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United States Environmental Protection Agency

# Evaluation of Fourth Generation Agent Sample Collection from Surfaces, Water, and Soil

**Office of Research and Development**
**Center For Environmental Solutions and Emergency Response**

**REPORT**

**for**

# Evaluation of Fourth Generation Agent Sample Collection from Surfaces, Water, and Soil

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## DISCLAIMER

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# Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development (ORD) conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation's environmental challenges. The Center's research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and groundwater, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

This report summarizes sampling results of a Fourth Generation Agent (FGA) to determine the sample collection efficiencies from environmental matrix types (e.g., surfaces, water, and soil). Sampling parameters included the evaluation of three different wipe materials and two different wipe wetting solvents to determine optimal sampling efficiencies for the tested surface matrix types. An unconventional sampling procedure involving two different strippable coatings applied to the contaminated surface was also evaluated. Three soil types, three water types (from drinking water utilities), and three surface types were also tested. The results are documented and discussed in this report.

Gregory Sayles, Director

Center for Environmental Solutions and Emergency Response

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| | |
|---|---|
| Ace | acetone |
| AMC | Army Material Command |
| ANOVA | Analysis of Variance |
| CAS | Chemical Abstract Services |
| CB | cotton ball |
| CCV | Continuing Calibration Verification |
| CESER | Center for Environmental Solutions and Emergency Response |
| CG | cotton gauze |
| CoC | Chain of Custody |
| CWA | Chemical Warfare Agent |
| D | deuterium |
| EPA | U.S. Environmental Protection Agency |
| FGA | Fourth Generation Agent |
| HD | sulfur mustard (bis(2-chloroethyl) sulfide) |
| HMRC | Hazardous Materials Research Center |
| HPLC | high performance liquid chromatography |
| HSMMD | Homeland Security and Materials Management Division |
| HSRP | Homeland Security Research Program |
| IDC | Initial Demonstration of Capability |
| IPA | isopropyl alcohol |
| L | Liter |
| LFB | laboratory fortified blank |
| LFSM | laboratory fortified sample matrix |
| LMB | laboratory method blank |
| LC-MS/MS | liquid chromatography - tandem mass spectrometry |
| LLOQ | lower limit of quantitation |
| LRB | laboratory record book |
| MDL | Method Detection Limit |
| MeOH | methanol |
| MRM | multiple reaction monitoring |
| NIST | National Institute of Standards and Testing |
| ORD | Office of Research and Development |
| P&A | Precision and Accuracy |
| PI | Principal Investigator |
| QA | quality assurance |
| QC | quality control |
| QL | Quantitation Limit |
| $R^2$ | coefficient of determination |
| RH | relative humidity |

| | |
|---|---|
| RL | reporting limit |
| RPG | rayon/polyester gauze |
| RSD | relative standard deviation |
| SAM | Selected Analytical Methods for Environmental Remediation and Recovery |
| SD | standard deviation |
| SOP | standard operating procedure |
| STREAMS | Scientific, Technical, Research, Engineering, and Modeling Support |
| TPCS | test parameter control sheet |
| VX | O-ethyl S-(2-[diisopropylamino]-ethyl) methylphosphonothioate |

## ACKNOWLEDGMENTS

This research is part of the U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program's (HSRP's) efforts to evaluate collection of a Fourth Generation Agent (FGA) from surfaces, water, and soil. This effort was directed by the principal investigator (PI) from the Office of Research and Development's (ORD's) Homeland Security and Materials Management Division (HSMMD) within the Center for Environmental Solutions and Emergency Response (CESER). The contributions of the following individuals have been a valued asset throughout this effort.

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# A. EXECUTIVE SUMMARY

The U.S. EPA's Homeland Security Research Program (HSRP) is charged with protecting human health and the environment from accidental and intentional releases of toxic chemicals. Fourth Generation Agents (FGAs) represent a significant homeland security threat. Procedures for collection of samples from different environmental matrices contaminated with FGAs require evaluation and development to ensure they can generate quality data for field and remediation operations.

This project evaluated sampling methods for recovery of the FGA, EA 6162 (which is also known as A-234), from various environmental matrices. Specific objectives for each testing phase included:

1. Evaluation of wipe sampling methods for recovery of EA 6162 samples from the surface of select urban construction materials.
2. Evaluation of strippable coatings for recovery of EA 6162 samples from the surface of select construction materials, particularly wood.
3. Evaluation of methods for analysis of EA 6162 in drinking water.
4. Evaluation of methods for analysis of EA 6162 in soil.

The test protocols were designed to evaluate all aspects of the wipe, water, soil, and strippable coating sample collection methods, including steps for sample collection, sample preservation, and sample preparation prior to analysis to assess for impacts on the accurate quantitation of EA 6162 contamination. Since low clean up levels might be desirable, samples were contaminated with nanogram (ng) to microgram (µg) levels of EA 6162.

After evaluation of various sampling parameters, surface wiping using cotton gauze with methanol provided the highest average recovery efficacies for each material (≥ 95% recovery for all materials) with low relative standard deviations (RSDs), as shown in Table ES 1. The low RSDs suggest good method reproducibility. Method Detection Limit (MDL) values for each material ranged from 0.014 to 0.026 nanograms per square centimeter (ng/cm$^2$) based on the 23 cm x 23 cm (529 cm$^2$) coupon.

Two strippable coatings (latex body paint and DETEX No. 1 Detergent) were evaluated for EA 6162 recovery from the surface of four materials (wood dimensional lumber, vinyl floor tile, ceramic floor tile, and plywood sheathing). Test material coupons were 2.5 cm x 4.0 cm in size. Each coating type was allowed to cure for 2 hours prior to removal. Using the DETEX coating, EA 6162 recovery from vinyl tile was 96% and from ceramic tile was 94%. A significantly lower amount of EA 6162 was recovered from wood dimensional lumber (46%) and plywood (24%), see Table ES 1. Absorption of EA 6162 into the wood dimensional lumber and plywood might have limited the ability of the DETEX coating to remove EA 6162. Compared to DETEX, latex body paint was more difficult to remove and yielded lower and more variable recovery of EA 6162.

Three domestic drinking water sources were evaluated for EA 6162 recovery. Water samples were directly analyzed by liquid chromatography – tandem mass spectrometry (LC-MS/MS), with no additional sample preparation. The average recovery for samples prepared at a concentration of 5.0 ng/mL was 95% to 100%, as shown in Table ES 1, with low relative standard deviations (RSDs). The measured MDL values ranged from 0.018 to 0.021 ng/mL based on a 40-mL water sample. EA 6162 was

also demonstrated to be stable in preserved drinking water samples when refrigerated for up to 28 days with recoveries > 95% for all measured time points.

Three soil types were evaluated for EA 6162 recovery; quartz sand was used as a control. An EA 6162 mass of 50 ng was applied to soil samples as an IPA solution and allowed to dwell on the soil for four time periods: 60 minutes, 24 hours, 7 days, and 14 days, prior to extraction with methanol and analysis by LC-MS/MS. As shown in Table ES 1, EA 6162 recoveries after a 60-minute dwell time ranged from Nebraska soil (77%) statistically significantly lower than Georgia soil (87% recovery) and sand (91% recovery); recovery from clean loam (83%) was not significantly different from any of the other two soils or sand. EA 6162 recoveries decreased for each of the three soil types over time, with the lowest average recovery (11%) observed for Georgia soil after 14 days, while recovery from sand did not decrease. The cause for the difference in EA 6162 recovery between soil types and over time was not studied. The measured MDL values for each soil type ranged from 0.041 nanograms/gram (ng/g) to 0.16 ng/g based on a nominal 5-g soil sample, perhaps reflecting differences in stability and extractability of EA 6162 from these soils. The results suggest that a particular soil type might influence method performance, therefore verification of method performance for a site-specific soil type is important.

***Table ES 1. Summary of EA 6162 Recovery Efficacy and MDLs***

| Testing | Sample Collection Approach | Material | Average EA 6162 Recovery | Recovery RSD | MDL |
|---|---|---|---|---|---|
| Phase 1 - Surface Wipe | Cotton Gauze Wipe with Methanol | Galvanized Steel | 96% | 3.9% | 0.019 ng/cm$^2$ |
| | | Vinyl Tile | 99% | 5.0% | 0.014 ng/cm$^2$ |
| | | Ceramic Tile | 95% | 1.9% | 0.026 ng/cm$^2$ |
| Phase 2 - Strippable Coating | DETEX Strippable Coating | Wood Dimensional Lumber | 46% | 11% | Not Determined |
| | | Vinyl Tile | 96% | 2.3% | |
| | | Ceramic Tile | 94% | 7.2% | |
| | | Plywood | 24% | 43% | |
| Phase 3 - Drinking Water | Direct Sample Analysis | Water Source 1 | 95% | 6.1% | 0.018 ng/mL |
| | | Water Source 2 | 100% | 1.1% | 0.021 ng/mL |
| | | Water Source 3 | 97% | 2.3% | 0.021 ng/mL |
| Phase 4 - Soils | Methanol Extraction | Nebraska Soil | 77% | 2.6% | 0.16 ng/g |
| | | Georgia Soil | 87% | 8.1% | 0.041 ng/g |
| | | Clean Loam | 83% | 11% | 0.087 ng/g |
| | | Sand | 91% | 0.87% | 0.031 ng/g |

## B. INTRODUCTION

The U.S. EPA is responsible for preparing for, responding to, and recovering from threats to public health, welfare, or the environment caused by actual or potential hazardous materials incidents. Hazardous materials include chemical, biological, and radiological substances, whether accidentally or intentionally released. Threat of release of a hazardous chemical agent into the environment is driving EPA's Homeland Security Research Program (HSRP) to systematically evaluate potential sampling and decontamination methods and technologies for chemical warfare agents (CWAs) and other toxic chemical threats. Fourth Generation Agents (FGAs) have emerged as a new class of toxic chemicals that represent a potential significant homeland security, chemical warfare, and terrorism threat. Like the CWA O-ethyl S-(2-[diisopropylamino]-ethyl) methylphosphonothioate (VX), FGAs bind to acetylcholinesterase to cause similar nerve agent poisoning symptoms [1] and persist under certain environmental conditions.

Procedures for collection of samples from different environmental matrices contaminated with FGAs require evaluation and development. FGAs are generally considered to have at least the same toxicity as other CWAs [1]. FGA sampling methods will need to be sufficiently sensitive to allow for detection and quantification of contamination during EPA homeland security response activities. With support from experts across EPA and other federal agencies, EPA's Homeland Security Research Program developed the guidance document *Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2022* [2]. Analytical methods are summarized in SAM that can be used for the analysis of a variety of contaminants in response to a homeland security incident. In SAM, methods for collecting wipe samples from a variety of surfaces (including nonporous, porous, permeable, and uneven surfaces) contaminated with hazardous chemicals [3] including organophosphate (OP) pesticides [4], CWAs [5 and 6], and CWA degradation products [7], have been developed and evaluated experimentally.

Analytical methods for the detection of FGAs are not reported in SAM. To ensure optimal usability of SAM methods, data demonstrating fit-for-purpose collection/recovery of FGAs from surfaces via wipe sampling and from other environmental matrices (e.g., water and soil) are needed. SAM methods should address complications that might arise from environmental sampling (e.g., matrix interferents, enhancement/suppression effects, etc.), since understanding these complications is important during response and remediation efforts. Furthermore, sampling processes (e.g., collection methods, sample size/volume, sample preservation steps, etc.) might affect analytical detection, and impact data that is provided to decision-makers during response, remediation, and recovery efforts. Accurately characterizing FGA hazards present at contaminated sites is enabled by an understanding of how to properly collect samples, preserve samples, and ensure that sampling strategies and procedures accurately measure concentration levels at contaminated sites, especially at very low FGA levels.

### *B.1 Project Objectives*

All testing was conducted using the FGA A-234, but for the purposes of this study it will be referred to as EA 6162 for the remainder of this report. This project evaluated sampling methods for recovery of EA 6162 from various environmental matrices. Specific project objectives included:

1. Evaluation of wipe sampling methods for recovery of EA 6162 samples from the surface of select, commonly used, urban materials.
2. Evaluation of strippable coatings for recovery of EA 6162 samples from the surface of select materials, particularly wood.
3. Evaluation of methods for analysis of EA 6162 in drinking water.
4. Evaluation of methods for analysis of EA 6162 in soil.

The test protocols were designed to evaluate aspects of the wipe, water, soil, and strippable coating sample collection methods, including steps for sample collection, sample preservation, and sample processing/preparation prior to analysis to assess for impacts to accurate quantitation of EA 6162 contamination. Since CWAs, including FGAs, can be extremely toxic, depending on route of exposure, studies were performed with low levels of EA 6162 quantitated with liquid chromatography – tandem mass spectrometry (LC-MS/MS).

Data quality objectives and performance criteria provided the requirements for determining the adequacy of data generated during this project. Valid data were assumed if the measurement quality objectives for the test measurements were met. To this end, accuracy was ensured by the calibration of the instruments used during testing, including the LC-MS/MS as described in Section D.6. The representativeness and uniformity of the test materials were critical attributes to assure reliable test results. Representativeness means that test samples were typical of commonly encountered materials and matrices (i.e., found in field, or "nonlaboratory", settings) in terms of quality, surface/matrix characteristics, etc. Uniformity means that all samples (per material/matrix) were essentially equivalent for the purposes of testing. Replicate coupon samples were obtained from a large enough quantity of material such that the replicate samples would have uniform characteristics. Samples were visually examined, and any with noticeable abnormalities on or within the sample were rejected from use as control or test samples. Except for cutting bulk materials to size to produce replicate coupons for testing, the test materials identified in Section D were used as received. No pretreatment, simulated fouling, accelerated aging, or other material conditioning steps were performed. Water samples were received from three municipal drinking water source providers, selected to have water quality characteristics typical of finished drinking water. Two soil samples were sourced from EPA's Office of Research and Development and a third soil was acquired from a commercial vendor. While three soils cannot be considered representative of the thousands of possible soil types, the composition of the three spanned several important soil characteristics that are discussed in the Materials and Methods and Results sections.

## *B.2 Test Facility Description*

All testing was performed at Battelle's Hazardous Materials Research Center (HMRC) located in West Jefferson, Ohio. The HMRC is certified to work with chemical surety material under a provisioning agreement with oversight by the U.S. Army Materiel Command (AMC; Provisioning Agreement Battelle-1). Wherever applicable and required, the reporting requirements of this agreement were followed.

## C. RESEARCH APPROACH

Project objectives were achieved through execution of a series of tests, completed in four parts, to evaluate EA 6162 sampling and recovery methods:

- Part 1 testing evaluated methods for recovery of EA 6162 from material surfaces via wipe sampling. The wipe sampling method that was evaluated for EA 6162 recovery was based on the method developed by Willison et al. for recovery of pesticides [4]. Surface material types included zinc-galvanized, low-carbon steel (Part 1A testing), peel-and-stick vinyl floor tile (Part 1B testing), and glazed ceramic tile (Part 1C testing), representative of material surfaces that may be commonly encountered in urban indoor settings. Low-carbon steel was used in lieu of stainless steel during previous EPA studies using a CWA [8 and 9] as low-carbon steel is more frequently encountered in urban settings, with common uses that include construction framing, fabrication of machine parts, vehicle bodies, and water transportation pipelines. Refer to Sections D.2 and E.1 for additional information.
- Part 2 testing evaluated methods for recovery of EA 6162 from a porous material via application of a strippable coating (i.e., spread-and-peel surface sampling media) to the material surface. Strippable coatings (specifically, body paint) have been evaluated, with varying degrees of success, for recovery of the CWAs VX and sulfur mustard (HD) from the surface of a variety of materials, including finished and unfinished wood flooring [12]. Strippable coating was evaluated during this project for recovery of EA 6162 from the surface of construction-grade lumber, peel-and-stick vinyl floor tile, glazed ceramic tile, and pine plywood sheathing. Refer to Sections D.3 and E.2 for additional information.
- Part 3 testing evaluated the analysis of EA 6162 in drinking water. EPA Method 538 [10] is a direct aqueous injection LC-MS/MS method for determination of selected nonvolatile chemical contaminants in drinking water. The experimental conditions in EPA Method 538 were demonstrated for the determination of EA 2192, a toxic byproduct of VX degradation, in water [11]. The adapted method is provided in EPA's SAM document for analysis of EA 2192 in both drinking water and nondrinking water samples [2]. During this project, the method evaluated for recovery of EA 6162 from drinking water samples was based on the adapted EPA Method 538 procedures [11]. Refer to Sections D.4 and E.3 for additional information.
- Part 4 testing evaluated methods for recovery of EA 6162 from soil. A microscale extraction method with subsequent LC-MS/MS analysis of extracts was evaluated for recovery and quantification of EA 6162 from soil. Refer to Sections D.5 and E.4 for additional information.

Experimental design, specific testing details, and test matrices for each part of testing, including wipe sampling, water sampling, soil sampling, and sampling via strippable coatings, are described in the following sections below.

# D. MATERIALS AND METHODS

## *D.1 Test Chemicals*

EA 6162 (Chemical Abstract Services [CAS] # 2387496-06-0) owned by the U.S. Government and held in Battelle's FGA inventory was used in testing. EA 6162 from the same lot was used for all testing. A stable-labeled analog of EA 6162 containing five deuterium atoms (D), in lieu of hydrogen (EA 6162-$d_5$), was also available for use. All EA 6162-$d_5$ originated from the same lot, also owned by the Government.

