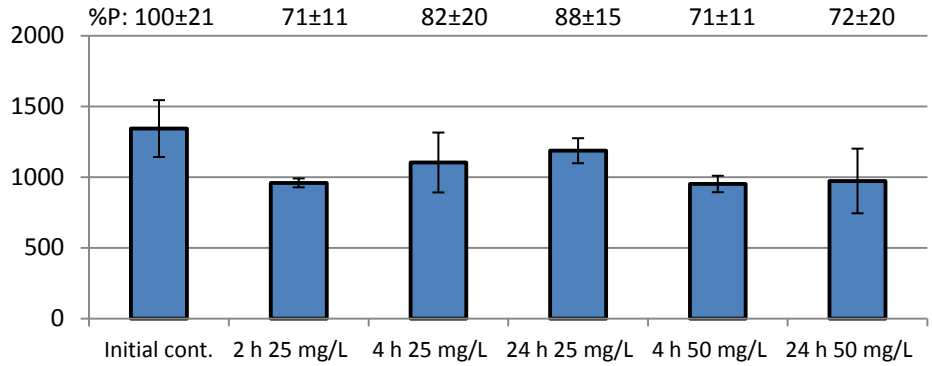

Figure 6. Hyperchlorination evaluation - percent persistence and chlordane remaining on PVC

As for the persistence and flushing evaluations, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 21 gives the p-values for comparisons of each possible set of coupons collected at the various hyperchlorination conditions. The data exhibiting significant differences are highlighted in gray. Overall, the statistical evaluation confirmed the visual observation of the data in the graphs. There were several

significant differences, but no two that were in succession to clarify the effect of the hyperchlorination. Instead the data seem to be indicating that hyperchlorination does not cause significant and repeatable decontamination of chlordane from PVC.

Table 21. Chlordane on PVC – Probability Value Matrix for Hyperchlorination Evaluation

Hyperchlorination Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
	2 h 25 mg/L FC	4 h 25 mg/L FC	24 h 25 mg/L FC	4 h 50 mg/L FC	24 h 50 mg/L FC
0 h	0.049	0.182	0.222	0.056	0.029
2 h 25 mg/L FC		0.152	0.019	0.427	0.464
4 h 25 mg/L FC			0.283	0.187	0.246
24 h 25 mg/L FC				0.006	0.178
4 h 50 mg/L FC					0.455

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences
FC-free chlorine

2.3 Results from Testing with Sodium Fluoroacetate on Cement Pipe Coupons

The following sections describe results from performing quality control, verification, and evaluation experimental design procedures for SFA on cement pipe coupons.

2.3.1 SFA on Cement Quality Control Results

Continuing calibration verification (CCV) samples were analyzed on the IC throughout each analysis set. After every 10 samples analyzed, a middle concentration calibration solution (0.5 mg/L) was reanalyzed and following each analysis set, the low calibration solution (0.1 mg/L) were reanalyzed. There were 34 middle concentration CCV samples analyzed and the recoveries ranged from 95% to 105% with an average of 99% and a standard deviation of 2%. Ten low calibration CCV samples were analyzed and the recoveries ranged from 95% to 126% with an average of 106% with a standard deviation of 12%. Overall, none of the middle level CCV samples were outside of the targeted range of recoveries and only two of the low level CCV samples were outside of the targeted range. In addition, 10% of the samples were split and 0.5 mg/L of chlordane was spiked into the sample extract to create LFM samples. Target recoveries for each of these QC samples were between 90% and 110%. The recoveries of the LFM samples are shown in Table 22.

For the LFM samples, the recoveries ranged from 78% to 238% with an average recovery of 119% and a standard deviation of 44%. Only five out of the 22 LFM samples were outside of the targeted range of 70% to 130% recovery and LFM results outside of the acceptable range were always accompanied with at least three other LFM samples that were within the targeted range. If the five outlying LFM results were removed, the average recovery would be 96% with a standard deviation of 11%. There was not a clear explanation as to why those five samples were over-recovered, but because of the number of samples that were within the acceptable range, no corrective action was made.

Table 22. SFA on Cement Laboratory Fortification Matrix Sample Results

Component of Testing	Laboratory Fortified Matrix (%R)
Step 1 - Surface Extraction Method Verification	92%
	98%
	78%
	94%
Step 2 - Surface Contamination Method Verification	90%
	88%
	86%
	184%
	90%
	191%
Persistence Evaluation	238%
	109%
	117%
	103%
Flushing Evaluation	184%
	97%
	88%
	119%
Hyperchlorination Evaluation	174%
	95%
	88%
	106%
Average	**119%**
Standard Deviation	**44%**

The bulk contamination solution was sampled at the beginning, middle, and end of the 2 h contamination time during the persistence evaluation, the flushing evaluation, and the hyperchlorination evaluation and the SFA measured quantitatively. Across those three experiments and three collection times during each experiment, the recovery of SFA from the 500 mg/L bulk contamination solution was 89%±2%. Therefore, most of the SFA remained available for adsorption throughout the duration of the 2 h contamination time period.

2.3.2 Method Verification Step 1: SFA on Cement Surface Extraction

Table 23 gives the results from the surface contamination extraction method verification for SFA on cement. When the SFA was spiked onto the cement coupon, some of the SFA adsorbed to the cement surface and some flowed through the cement and adsorbed to the polycarbonate backing on which the cement was mounted. The cement and backing were extracted separately and the results were reported for both the cement and the backing for all five components of the testing. Table 23 gives the results including the amount of SFA

spiked onto the coupons, the amount extracted from the backing and cement, the total recovery, and the standard deviation. Overall, the total recovery ranged from 68% to 91% with standard deviations across the five replicates of less than 28%, indicating that SFA could be extracted and measured from the cement coupons. The amounts recovered from the backing and cement show that considerably more SFA adsorbed to the cement surface than being adsorbed to the polycarbonate backing. This is consistent with the chemical characteristics of SFA, as preferential adsorption would be expected from a highly non-polar organic chemical as opposed to SFA, a salt. The concentration of each spiking solution was confirmed using IC. The low, middle, and high spiking solutions had average percent recoveries of 93% ±1% of the target concentration levels.

Table 23. SFA on Cement Surface Contamination Extraction

Spike Level	Amount spiked (µg)	Avg. amount recovered from cement(µg)	Avg. amount recovered from backing (µg)	Avg. total recovered (µg)	Total % Recovery	SD
Low level	10	6.9	2.2	9.1	91%	28%
Mid level	50	25	11	36	72%	10%
High level	500	220	130	340	68%	3%

Five replicates were spiked and extracted at each concentration level.