All testing involved the use of a solution of EA 6162. EA 6162 and EA 6162-$d_5$ purity were determined by dissolving a known volume of the neat chemical into a known volume of solvent and using the EA 6162 density (1.123 g/mL) to calculate the final concentration (target 1,000 µg/mL concentration). Solutions were analyzed on a gas chromatograph with a flame ionization detector to determine the relative abundance of EA 6162 or EA 6162-$d_5$ as determined by peak area and reported as percent purity. Solvent blanks were used to correct for possible solvent contaminants. Measured purities are shown in Table 1. Chemical purity was greater than 95% over the course of testing.

***Table 1. EA 6162 Purity Measurements***

| Analyte | Lot Number | Analysis Date | Purity |
|---|---|---|---|
| EA 6162 | S3127-1 | 9/25/20 | 97.8% |
| EA 6162 | S3127-1 | 4/8/21 | 97.8% |
| EA 6162 | S3127-1 | 11/11/21 | 97.8% |
| EA 6162-$d_5$ | C2973-1 | 8/12/20 | 98.6% |
| EA 6162-$d_5$ | C2973-1 | 4/8/21 | 96.9% |
| EA 6162-$d_5$ | C2973-1 | 11/11/21 | 97.1% |

Malathion (CAS # 121-75-5, part # 36143-100 mg, Sigma-Aldrich, St. Louis, MO, 99.2% purity) was used as a recovery surrogate for all testing.

## *D.2 Part 1-Wipe Sampling*

Coupons used as test and control samples during all wipe sample collection testing measured 23 cm x 23 cm. The three test materials were galvanized steel, vinyl tile, and ceramic tile. Type 304 stainless steel was used as a control material. Refer to Table 2 for information on all test materials.

***Table 2. Part 1 Test Materials***

| Material | Description | Supplier | Part # | Preparation |
|---|---|---|---|---|
| Galvanized Steel | • Zinc-galvanized low-carbon steel sheet<br>• 12 inch x 12 inch, 0.024 inch thick | McMaster-Carr (Aurora, OH) | 8943K113 | • Cut to 23 cm x 23 cm<br>• Wiped with isopropyl alcohol (IPA)-soaked wipe upon receipt to remove any residual cutting oil/grease<br>• Cleaned with air to remove dust/debris |
| Vinyl Tile | • Armstrong Flooring Terraza Grand Arctic White Peel and Stick Vinyl Tile<br>• 18 inch x 18 inch, 2 mm thick | Lowe's (Mooresville, NC) | 737982 | • Cut to 23 cm x 23 cm<br>• Cleaned with air to remove dust/debris |
| Ceramic Tile | • Chilo Gray Glazed Ceramic Floor Tile<br>• 12 inch x 12 inch, 8 mm thick | Lowe's (Mooresville, NC) | 683041 | • Cut to 23 cm x 23 cm<br>• Cleaned with air to remove dust/debris |
| Stainless Steel | • Type 304 stainless steel<br>• 9 inch by 9 inch, 0.024 inch thick | Adept Products, Inc. (West Jefferson, OH) | Custom | • Wiped with IPA-soaked wipe upon receipt to remove any residual cutting oil/grease<br>• Air-dried prior to use |

Three different wipe types were evaluated:

- Medtronic Curity™ non-sterile, non-woven, rayon/polyester (RPG) gauze sponge, 2 inch x 2 inch (part # 22-037-921, Fisher Scientific, Pittsburgh, PA). Evaluated during a previous EPA study [13] for recovery of CWAs from the surface of coatings.
- Dukal™ sterile woven cotton gauze (CG) sponge, 4 inch x 4 inch (part # 22-415-507, Fisher Scientific). Evaluated for recovery of pesticides by Willison et al. [4].
- Medique Swisspers™ triple-sized cotton balls (CBs), approximately 1 inch across (part # 19-090-703, Fisher Scientific). Evaluated for recovery of pesticides by Willison at al. [4].

Two wipe wetting solvents were evaluated:

- Isopropanol (CAS # 67-63-0, Optima™ grade, part # A464-4, > 99.9%, Fisher Scientific). Isopropyl alcohol (IPA) generally demonstrates compatibility with a variety of material types (i.e., materials are not degraded, weakened, or otherwise damaged by contact with or immersion in IPA), and IPA was evaluated as a wipe wetting solvent by Willison et al. for recovery of pesticides [4].
- Methanol (CAS # 67-56-1, Optima grade, part # A456-4, Fisher Scientific, or similar). FGAs generally demonstrate high solubility in methanol.

During Part 1, testing solutions of EA 6162 were prepared in IPA and in water (High Performance Liquid Chromatography grade, part # W5SK-4, Fisher Scientific). IPA solutions were prepared once and stored at -20 ± 10 °C. Water solutions were prepared daily prior to use. Sixteen discrete 1µL droplets of EA 6162 solutions were applied approximately 5 cm apart

in a 4 x 4 pattern across the 23 cm x 23 cm coupon surface, see Figure 1. Droplets were delivered using a 50-µL Hamilton syringe (part # 80985, Hamilton, Reno, NV) fitted into a repeating dispenser (part # PB600-1, Hamilton). The dispenser delivers 1/50$^{th}$ of the syringe volume with each actuation; thus, each activation of the dispenser delivered a 1-µL droplet.

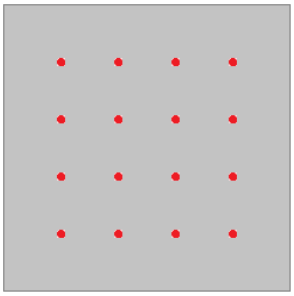


***Figure 1. Part 1 FGA Coupon Spike Pattern***

The IPA solution quickly spread out on the galvanized steel surface when applied and evaporated within several seconds. The aqueous solution beaded on the galvanized steel surface when applied and took approximately ten minutes to evaporate. Example photos of both solutions applied to galvanized steel are shown in Figure 2; some droplets are circled in red. The IPA droplets spread to approximately 5 - 10 mm in diameter while the water droplets remained approximately 1 - 2 mm in diameter.

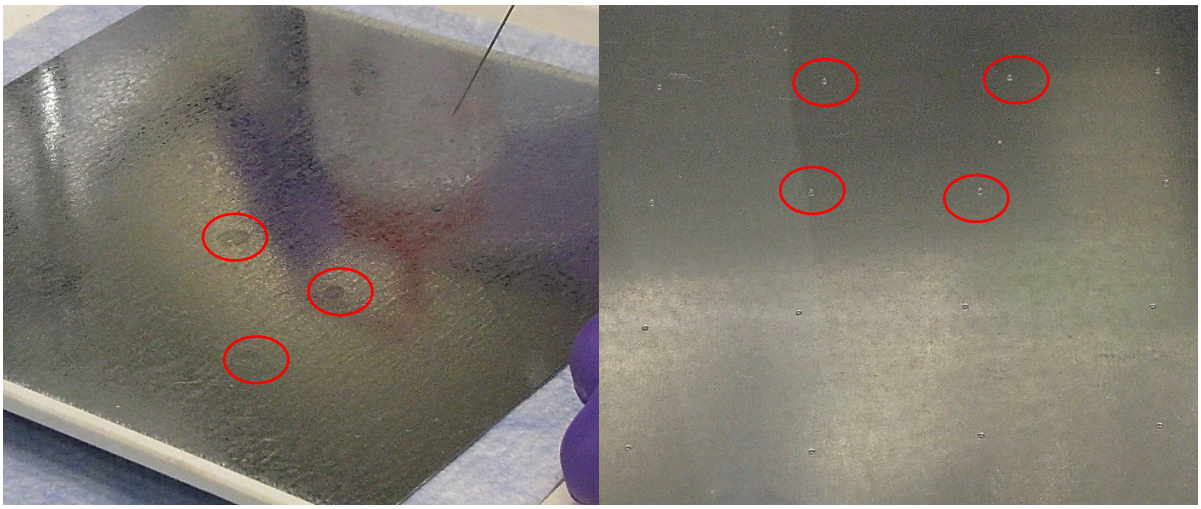


***Figure 2. EA 6162 Solution Applied to Galv. Steel – IPA (Left) and Aqueous (Right)***

Only the aqueous EA 6162 solution was applied to vinyl tile and ceramic tile. The solution beaded on the vinyl tile and ceramic tile surfaces when applied and took less than 30 minutes to evaporate; droplets were difficult to see, so evaluation of evaporation progress was only assessed at the 30-minute timepoint. Example photos of the EA 6162 solutions applied to vinyl tile and ceramic tile are shown in Figure 3; droplets have been circled in red.

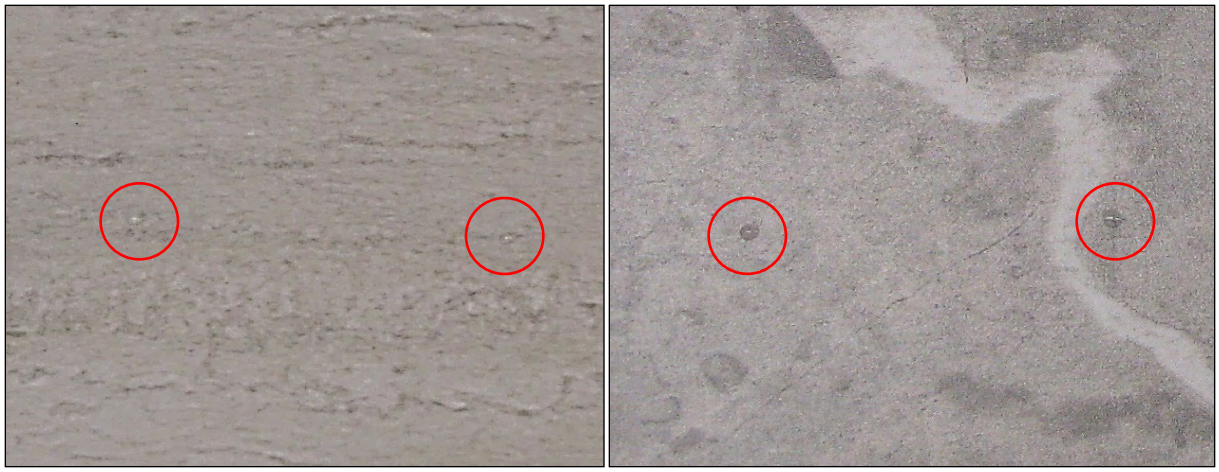


***Figure 3. Aqueous EA 6162 Solution Applied to Vinyl Tile (Left) and Ceramic Tile (Right)***

The droplets of EA 6162 solution were allowed to dwell on the coupon for various time intervals, see Section E.1. After the dwell time, one of three wipe types was wetted with one of two wetting solvents and used to collect a wipe sample from the surface of each coupon. The wipe wetting volumes used for each wipe type were 1.5 mL for the rayon/polyester gauze wipe and 3.0 mL for the cotton gauze and cotton ball wipes. Collection of wipe samples involved three wiping passes in horizontal, vertical, and diagonal directions, with the wipe folded between each pass so that the applied surface of the wipe was inside the fold. A fourth "pass" was along the perimeter of the coupon. As wipes were moved across the surface of a coupon, the leading edge of the wipe was maintained. Figure 4 illustrates the wipe sampling pattern. Since the cotton balls could not be folded, they were turned slightly between each of the four wipe passes (horizontal, vertical, diagonal, perimeter).

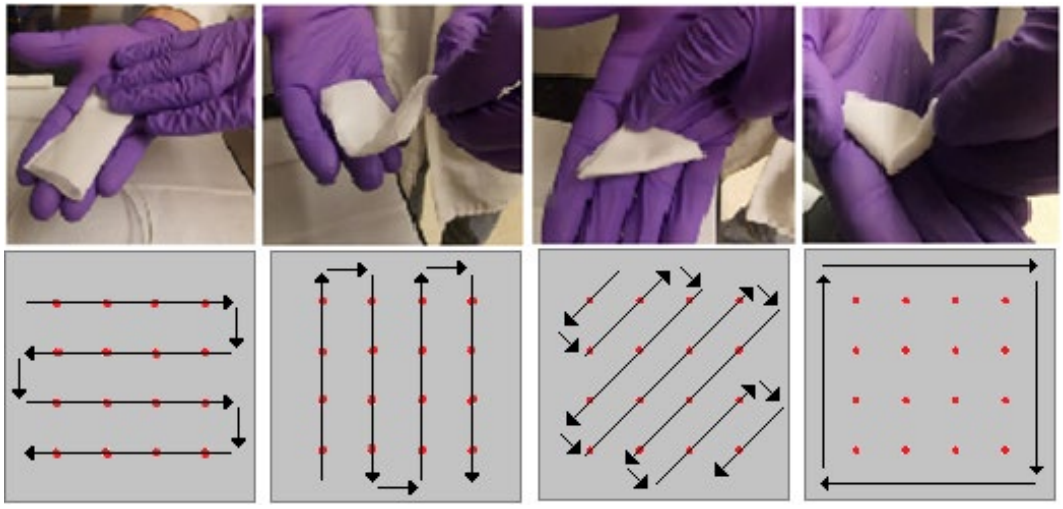


***Figure 4. Part 1 Wipe Sampling Pattern***

Following collection, the wipes were spiked with malathion as a surrogate dissolved in IPA solvent (to assess efficiency of EA 6162 extraction from the wipe) by applying sixteen 1-µL droplets of malathion solution directly on the wipes. The wipes were then extracted with 15 mL of methanol inside of a 60-mL wide-mouth glass jar (part # 05-719-51, Fisher Scientific). The

cotton gauze and cotton ball wipes absorbed most of the 15 mL of methanol used to extract the wipes. To ensure proper wipe extraction, the wipes were compressed into a corner of the extraction jar to completely submerge the wipe in extraction solvent using a 20-mL disposable syringe (part # 14-823-16J, Fisher Scientific). Only the solvent was aspirated into and out of the syringe and through the wipe three times prior final solvent extraction from the wide-mouth jar. The solvent was aliquoted in to autosampler vials. After the first recovery efficacy trial, a 30-mL disposable syringe (7510A655, McMaster-Carr) was used to improve the cotton ball extraction using the same procedure described above.

An initial study was performed to determine the volume of solvent applied to each wipe type that was retained following wiping. The rayon/polyester gauze retained 0.94 mL of methanol and 0.82 mL of IPA. The cotton gauze retained 2.1 mL of methanal and 2.0 mL of IPA. The cotton ball retained 2.3 mL of methanol and 2.2 mL of IPA. These volumes were added to the 15 mL of extraction solvent when calculating the recovered mass of EA 6162; see Equation 2.

Following collection of the first wipe sample, a repeat wipe sample was obtained for each coupon using a new wetted wipe. The additional wipe sample was extracted separately from the first wipe. The amount of EA 6162 recovered in the first and second wipe samples was compared to assess the potential for increased EA 6162 recovery through composite sampling. The wipes in extraction solvent were then sonicated at 40 kiloHertz (kHz) for 10 minutes. Following sonication, an aliquot of each wipe extract was transferred to glass vials. Extract samples were prepared for LC-MS/MS analysis by mixing 100 µL of extract with 900 µL of internal standard solution (see Section D.6). This preparation resulted in a 10-fold dilution of the extract sample.

### *D.3 Part 2-Strippable Coating*

Coupons used as test and control samples during all strippable coating sample collection testing measured 2.5 cm x 4.0 cm. The four test materials were wood, plywood, vinyl tile, and ceramic tile. Type 304 stainless steel was used as a control material. Refer to Table 3 for information on wood dimensional lumber and plywood. Refer to Table 2 for information on vinyl tile, ceramic tile, and stainless steel.

***Table 3. Part 2 Test Materials***

| Material | Description | Supplier | Part # | Preparation |
|---|---|---|---|---|
| Wood | • Kiln-dried dimensional lumber<br>• 2 inch x 6 inch x 10 feet | Home Depot (Atlanta, GA) | 161721 | • Cut to 2.5 cm x 4.0 cm<br>• Cleaned with air to remove dust/debris |
| Plywood | • Pine plywood sheathing<br>• 3 ply construction<br>• 11/32 inch x 4 feet x 8 feet | Lowe's (Mooresville, NC) | 12246 | • Cut to 2.5 cm x 4.0 cm<br>• Cleaned with air to remove dust/debris |

Two different strippable coatings were evaluated:

- Latex body paint (part #010538, Liquid Latex Fashions, Montgomeryville, PA, white color)
- DETEX No. 1 Detergent - Latex Decontaminant (Sherman Chemicals, Dorset, UK)

Aqueous solutions of EA 6162 were prepared daily prior to use. No degradation of EA 6162 was observed during this evaluation. Sixteen discrete 1-µL droplets of EA 6162 solution were applied approximately 5 mm apart in a 4 x 4 pattern (see Figure 1) across the 2.5 cm x 4.0 cm coupon surface. Droplets were delivered using a 50-µL Hamilton syringe fitted into a repeating dispenser. A piece of masking tape (part # 76505A411, McMaster-Carr) was applied to the edge of each coupon prior to spiking and coating application to aid in lifting the dried coating from the surface.

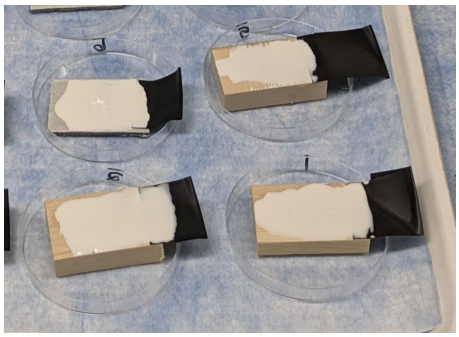
EA 6162 solution beaded on all vinyl tile, ceramic tile, and stainless-steel coupons after spiking while the droplets soaked into and/or spread on all wood dimensional lumber and plywood coupons; see Figure 5. Note that the plywood photo was taken immediately after solution application, before the droplets soaked into the wood. The solution was visibly dry on all coupons after the allotted 30-minute dry time. A residue was observed on the stainless-steel coupons after the dry time; the residue was not present after removal of the strippable coating for all testing.

A 0.5 mL volume of strippable coating was added to the coupon using a calibrated repeater pipette (part # 4982000322, Eppendorf, Hamburg, Germany). Coatings were spread as evenly as possible across the entire coupon surface using a disposable plastic putty knife (part # 3546A411, McMaster-Carr), with one putty knife used per coupon, then disposed. Every effort was made to ensure the putty knife did not contact the coupon surface. Coatings were allowed to dry for at least 2 hours prior to removal. Figure 6 shows example test material coupons after strippable coating was applied and spread across the surface. Figure 6 also shows examples of removal of each coating type after the 2-hour dry time. Broad-tipped disposable polystyrene forceps (part # 5378-0042, Burkle, Inc., Greensboro, NC) were used to remove the coating. The DETEX turned a darker yellow after drying, providing a visible indication once drying was complete. DETEX was less stretchable and easier to remove than the latex body paint since the DETEX also did not adhere as strongly to the test material surface. Latex body paint may be more difficult to work with in the field due to the difficulty with removal from some surfaces, such as the wood dimensional lumber and plywood.

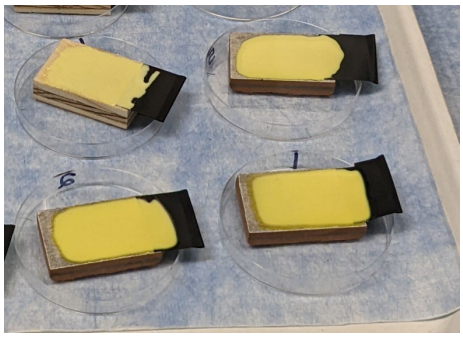
Extraction of the removed coating used 10 mL of solvent (methanol, acetone, or 3:7 methanol:acetone mixture) as described in Sections E.2 and F.2. Following removal of the strippable coating each coupon was placed in 15 mL of solvent (methanol, acetone, or 3:7 methanol:acetone mixture) to extract any residual EA 6162. Coupons were placed into the extraction solvent with the spiked side facing down. Extraction of the DETEX with acetone resulted in a yellow-tinted solution. The vinyl tile partly dissolved in the acetone during coupon extraction, and the acetone extracts of the plywood coupons were yellow in color. Extraction of nontarget compounds from vinyl tile and plywood may have negatively impacted the response

of the malathion internal standard. Based on these observations, acetone may not be the optimum solvent for the extraction of vinyl tile and plywood. Note that during field use the material substrate would not be solvent extracted, only the strippable coating would be extracted.

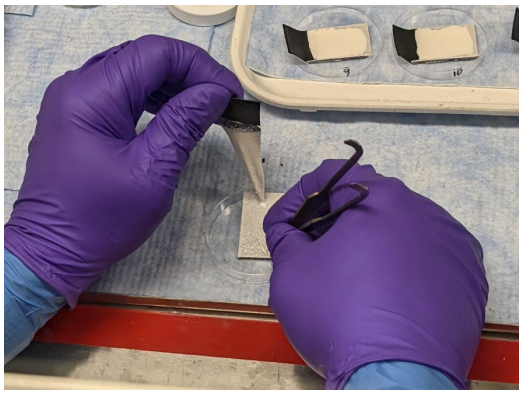

***Figure 5. EA 6162 Solution Droplets Applied to Strippable Coating Coupons***



Latex Body Paint on Vinyl Tile
and Wood Dimensional Lumber



DETEX on Plywood and
Ceramic Tile



Latex Body Paint Removal
from Vinyl Tile

DETEX Removal from
Stainless Steel

***Figure 6. Strippable Coatings After Application and During Removal***

### *D.4 Part 3-Water Testing*

Source waters from three municipal drinking water utilities were used for this evaluation and are designated as: Water Source 1, Water Source 2, and Water Source 3. Water quality parameters were provided by the supplier for each water source sample; see Table 4.

Upon receipt, water samples were preserved based on EPA Method 538 methodologies [11] by adding 10 mL of 2M ammonium acetate and 2 mL of 32 g/L 2-mercaptopyridine N-oxide sodium (sodium omadine) to a 1-L sample, in this case by adding the preservatives to a 1-L volumetric flask and filling the flask to the 1-L mark with source sample water. The water was well mixed and transferred to a clean 1-L glass bottle (Thermo Scientific™, Waltham, MA, certified amber Boston Round, part # 349-1000). Preserved water samples were stored in a refrigerator maintained at 2-8 °C. Immediately following preservation, water samples were tested once for pH using a pH meter (model # Orion Star A221, Thermo Scientific™) and free chlorine using check test strips (part # 481026, SenSafe, Rockhill, SC). See Table 4 for information regarding receipt and preservation. Testing with water samples began within one week of receipt.

***Table 4. Part 3-Water Source Information***

| Parameter | Water Source 1 | Water Source 2 | Water Source 3 |
|---|---|---|---|
| Sample Date | 5/5/2021 | 5/18/2021 | 5/26/2021 |
| pH | 8.9 | 7.3 | 7.3 |
| Total Hardness (mg/L as $CaCO_3$) | 122 | 300 | 106 |
| Free Chlorine (mg/L) | 1.20 | 0.37 | 3.7 |
| Turbidity (ntu) | 0.05 | 0.26 | 0.10 |
| Specific Conductance (uS/cm) | 360 | 537 | 286 |
| Alkalinity (mg/L as $CaCO_3$) | 75 | 260 | 79 |
| TOC (mg/L) | 0.599 | Not provided | 1.3 |
| Receipt Date | 5/6/2021 | 5/20/2021 | 5/27/2021 |
| Water Temperature at Receipt (°F) | 36.4 | 36.5 | 33.5 |
| Preservation Date | 5/7/2021 | 5/20/2021 | 6/1/2021 |
| pH Post Preservation | 7.5 | 7.5 | 7.4 |
| Free Chlorine Post Preservation (mg/L) | 0 | 0 | 0 |

All water samples were prepared for LC-MS/MS analysis by mixing 100 µL of water sample with 100 µL of methanol and 800 µL of internal standard solution (see Section D.6); 100 µL of methanol was used to matrix-match the water samples with the prepared calibration standards. This preparation resulted in a 10-fold dilution of the water sample.

### *D.5 Part 4-Soil Testing*

Soil from three different sources were used in testing:

- Nebraska Aglands AP – Nebraska soil, obtained from EPA Office of Research and Development, with a composition of 5.1% sand, 57.5% silt, 31.7% clay, 1.9% total organic carbon (TOC), and pH 5.5 when measured in a 1:1 soil: water mix.
- Georgia BT2 – Georgia soil, obtained from EPA Office of Research and Development with a composition of 46% sand, 22% silt, 32% clay, 0.2% TOC, and pH 5.0 when measured in 1:1 soil: water mix.
- Clean Loam Soil – part # CLNLOAM6, lot # LRAD0102, Supelco, Burlington, MA. Composition unknown, 38.5 mg/kg TOC.

Purified quartz sand (part # 3382-05, JT Baker, Inc., Phillipsburg, NJ) was used as a control throughout testing. A visual inspection of the soils was performed upon receipt, and the Nebraska soil particles were found to be nonuniform, with large particles present. A mortar and pestle were used to grind the soil into smaller, more uniform particles. Before and after grinding photos are shown in Figure 7. The Georgia soil, clean loam soil, and quartz sand particles were fine and uniform and did not require any grinding; photos are shown in Figure 8.


***Figure 7. Nebraska Soil Before and After Grinding***

**Georgia**

**Clean Loam**

**Sand**

***Figure 8. Georgia Soil, Clean Loam, and Sand - As Received***

An evaluation of the moisture content of the Nebraska and Georgia soils was performed by weighing out 5 g of each soil, heating the soils to 100 °C for 24 hours, then weighing the dried soil. The moisture content was determined for both soils, Nebraska soil at 3.2% and Georgia soil at 1.3%. To make the soils more field representative, all soil types were moistened with distilled water before testing. Large batches (200 g) of each of the three test soils and the sand control were slowly wetted with water (in 5-mL increments) and mixed, with a total of 20 mL water added to each soil (equating to 9% added water by weight). The wetted soils were tumbled and mixed for over 48 hours to make sure the soils were adequately mixed and evenly moistened. After tumbling, the soils appeared fairly uniform. The Nebraska soil and clean loam were clumpy, and a mortar and pestle as well as a spatula were used to break up the soils into smaller particles. Photos of the moistened soils after tumbling and breaking apart are shown in Figure 9.

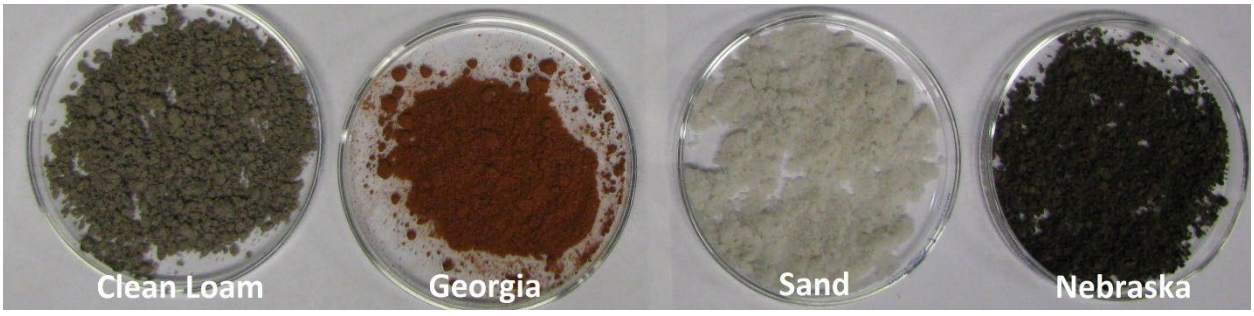



***Figure 9. Part 4-Soils after Water Addition***

Figure 10 shows the approximate location of each test material on the United States Department of Agriculture texture triangle from the National Soils Survey Handbook. Note that the Nebraska soil sand, silt, and clay components should sum to 100% but do not. The clean loam was located near the center of the loam region of the texture triangle since particle sizes for sand, silt, and clay were not provided by the vendor. The quartz sand was located at the 100% sand vertex of the triangle. Each test material was located in a distinct area of the texture triangle.

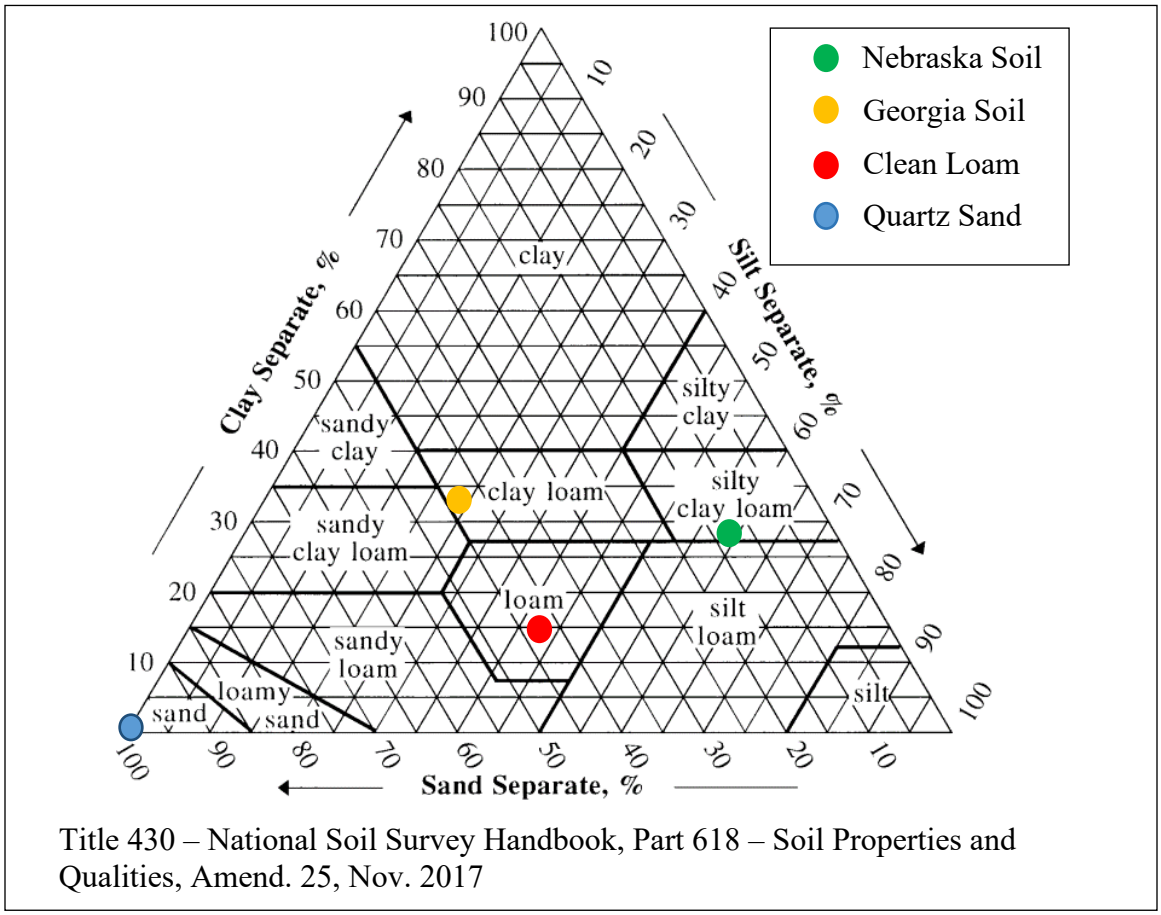



***Figure 10. Texture Triangle and Particle-Size Limits***

Testing for the recovery of EA 6162 from soils involved preparation of glass vials (16 mL, part # # 03-340-60D, Fisher Scientific) containing 5.0 ± 0.5 g of soil. Soils were spiked with EA 6162 by applying one 10-µL droplet of dilute EA 6162 solution in IPA onto the soil. The vials were capped and gently shaken side-to-side to move the spiked soil around. The 10-µL droplet of EA 6162 solution in IPA was visible in the Nebraska and Georgia soils after spiking but was not visible in the clean loam or the sand after spiking. The droplet was not visible in any of the soils after the EA 6162 dwell time (see Section E.4). Photos of the spiked soils are shown in Figure 11.

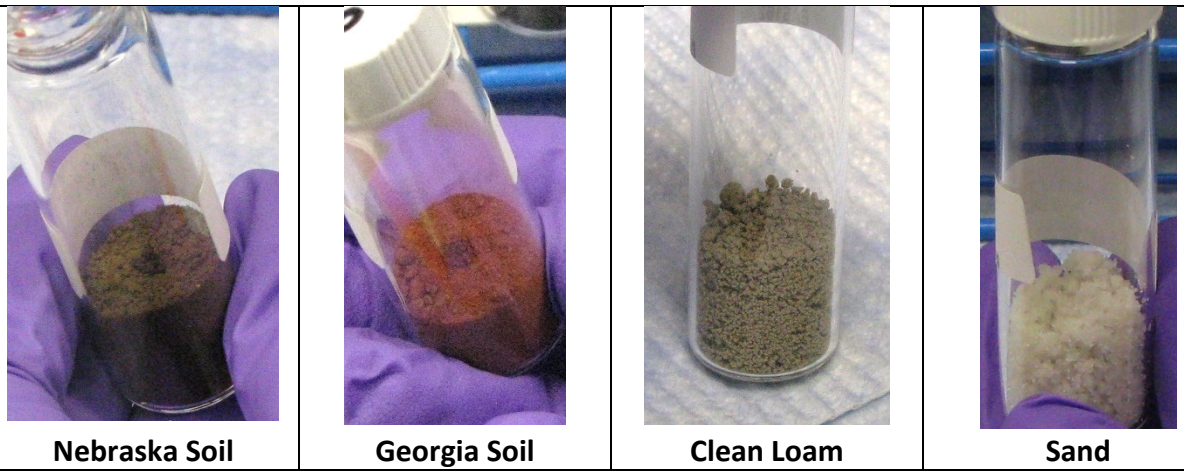



***Figure 11. Part 4-EA 6162 Solution Applied to Soils***

After the dwell time, the vials of soil were spiked with 50 ng of malathion as a surrogate by applying 10 µL of malathion solution (5.0 µg/mL in IPA) onto the soil. Following surrogate addition, the soil vials were immediately extracted with 10 mL of methanol, then sonicated at 40 kHz for 10 minutes. Following sonication, a 3-mL disposable Luer lock syringe (part # 14-823-282, Fisher Scientific) was used to filter an aliquot of each extract through a 1-µm polytetrafluoroethylene filter (part # 309657, BD) into a glass vial. Filtered extract samples were prepared for LC-MS/MS analysis by mixing 100 µL of extract with 900 µL of internal standard solution (see Section D.6). This preparation resulted in a 10-fold dilution of the extract sample.