2.3.3 Method Verification Step 2: SFA on Cement Surface Contamination

This verification indicates if a contaminant will adsorb to the cement surface containing biofilm in the event that it is exposed to a bulk solution. Table 24 gives the results from the surface contamination method verification for SFA on cement including the amount of SFA extracted from each part of the coupon after a two hour exposure to 1 mg/L SFA. Overall, an average of 55 µg ± 17 µg was adsorbed to the coupon surfaces (cement and backing combined) out of a total of 500,000 µg of SFA that was available in the bulk contamination solution (0.011%). These data indicated that SFA adsorbed to the cement coupon following exposure to the bulk contamination solution in a similar way as it did during the surface extraction method verification, with more SFA having adsorbed to the cement surface than to the polycarbonate backing.

During this verification, one set of coupons was contaminated as described above only with a 100 mg/L SFA contamination solution (which had been used to contaminate coupons at a detectable level during some method development work), but the SFA was not able to be detected following extraction. This set of coupons had been contaminated following growth of biofilm as described in Section 1.2. Prior to that, another method was being used to grow biofilm. Because of the lack of measured SFA, it was suspected that in prior experiments, biofilm had not been grown on the coupons and now that it had been, SFA was not adsorbing as readily. The method verification was repeated using a 500 mg/L SFA contamination solution. Within this set of coupons, two coupons were included (only two because of the limited capacity of the AR) that had no biofilm growth in order to get some indication as to whether biofilm growth played a role in the lack of adsorption of SFA. The two coupons without biofilm had 4-5 times the amount of

SFA (225 µg SFA) adsorbed onto the cement surface of the biofilmed coupons (49 µg). While this observation is based on very little data, it suggests that at least for SFA, an ionic bonding chemical, that biofilm hinders its adsorption to cement surfaces. More research would be required to further characterize the behavior of this and other contaminants with biofilms.

Table 24. SFA on Cement Surface Contamination

Contaminated Coupon	Amount Recovered from Cement (µg)	Amount Recovered from Backing (µg)	Total Amount Recovered from Coupon (µg)
#1	37	3.0	40
#2	73	10	83
#3	40	5.8	46
#4	48	6.7	55
#5	46	5.2	51
Avg.	49	6.2	55
St. Dev.	14	2.7	17
%RSD	29%	43%	31%

2.3.4 SFA on Cement Persistence Evaluation

Figure 7 shows the results from the persistence evaluation for SFA on the cement coupon surfaces as well as the polycarbonate backing. The vertical axes show the amount of SFA remaining on the coupons after each time period (shown across the horizontal axis) during which fresh tap water is flowing through the AR and the AR is rotating at 100 rpm (1 ft/s). The average free chlorine concentration in the tap water during this evaluation was 1.46 mg/L ± 0.12 mg/L, the average pH was 7.9 ± 0.2, and average temperature was 24.0°C ± 1.0 °C. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The error bars on the graphs are the standard deviations of the remaining SFA on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graphs.

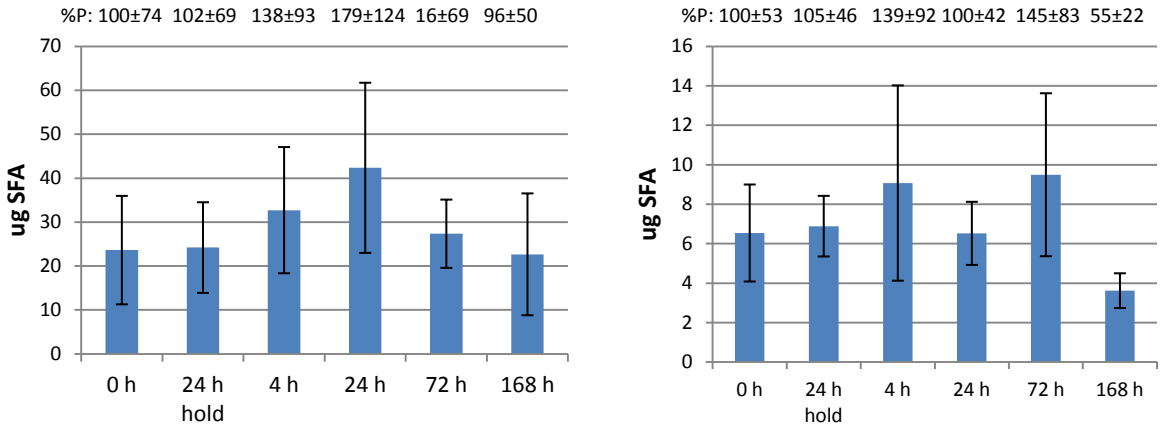

Figure 7. Persistence evaluation - percent persistence and SFA remaining on cement (left) and backing (right)

Table 25 gives the p-values for comparisons of each possible set of coupons collected at the various time periods. There was only one significant difference across all of the

Table 25. SFA on Cement – Probability Value Matrix for Persistence Evaluation

	Persistence Evaluation Times	probability (p) values (< 0.05 - significant difference)				
		24 h hold	4 h	24 h	72 h	168 h
Cement	0 h	0.457	0.290	0.142	0.384	0.342
	24 h hold		0.305	0.079	0.370	0.420
	4 h			0.328	0.309	0.270
	24 h				0.129	0.159
	72 h					0.370
Backing	0 h	0.328	0.219	0.497	0.225	0.125
	24 h hold		0.268	0.429	0.241	0.056
	4 h			0.234	0.427	0.122
	24 h				0.115	0.045
	72 h					0.077

Read as matrix, for times at left, read right for p-value to determine possible differences.
Light shading – significant differences

combinations of data sets and it is highlighted in gray. For neither cement nor the backing did the levels of residual SFA change significantly due to the scenarios tested during this evaluation. The only significant difference between coupon collection periods was a decrease in residual SFA on the backing between the 24 h after flow was resumed and the 168 h sample. However, the 72 h sample collected in between those two did not exhibit a significant difference, further exemplifying the scattered nature of the results. The %P after the persistence evaluation was 96% ±50% for the cement and 55% ±22% for the backing.