### *D.6 Quantitative Analysis by LC-MS/MS*

EA 6162 samples from each part of testing were analyzed using reversed-phase high performance liquid chromatography (HPLC) and multiple reaction monitoring (MRM) mass spectrometry on an AB Sciex (Toronto, CA) 5500 triple quadrupole MS coupled to a Shimadzu (Kyoto, Japan) 20 XR series LC. Labeled EA 6162 was used as the internal standard for quantitation of EA 6162. Labeled malathion (malathion-$d_{10}$, part # 777498, Sigma-Aldrich, purity = 99.6%) was used as the internal standard for quantitation of malathion. Internal standards were added to calibration standards, controls, and test sample extracts just prior to LC-MS/MS analysis using a solution of EA 6162-$d_5$ and malathion-$d_{10}$ in water as the sample diluent. LC method parameters are shown in Table 5, and MS/MS parameters are shown in Table 6.

***Table 5. LC Method Parameters***

| | |
|---|---|
| **Autosampler** | Shimadzu SIL20ACXR |
| **Column** | Restek™ (Bellefonte, PA) Allure PFPP, 5 µm, 50 x 2.1 mm |
| **Mobile Phase A** | 2 mM ammonium formate/2 mM formic acid in water |
| **Mobile Phase B** | 2 mM ammonium formate/2 mM formic acid in methanol |
| **Flow Rate** | 0.3 mL/min |
| **Injection Volume** | 10 µL |
| **Run Time** | 5.5 minutes |
| **Sample Temperature** | 7 °C |
| **Autosampler Wash** | 90% acetonitrile |

| | |
|---|---|
| **Column Temperature** | Ambient (21 ± 2°C) |

***Table 6. MS/MS Method Parameters***

| | |
|---|---|
| **Software** | Analyst 1.7 |
| **Ionization Mode** | Positive Electrospray |
| **Scan Mode** | MRM |
| **EA 6162 Transition** | 225.1 > 74.1 |
| **EA 6162-$d_5$ Transition** | 230.1 > 74.1 |
| **Malathion Transition** | 331.1 > 126.9 |
| **Malathion-$d_{10}$ Transition** | 341.1 > 132.0 |
| **Curtain Gas** | 20 psi |
| **Collision Gas** | 6 pounds per square inch (psi) |
| **Ion Spray** | 5500 volts |
| **Temperature** | 500 °C |
| **Ion Source (GS1)** | 50 psi |
| **Ion Source (GS2)** | 50 psi |
| **Interface Heater** | On |
| **Entrance Potential** | 8 volts |
| **Cell Exit Potential** | 12 volts |

Stock solutions of all test chemicals were prepared in isopropyl alcohol. Stock solution concentrations were calculated using Equation 1:

$$C_A = \frac{V_A \times D_A \times P_A}{V_S} \qquad \text{Equation 1}$$

where:

$C_A$ = analyte concentration in the solution (mg/mL)

$V_A$ = volume of analyte added to the solvent (µL)

$D_A$ = density of the analyte (mg/µL)

$P_A$ = purity of the analyte (refer to Table 1)

$V_S$ = volume of the solvent (mL)

Stock solutions were used to prepare calibration standards. Table 7 provides a summary of the calibration standard concentrations and continuing calibration verification (CCV) standard concentrations. The expiration date for EA 6162 calibration standards was six months from the date of preparation. The expiration date for CCV standards was one month from the date of preparation. Calibration standards and CCVs were stored at -20 ± 10 °C. The signal-to-noise ratio of the lowest calibration standard was required to be 3:1 at minimum. Example calibration curves for EA 6162 and malathion are shown in Appendix A.

***Table 7. LC-MS/MS Calibration Curve and CCV Levels***

| Calibration Level | EA 6162 (ng/mL) | Malathion (ng/mL) | Internal Standards (ng/mL) |
|---|---|---|---|
| 1 | 0.01 | 0.01 | 0.5 |
| 2 | 0.02 | 0.02 | 0.5 |
| 3 | 0.04 | 0.04 | 0.5 |
| 4 | 0.2 | 0.2 | 0.5 |
| 5 | 0.4 | 0.4 | 0.5 |
| 6 | 1.0 | 1.0 | 0.5 |
| 7 | 3.5 | 3.5 | 0.5 |
| Low CCV | 0.01 | 0.01 | 0.5 |
| High CCV | 1.0 | 1.0 | 0.5 |

CCVs were prepared by a second analyst and were analyzed prior to sample analysis and after no more than every ten samples. Quantifiable samples bracketed by a failing CCV were reanalyzed. Calibration standards and CCVs were matrix-matched to the samples as closely as possible. For example, test samples in IPA prepared for analysis by a 10-fold dilution in water were quantitated using calibration standards and CCVs prepared in 10% IPA. Note that due to this 10-fold dilution performed during all analytical sample preparation, the LC-MS/MS lower limit of quantitation (LLOQ) for EA 6162 was equal to the Level 1 calibration standard concentration multiplied by 10, i.e., 0.10 ng/mL.

A linear regression was used to describe the data with $1/x^2$ weighting with the origin excluded. The lowest calibration standard and low CCV were required to calculate back (using the established calibration curve) to within ± 25% of the nominal standard concentration. The remaining calibration standards and high CCV were required to calculate to within ± 15% of the nominal standard concentration. Table 8 provides a summary of the analytical run acceptance criteria for various parameters.

***Table 8. Analysis Performance Parameters and Acceptance Criteria***

| Parameter | Criterion |
|---|---|
| Calibration curve coefficient of determination ($r^2$) | ≥ 0.990 |
| Acceptance limit for lowest calibration standard processed against curve | 75 - 125% |
| Acceptance limit for remaining calibration standards processed against curve | 85 - 115% |
| Solvent blank | < lowest calibration standard (0.01 ng/mL) |
| Acceptance limit for lowest CCV | 75 - 125% |
| Acceptance limit for remaining CCVs | 85 - 115% |
| Signal-to-noise ratio for quantitation ion for the lowest calibration standard (0.01 ng/mL) | Minimum of 3:1 |
| Retention time for target compound and internal standard | ± 0.1 min. as same compounds in mid-level calibration standard |
| Internal standard area in samples | 50% to 150% area of nearest passing calibration standard or passing CCV |

All testing met the analysis acceptance criteria, except as discussed in Section F. Calibration and CCV acceptance criteria were tighter than the criteria for EPA Method 538 whereas internal standard response acceptance was the same.

The concentration of analyte in samples was interpolated using the analyte area/internal standard area ratio and the regression equation generated from calibration standards. Samples that quantitated below the lowest calibration standard concentration, or displayed area counts below the area counts of the lowest concentration on the calibration curve, were reported as less than the LLOQ corrected to account for the sample dilution factor (e.g., < 0.10 ng/mL). Samples that quantitated above the highest calibration standard were diluted using calibrated positive displacement pipettes and reanalyzed. If the internal standard area was outside the acceptance range of 50% - 150%, the sample dilution was increased to reduce matrix effects and the sample reanalyzed until a passing internal standard area was obtained. All data was reported to two significant figures.

### *D.7 Calculations*

The mass of EA 6162 recovered from spike controls, sample wipes, soils, strippable coatings, and strippable coating coupons via solvent extraction was determined according to Equation 2:

$$Mass_{Rec} = Conc_{Ext} \times DF \times Vol_{Ext} / 1{,}000 \qquad \text{Equation 2}$$

where:

$Mass_{Rec}$ = EA 6162 mass recovered (µg)

$Conc_{Ext}$ = EA 6162 sample extract concentration (ng/mL) determined by LC-MS/MS analysis

DF = dilution factor (minimum 10-fold dilution)

$Vol_{Ext}$ = Volume of extraction solvent (mL)

1,000 = Conversion of ng to µg

The concentration of EA 6162 in water samples was determined according to Equation 3:

$$Conc_{Wat} = Conc_{Sample} \times DF_{Ext} \qquad \text{Equation 3}$$

where:

$Conc_{Water}$ = EA 6162 concentration in water sample (ng/mL)

$Conc_{Sample}$ = EA 6162 in analytical sample (ng/mL) determined by LC-MS/MS analysis

DF = dilution factor

Note that for Part 3 testing, all water samples received only a 10-fold dilution as no sample concentrations were above the calibration curve highest standard.

Reporting limits (RLs) for Part 1, 3, and 4 testing were based on the LLOQ and the extraction solvent volume. The Part 3 water RL was equal to the LLOQ.

- Part 1 cotton gauze and cotton ball RL = 0.10 ng/mL x 17 mL solvent = 1.7 ng
- Part 1 rayon/polyester gauze RL = 0.10 ng/mL x 16 mL solvent = 1.6 ng
- Part 2 coating RL = 0.10 ng/mL x 10 mL solvent = 1.0 ng
- Part 2 coupon RL= 0.10 ng/mL x 15 mL solvent = 1.5 ng
- Part 3 RL = 0.1 ng/mL
- Part 4 RL = 0.10 ng/mL x 10 mL solvent = 1.0 ng

# E. TESTING APPROACH

Part 1, 2, 3 and 4 testing utilized data quality indicators to help evaluate the adequacy of test results; refer to Section G. Data quality indicators included the following:

- Laboratory fortified blanks (LFBs) were identical to test material samples except that an inert control material was used. LFBs were used to assess the analysis of EA 6162 and surrogate independent of test material matrix.
- Laboratory method blanks (LMBs) consisted of test or control materials that were not spiked with EA 6162. LMBs were used to assess potential interfering compounds associated with test materials and possible cross contamination during testing. Surrogate was typically added to the LMB samples.
- Laboratory fortified sample matrix (LFSM) samples were prepared by post-spiking EA 6162 and surrogate into a sample collected from unspiked test or control material. LFSMs were used to assess the analysis of EA 6162 and surrogate in the presence of test material or control matrix independent of sample collection.
- Spike controls consisted of clean solvent spiked with EA 6162 and surrogate at the same level as the test samples, LFBs, LMBs, and LFSM samples. For each testing trial, all EA 6162 percent recoveries and surrogate percent recoveries were calculated relative to the average measured concentration of EA 6162 and surrogate in the spike controls for that trial.

Part 3 did not include LFSM samples. Parts 1, 3, and 4 also included Initial Demonstration of Capability (IDC) testing using a control material. IDC tests included four Precision and Accuracy (P&A) samples spiked with EA 6162 and surrogate at a “higher” mass level and seven Quantitation Limit (QL) samples spiked with EA 6162 and surrogate at a “lower” mass level. P&A were spiked such that sample concentrations were near the midpoint of the LC-MS/MS calibration curve. QL samples were spiked so that sample concentrations were just above the LC-MS/MS LLOQ. The P&A study was performed to demonstrate laboratory capability. QLs represented the smallest detectable concentration of analyte greater than the method detection limit. Both the P&A and QL results needed to achieve program goals of precision (average recovery) and bias (RSD).

## *E.1 Part 1-Wipe Sampling*

Four separate wipe collection studies were performed during Part 1:

1. IDC for wipe sampling
2. Part 1A wipe collection from galvanized steel
3. Part 1B wipe collection from vinyl tile
4. Part 1C wipe collection from ceramic tile

The IDC was performed using the same procedure/method planned for use during recovery efficacy testing except that stainless steel was used as the test material. The IDC included the preparation of P&A samples and QL samples; see Table 9 for the IDC test matrix. P&A coupon samples were spiked with 75 ng of EA 6162 (16 x 1-µL of 4.7 µg/mL solution). Following collection, P&A wipes were spiked with 75 ng of surrogate (16 x 1-µL of 4.7 µg/mL solution). QL samples were spiked with 3.0 ng of EA 6162 (16 x 1-µL of 0.19 µg/mL solution). Following collection, QL wipes were spiked with 3.0 ng of surrogate (16 x 1-µL of 0.19 µg/mL solution).

LMBs consisted of wipe samples collected from blank test materials. Following collection but prior to extraction, the wipes were spiked with 75 ng surrogate. Two sets of spike controls were prepared to match the P&A and QL EA 6162 and surrogate levels. For the spike controls, EA 6162 and surrogate were spiked directly into solvent in a glass jar. The spike control replicates were prepared at the start, middle, and end of each test, bracketing sample spiking. Following preparation, spike controls were sonicated, aliquoted and analyzed as described in Section D.2.

The IDC matrix was executed three times, once per wipe type. As noted in Section D.2, all wipes and spike controls were extracted in 15 mL of methanol.

***Table 9. Part 1 IDC Test Matrix***

| Sample Type | Material | Wetting Solvent | EA 6162 Spike Mass (ng) | Surrogate Spike Mass (ng) | Replicates |
|---|---|---|---|---|---|
| P&A | Stainless Steel | Methanol | 75 | 75 | 4 |
| P&A | Stainless Steel | IPA | 75 | 75 | 4 |
| QL | Stainless Steel | Methanol | 3.0 | 3.0 | 7 |
| QL | Stainless Steel | IPA | 3.0 | 3.0 | 7 |
| LMB | Stainless Steel | Methanol | None | 75 | 1 |
| LMB | Stainless Steel | IPA | None | 75 | 1 |
| LMB | Galvanized Steel | Methanol | None | 75 | 1 |
| LMB | Galvanized Steel | IPA | None | 75 | 1 |
| Spike Control | None | NA | 75 | 75 | 3 |
| Spike Control | None | NA | 3.0 | 3.0 | 3 |

NA = not applicable

The Part 1A recovery efficacy test matrix for galvanized steel is shown in Table 10 and was executed three times for galvanized steel, once per wipe type (RPG, CG, and CB) to provide one iteration of wipe recovery testing. A total of three Part 1A recovery test iterations were performed using different EA 6162 dwell times on the galvanized steel for each iteration; two different EA 6162 solutions were used for spiking:

- Iteration 1 – 60-minute dwell time spiked with EA 6162 in IPA
- Iteration 2 – 1-minute dwell time spiked with EA 6162 in IPA
- Iteration 3 – 30-minute dwell time spiked with EA 6162 in water

This procedure resulted in a total of nine Part 1A trials using galvanized steel. All galvanized steel test coupons were spiked with 10 µg of EA 6162 (16 x 1-µL of 625 µg/mL in either IPA or

water solution). Two wipe samples were collected in series from each test sample coupon; each wipe was extracted separately. Following collection, test sample wipes were spiked with 10 µg of surrogate (16 x 1-µL of 625 µg/mL in IPA solution). LFSM controls consisted of wipe samples collected from unspiked test coupons. Following collection but prior to extraction, the LFSM wipes were spiked with the same amount of EA 6162 and surrogate as used for the test samples.

LFBs consisted of wipe samples collected from unspiked stainless-steel coupons used as a control material. Following collection but prior to extraction, the wipes were spiked with EA 6162 and surrogate. One set of LFBs was spiked at the same level as the LFSMs. A second set of LFBs was spiked with 3 ng of EA 6162 (16 x 1-µL 190 ng/mL in IPA solution) as well as 3 ng of surrogate (16 x 1-µL 190 ng/mL in IPA solution).

One set of galvanized steel LMBs was spiked with 10 µg of surrogate prior to extraction. A second set of galvanized steel LMBs and a set of stainless steel LMBs were collected but not spiked with surrogate. Two sets of spike controls were prepared to match the 10 µg and 3.0 ng EA 6162 and surrogate levels. For the spike controls, EA 6162 and surrogate were spiked directly into solvent in a glass jar. As noted in Section D.2, all wipes and spike controls were extracted in 15 mL of methanol. All samples spiked at the 10-µg level were diluted 1,000-fold prior to analysis.

***Table 10. Part 1A Wipe Recovery Efficacy Test Matrix***

| Sample Type | Material | Wetting Solvent | EA 6162 Spike Mass | Surrogate Spike Mass | Number Wipes | Reps |
|---|---|---|---|---|---|---|
| Efficacy | Galvanized Steel | Methanol | 10 µg | 10 µg | 2 | 5 |
| Efficacy | Galvanized Steel | IPA | 10 µg | 10 µg | 2 | 5 |
| LFSM | Galvanized Steel | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFSM | Galvanized Steel | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 3.0 ng | 3.0 ng | 1 | 3 |
| LFB | Stainless Steel | IPA | 3.0 ng | 3.0 ng | 1 | 3 |
| LMB | Galvanized Steel | Methanol | None | 10 µg | 1 | 1 |
| LMB | Galvanized Steel | IPA | None | 10 µg | 1 | 1 |
| LMB | Stainless Steel | Methanol | None | None | 1 | 1 |
| LMB | Stainless Steel | IPA | None | None | 1 | 1 |
| LMB | Galvanized Steel | Methanol | None | None | 1 | 1 |
| LMB | Galvanized Steel | IPA | None | None | 1 | 1 |
| Spike Control | None | NA | 10 µg | 10 µg | NA | 5 |
| Spike Control | None | NA | 3.0 ng | 3.0 ng | NA | 3 |

NA = not applicable

The Part 1B recovery efficacy test matrix for vinyl tile is shown in Table 11. Only the rayon/polyester gauze and cotton gauze wipes were evaluated for EA 6162 recovery from vinyl tile, therefore the test matrix was executed two times, once per wipe type. Each recovery test was performed with only a 30-minute dwell time using an aqueous EA 6162 spiking solution, resulting in a total of two trials using vinyl tile.

***Table 11. Part 1B Wipe Recovery Efficacy Test Matrix***

| Sample Type | Material | Wetting Solvent | EA 6162 Spike Mass | Surrogate Spike Mass | Number Wipes | Reps |
|---|---|---|---|---|---|---|
| Efficacy | Vinyl Tile | Methanol | 10 µg | 10 µg | 2 | 5 |
| Efficacy | Vinyl Tile | IPA | 10 µg | 10 µg | 2 | 5 |
| LFSM | Vinyl Tile | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFSM | Vinyl Tile | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 15 ng | 15 ng | 1 | 3 |
| LFB | Stainless Steel | IPA | 15 ng | 15 ng | 1 | 3 |
| LMB | Vinyl Tile | Methanol | None | 75 ng | 1 | 1 |
| LMB | Vinyl Tile | IPA | None | 75 ng | 1 | 1 |
| LMB | Stainless Steel | Methanol | None | None | 1 | 1 |
| LMB | Stainless Steel | IPA | None | None | 1 | 1 |
| LMB | Vinyl Tile | Methanol | None | None | 1 | 1 |
| LMB | Vinyl Tile | IPA | None | None | 1 | 1 |
| Spike Control | None | NA | 10 µg | 10 µg | NA | 5 |
| Spike Control | None | NA | 15 ng | 15 ng | NA | 3 |
| Spike Control | None | NA | None | 75 ng | NA | 3 |

NA = not applicable

The Part 1C recovery efficacy test matrix for ceramic tile is shown in Table 12; this test matrix mirrored the Part 1B test matrix. Only the rayon/polyester gauze and cotton gauze wipes were evaluated for EA 6162 recovery from ceramic tile. Therefore, the test matrix was executed two times, once per wipe type. Each recovery test was performed with only a 30-minute dwell time using an aqueous EA 6162 spiking solution, resulting in a total of two trials using ceramic tile.

***Table 12. Part 1C Wipe Recovery Efficacy Test Matrix***

| Sample Type | Material | Wetting Solvent | EA 6162 Spike Mass | Surrogate Spike Mass | Number Wipes | Reps |
|---|---|---|---|---|---|---|
| Efficacy | Ceramic Tile | Methanol | 10 µg | 10 µg | 2 | 5 |
| Efficacy | Ceramic Tile | IPA | 10 µg | 10 µg | 2 | 5 |
| LFSM | Ceramic Tile | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFSM | Ceramic Tile | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | IPA | 10 µg | 10 µg | 1 | 3 |
| LFB | Stainless Steel | Methanol | 15 ng | 15 ng | 1 | 3 |
| LFB | Stainless Steel | IPA | 15 ng | 15 ng | 1 | 3 |
| LMB | Ceramic Tile | Methanol | None | 75 ng | 1 | 1 |
| LMB | Ceramic Tile | IPA | None | 75 ng | 1 | 1 |
| LMB | Stainless Steel | Methanol | None | None | 1 | 1 |
| LMB | Stainless Steel | IPA | None | None | 1 | 1 |
| LMB | Ceramic Tile | Methanol | None | None | 1 | 1 |
| LMB | Ceramic Tile | IPA | None | None | 1 | 1 |
| Spike Control | None | NA | 10 µg | 10 µg | NA | 5 |
| Spike Control | None | NA | 15 ng | 15 ng | NA | 3 |
| Spike Control | None | NA | None | 75 ng | NA | 3 |

NA = not applicable

All vinyl tile and ceramic tile test coupons were spiked with 10 µg of EA 6162 (16 x 1-µL of 625 µg/mL in either IPA or water solution). Two wipe samples were collected in series from each test sample coupon; each wipe was extracted separately. Following collection, test sample wipes were spiked with 10 µg of surrogate (16 x 1-µL of 625 µg/mL in IPA solution). LFSM samples were prepared in the same manner as for galvanized steel coupon trials. One set of LFB samples was spiked at the same level as the LFSMs. A second set of LFBs was spiked with 15 ng of EA 6162 (16 x 1-µL 940 ng/mL in IPA solution), as well as 15 ng of surrogate (16 x 1-µL 940 ng/mL in IPA solution), an increase in EA 6162 and surrogate mass loadings compared to the 3 ng LFB spikes conducted for the galvanized steel trials. The increased mass loading was performed due to elevated EA 6162 and surrogate recoveries observed for the 3 ng spikes performed in the galvanized steel trials. The elevated recoveries were attributed to possible cross contamination from the 10 µg spikes conducted for other test samples (see discussion in Section F.1). Separate syringes were also used to spike the two different mass levels for the vinyl tile and ceramic tile trials.

One set of galvanized steel LMBs was spiked with 75 ng of surrogate prior to extraction, a decrease in surrogate spike level compared to the galvanized steel trials. The surrogate spike level was reduced to allow these LMB samples to be analyzed with a 10-fold dilution. A second set of galvanized steel LMBs and a set of stainless steel LMBs were collected but not spiked with surrogate. Two sets of spike controls were prepared to match the 10 µg and 15 ng EA 6162 and surrogate levels; a third set of spike controls was prepared to match the 75 ng surrogate level. For the spike controls, EA 6162 and surrogate were spiked directly into solvent in a glass jar. As

noted in Section D.2, all wipes and spike controls were extracted in 15 mL of methanol. All samples spiked at the 10-µg level were diluted 1,000-fold prior to LC-MS/MS analysis.

An MDL study was also performed, using 40 CFR Part 136 Appendix B Rev 2, except limited LMBs were prepared for each test matrix. A total of eight replicates were prepared over three days. MDL samples were prepared in the same manner as the recovery efficacy samples; however, the coupons were spiked with 75 ng of EA 6162 applied as 16 x 1 µL drops of a 4.7 µg/mL water solution and allowed to dwell on the coupons for 30 minutes. The test matrix is shown in Table 13. Two wipes were collected in series in the same manner as the recovery efficacy samples. One LMB was prepared for each material type on each day of MDL sample preparation. The 75 ng of surrogate (10 µL of a 7.5 µg/mL in IPA) was applied to the wipes just before solvent extraction. One set of three spike controls was prepared on each day of MDL sample preparation. All wipe extract analyses were also performed on three separate days.

***Table 13. Part 1 MDL Study Test Matrix***

| Sample Type | Material | EA6162 Spike Mass (ng) | Surrogate Spike Mass (ng) | Number Wipes | Reps |
|---|---|---|---|---|---|
| MDL | Vinyl Tile | 75 | 75 | 2 | 8 |
| MDL | Ceramic Tile | 75 | 75 | 2 | 8 |
| MDL | Galvanized Steel | 75 | 75 | 2 | 8 |
| LFB | Stainless Steel | 75 | 75 | 1 | 8 |
| LMB | Vinyl Tile | None | 75 | 1 | 3 |
| LMB | Ceramic Tile | None | 75 | 1 | 3 |
| LMB | Galvanized Steel | None | 75 | 1 | 3 |
| Spike Control | None | 75 | 75 | NA | 9 |

NA = not applicable

### *Part 2-Strippable Coating*

Two separate studies were performed during Part 2 testing using each strippable coating:

1. Strippable coating extraction efficiency, and
2. Strippable coating recovery efficacy.

For the extraction efficiency samples, the EA 6162 spike level was 10 µg (16 x 1.0 µL drops of a 625 µg/mL solution in water) onto 304 stainless-steel coupons. The first extraction efficiency trial used 10 µg of malathion for the surrogate (1 x 16 µL of a 625 µg/mL solution in IPA). For all remaining trials the surrogate level was reduced to 250 ng of malathion (10 µL of a 25 µg/mL solution in IPA) to ensure that the surrogate would be within the instrument calibration range regardless of the sample dilution. Three different solvents were evaluated for extraction efficiency: methanol, acetone (CAS # 67-64-1, Optima grade, part # A929SK-4, Fisher Scientific), and a 3:7 methanol:acetone mixture (MeOH:Ace). Once the strippable coating was removed, surrogate was spiked onto the coating and onto the stainless-steel coupon. The coating and the stainless-steel coupon were immediately placed in separate jars of solvent for extraction. LMB

samples consisted of samples collected from blank stainless-steel coupons with applied strippable coating. Following collection but prior to extraction, the removed LMB coating and LMB coupon were spiked with surrogate and extracted separately in solvent.

See Table 14 for a summary of the extraction efficiency test matrix. A total of five replicates were performed for each solvent type. Table 14 testing was performed twice, once for the latex body paint (designated Trial EE 1) and once for DETEX (designated Trial EE 2).

***Table 14. Strippable Coating Extraction Efficiency Test Matrix***

| Sample Type | EA 6162 Spike Mass (µg) | EA 6162 Dwell (Minutes) | Extraction Solvent | Coating | Coupon |
|---|---|---|---|---|---|
| Extraction Efficiency | 10 | 30 | Methanol | 5 | 5 |
| LMB | NA | NA | Methanol | 1 | 1 |
| Extraction Efficiency | 10 | 30 | Acetone | 5 | 5 |
| LMB | NA | NA | Acetone | 1 | 1 |
| Extraction Efficiency | 10 | 30 | 3:7 MeOH:Ace | 5 | 5 |
| LMB | NA | NA | 3:7 MeOH:Ace | 1 | 1 |
| Spike Control | 10 | NA | Methanol | 3 | |

The extraction solvent used during strippable coating recovery efficacy testing was selected based on the results of the extraction efficiency testing.

Strippable coating recovery efficacy (Table 15 and Table 16) was performed following identical procedures and spike levels described for extraction efficiency testing and used the selected solvent from the solvent extraction evaluation testing. Table 15 shows the recovery efficacy matrix for vinyl tile and wood dimensional lumber, and Table 16 shows the recovery efficacy matrix for ceramic tile and plywood. Table 15 testing was performed twice, once for latex body paint (Trial Eff 1) and once for DETEX (Trial Eff 3). Table 16 testing was also performed twice, once for latex body paint (Trial Eff 2) and once for DETEX (Trial Eff 4). The LFB was prepared in the same manner as the test materials, except 304 stainless steel was used. LFSMs consisted of unspiked coated coupons. Following removal of the strippable coating, the coating and test material coupon were each spiked with 10 µg of EA 6162 (applied as 16 x 1.0 µL drops of a 625 µg/mL solution), allowed to sit for 10 minutes, spiked with surrogate, and then solvent extracted. All samples spiked at the 10-µg level were diluted 1,000-fold prior to LC-MS/MS analysis

***Table 15. Wood Dimensional Lumber and Vinyl Tile Strippable Coating Recovery Efficacy Test Matrix***

| Sample Type | Material | EA 6162 Spike Mass (µg) | EA 6162 Dwell (Minutes) | Coating | Coupon |
|---|---|---|---|---|---|
| Efficacy | Wood Dimensional Lumber | 10 | 30 | 5 | 5 |
| Efficacy | Vinyl Tile | 10 | 30 | 5 | 5 |
| LFB | Stainless Steel | 10 | 30 | 3 | 3 |
| LFSM | Wood Dimensional Lumber | 10 | 10 | 1 | 1 |
| LFSM | Vinyl Tile | 10 | 10 | 1 | 1 |
| LFSM | Stainless Steel | 10 | 10 | 1 | 1 |
| LMB | Wood Dimensional Lumber | NA | NA | 1 | 1 |
| LMB | Vinyl Tile | NA | NA | 1 | 1 |
| LMB | Stainless Steel | NA | NA | 1 | 1 |
| Spike Control | None | 10 | NA | 3 | |

***Table 16. Ceramic Tile and Plywood Strippable Coating Recovery Efficacy Test Matrix***

| Sample Type | Material | EA 6162 Spike Mass (µg) | EA 6162 Dwell (Minutes) | Coating | Coupon |
|---|---|---|---|---|---|
| Efficacy | Ceramic Tile | 10 | 30 | 5 | 5 |
| Efficacy | Plywood | 10 | 30 | 5 | 5 |
| LFB | Stainless Steel | 10 | 30 | 3 | 3 |
| LFSM | Ceramic Tile | 10 | 10 | 1 | 1 |
| LFSM | Plywood | 10 | 10 | 1 | 1 |
| LFSM | Stainless Steel | 10 | 10 | 1 | 1 |
| LMB | Ceramic Tile | NA | NA | 1 | 1 |
| LMB | Plywood | NA | NA | 1 | 1 |
| LMB | Stainless Steel | NA | NA | 1 | 1 |
| Spike Control | None | 10 | NA | 3 | |

### *E.3 Part 3-Water Testing*

Four separate studies were performed during Part 3 testing using each of the water sources:

1. Water IDC;
2. Water recovery efficacy;
3. Water storage study; and
4. Water Method Detection Limit (MDL).

The Initial Demonstration of Capability included the preparation of P&A samples and QL samples. For the P&A samples the EA 6162 and surrogate spike levels were both 200 ng. Spiking

used 16 µL of a 12.5 µg/mL solution of EA 6162 or surrogate in IPA into 40 mL of water, resulting in a final water concentration of 5.0 ng/mL for each P&A sample; refer to Table 17.

***Table 17. Water IDC Test Matrix***

| Sample Type | Water Type | EA 6162 Concentration (ng/mL) | Surrogate Concentration (ng/mL) | Reps |
|---|---|---|---|---|
| P&A | HPLC Water | 5.0 | 5.0 | 4 |
| QL | HPLC Water | 0.50 | 0.50 | 7 |
| LMB | HPLC Water | NA | 5.0 | 1 |
| LMB | Source 1 | NA | 5.0 | 1 |

NA = not applicable

For the QL samples the EA 6162 and surrogate spike level were both 20 ng. Spiking used 16 µL of a 1.25 µg/mL solution of EA 6162 or surrogate in IPA into 40 mL of water, resulting in a final water concentration of 0.50 ng/mL for each QL sample; refer to Table 17. After performing the required 10-fold dilution, the QL concentration was five times the instrument LLOQ; the QL concentration was selected to ensure adequate instrument response. HPLC water was used for one LMB. LMB analysis was also planned for water from each source; however, only Water Source 1 was available when the IDC testing was performed.

To test the efficacy of recovering EA 6162 from each of the three water sources, 200 ng of EA 6162 and surrogate were each spiked into 40 mL of water for a final concentration of 5.0 ng/mL; refer to Table 18. The 5.0 ng/mL final concentration was selected to be equivalent to 10-times the QL. Spiking used 16 µL of a 12.5 µg/mL solution of EA 6162 or surrogate in IPA. HPLC water was used for the LFB, which was prepared and analyzed in the same manner as three water source samples. Note that because each water sample was received from the municipal providers on a different date, a separate set of LFBs and LMBs was prepared and analyzed each time a recovery efficacy test was performed.

***Table 18. Water Recovery Efficacy Test Matrix***

| Sample Type | Water Type | EA 6162 Concentration (ng/mL) | Surrogate Concentration (ng/mL) | Reps |
|---|---|---|---|---|
| Efficacy | Source 1 | 5.0 | 5.0 | 5 |
| Efficacy | Source 2 | 5.0 | 5.0 | 5 |
| Efficacy | Source 3 | 5.0 | 5.0 | 5 |
| LMB | Source 1 | NA | 5.0 | 1 |
| LMB | Source 2 | NA | 5.0 | 1 |
| LMB | Source 3 | NA | 5.0 | 1 |
| LFB | HPLC Water | 5.0 | 5.0 | 5 |
| LMB | HPLC Water | NA | 5.0 | 1 |

An EA 6162 stability study was performed over the course of 28 days of refrigerated storage. Refer to the below steps and Table 19 for sample preparation:

1. A single 100-mL volumetric flask was used to measure each water source sample. LMBs were also be prepared in a 100-mL volumetric flask;
2. 0.5 µg of EA 6162 was spiked into the 100 mL volumetric flask (equal to 5.0 ng/mL in the water samples). Spiking used 20 µL of a 25 µg/mL solution of EA 6162 in IPA;
3. The 100 mL spiked sample was well mixed and divided into 10-mL aliquots for either immediate analysis (Day 0) or storage for the stability study. LMBs were also divided into 10-mL aliquots. A 22-mL Polyseal vial (part # GLC-01007, Qorpak, Clinton, PA) was used for aliquot storage;
4. Aliquots for the stability study were stored in a refrigerator maintained at 2-8 °C for 7, 14, and 28 days. Note that Source 3 was held for 33 days instead of 28 days;
5. Surrogate spiking was performed into the 10-mL aliquots just prior to analysis. Spiking used 20 µL of a 2.5 µg/mL solution of surrogate in IPA (equal to 5.0 ng/mL in the water samples); and
6. Seven analytical samples were taken from each 10-mL water aliquot for LC-MS/MS analysis.

One LMB was prepared and stored for each day of analysis. Note that LFBs were only prepared for Day 0 analysis, so are designated as N/A in Table 19.