2.3.5 SFA on Cement Flushing Evaluation

Figure 8 shows the results from the flushing evaluation for SFA on the cement coupon surfaces as well as the polycarbonate

backing. As was the case for the persistence evaluation, the vertical axes show the amount of SFA remaining on the coupons after each time period and flushing condition that is shown across the horizontal axes. The average free chlorine concentration in the tap water during this evaluation was 1.62 mg/L ± 0.18 mg/L, the average pH was 7.8 ± 0.1, and average temperature was 23.8 °C ± 0.9 °C. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP. The error bars on the graphs are the standard deviations of the remaining

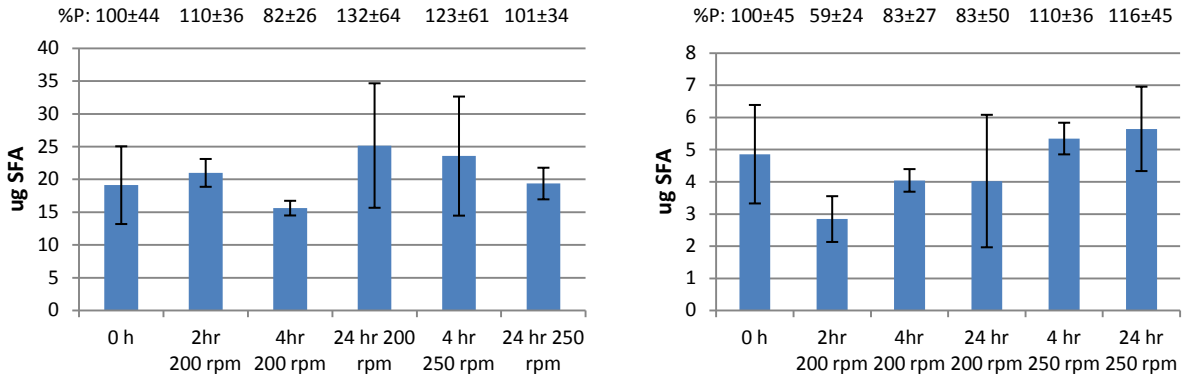

Figure 8. Flushing evaluation - percent persistence and SFA remaining on cement (left) and backing (right).

SFA on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graphs.

As for the persistence evaluation, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 26 gives the p-values for comparisons of each possible set of coupons collected at the various flushing conditions. There was only one significant difference across the various flushing scenarios and it was highlighted in gray. The statistical data indicated that there was only one significant difference across the cement and backing data. This data suggests that SFA is not decontaminated effectively by increasing the duration of flushing and flow velocity past the cement pipe coupons. The %P after the flushing evaluation was 101% ±34% for the cement and 116% ±45% for the backing.

Table 26. SFA on Cement – Probability Value Matrix for Flushing Evaluation

	Flushing Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
		2hr - 200 rpm	4hr - 200 rpm	24 hr - 200 rpm	4 hr - 250 rpm	24 hr - 250 rpm
Cement	0 h	0.242	0.368	0.154	0.243	0.064
	2hr - 200 rpm		0.074	0.243	0.327	0.002
	4hr - 200 rpm			0.058	NA	0.099
	24 hr - 200 rpm				0.441	0.061
	4 hr - 250 rpm					0.105
Backing	0 h	0.030	0.500	0.125	0.330	0.309
	2hr - 200 rpm		0.156	0.169	0.086	0.063
	4hr - 200 rpm			0.058	NA	0.190
	24 hr - 200 rpm				0.181	0.179
	4 hr - 250 rpm					0.301

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences

2.3.6 SFA on Cement Hyperchlorination Evaluation

Figure 9 shows the results from the hyperchlorination evaluation for SFA on the cement coupon surfaces as well as the polycarbonate backing as was done for the persistence and flushing evaluations. The columns at the far left side of the graphs represent the initial contamination level (as measured on the contaminated control coupons) and each successive column represents the time periods and experimental conditions defined by the PDEDP, specifically, the amount of time that the coupons were exposed to either 25 mg/L or 50 mg/L free chlorine. The error bars on the graphs are the standard deviations of the remaining SFA on the three coupons that were collected at each time period. The %P that corresponds with each time period is given across the top of the graphs.

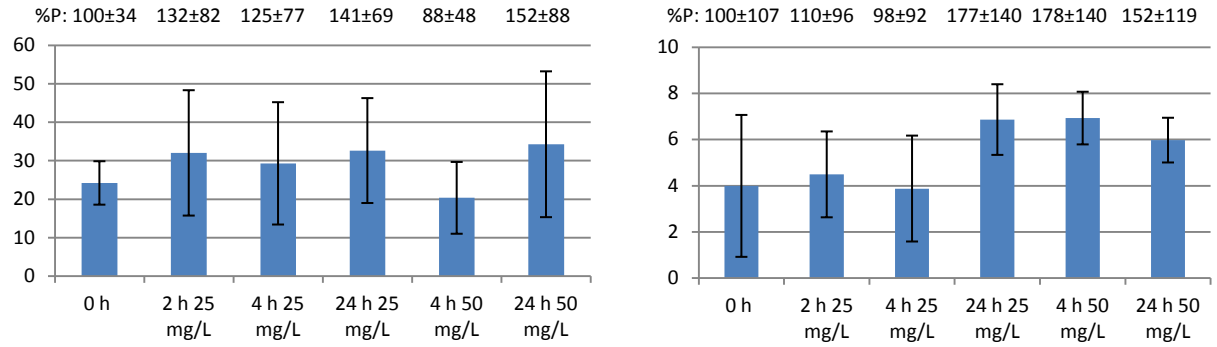

Figure 9. Hyperchlorination evaluation - percent persistence and SFA remaining on cement (left) and backing (right)

As for the persistence and flushing evaluations, statistical analyses were performed using t-tests to further clarify any differences between the data from each flushing scenario. Table 27

Table 27. SFA on Cement – Probability Value Matrix for Hyperchlorination Evaluation

	Hyperchlorination Evaluation Conditions	probability (p) values (< 0.05 - significant difference)				
		2 h 25 mg/L FC	4 h 25 mg/L FC	24 h 25 mg/L FC	4 h 50 mg/L FC	24 h 50 mg/L FC
Cement	0 h	0.298	0.298	0.163	0.369	0.206
	2 h 25 mg/L FC		0.415	0.399	0.090	0.098
	4 h 25 mg/L FC			0.085	0.108	0.162
	24 h 25 mg/L FC				0.047	0.358
	4 h 50 mg/L FC					0.048
Backing	0 h	0.430	0.485	0.048	0.153	0.168
	2 h 25 mg/L FC		0.047	0.064	0.016	0.221
	4 h 25 mg/L FC			0.059	0.016	0.187
	24 h 25 mg/L FC				0.492	0.248
	4 h 50 mg/L FC					0.285

Read as matrix, for conditions at left, read right for p-value to determine possible differences.
Light shading – significant differences
FC – free chlorine

gives the p-values for comparisons of each possible set of coupons collected at the various hyperchlorination conditions. The data exhibiting significant differences are highlighted in gray. There were several significant differences between the data sets from some of the experimental scenarios, but no clear trends indicating that hyperchlorination was an effective means for decontaminating SFA from the surface of cement pipes. The %P after the hyperchlorination evaluation was 152% ±88% for the cement and 152% ±119% for the backing.

3 RESULTS SUMMARY

The objective of this project was to develop a PDEDP that could be used across laboratories to performed pipe decontamination research. In addition, data was to be collected pertaining to the adsorption, persistence, and possible decontamination approaches to chlordane and sodium fluoroacetate on cement-line pipe and/or PVC. Several key points of summary are given below.

3.1 Experimental Design Protocol Development

The development and testing of the PDEDP was successfully accomplished. Use of the annular reactor proved to be an effective means of reproducibly simulating the flow of water past pipe materials. The surface extraction and surface contamination method verification steps were necessary to demonstrate whether or not a selected contaminant can be studied (if it cannot be extracted it will be difficult to study its decontamination behavior) and if it is a viable threat (if a contaminant will not partition onto a pipe from an aqueous solution, it may not be a decontamination concern). These method verification steps were demonstrated with a limited number of replicates for chlordane and SFA. Each of these method verifications could be more rigorously tested by including more replicates and additional separate experiments and optimized (sonication time, solvent, etc.) in order to provide additional information on the reproducibility of the pipe material coupon extraction for the selected pipe material type and contaminant as well as to more accurately determine the extent of and reproducibility of the contamination step.

The persistence evaluation was a beneficial component of the PDEDP as it mimicked rather typical conditions in a water distribution system and it was compared with the flushing evaluation at higher flow velocities to determine if there was increased efficacy at higher flow velocities. Additional information could be gleaned during this evaluation by controlling the water quality parameters in order to study how water quality parameters impact contaminant adsorption and decontamination efficacy. Lastly, the hyperchlorination evaluation allowed for collection of data using a chemical decontamination approach. These results were compared with the persistence and flushing evaluations. Additional work could be performed to include multiple other pipe decontamination chemicals to compare the effectiveness of those approaches with hyperchlorination. Regardless of the additional work that could be performed, each of the PDEDP steps was successfully demonstrated and the combined results proved to be a useful data set.

3.2 Persistence and Decontamination Testing

3.2.1 Chlordane on Cement

The surface extraction method verification confirmed that chlordane could be extracted from the surface of cement after direct contamination of the cement coupon and the surface contamination method verification confirmed that a cement coupon could be contaminated with chlordane by exposing to a solution of contaminated water. The results from the persistence and flushing evaluations for the cement exhibited very similar results. The %P after 24 h for the persistence evaluation (AR operated at 100 rpm (1 ft/s)) was 9% ± 3% and the %P after 24 h during the flushing evaluation (AR operated at 200 rpm (1.64 ft/s)) was 6% ± 1%. However, during the flushing evaluation, a further decrease was noted during the next 4 h of the AR operating at 250 rpm (1.91 ft/s), taking the %P to 2% ± 1% for the flushing evaluation. These results suggest that the flow velocity past the pipe materials may have less to do with the decontamination efficacy than the duration of the flow past the contaminated pipe.

Results from the hyperchlorination evaluation showed that hyperchlorination without flow is not an effective means of decontaminating chlordane from cement. This result was unexpected as free chlorine would be expected to oxidize the chlordane from the surface of the cement. These data suggest oxidation was not occurring to the extent that was anticipated and flushing with water with a concentration of 1-2 mg/L of free chlorine was much more effective at decontaminating the pipe materials than water with a free chlorine concentration of 25 mg/L and 50 mg/L.

3.2.2 Chlordane on PVC

The surface extraction method verification confirmed that chlordane could be extracted from the PVC surface after direct contamination of the PVC coupon and the surface contamination method verification confirmed that a PVC coupon could be contaminated with chlordane by exposing to a solution of contaminated water. The results from the persistence and flushing evaluations for the PVC exhibited very similar results. The %P after 24 h for the persistence evaluation (AR operated at 100 rpm (1 ft/s)) was 14% ± 4% and the %P after 24 h during the flushing evaluation (AR operated at 200 rpm (1.64 ft/s)) was 14% ± 6%. However, during the persistence evaluation, a further decrease was noted between 24 and 168 h, taking the %P to 5% ± 3% for the overall persistence evaluation. As for the chlordane on cement results, these results suggest that the flow velocity past the pipe materials may have less to do with the decontamination efficacy than the duration of the flow past the contaminated pipe. Again, as for the chlordane on cement testing, results from the hyperchlorination evaluation unexpectedly showed that hyperchlorination without flow is not an effective means of decontaminating chlordane from PVC.

3.2.3 Sodium Fluoroacetate on Cement

The surface extraction method verification confirmed that SFA could be extracted from the surface of cement after direct contamination of the cement coupon and the surface contamination method verification confirmed that a cement coupon could be contaminated with SFA by exposing to a solution of contaminated water. The results from the persistence, evaluation, and hyperchlorination evaluations suggest that these approaches were not effective in

decontaminating SFA from cement. These results are exemplified by the %Ps. After the persistence evaluation (AR operated at 100 rpm (1 ft/s)) the %P was 96% ± 50%, after the flushing evaluation, 101% ±34%, and after the hyperchlorination study, 152% ±88%.

3.3 Future Research Needs

The water system decontamination research area is one with many facets to be explored. This work has laid the framework for a PDEDP that can be adapted to accommodate other research priorities. Below are a few possible areas for further study:

- Importance of biofilm to pipe decontamination research – During the SFA surface contamination method verification step, two cement coupons without biofilm (only two because of the limited capacity of the AR and that the impromptu experiment was outside the context of the PDEDP) were contaminated with SFA along with the coupons containing biofilm. For these two coupons, approximately five times as much SFA was adsorbed to the non-biofilm coupons. This very limited data set suggested that the presence or absence of biofilm could significantly impact the results of pipe adsorption/decontamination research. More rigorous experimentation could be performed to better characterize the role of biofilm.
- Broadening of adsorption/decontamination data set by expanding on list of chemical contaminants tested using the PDEDP (e.g., organophosphates as available toxic chemicals and simulated chemical agents, metals to simulate heavy metal or radiological contamination).
- Study of adsorption/decontamination of biological organisms using the PDEDP.
- Use of additional pipe materials with additional chemicals and biological organisms as well as additional chemical pipe cleaning materials as possible decontamination agents.
- Scaling up of AR experiments into experiments with real pipe using a pipe loop in order to study how well the AR experiments translate into scenarios with real pipe.
- Study of risk assessment questions addressing how much persistence of various chemicals is acceptable.