***Table 19. Water Stability Study Test Matrix***

| Sample Type | Water Type | EA 6162 Concentration (ng/mL) | Day / Analytical Samples per 10-mL Aliquot | | | |
|---|---|---|---|---|---|---|
| | | | Day 0 | Day 7 | Day 14 | Day 28 |
| Stability | Source 1 | 5.0 | 7 | 7 | 7 | 7 |
| Stability | Source 2 | 5.0 | 7 | 7 | 7 | 7 |
| Stability | Source 3 | 5.0 | 7 | 7 | 7 | 7[1] |
| LMB | Source 1 | NA | 1 | 1 | 1 | 1 |
| LMB | Source 2 | NA | 1 | 1 | 1 | 1 |
| LMB | Source 3 | NA | 1 | 1 | 1 | 1 |
| LFB | HPLC Water | 5.0 | 7 | N/A | N/A | N/A |
| LMB | HPLC Water | NA | 1 | N/A | N/A | N/A |

[1] Held for 33 days.

MDL studies were performed, using 40 CFR Part 136 Appendix B Revision 2 for all water sources, except limited LMBs were prepared for each test matrix; see Table 20. Eight separate samples were prepared and analyzed over three days using all three water source samples, with three samples prepared on Day 1, three samples on Day 2, and two samples on Day 3. Eight LFBs were also prepared over three days in the same manner as the MDL samples. One LMB was prepared for each of the three days that samples were prepared (note that this differs from 40 CFR Part 136 Appendix B Rev 2 which requires an equivalent number of blank samples). The MDL spike level was selected based on the IDC testing results. Spiking used 16 µL of a 0.125 µg/mL solution of EA 6162 in IPA into 40 mL of water for a final concentration of 0.050 ng/mL.

The surrogate was spiked at the same level as the recovery efficacy study. Because IDC, recovery efficacy, and stability samples required a 10-fold dilution prior to analysis to get EA 6162 and surrogate concentrations within the LC-MS/MS calibration curve, MDL samples were also diluted 10-fold prior to analysis.

***Table 20. Water MDL Study Test Matrix***

| Sample Type | Water Type | EA 6162 Concentration (ng/mL) | Surrogate Concentration (ng/mL) | Reps |
|---|---|---|---|---|
| MDL | Source 1 | 0.050 | 5.0 | 8 |
| MDL | Source 2 | 0.050 | 5.0 | 8 |
| MDL | Source 3 | 0.050 | 5.0 | 8 |
| LMB | Source 1 | NA | 5.0 | 3 |
| LMB | Source 2 | NA | 5.0 | 3 |
| LMB | Source 3 | NA | 5.0 | 3 |
| LFB | HPLC Water | 0.050 | 5.0 | 8 |
| LMB | HPLC Water | NA | 5.0 | 3 |

### *E.4 Part 4-Soil Testing*

Three separate studies were performed during Part 4 testing using each of the three soil types:

1. Soil IDC;
2. Soil recovery efficacy; and
3. Soil MDL.

The IDC included the preparation of P&A samples and QL samples using 5.0 ± 0.5 g of sand for all testing. For the P&A samples the EA 6162 and surrogate spike levels were both 50 ng. Spiking used 10 µL of a 5.0 µg/mL solution of EA 6162 in IPA onto the sand surface. The EA 6162 was allowed to dwell for 60 minutes; 50 ng of surrogate (10 µL of a 5.0 µg/mL surrogate solution in IPA) was then added to the sand surface just prior to solvent extraction. Four P&A replicates were prepared and analyzed. The QL samples were prepared in the same manner as the P&A samples but spiked with only 10 ng of EA 6162 (10 µL of 1.0 µg/mL EA 6162 solution in IPA) with a 60-minute EA 6162 dwell time and 10 ng of surrogate (10 µL of 1.0 µg/mL solution in IPA) added just prior to solvent extraction. Seven QL replicates were prepared and analyzed. The IDC test matrix is shown in Table 21. The LMB samples included each test soil, as well as the sand, spiked with 50 ng of surrogate just prior to solvent extraction. Two sets of spike controls were prepared to match P&A and QL EA 6162 and surrogate levels. For the spike controls, EA 6162 and surrogate were spiked directly into solvent in a glass jar. The spike control replicates were prepared at the start, middle, and end of each test, bracketing sample spiking. Following preparation, spike controls were sonicated, aliquoted and analyzed as described in Section D.5.

***Table 21. Soil IDC Test Matrix***

| Sample Type | Material | EA 6162 Spike Mass (ng) | Surrogate Spike Mass (ng) | Reps |
|---|---|---|---|---|
| P&A | Sand | 50 | 50 | 4 |
| QL | Sand | 10 | 10 | 7 |
| LMB | Sand | NA | 50 | 1 |
| LMB | Nebraska Soil | NA | 50 | 1 |
| LMB | Georgia Soil | NA | 50 | 1 |
| LMB | Clean Loam | NA | 50 | 1 |
| Spike Control | None | 50 | 50 | 3 |
| Spike Control | None | 10 | 10 | 3 |

To test the efficacy of recovering EA 6162 from each of the soils, 50 ng of EA 6162 was added to 5.0 ± 0.5 g of soil. The effect of EA 6162 dwell time on soil recovery was evaluated, with four different dwell times tested: 60 minutes, 24 hours, 7 days, and 14 days. Surrogate was added to each soil sample just prior to solvent extraction. Five replicates of each soil were prepared and analyzed for each trial. The recovery efficacy matrix shown in Table 22 was completed a total of four times, once for each EA 6162 dwell time. LFBs were prepared in triplicate in the same manner as the recovery efficacy soils, except the sand control was used. To generate LFSMs, blank soils were extracted and filtered as described in Section D.5. A 1.0-mL aliquot of the filtered extract was then spiked with 10 µL of 0.5 µg/mL EA 6162 in IPA solution and 10 µL of 0.5 µg/mL surrogate in IPA solution, equivalent to 5 ng of each analyte. One LMB was prepared for each soil type and sand. Two sets of spike controls were prepared to match the EA 6162 and surrogate levels for the recovery efficacy and LFSB samples. For the spike controls, EA 6162 and surrogate were spiked directly into solvent in a glass jar. The spike control replicates were prepared at the start, middle, and end of each test, bracketing sample spiking. Following preparation, spike controls were sonicated, aliquoted and analyzed as described in Section D.5.

***Table 22. Soil Recovery Efficacy Test Matrix***

| Sample Type | Material | EA 6162 Spike Mass (ng) | Surrogate Spike Mass (ng) | Reps |
|---|---|---|---|---|
| Efficacy | Nebraska Soil | 50 | 50 | 5 |
| Efficacy | Georgia Soil | 50 | 50 | 5 |
| Efficacy | Clean Loam | 50 | 50 | 5 |
| LFB | Sand | 50 | 50 | 3 |
| LFSM | Nebraska Soil | 5.0 | 5.0 | 3 |
| LFSM | Georgia Soil | 5.0 | 5.0 | 3 |
| LFSM | Clean Loam | 5.0 | 5.0 | 3 |
| LMB | Nebraska Soil | None | 50 | 1 |
| LMB | Georgia Soil | None | 50 | 1 |
| LMB | Clean Loam | None | 50 | 1 |
| LMB | Sand | None | 50 | 1 |
| Spike Control | None | 50 | 50 | 5 |
| Spike Control | None | 5.0 | 5.0 | 3 |

An MDL study was also performed, using 40 CFR Part 136 Appendix B Rev 2, except limited LMBs were prepared for each test matrix. A total of eight replicates were prepared over three days. MDL samples were prepared in the same manner as the recovery efficacy samples, however, the soils and sand were spiked with 2.0 ng of EA 6162 applied as 10 µL of a 0.20 µg/mL IPA solution and allowed to dwell on the soil for 60 minutes. The test matrix is shown in Table 23. One LMB was prepared for each soil type on each day of MDL sample preparation. The same mass of surrogate as used for the recovery testing was applied to the soils just before solvent extraction. One set of three spike controls was prepared on each day of MDL sample preparation. All solvent extract analyses were also performed on three separate days.

***Table 23. Soil MDL Study Test Matrix***

| Sample Type | Material | EA 6162 Spike Mass (ng) | Surrogate Spike Mass (ng) | Reps |
|---|---|---|---|---|
| MDL | Nebraska Soil | 2.0 | 50 | 8 |
| MDL | Georgia Soil | 2.0 | 50 | 8 |
| MDL | Clean Loam | 2.0 | 50 | 8 |
| LFB | Sand | 2.0 | 50 | 8 |
| LMB | Nebraska Soil | None | 50 | 3 |
| LMB | Georgia Soil | None | 50 | 3 |
| LMB | Clean Loam | None | 50 | 3 |
| Spike Control | None | 2.0 | 50 | 9 |

# F. RESULTS

## *F.1 Part 1-Wipe Sampling*

***Initial Demonstration of Capability***. Several iterations of IDC tests were performed to evaluate potential factors affecting recovery limits. For the IDC tests, the EA 6162 average for P&A samples was required to be within ± 30% of the spike control average with an RSD of ≤ 20% between replicates. The EA 6162 average for QL samples was required to be within ± 20% of the spike control average with an RSD of ≤ 30% between replicates. The average EA 6162 mass recovered for the IDC tests are presented in Table 24 for the first iteration. EA 6162 recoveries for the P&A and QL samples did not meet the acceptance criteria. Additionally, most of the 3.0-ng QL results were below the LLOQ of the LC-MS/MS; the results were extrapolated and represent estimates of unknown accuracy. Further evaluation was needed to demonstrate EA 6162 recovery efficacy with galvanized steel.

***Table 24. Part 1 IDC EA 6162 Mass Recovered***

| Test | Wipe Type | Sample Type | Average EA 6162 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| P&A | RPG | IPA Wipe | 11 | 14% | 29% |
| | | Methanol Wipe | 25 | 33% | 25% |
| | CG | IPA Wipe | 24 | 33% | 12% |
| | | Methanol Wipe | 23 | 31% | 22% |
| | CB | IPA Wipe | 25 | 34% | 27% |
| | | Methanol Wipe | 11 | 15% | 78% |
| QL | RPG | IPA Wipe | 0.59[2] | 23% | 26% |
| | | Methanol Wipe | 1.4[2] | 52% | 27% |
| | CG | IPA Wipe | 1.1[2] | 40% | 14% |
| | | Methanol Wipe | 1.5[2] | 53% | 18% |
| | CB | IPA Wipe | 1.0[2] | 40% | 14% |
| | | Methanol Wipe | 0.29[2] | 11% | 51% |

[1] 4 P&A replicates, 7 QL replicates [2] Estimated results < LLOQ

IDC recovery efficacy tests were developed to improve recovery results and assess average EA 6162 mass recovered from surfaces and are presented in Tables 25 through Table 27. The average recovered EA 6162 mass for the first wipe (Wipe 1) and the second wipe (Wipe 2) as well as the summed mass for the two wipes (Total) are reported. Note that all data are reported to two significant figures; therefore, the total masses may not always appear to sum correctly due to rounding of data. Iteration 1 spiking used a solution of EA 6162 prepared in IPA and allowed to dwell on the galvanized steel surface for 60 minutes. Relatively low EA 6162 recoveries (ranging from 36% to 60% using two wipes on the tested surface) were observed during Iteration 1, regardless of wipe type or wipe solvent. Collection of the second wipe

increased the recovered EA 6162 mass by only 6% to 14%. RSD values for the total recovered EA 6162 mass were also reported. Despite low EA 6162 recoveries, low RSD values were established for all wipe types/solvent combinations, indicating good reproducibility for the collection method.

***Table 25. EA 6162 Mass Recovered from Galvanized Steel after 60-minute Dwell Time***

| Wipe Type | Sample Type | Average Wipe 1 Mass (µg) [1] | Average Wipe 2 Mass (µg) [1] | Total Mass (µg) | Wipe 1 Recovery vs Spike Control | Total Recovery vs Spike Control | Total RSD |
|---|---|---|---|---|---|---|---|
| RPG | IPA Wipe | 2.1 | 1.4 | 3.4 | 22% | 36% | 9.4% |
| | Methanol Wipe | 3.7 | 0.86 | 4.5 | 39% | 48% | 9.5% |
| CG | IPA Wipe | 4.1 | 1.3 | 5.4 | 46% | 60% | 8.6% |
| | Methanol Wipe | 4.5 | 0.51 | 5.0 | 50% | 56% | 11% |
| CB | IPA Wipe | 2.6 | 1.3 | 3.9 | 26% | 39% | 7.9% |
| | Methanol Wipe | 3.3 | 1.2 | 4.4 | 33% | 44% | 22% |

[1] 5 replicates

A shorter dwell time of 1 minute on the galvanized steel surface was selected for Iteration 2 testing and included spiking a solution of EA 6162 prepared in IPA. The shorter dwell time resulted in much improved EA 6162 recovery, ranging from 71% to 103%, as shown in Table 26. Collection of the second wipe increased the recovered EA 6162 mass by 8% to 26%. Higher average total recoveries were observed when using methanol as a wipe-wetting solvent than when using IPA.

***Table 26. EA 6162 Mass Recovered from Galvanized Steel after 1-minute Dwell Time***

| Wipe Type | Sample Type | Average Wipe 1 Mass (µg) [1] | Average Wipe 2 Mass (µg) [1] | Total Mass (µg) | Wipe 1 Recovery vs Spike Control | Total Recovery vs Spike Control | Total RSD |
|---|---|---|---|---|---|---|---|
| RPG | IPA Wipe | 5.2 | 1.9 | 7.2 | 52% | 71% | 7.6% |
| | Methanol Wipe | 8.8 | 0.86 | 9.7 | 88% | 96% | 4.5% |
| CG | IPA Wipe | 5.7 | 2.5 | 8.2 | 59% | 85% | 4.3% |
| | Methanol Wipe | 8.6 | 1.3 | 9.9 | 89% | 103% | 1.4% |
| CB | IPA Wipe | 5.5 | 2.4 | 7.9 | 56% | 81% | 3.9% |
| | Methanol Wipe | 7.2 | 2.2 | 9.4 | 74% | 96% | 11% |

[1] 5 replicates

A 1-minute dwell time was a poor representation of a contamination incident following sampling. Longer dwell times were evaluated; however these longer dwell times were still shorter than a true contamination incident, but based on experimental design and the scope of this effort. An intermediate dwell time of 30 minutes was selected for Iteration 3 testing. Water was used to prepare the EA 6162 spiking solution instead of IPA to reduce the area of contamination; refer to Figure 11 to see the reduced spread of water on the galvanized steel compared to IPA. Note that EA 6162 was known to be stable in water prior to this testing and demonstrated to be stable in drinking water during this evaluation (see Section F.3). Iteration 3

resulted in similar EA 6162 recoveries compared to Iteration 2, indicating that the 30-minute dwell time with an aqueous EA 6162 spiking solution provided an acceptable spiking approach. An aqueous spiking solution appeared to have solved our issues with low recoveries and high variability that was observed in our initial IDC and in our IDC that used IPA as a spiking solution with the longer dwell time (60 minutes). The aqueous spiking solution results at the 30-minute dwell time more closely matched our results with IPA spiking solution and the very short (1-minute) dwell time. Collection of the second wipe increased the recovered EA 6162 mass by 11% to 32%. Higher average recoveries were again observed for methanol wipe solvent compared to IPA, with the second IPA recovering more EA 6162 presumably due to more EA 6162 remaining on these coupons after the first wipe was collected. Note that the low recoveries for the cotton ball wipes are driven by the high average spike control mass for these samples (see Table 28).

***Table 27. EA 6162 Mass Recovered from Galvanized Steel after a 30-minute Dwell Time***

| Wipe Type | Sample Type | Average Wipe 1 Mass (µg) [1] | Average Wipe 2 Mass (µg) [1] | Total Mass (µg) | Wipe 1 Recovery vs Spike Control | Total Recovery vs Spike Control | Total RSD |
|---|---|---|---|---|---|---|---|
| RPG | IPA Wipe | 3.9 | 2.8 | 6.7 | 43% | 75% | 5.6% |
| | Methanol Wipe | 7.8 | 1.1 | 8.9 | 87% | 99% | 6.4% |
| CG | IPA Wipe | 6.2 | 1.7 | 7.9 | 62% | 80% | 15% |
| | Methanol Wipe | 8.4 | 1.1 | 9.5 | 85% | 96% | 3.9% |
| CB | IPA Wipe | 5.1 | 2.4 | 7.5 | 37% | 55% | 10% |
| | Methanol Wipe | 8.2 | 2.9 | 11 | 60% | 81% | 8.6% |

[1] 5 replicates

A fixed effects Analysis of Variance (ANOVA) model was fitted separately to the total mass recovery data for each Part 1A testing iteration (see detailed discussion in Appendix B). For Iteration 1, the highest average total mass recovered was observed for the cotton gauze IPA wipe and for the cotton gauze methanol wipe; these recoveries differed significantly from both the rayon/polyester gauze with IPA wipes and cotton ball wipes with IPA wipes. No other conditions differed significantly. For Iteration 2, the average total mass recovery for methanol was significantly higher than the average total mass recovery of the IPA for all wipe types. There were no significant differences detected between conditions with different wipe types when the wetting solvents were the same. For Iteration 3, the cotton ball wipe with the methanol had the highest average total mass recovery and was statistically significantly different from all five other conditions. The cotton gauze wipe with methanol had the second highest average total mass recovery and was significantly different from all wipe types using IPA. The rayon/polyester gauze wipe with methanol had the third highest mean total mass recovery and was significantly different from rayon/polyester gauze with IPA, which had the lowest average total mass recovery. The total recovery vs spike control data exhibit rayon/polyester gauze and cotton gauze wipe recoveries in methanol as higher than cotton ball suggesting higher variability in the cotton ball recovery data. Rayon/polyester gauze and cotton

gauze were selected for use in Part 1B and 1C based on the results of the Part 1A testing as well as the greater ease of use for these two wipe types compared to the cotton balls.