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APPENDIX
Experimental Design Protocol

United States Environmental Protection Agency

National Homeland Security
Research Center

Water Infrastructure Protection Division

Experimental Design Protocol for the Study of Chemical Contaminant Persistence and Decontamination in Drinking Water Pipes

Experimental Design Protocol for the Study of Chemical Contaminant Persistence and Decontamination in Drinking Water Pipes

February 10, 2012

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LIST OF ABBREVIATIONS

AR	annular reactor
ASTM	ASTM International
AWWA	American Water Works Association
cm	centimeters
cfu	colony forming units
EPA	U.S. Environmental Protection Agency
°C	degrees Celsius
F	flushing
ft/s	feet per second
h	hour
HC	hyperchlorination
HPC	heterotrophic plate counts
IC	ion chromatography
IS	internal standard
in.	inch
g	gram
GC-MS	gas chromatographic mass spectrometry
K_{ow}	octanol-water partitioning coefficient
KOH	potassium hydroxide
LOQ	limit of quantitation
LFM	laboratory fortified matrix
L	liter
Lpm	liter per minute
µL	microliter
µg	microgram
mA	milliamp
mM	millimolar
mg	milligrams
mm	millimeters
mL	milliliters
min	minute
ng	nanogram
NHSRC	National Homeland Security Research Center
QAPP	Quality Assurance Project Plan
QC	quality control
%R	percent recovery
%P	percent persistence
PE	persistence evaluation
PDEDP	Persistence and Decontamination Experimental Design Protocol
PVC	polyvinyl chloride
rpm	revolutions per minute
s	second
SIM	selected ion monitoring
SPME	solid phase micro extraction

INTRODUCTION

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) conducts research to protect, detect, respond to, and recover from terrorist attacks on the nation's water and wastewater infrastructure. The objective of this project was the development and testing of a standardized Persistence and Decontamination Experimental Design Protocol (PDEDP) to quantitatively determine the adherence and persistence of individual priority contaminants to the wetted surfaces of various drinking water pipe materials. This experimental design also addresses testing of techniques for decontaminating affected pipe surfaces if the contaminant persists. The experimental design can be implemented in a consistently reproducible fashion across different laboratories for various contaminants and pipe materials. The PDEDP is used to gain additional experimental information about the adsorption of specific contaminants to various drinking water pipe materials and to test various methods to destroy, reduce, or remove adsorbed contaminants.

Multiple research studies have already been conducted to determine the adsorption of particular chemical, biological, and radiological contaminants to drinking water pipe materials and test various methods to destroy, reduce, or remove adsorbed contaminants[3-5]. While useful data have resulted from studies conducted to date, often the differing designs of previous studies limit the usability and comparability of the data. This document describes a proposed experimental design that could be used to generate contaminant persistence and decontamination data for water utilities and other decision-makers with decontamination responsibility in the instance of an intentional or natural contamination of a drinking water system. This experimental design could also provide a means to generate data that are comparable to that which has been published in the peer-review literature.

One of the most significant factors in this experimental design is the use of an annular reactor (AR) as the device used to simulate flow past coupons of materials that represent drinking water pipe surfaces. The AR simulates pipe flow with a variable speed motor that drives an inner rotating cylinder, providing surface shear between pipe surface coupons and water within the AR. Twenty removable slide coupons of relevant materials can be mounted within the reactor. There are benefits and drawbacks of using the AR as the flow simulator. The main drawback of using the AR is that actual pipe sections cannot be used as in some previous studies; pipe material coupons either need to be purchased from the AR manufacturer or pipe materials need to be attached to a standard backing that can be inserted into the AR.

Several benefits of using the AR outweigh these drawbacks, including the following:

- Provides option of altering rotational speed to simulate various flow velocities, and therefore shear, to allow simulation of both flushing and decontamination conditions
- Injection ports facilitate the precise alteration of water chemistry
- The AR manufacturer offers coupons with several common pipe materials, such as cement lined and polyvinyl chloride (PVC). Cement lined coupons meet requirements of the C150-07 American Society for Testing and Materials (ASTM) Standard Specification for Portland Cement[1] and the thickness of the concrete is at least 1.6 millimeters (mm), as specified in American Water Works

Association (AWWA) C104-03 Standard for Cement-Mortar Lining for Ductile-Iron Pipe and Fittings for Water[2].

- ARs are commercially available, providing ease of repeatability across laboratories, as opposed to requiring the fabrication of flow cells at each laboratory
- Several decontamination projects described in the literature have used the AR,[2-5] making it possible to replicate the experimental conditions found in the literature

Overall, the measurement of persistence and decontamination of contaminants from pipe material coupons is going to be challenging because of the small amounts of contaminant that are to be recovered from coupon surfaces. To ensure the accuracy and precision of persistence and decontamination data, it is important that as many experimental factors as possible be controlled. The AR provides the best approach to providing experimental conditions that are adequately controlled to attain usable persistence and decontamination data.

The following experimental design is meant to be generic, since it is intended for use with various contaminants and pipe materials. Note that before following this experimental design, the laboratory being used must be capable of measuring the contaminant used for contaminating the pipe material and have at least one AR and an adequate number of AR coupons of the desired pipe material.

A1 EXPERIMENTAL DESIGN

A1.1 Experimental Reactor System

For the persistence and decontamination experiments described in this experimental design, the conditions within operational drinking water pipes are to be simulated in annular reactors (AR) (BioSurface Technologies Corporation, Bozeman, MT). The ARs consist of a glass outer cylinder and a rotating polycarbonate inner cylinder with 20 flush mounted rectangular coupons that can be manufactured from materials such as polyvinyl chloride (PVC), steel, and concrete and obtained from the manufacturer of the AR. These pipe material coupons, which have surfaces that are .55 inch (in) (14 millimeters (mm)) × 5.8 in. (148 mm), simulate the inner surface of drinking water pipes. Shear stress is to be applied to the coupon surfaces by setting the inner cylinder rotation to 100 revolutions per minute (rpm), which produces shear similar to 1 foot (ft)/second (s) (30.5 centimeter (cm)/s) flow in a 6 in. (15.2 cm) pipe[5]. During normal operation, the flow of drinking water through the AR (connected directly to the tap) is to be maintained at a mean velocity of 200 mL/min so that mean the residence time of the water in the AR is five minutes. This rapid flow velocity prevents the depletion of chlorine level over the course of the experiments. The short residence time decreases the chance that desorbing contaminant could re-contaminate an AR surface. The pH, temperature, and free chlorine concentration of the drinking water are to be measured daily. The ARs are to always be operated in the dark by covering them completely with aluminum foil or another opaque material. Some contaminants may adsorb onto the polycarbonate components of the AR and affect the amount of contaminant that is available for coupon contamination. To control against this adsorption negatively impacting experiments, the bulk contamination solution is to be monitored to ensure

that an adequate concentration of contaminant is maintained to achieve pipe coupon contamination.

Prior to any persistence or decontamination experiments, a biofilm is to be grown on the coupons by submerging the required number of coupons into a container that allows recirculation of dechlorinated tap water (outlet near the top of the container and inlet near the bottom of the container) fortified with 1 gram (g) of yeast extract. This water is to be kept in the dark and be recirculated using a pump for four days with an additional 1 g of yeast added after two days. The biofilm growth is to be measured, using heterotrophic plate counts (HPC), on one of the 20 pipe material coupons in the AR. The four-day time period for biofilm growth also serves to condition the pipe material coupons in flowing water prior to coupon contamination. Note that the extent of biofilm growth on the pipe material coupons can have a significant effect on how much contaminant is adsorbed to the pipe coupon so it is important to confirm its presence.

A1.2 Pipe Coupon Contamination Method Verification Experiments

The generation of persistence and decontamination data from this experimental design includes contamination of coupons by exposing them to a bulk solution of at least one contaminant. Thereafter, the persistence of that contaminant on the coupons and/or the application of a decontamination approach are to be investigated to determine both the propensity of the contaminant to persist on the coupons and the effectiveness of decontamination approaches in removing the contaminant from the coupon surface. The usefulness of results from such experiments relies on the accuracy of the required contaminant measurements. In order to be confident in these measurements, two important questions need to be answered about the approach to contaminant measurement.

- When adsorbed to the coupon surface, how well can the contaminant be extracted from that surface?

- When a coupon has been exposed to a bulk solution at a given concentration, how much of the contaminant is adsorbed to the coupon surface?

To answer these two questions, two method verification steps make up the first two steps of the experimental design. First, the surface contamination extraction method is to be validated. Second, the coupon surface contamination method is to be validated. If the contaminant is able to be extracted from the surface of the coupon and it is able to be deposited onto the coupon surface from the bulk solution, the experimental design can proceed to experiments that seek information about contaminant persistence and, if the contaminant is persistent, the effectiveness of various decontamination approaches.

A1.2.1 Method Verification Step 1: Surface Contamination Extraction

The purpose of this step is to determine whether it is possible to extract the contaminant if adsorbed to a pipe material surface. The surface contamination extraction method verification includes the extraction of the entire coupon by placing each coupon in a test tube (BD Falcon

#352045, BD Biosciences, San Jose, CA) filled with an appropriate extraction solution, depending on the characteristics of the contaminant. If the contaminant requires an organic solvent, a glass test tube may need to be used (Fisher #14-962-26H Fisherbrand, Pittsburgh, PA). After inserting the coupon, the test tube is to be sealed with a cap and sonicated for 10 minutes, solvent decanted and replaced with fresh solvent, and then sonicated for another 10 minutes. The decanted solvents are to be combined. For pipe material coupons with a significant amount of corrosion or other loose particles, the contaminant may be bound to that component of the pipe that could separate from the coupon during sonication. The coupon is to be removed and the resulting solution is to be centrifuged and supernatant solution collected for analysis. For organic chemicals, the extraction solution is to be an organic solvent that may be concentrated using nitrogen evaporation prior to analysis using a gas chromatographic mass spectrometer (GC-MS) or other appropriate detection device. For biological organisms, ATSM Type I water should be the extraction solvent and membrane filtration should be used to measure the biological organisms via plate enumeration.

The verification requires 20 coupons of the applicable material type with a biofilm developed as described in Section A1.1. These coupons are to be removed from the biofilm growth container after the four day long biofilm development (in uncontaminated water) and allowed to air dry until water droplets are not visible on the surface, but the surface is still damp. This drying step is to ensure that the contaminant is added to the coupon surface and not the water remaining on the coupon surface. The required drying time is to be documented and used for other surface contamination extraction and measurement verifications. For this phase of the evaluation, each coupon (including blanks) is to be cut approximately in half with scissors and five drops of stock solution applied directly to each smaller coupon (total volume of 15 µL) using a micropipette (Eppendorf Research Plus, Eppendorf International, Hauppauge, NY or equivalent) approximately 10 mm apart. If the contaminant is water soluble, the stock solution should be prepared in ASTM Type I water (for contaminants insoluble in water, an appropriate solvent is to be used). The concentration of the stock solution depends on the quantitation limit of the analytical technique that is available for the contaminant. For example, if the quantitation limit of the applicable analytical technique is 0.1 µg/mL, and the final extraction solution is concentrated to 10 mL, then the minimum amount of contaminant that would be removed and measured from the coupon surface would be 1 µg in 0.075 mL; which corresponds to a contaminant stock concentration (from which the drops originate) of 0.33 µg/mL. Because measuring the contaminant in this scenario would require a 100% recovery and the results would still be at the quantitation limit, this scenario would not be preferable as measurements near the detection limit are likely to be imprecise. Instead, the contaminant stock solutions are to be prepared at concentrations 10, 50, and 500 times higher to provide data that indicates what concentration range provides the best likelihood of precise measurements which corresponds with precise extraction recoveries. Precise extraction recoveries allow for the determination of any differences between experimental conditions (i.e., in this case, contaminant concentration). Using a range of stock solution concentration also provides information about how the extraction recovery varies with concentration. The concentration of the stock solution is to be confirmed with the appropriate analytical method. The drops of contaminant stock solution are to be applied to each coupon as shown in Figure 1.

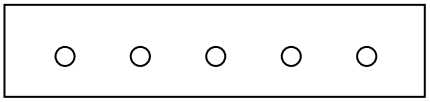

Figure 1. Schematic of drops of contaminant solution across coupon surface

Each concentration is to be applied to five coupons (for a total of 15 coupons). The coupon should air dry until the drops are not visible on the surface. This drying step ensures that the contaminant is on the surface of the coupon (and not still in a droplet of solution) prior to the extraction procedure. The required drying time is to be documented and used for other surface contamination extraction and measurement verifications. Five non-contaminated coupons should also be measured to determine any possible interference. Table 1 gives an overview of the steps included in the surface contamination extraction and measurement method verification.