Part 1B and 1C testing included wipe sample collection from vinyl tile and ceramic tile using only two wipe types. Based on the results of Part 1A, each recovery test was performed using a 30-minute dwell time and an aqueous EA 6162 spiking solution.

Spike controls were used to evaluate recoveries directly from wipe materials. Spike control averages were required to be within ± 20% of the nominal EA 6162 spike mass with an RSD of ≤ 30% between replicates. The average EA 6162 spike control results are shown in Table 28. Results shown in red were outside the required tolerances. The high average recovery and RSD for the Iteration 3 cotton gauze 3.0-ng spike control was due to one of the three spike control replicates having a 243% recovery; the other two replicates had acceptable recovery. The likely cause of the high recovery was attributed to carryover following preparation of 10-µg spike control. See also the LFB recovery discussion below. The cause for the high average recovery for the Iteration 3 cotton ball 10-µg spike control is not known; the results for all five replicates were similar, with an RSD of 6.0% indicating that a single spiking error did not occur.

***Table 28. Part 1A Spike Control Results***

| Test | Wipe Type | Spike Control Nominal Mass | Average EA6162 Mass [1] | Average Recovery vs Spike Mass | RSD |
|---|---|---|---|---|---|
| IDC | RPG | 75 ng | 77 ng | 102% | 2.2% |
| | | 3.0 ng | 2.6 ng | 87% | 2.7% |
| | CG | 75 ng | 74 ng | 98% | 4.8% |
| | | 3.0 ng | 2.8 ng | 92% | 3.6% |
| | CB | 75 ng | 72 ng | 95% | 8.2% |
| | | 3.0 ng | 2.6 ng | 85% | 4.2% |
| Iteration 1 | RPG | 10 µg | 9.5 µg | 95% | 7.3% |
| | | 3.0 ng | 3.0 ng | 98% | 3.2% |
| | CG | 10 µg | 9.0 µg | 90% | 5.3% |
| | | 3.0 ng | 2.9 ng | 96% | 2.5% |
| | CB | 10 µg | 10 µg | 100% | 11% |
| | | 3.0 ng | 3.5 ng | 115% | 15% |
| Iteration 2 | RPG | 10 µg | 10 µg | 101% | 6.6% |
| | | 3.0 ng | 3.2 ng | 105% | 8.5% |
| | CG | 10 µg | 9.7 µg | 97% | 5.3% |
| | | 3.0 ng | 3.4 ng | 111% | 19% |
| | CB | 10 µg | 9.8 µg | 98% | 5.4% |
| | | 3.0 ng | 3.5 ng | 116% | 12% |
| Iteration 3 | RPG | 10 µg | 9.0 µg | 90% | 11% |
| | | 3.0 ng | 3.3 ng | 107% | 8.5% |
| | CG | 10 µg | 9.9 µg | 99% | 3.5% |
| | | 3.0 ng | 4.6 ng | 152% | 52% |

| | | | | | |
|---|---|---|---|---|---|
| | CB | 10 µg | 14 µg | 137% | 6.0% |
| | | 3.0 ng | 3.1 ng | 101% | 8.4% |

[1] 3 replicates for 3.0 ng and 75 ng spikes and 5 replicates for 10 µg spikes

The average recovery of all replicate LFSMs and LFBs were required to be within ± 20% of the spike control average with an RSD of ≤ 30% between replicates. The average Part 1A galvanized steel LFSM results are shown in Table 29. Two LFB spike levels were evaluated, 10-µg and 3.0-ng levels. The average Part 1A stainless steel LFB results at the 10-µg spike level are shown in Table 30. The average Part 1A stainless steel LFB results at the 3.0-ng spike level are shown in Table 31. Results shown in red were outside the required tolerances. The high LFB recoveries spiked with 3.0 ng of EA 6162 may be related to co-preparation of samples spiked at 10 µg of EA 6162. While every effort was made to prevent cross-contamination, including single use of forceps for handling wipes and pipettes, some contamination did occur as evidenced by low level detection of EA 6162 in the LMBs (see Table 32). In some instances, the LMB levels were on a par with (or higher than) the spike levels for the LFBs. Problems with low level contamination were not isolated to EA 6162, as high recoveries also occurred for the 3.0 ng surrogate spikes as shown in Table 33.

***Table 29. Part 1A LFSM Results***

| Test | Wipe Type | Wipe Solvent | Average EA 6162 Mass (µg) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| Iteration 1 | RPG | IPA | 9.5 | 100% | 8.0% |
| | | Methanol | 9.5 | 100% | 1.3% |
| | CG | IPA | 9.5 | 106% | 7.3% |
| | | Methanol | 9.5 | 106% | 5.2% |
| | CB | IPA | 9.7 | 97% | 5.3% |
| | | Methanol | 10 | 103% | 6.9% |
| Iteration 2 | RPG | IPA | 10 | 103% | 4.2% |
| | | Methanol | 10 | 100% | 7.7% |
| | CG | IPA | 10 | 107% | 6.3% |
| | | Methanol | 9.9 | 103% | 8.8% |
| | CB | IPA | 10 | 102% | 7.2% |
| | | Methanol | 10 | 106% | 3.5% |
| Iteration 3 | RPG | IPA | 9.3 | 103% | 8.5% |
| | | Methanol | 9.0 | 100% | 2.2% |
| | CG | IPA | 10 | 106% | 4.1% |
| | | Methanol | 10 | 102% | 4.5% |
| | CB | IPA | 13 | 93% | 2.6% |
| | | Methanol | 13 | 98% | 4.3% |

[1] 3 replicates

***Table 30. Part 1A LFB Results: 10-µg Spike***

| Test | Wipe Type | Wipe Solvent | Average EA 6162 Mass (µg) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| Iteration 1 | RPG | IPA | 9.4 | 99% | 5.8% |
| | | Methanol | 9.3 | 98% | 8.4% |
| | CG | IPA | 9.2 | 102% | 9.6% |
| | | Methanol | 9.5 | 105% | 9.9% |
| | CB | IPA | 10 | 101% | 4.5% |
| | | Methanol | 10 | 101% | 5.4% |
| Iteration 2 | RPG | IPA | 10 | 100% | 3.6% |
| | | Methanol | 10 | 103% | 3.2% |
| | CG | IPA | 11 | 118% | 11% |
| | | Methanol | 10 | 105% | 2.0% |
| | CB | IPA | 9.9 | 102% | 4.7% |
| | | Methanol | 11 | 113% | 14% |
| Iteration 3 | RPG | IPA | 10 | 112% | 9.2% |
| | | Methanol | 11 | 117% | 2.6% |
| | CG | IPA | 11 | 106% | 2.6% |
| | | Methanol | 9.7 | 97% | 3.5% |
| | CB | IPA | 13 | 96% | 6.0% |
| | | Methanol | 13 | 93% | 8.9% |

[1] 3 replicates

***Table 31. Part 1A LFB Results: 3.0-ng Spike***

| Test | Wipe Type | Wipe Solvent | Average EA 6162 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| Iteration 1 | RPG | IPA | 2.9 | 97% | 3.5% |
| | | Methanol | 3.4 | 114% | 4.8% |
| | CG | IPA | 3.5 | 119% | 6.6% |
| | | Methanol | 3.5 | 120% | 12% |
| | CB | IPA | 4.4 | 126% | 33% |
| | | Methanol | 8.8 | 250% | 14% |
| Iteration 2 | RPG | IPA | 3.4 | 105% | 8.1% |
| | | Methanol | 3.6 | 113% | 5.2% |
| | CG | IPA | 3.8 | 111% | 10% |
| | | Methanol | 4.2 | 126% | 14% |
| | CB | IPA | 3.6 | 102% | 4.7% |
| | | Methanol | 3.7 | 107% | 4.1% |
| Iteration 3 | RPG | IPA | 3.2 | 99% | 4.9% |
| | | Methanol | 3.2 | 97% | 7.6% |
| | CG | IPA | 6.2 | 134% | 15% |
| | | Methanol | 7.0 | 151% | 55% |
| | CB | IPA | 3.0 | 98% | 8.2% |
| | | Methanol | 3.6 | 116% | 16% |

[1] 3 replicates

LMBs were required to have no quantifiable EA 6162 or malathion results above the method reporting limit of 1.6 ng for RPG wipes or 1.7 ng for CG and CB wipes. These reporting limits were approximately 6,000-fold lower than the 10-µg spike level used for the wipe testing. Most Part 1A LMB results were below the reporting limit, but several LMBs from Part 1A testing had low-level, quantifiable results which are shown in Table 32. As discussed for the LFB samples, every effort was made during testing to prevent potential cross contamination. However, these results may be related to the high EA 6162 and malathion spike levels. Because the spike levels were so much higher than the LMB detections, the Part 1A wipe sampling results should not have been negatively impacted by any low-level cross contamination.

***Table 32. Part 1A LMB Results Above Reporting Limit***

| Test | Wipe Type | Test Material | Wipe Solvent | Analyte | Mass (ng) |
|---|---|---|---|---|---|
| Iteration 1 | CG | Stainless Steel | Methanol | Malathion | 1.7 |
| | CB | Stainless Steel | IPA | EA 6162 | 13 |
| | | Galvanized Steel | IPA | Malathion | 8.5 |
| | | Stainless Steel | IPA | Malathion | 11 |
| | | Galvanized Steel | Methanol | Malathion | 3.3 |
| | | Stainless Steel | Methanol | Malathion | 3.5 |
| Iteration 3 | RPG | Galvanized Steel | IPA | EA 6162 | 1.8 |
| | CG | Galvanized Steel | IPA | EA 6162 | 2.3 |
| | | Galvanized Steel | Methanol | EA 6162 | 4.1 |
| | | Stainless Steel | Methanol | EA 6162 | 3.0 |
| | | Galvanized Steel | Methanol | Malathion | 13 |
| | | Stainless Steel | Methanol | Malathion | 1.9 |

The average malathion surrogate recovery was required to be within ± 20% of the spike control surrogate average with an RSD of ≤ 30% between replicates. All the IDC and Part 1A average surrogate recoveries for the 10-µg spike level met the required tolerances. Several average surrogate recoveries at the 3-ng spike level did not meet the required tolerances; the results for these samples are shown in Table 33. Note that the QL and LFB surrogate recoveries were calculated against the surrogate spike control results, while the spike control surrogate recoveries were calculated against the nominal 3.0-ng spike mass. These out-of-tolerance average recoveries were all greater than the upper limit of 120%. The high recoveries again point to the potential for cross contamination when working with both high spike masses (10 µg) and low spike masses (3.0 ng) within the same sample set. The high surrogate recoveries for these control samples do not indicate any potential problems with the Part 1A wipe samples.

The average and standard deviation for the recovered malathion 10 µg spike mass for all samples were calculated to compare actual surrogate recoveries to the acceptance range of ± 20% of the spike control average. The average mass was 11.4 µg, which represented a recovery of 102% compared to the average recovered malathion for all spike controls (11.2 µg). The surrogate SD was 0.73 µg, resulting in acceptance limits of 89% - 115% based on ± 2 SD around the average recovery, narrower recovery limits than the ± 20% limit used for this testing. Note

that these limits are not necessarily how EPA would establish control limits but are useful for evaluating this data set.

***Table 33. Part 1 IDC and Part 1A Out-of-Tolerance Surrogate Results***

| Test | Wipe Type | Sample Type | Wipe Solvent | Spike Nominal Mass (ng) | Average Malathion Mass (ng) | Average Recovery vs Spike Controls | RSD |
|---|---|---|---|---|---|---|---|
| IDC | RPG | Spike Control | NA | 3.0 | 3.7 | 123% | 1.9% |
| | CG | QL | IPA | 3.0 | 4.6 | 148% | 19% |
| | | QL | Methanol | 3.0 | 4.0 | 129% | 19% |
| | CB | QL | Methanol | 3.0 | 4.1 | 137% | 10% |
| Iteration 1 | CG | Spike Control | NA | 3.0 | 3.8 | 126% | 4.9% |
| | | LFB | IPA | 3.0 | 6.2 | 162% | 15% |
| | CB | Spike Control | NA | 3.0 | 4.0 | 133% | 1.3% |
| | | LFB | IPA | 3.0 | 6.3 | 156% | 24% |
| | | LFB | Methanol | 3.0 | 9.6 | 237% | 65% |
| Iteration 2 | RPG | LFB | Methanol | 3.0 | 4.4 | 128% | 7.0% |
| | CG | Spike Control | NA | 3.0 | 3.8 | 125% | 6.0% |
| | | LFB | Methanol | 3.0 | 5.1 | 135% | 3.9% |
| | CB | Spike Control | NA | 3.0 | 4.1 | 134% | 15% |
| Iteration 3 | RPG | LFB | IPA | 3.0 | 4.4 | 121% | 4.4% |
| | CB | LFB | IPA | 3.0 | 4.4 | 126% | 5.8% |
| | | LFB | Methanol | 3.0 | 4.6 | 131% | 10% |

Throughout all testing, temperature and relative humidity (RH) in the test environment were monitored but not controlled. Average temperature and relative humidity results for IDC and Part 1A testing are shown in Table 34. Temperature was very consistent across all testing while RH decreased. The decrease in RH was associated with tests being performed during the winter months. The lower RH would not have positively impacted wipe collection results as EA 6162 is stable in the presence of water.

***Table 34. Part 1 IDC and Part 1A Environmental Conditions***

| Test | Wipe Type | Average Temperature (°C) | Average RH (%) |
|---|---|---|---|
| IDC | RPG | 22 | 32 |
| | CG | 22 | 49 |
| | CB | 22 | 36 |
| Iteration 1 | RPG | 22 | 51 |
| | CG | 22 | 49 |
| | CB | 22 | 40 |
| Iteration 2 | RPG | 22 | 15 |
| | CG | 22 | 15 |
| | CB | 21 | 27 |
| Iteration 3 | RPG | 21 | 15 |
| | CG | 21 | 16 |
| | CB | 21 | 15 |

The average of the spike controls was required to be within ± 20% of the nominal EA 6162 spike mass with an RSD of ≤ 30% between replicates. The average Part 1B and 1C EA 6162 spike control results are shown in Table 35. The average results for Part 1B vinyl tile and Part 1C ceramic tile LFSM samples are shown in Table 36. The average results for Part 1B and Part 1C stainless steel LFB results for the 10-µg spike level are shown in Table 37. The average results for Part 1B and Part 1C stainless steel LFB results for the 15-ng spike level are shown in Table 38. All results were within the required tolerances. The 15 ng spike controls were used to calculate the recoveries for the low level LFBs. Results shown in red were outside the required tolerances; for both of these 15 ng spike controls one of the three replicates was 2-3x the expected level leading to the high average recovery and high RSD. The surrogate recovery was acceptable for the two spike controls. High EA 6162 recovery was not observed for the associated LFB samples (refer to Table 38). No assignable cause was found for the high spike level observed for these replicates. For both the vinyl tile and ceramic tile trials with rayon/polyester gauze wipes, exclusion of the high replicate resulted in average recoveries and RSDs within acceptance limits (values in parentheses). Based on the available evidence, these were isolated deviations and did not impact the overall conclusions from the results of testing.

***Table 35. Part 1B and 1C Spike Control Results***

| Testing Part | Wipe Type | Spike Control Nominal Mass | Average EA 6162 Mass [1] | Average Recovery | RSD |
|---|---|---|---|---|---|
| 1B | RPG | 10 µg | 9.5 µg | 95% | 2.4% |
| | | 15 ng | 25 (14) ng | 167% (92%) | 78% (4.0%) |
| 1B | CG | 10 µg | 9.3 µg | 93% | 4.8% |
| | | 15 ng | 15 ng | 97% | 14% |
| 1C | RPG | 10 µg | 11 µg | 108% | 4.2% |
| | | 15 ng | 20 (15) ng | 132% (98%) | 45% (1.6%) |
| 1C | CG | 10 µg | 10 µg | 100% | 3.8% |
| | | 15 ng | 14 ng | 96% | 5.0% |

[1] 3 replicates for 15 ng spikes and 5 replicates for 10 µg spikes

The average recovery of all replicate LFSM and LFB post-spiked control samples were required to be within ± 20% of the spike control average with an RSD of ≤ 30% between replicates. Note the improved LFB performance for the 15-ng spike compared to the 3-ng spike used in Part 1A where five sets of LFBs had average recoveries greater than 120% (see Table 31). These results indicate that 15 ng was a more appropriate LFB spike level under the test conditions.