Table 1. Surface Contamination Extraction Method Verification (Step 1)

Step	Description
1A	Develop biofilm on 20 pipe material coupons (confirm with heterotrophic plate count) and allow coupons to air dry
1B	Determine contaminant stock solution concentration required for detection with 100% contaminant recovery (depends on quantitation limit of contaminant measurement technique)
1C	Prepare contaminant stock solutions at 10, 50, and 500 times (×) the concentration required for attaining detection limit with 100% recovery and confirm the concentration
1D.1	Leave five coupons unspiked for blank analyses
1D.2	Spike five drops of the 10× stock solution on five coupons and air dry
1D.3	Spike five drops of the 50× stock solution on five coupons and air dry
1D.4	Spike five drops of the 500× stock solution on five coupons and air dry
1E	Extract contaminant from all coupons and calculate recovery

The percent recovery (%R) should be calculated using the following equation

$$\%R = \frac{C_R}{C_o} \times 100$$

where C_R is the mass of contaminant (or number of organisms) recovered from the coupon surface and C_o is the mass of contaminant (or number of organisms) originally dispensed onto the coupon surface. The percent recovery data is to be evaluated to determine if the extraction recovery is adequate for obtaining useful contaminant persistence and decontamination data and how the extraction recovery varies with the concentration level of the contaminant applied to the coupons. Following evaluation of the data, it may be necessary to repeat experiments with additional replicates to clarify the results.

A1.2.2 Method Verification Step 2: Surface Contamination

Step 2 involves validating a method to contaminate the surface of the pipe material coupons in a way that simulates an actual intentional contamination of a water distribution system. The surface contamination method to be validated incorporates:

- Preparing coupons with biofilm
- Exposing the coupons to contaminated water (100 mg/ liter (L) or 10^6 CFU/mL, depending on contaminant) in the AR without flow (batch mode)
- Extraction of the contaminant from the coupon using the method validated in Step 1.

To begin the verification, 10 coupons are to be prepared with a biofilm. The coupons are to be loaded in the AR. Then, contaminant is to be added to the AR so that the bulk solution becomes contaminated to the above-stated concentration levels. During this time, the AR is to be operating as described in Section A1.1, but the flow through the AR is to be stopped to increase the contact time between the contaminated water and the coupons. Two hours following the contamination of the water, the coupons are to be removed, rinsed twice with 25 mL of ASTM Type I water, and then extracted and analyzed following the surface contamination extraction and measurement method validated as described in Section A1.2.1. This rinse step is to ensure that the contaminant is extracted from the surface of the coupon and is not just an artifact of residual contamination solution on the surface of the coupon. It is possible that a slow adsorbing contaminant would have to be exposed to the coupons for a longer time or that a higher concentration contamination solution would need to be used. The bulk solution is to be sampled at the start of the contamination time period, at the half-way point, and at the end and the concentration of contaminant confirmed via the appropriate measurement technique to confirm the availability of the contaminant for adsorption.

The extent of surface contamination is to be evaluated to determine whether the level of contamination and precision of these results are adequate for obtaining useful contaminant persistence and decontamination data. Following evaluation of the data, it may be necessary to repeat experiments with additional replicates, increased contamination times, or increased contamination solution concentrations to clarify the results. This verification may have to be repeated for additional coupon material and/or contaminant combinations.

A1.3 Evaluation of Contaminant Persistence

This section describes the approach to evaluating the persistence of a contaminant on various pipe coupon materials. Table 2 provides an overview of the persistence evaluation (PE). Once validated that a contaminant can be extracted from the surface of a coupon and a pipe coupon can be contaminated with contact with a bulk contaminant solution, the persistence of that contaminant on the pipe surface can be evaluated. For each combination of coupon material and contaminant, 20 coupons should be prepared with biofilm as described in Section A1.1.

Table 2. Persistence Evaluation

PE Step	Description	Coupons removed (20 total)
PE 1	Develop biofilm (confirm with heterotrophic plate count) on 20 coupons; remove two coupons as blanks	2
PE 2	Stop flow through the AR, inject enough contaminant into the AR to make the bulk concentration within the AR 100 mg/L of contaminant; wait 2 hours (concentration and time could vary depending on results of surface contamination verification)	0
PE 3	Sample bulk contaminant solution at start, half-way point, and end of contamination period and measure bulk water contaminant concentrations	0
PE 4	Following 2 hour contamination period, remove three coupons as control coupons; extract and determine residual surface contaminant concentration	3
PE 5	Stop AR rotation to simulate stopped flow. Replace bulk contamination solution with uncontaminated water and remain at stopped flow for 24 hours; collect three coupons, extract and determine residual surface contaminant concentration.	3
PE 6	Restart the AR rotation and flow through the AR. Remove three coupons at 4 hours, 1 day, 3 days, and 7 days after restart of AR rotation and flow; extract and determine residual surface contaminant concentration	12
PE 7	Calculate percent persistence for all coupons by comparing to control coupons	0

Two coupons with biofilm should be collected as non-contaminated blanks and the rest of the coupons contaminated with a bulk solution following the validated surface contamination method as described in Section A1.1. Immediately following the coupon contamination step, three coupons are to be removed to serve as control coupons. The amount of contaminant on the surface of these control coupons is to be compared with the amount remaining on the coupons that are left in the AR for various lengths of time following the removal of the control coupons. Collectively, the coupons removed from the AR during this part of the evaluation are to be referred to as the persistence evaluation (PE) coupons.

Thereafter, a stopped flow scenario is to be evaluated by stopping the rotation of the AR and stopping the flow of water through the AR (after the contaminant water is replaced by uncontaminated drinking water). This stopped flow scenario is to be held for 24 hours, which is when three PE coupons are to be removed. After that 24 hour period, the flow of drinking water and AR rotation should be resumed to normal operating conditions as described in Section A1.1. Following the stopped flow scenario, sets of three PE coupons are to be collected from the AR at four different time increments (4 hours, 1 day, 3 days, and 7 days) following the resumption of flow. Following the removal of each of these sets of PE coupons, they are to be extracted and the amount of contaminant on the coupon surfaces compared with the amount on the control coupons collected just after the coupon contamination step. This comparison can be made by calculating the percent persistence (%P) of the contaminant on the coupons as described by the following equation:

$$\%P = \frac{C_{PE}}{C_C} \times 100$$

where C_{PE} is the mass of contaminant (or number of organisms) recovered from the PE coupon surface and C_C is the average mass of contaminant (or number of organisms) originally measured from the surfaces of the control coupon surfaces. The %P data should be evaluated to determine whether the %P at the various time periods is adequate to consider evaluation using various approaches to decontamination of contaminants that are persistent on pipe surfaces. It should be noted that the evaluation of persistence needs to be performed separately for each combination of contaminant and coupon material. In addition, the uncertainty of each of the individual measurements required to calculate the %P (i.e., uncertainty in the analytical measurements required to determine C_{PE} and C_C) is to be used to propagate the uncertainty in the %P calculation. The uncertainty is to be used to determine the adequacy of the %P in making comparisons between the various time increments evaluated during the persistence evaluation. Upon evaluation of the %P, additional replicates may need to be evaluated in order to attain low enough relative uncertainties in order to determine significant differences.

A1.4 Evaluation of Decontamination Approaches

For those contaminant and pipe material combinations that are determined to be persistent, this section describes the evaluation of two approaches to decontaminating pipe, flushing (F) and hyperchlorination (HC). Table 3 provides an overview of the flushing evaluation and Table 4 provides an overview of the HC evaluation. However, the same general evaluation could be performed for other decontamination approaches that alter the makeup of the available tap water. As was the case for the persistence evaluation, a biofilm is to be grown on

Table 3. Evaluation of Flushing as a Decontamination Approach

Step	Description	Coupons removed (20 total)
F 1	Develop biofilm (confirm with heterotrophic plate count) on 20 coupons of the same material; remove two coupons as blanks	2
F 2	Inject enough contaminant into the AR to make the bulk concentration within the AR 100 mg/L of contaminant; wait 2 hours (concentration and time could vary depending on results of surface contamination verification)	0
F 3	Sample bulk contaminant solution at start, half-way point, and end of contamination time and measure bulk water contaminant concentrations	0
F 4	Following 2 hour contamination period, replace bulk contamination solution with uncontaminated water and remove three coupons as contaminated control coupons	3
F 5	Increase AR rotational velocity to 200 rpm (1.64 ft/s) from original velocity of 100 rpm (1 ft/s)	0
F 6	Remove three coupons at 2 hours, 4 hours, and 1 day following increase in rotational velocity	9
F 7	Increase AR rotational velocity to 250 rpm from 200 rpm	0
F 8	Remove three coupons at 4 hours and 1 day following increase in rotational velocity to 250 rpm (1.91 ft/s)	6
F 9	Calculate percent persistence for all coupons by comparing with control coupons	0

20 coupons of the desired material as described in Section A1.1. Thereafter, two coupons are to be collected as blanks and 18 coupons are to be contaminated using the validated surface contamination method. Following contamination, three contaminated coupons are to be removed to serve as the control coupons. The amount of contaminant on the surface of these control coupons should be compared with the amount remaining on the coupons that are left in the AR (operated under increased flow conditions to simulate flushing). These coupons are to be referred to as the decontamination evaluation (DE) coupons.

Specifically, following coupon contamination, the AR inner cylinder rotation is to be raised from 100 rpm (1 ft/s) to 200 rpm (1.64 ft/s), which corresponds to a water velocity of 0.5 ms^{-1} (1.64 ft/s) in a 15.2 cm (6 in.) pipe[3]. This increased rotational speed is to be held for one day. Sets of three DE coupons are to be collected from the AR at three different time increments (2 hour, 4 hours, and 1 day) following the coupon contamination. Then, the rotational speed is to be increased again to 250 rpm (1.91 ft/s) and held for another day, with the collection of three DE coupons after 4 hours and after 1 day of 250 rpm conditions. Following the removal of each set of three DE coupons, the coupons are to be extracted and the amount of contaminant on the coupon compared with the amount on the control coupons collected just after the surface contamination step. This comparison is to be made by calculating the %P of the contaminant originally on the coupons, as described in the previous section. As was the case for the persistence evaluation, the evaluation of decontamination approaches needs to be performed separately for each combination of contaminant and coupon material.

The evaluation of hyperchlorination as a decontamination approach is to be performed as shown in Table 4. The evaluation is to start in a similar way as for the flushing evaluation. However, instead of increasing the rotational velocity of the AR, the rotation of the AR is to be stopped and the drinking water flow through the AR is to also be stopped to simulate a stopped flow scenario. The free chlorine concentration is to then be increased first to 25 mg/L and then to 50 mg/L after several increments of time after which DE coupons are to be collected from the AR. Note that other chemical decontamination approaches could be evaluated in the same way as hyperchlorination if that decontaminant was added in place of the increased free chlorine.

Table 4. Evaluation of Hyperchlorination as a Decontamination Approach

Step	Description	Coupons removed (20 total)
HC 1	Develop biofilm (confirm with heterotrophic plate count) on 20 coupons of same material; remove two coupons as blanks	2
HC 2	Inject enough contaminant into the AR to make bulk concentration within AR 100 mg/L of contaminant; wait 2 hours (contaminant concentration and time could vary depending on results of surface contamination verification)	0
HC 3	Sample bulk contaminant solution at start, half-way point, and end of contamination time and measure bulk water contaminant concentrations	0
HC 4	Following the 2 hour contamination period, remove three coupons as control coupons; extract and determine residual surface contaminant concentration	3
HC 5	Following 2 hour contamination period, stop flow through AR and stop rotation of the AR; increase the free chlorine concentration to 25 mg/L from original concentration of 1 mg/L	0
HC 6	Remove three coupons at 2 hours, 4 hours, and 1 day following increase in free chlorine concentration	9
HC 7	Increase free chlorine concentration to 50 mg/L	0
HC8	Remove three coupons at 4 hours and 1 day following increase in free chlorine concentration to 50 mg/L	6
HC 9	Calculate %P for all coupons by comparing with control coupons	0

Sections A2-A10

Sections A2-A10 of the prospective QAPP will be very dependent on the selection of the contaminant that is to be used for the testing of this experimental design. The section headings are shown below:

- Sampling Methods
- Sample Handling and Custody
- Analytical Methods
- Quality Control
- Instrument/Equipment Testing, Inspection, and Maintenance
- Instrument/Equipment Calibration and Frequency
- Inspection/Acceptance of Supplies and Consumables
- Non-direct Measurements
- Data Management.

Therefore, these sections will need to be completed pending selection of a contaminant (or contaminants) to be tested.

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