***Table 36. Part 1B and 1C LFSM Results***

| Testing Part | Wipe Type | Wipe Solvent | Average EA 6162 Mass (µg) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| 1B | RPG | IPA | 9.9 | 104% | 3.2% |
| | | Methanol | 10 | 105% | 2.7% |
| 1B | CG | IPA | 10 | 107% | 3.9% |
| | | Methanol | 9.6 | 103% | 4.7% |
| 1C | RPG | IPA | 11 | 102% | 11% |
| | | Methanol | 11 | 102% | 3.5% |
| 1C | CG | IPA | 10 | 104% | 4.3% |
| | | Methanol | 10 | 102% | 2.0% |

[1] 3 replicates

***Table 37. Part 1B and 1C LFB Results: 10-µg Spike***

| Testing Part | Wipe Type | Wipe Solvent | Average EA 6162 Mass (µg) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| 1B | RPG | IPA | 9.5 | 99% | 1.8% |
| | | Methanol | 9.7 | 101% | 3.0% |
| 1B | CG | IPA | 9.6 | 102% | 4.1% |
| | | Methanol | 10 | 108% | 8.0% |
| 1C | RPG | IPA | 11 | 104% | 7.2% |
| | | Methanol | 11 | 105% | 2.3% |
| 1C | CG | IPA | 11 | 106% | 2.8% |
| | | Methanol | 10 | 103% | 6.7% |

[1] 3 replicates

***Table 38. Part 1B and 1C LFB Results: 15-ng Spike***

| Testing Part | Wipe Type | Wipe Solvent | Average EA 6162 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| 1B | RPG | IPA | 15 | 107%[2] | 6.9% |
| | | Methanol | 14 | 102%[2] | 1.7% |
| 1B | CG | IPA | 15 | 103% | 5.2% |
| | | Methanol | 15 | 106% | 10% |
| 1C | RPG | IPA | 15 | 101%[2] | 9.2% |
| | | Methanol | 15 | 101%[2] | 7.3% |
| 1C | CG | IPA | 14 | 96% | 11% |
| | | Methanol | 15 | 102% | 0.55% |

[1] 3 replicates [2] Recovery calculated using average of two 15-ng spike control replicates

LMBs were required to have no quantifiable EA 6162 results above the method reporting limit of 1.6 ng for RPG wipes and 1.7 ng for cotton gauze and cotton ball wipes. Only two LMB results (from Part 1C for RPG wipes) had quantifiable results. The results for these samples are shown in Table 39. As with the Part 1A LMB detections, because the recovery efficacy spike levels were

so much higher, the Part 1C wipe sampling results should not have been negatively impacted by any low-level cross contamination.

***Table 39. Part 1C LMB Results Above Reporting Limit***

| Material | Wipe Type | Test Material | Wipe Solvent | Analyte | Mass (ng) |
|---|---|---|---|---|---|
| Ceramic Tile | RPG | Ceramic Tile | IPA | EA 6162 | 1.7 |
| | | Stainless Steel | Methanol | EA 6162 | 3.2 |

The average malathion surrogate recovery was required to be within ± 20% of the spike control surrogate average with an RSD of ≤ 30% between replicates. Only one set of surrogate results did not meet the required tolerances, with an average recovery of 130% (20% RSD) for the vinyl tile LFSM samples using the rayon/polyester gauze wipes and methanol. The cause of this slightly high recovery was not determined; since the average EA 6162 recovery was acceptable for this sample set (see Table 36) there was no impact to the data. Note the improved performance resulting from increasing the 3.0-ng spike to 15 ng, compared to Part 1A testing where the average surrogate recoveries were greater than 120% for multiple sample sets using a 3.0-ng spike (see Table 31).

Throughout all testing, temperature and RH in the test environment were monitored but not controlled. Average temperature and relative humidity results are shown in Table 40. While the RH did vary between testing, no impact to the wipe recovery results was expected.

***Table 40. Part 1B and 1C Environmental Conditions***

| Testing Part | Wipe Type | Average Temperature (°C) | Average RH (%) |
|---|---|---|---|
| 1B | RPG | 21 | 23 |
| 1B | CG | 20 | 17 |
| 1C | RPG | 21 | 15 |
| 1C | CG | 21 | 39 |

The average EA 6162 mass recovered for the vinyl tile recovery efficacy testing is shown in Table 41 and for the ceramic tile recovery efficacy testing is shown in Table 42. The average recovered EA 6162 mass for the first wipe (Wipe 1) and the second wipe (Wipe 2) as well as the summed mass for the two wipes (Total) are reported. Looking at both vinyl tile and ceramic tile, collection of the second wipe increased the recovered EA 6162 mass by 11% to 27%. RSD values for the total recovered EA 6162 mass were also reported. The low RSD values for all wipe types/solvent combinations for both vinyl tile and ceramic tile indicated good reproducibility for the collection method from these materials.

***Table 41. EA 6162 Mass Recovered from Vinyl Tile***

| Wipe Type | Sample Type | Average Wipe 1 Mass (µg) [1] | Average Wipe 2 Mass (µg) [1] | Total Mass (µg) | Wipe 1 Recovery vs Spike Control | Total Recovery | Total RSD |
|---|---|---|---|---|---|---|---|
| RPG | IPA Wipe | 3.4 | 2.1 | 5.5 | 35% | 58% | 16% |
| | Methanol Wipe | 7.2 | 1.5 | 8.7 | 75% | 91% | 5.1% |
| CG | IPA Wipe | 4.7 | 2.5 | 7.2 | 50% | 77% | 7.5% |
| | Methanol Wipe | 7.6 | 1.7 | 9.3 | 81% | 99% | 5.0% |

[1] 5 replicates

***Table 42. EA 6162 Mass Recovered from Ceramic Tile***

| Wipe Type | Sample Type | Average Wipe 1 Mass (µg) [1] | Average Wipe 2 Mass (µg) [1] | Total Mass (µg) | Wipe 1 Recovery vs Spike Control | Total Recovery | Total RSD |
|---|---|---|---|---|---|---|---|
| RPG | IPA Wipe | 4.2 | 2.6 | 6.8 | 38% | 63% | 9.3% |
| | Methanol Wipe | 8.2 | 1.9 | 10 | 75% | 93% | 6.6% |
| CG | IPA Wipe | 6.4 | 2.0 | 8.4 | 63% | 83% | 6.7% |
| | Methanol Wipe | 8.5 | 1.0 | 9.5 | 84% | 95% | 1.9% |

[1] 5 replicates

The average EA 6162 mass recovered from galvanized steel for rayon/polyester gauze and cotton gauze wipes (Part 1A Iteration 3 data shown in Table 27) was included in a fixed effects ANOVA model that was fitted to the total mass recovery data over all materials (galvanized steel, vinyl tile, and ceramic tile), wipes (rayon/polyester gauze and cotton gauze), and solvents (IPA and methanol), see Appendix C. For both the rayon/polyester gauze and cotton gauze wipes, methanol resulted in significantly better recoveries of EA 6162 from all three materials than IPA. Also, there was no difference between the performance of rayon/polyester gauze wipes with methanol and cotton gauze wipes with methanol.

MDL replicates were prepared on three separate dates for all three material types, with the sample analyses on three separate dates. MDL sample collection used only cotton gauze wipes wetted with methanol. Table 43 shows the standard deviation for the eight replicates analyzed for each material. Note that the concentration was calculated based on the recovered mass of EA 6162 divided by the nominal coupon area of 529 $cm^2$. The MDL was calculated by multiplying the standard deviation by 2.998, the Student's single-tailed $99^{th}$ percentile t-value for eight replicates. The MDL values were consistent for all three materials, ranging from 0.014 to 0.019 $ng/cm^2$. Each MDL value was greater than or equal to 10% of the 0.14 $ng/cm^2$ spike level and below the spike level, indicating that the selected EA 6162 spike concentration was not too high. Earlier versions of 40 CFR Part 136 Appendix B had this requirement (that MDLs be below the spike level but above 10% of the spike level) to prevent the use of high spike concentrations, which can result in low variance for the results, creating artificially low MDLs. For all the laboratory method blanks, no EA 6162 chromatographic peak was detected (results not shown). Since EA 6162 was not detected in the LMBs, it is reasonable to conclude that EA 6162 would not be detected in any the required MDL blanks, or to exceed any of the calculated

MDLs; thus, limiting the number of LMBs collected during this study would not impact the final reported MDL.

***Table 43. Part 1 EA 6162 MDL Results***

| Material | SD ($ng/cm^2$) | MDL ($ng/cm^2$) | Percent of Spike Level | Average Surrogate Recovery |
|---|---|---|---|---|
| Galvanized Steel | 0.0062 | **0.019** | 13% | 93% |
| Vinyl Tile | 0.0048 | **0.014** | 10% | 90% |
| Ceramic Tile | 0.0086 | **0.026** | 18% | 90% |

The results of iterative testing for Part 1 indicated that the developed sample collection method using either cotton gauze wipes or rayon/polyester gauze wipes wetted with methanol, followed by methanol extraction, and analysis by LC-MS/MS allowed for recovery of EA 6162 from surfaces at the levels investigated.

### *F.2 Part 2 Strippable Coating*

The average EA 6162 and malathion surrogate spike control results are shown in Table 44. The mean of the spike controls was required to be within ± 20% of the of the nominal EA 6162 spike mass with an RSD of ≤ 30% for replicates. The third malathion replicate for trials Eff 1 and for Eff 3 both appear to have been accidentally double spiked, leading to elevated average surrogate recovery and high RSD values (shown in red). Exclusion of the high replicate resulted in average recoveries and RSDs within acceptance limits (values in parentheses). Based on the available evidence, these were isolated deviations and did not impact the conclusions stemming from testing.

***Table 44. Part 2 Spike Control Results***

| Trial | EA 6162 | | | Malathion | | |
|---|---|---|---|---|---|---|
| | Average Mass (µg) [1] | Average Recovery | RSD | Average Mass (µg) [1] | Average Recovery | RSD |
| EE 1 | 9.6 | 96% | 1.7% | 9.8 | 98% | 8.3% |
| EE 2 | 9.4 | 94% | 3.2% | 0.24 | 96% | 4.3% |
| Eff 1 | 9.4 | 94% | 2.0% | 0.31 (0.23) | 124% (93%) | 43% (1.5%) |
| Eff 2 | 9.1 | 91% | 1.7% | 0.23 | 93% | 7.7% |
| Eff 3 | 9.5 | 95% | 2.6% | 0.29 (0.22) | 117% (87%) | 43% (7.4%) |
| Eff 4 | 9.9 | 99% | 2.5% | 0.22 | 90% | 5.2% |

[1] 3 replicates

The LFSM samples were required to be within ± 20% of the spike control average. The LFSM results for the latex body paint are shown in Table 45 and for DETEX in Table 46. The LFSM mass was 10 µg. However, the recoveries were calculated against the average spike control mass shown in Table 44. The only matrices that did not have EA 6162 recoveries within the acceptance limits was wood, with a recovery of 71% (Trial Eff 1) and 51% (Trial Eff 3), and plywood with a recovery of 78% (Trial Eff 4). These lower recoveries indicate a potential

stronger interaction of EA 6162 with wood dimensional lumber and plywood than the other three test materials, leading to a lower solvent extraction efficiency under the test conditions.

***Table 45. Part 2 Latex Body Paint LFSM Results***

| Trial | Material | Test Item | EA 6162 Mass (µg) | Recovery vs Spike Control |
|---|---|---|---|---|
| Eff 1 | Wood Dimensional Lumber | Coating | 9.0 | 95% |
| | | Coupon | 6.7 | 71% |
| | Vinyl Tile | Coating | 8.6 | 92% |
| | | Coupon | 9.1 | 96% |
| | Stainless Steel | Coating | 9.0 | 96% |
| | | Coupon | 9.3 | 99% |
| Eff 2 | Ceramic Tile | Coating | 8.9 | 98% |
| | | Coupon | 8.9 | 98% |
| | Plywood | Coating | 8.9 | 99% |
| | | Coupon | 8.2 | 91% |
| | Stainless Steel | Coating | 8.9 | 99% |
| | | Coupon | 9.4 | 103% |

***Table 46. Part 2 DETEX LFSM Results***

| Trial | Material | Test Item | EA 6162 Mass (µg) | Recovery vs Spike Control |
|---|---|---|---|---|
| Eff 3 | Wood Dimensional Lumber | Coating | 9.2 | 96% |
| | | Coupon | 4.9 | 51% |
| | Vinyl Tile | Coating | 9.5 | 100% |
| | | Coupon | 9.3 | 98% |
| | Stainless Steel | Coating | 9.3 | 98% |
| | | Coupon | 9.8 | 102% |
| Eff 4 | Ceramic Tile | Coating | 10 | 101% |
| | | Coupon | 10 | 102% |
| | Plywood | Coating | 9.6 | 96% |
| | | Coupon | 7.8 | 78% |
| | Stainless Steel | Coating | 9.6 | 97% |
| | | Coupon | 9.4 | 94% |

The LMBs were required to have no quantifiable EA 6162 results above the reporting limit of 1.0 ng (0.0010 µg) for coatings and 1.5 ng (0.0015 µg) for coupons. Latex body paint LMBs from the latex body paint extraction efficiency trial contained EA 6162 above the quantitation limit as shown in Table 47. The reason for the LMB detections is not known. Since the associated test samples all contained EA 6162 at greater than 1.0 µg, these low-level LMB detections did not indicate any problems with the sample data.

***Table 47. Part 2 LMB Results Above Reporting Limit***

| Trial | Test Item | Solvent | EA 6162 Mass (µg) |
|---|---|---|---|
| EE 1 | Latex Body Paint | Methanol | 0.0019 |
| EE 1 | Latex Body Paint | Acetone | 0.0084 |
| EE 1 | Latex Body Paint | 3:7 MeOH:Ace | 0.0046 |

Elevated EA 6162 quantitation limits were reported for plywood coupon and vinyl tile coupon LMBs due to a required 100-fold dilution to minimize suppression of the surrogate internal standard. Both these materials had a 15-ng quantitation limit for the blanks. In all cases, the test sample coupon results were above 15 ng, so there was no impact to the reported data. Samples analyzed immediately after DETEX LMB coating samples exhibited suppression of both the EA 6162-$d_5$ and malathion-$d_{10}$ internal standards. Analysis of a solvent blank after the DETEX LMB coating samples rectified this problem. This suppression was only observed for the DETEX LMB coating samples, which were diluted 10-fold prior to analysis, compared to test samples which were diluted 100- or 400-fold prior to analysis. The internal standard suppression problem may be related to observed yellow color in DETEX coating extracts.

Average malathion surrogate recoveries were required to be within ± 20% of the spike control average with an RSD of ≤ 30% across replicates. While all replicate sets met the ≤ 30% RSD requirement, numerous samples had surrogate recoveries < 80%; see Table 48. Of the thirty low surrogate recoveries, ten were for latex body paint coatings and another nine were for plywood and wood dimensional lumber coupons. The lower surrogate recoveries for these materials may have been related to interactions of malathion with the test material, such as adsorption into porous materials. Additionally, the surrogate application and extraction procedure may have had a role in the lower recoveries for these nineteen samples as well as other samples. A review of the procedure during testing did not identify any changes were required.

Suppression of the malathion-$d_{10}$ internal standard response was observed for vinyl coupon extracts and plywood coupon extracts. A 100-fold minimum dilution for vinyl coupon extracts resulted in acceptable malathion-$d_{10}$ response. However, all plywood coupon extracts had a malathion-$d_{10}$ response ranging from 22% - 44% of the nearest standard response. A larger dilution for these samples may have improved response but ran the risk of diluting out the surrogate, so surrogate recovery data were reported using the internal standard response.

***Table 48. Part 2 Sample Surrogate Recovery Less Than 80%***

| Trial | Test Item | Sample ID | Malathion Mass (µg) | Recovery vs Spike Control |
|---|---|---|---|---|
| EE 2 | DETEX | Acetone Replicate 5 | 0.16 | 68% |
| | | 3:7 MeOH:Ace Rep 2 | 0.14 | 61% |
| | Stainless Steel Coupon | Acetone Replicate 4 | 0.13 | 53% |
| | | Acetone Replicate 5 | 0.19 | 79% |
| | | 3:7 MeOH:Ace Rep 1 | 0.17 | 72% |
| | | Acetone LMB | 0.14 | 59% |
| Eff 1 | Latex Body Paint | Wood Replicate 1 | 0.17 | 74% |
| | | Wood Replicate 2 | 0.17 | 72% |
| | Wood Dimensional Lumber Coupon | Wood Replicate 2 | 0.17 | 72% |
| | | Wood Replicate 3 | 0.18 | 79% |
| | | Wood Replicate 5 | 0.18 | 75% |
| | | Wood LMB | 0.16 | 69% |
| Eff 2 | Latex Body Paint | Ceramic Tile Replicate 1 | 0.17 | 71% |
| | | Plywood Replicate 4 | 0.18 | 77% |
| | | Stainless Steel Replicate 1 | 0.18 | 78% |
| | | Stainless Steel Replicate 3 | 0.18 | 78% |
| | | Ceramic Tile LMB | 0.17 | 72% |
| | | Plywood LMB | 0.17 | 72% |
| | | Ceramic Tile LFSM | 0.12 | 52% |
| | | Plywood LFSM | 0.18 | 76% |
| | Plywood Coupon | Plywood Replicate 1 | 0.16 | 71% |
| | | Plywood Replicate 5 | 0.13 | 57% |
| | Stainless Steel Coupon | Stainless Steel Replicate 3 | 0.17 | 72% |
| | | Stainless Steel LFSM | 0.17 | 75% |
| Eff 3 | DETEX | Vinyl Tile Replicate 1 | 0.17 | 77% |
| | | Vinyl Tile LMB | 0.17 | 77% |
| Eff 4 | Plywood Coupon | Plywood Replicate 1 | 0.17 | 77% |
| | | Plywood Replicate 2 | 0.15 | 67% |
| | | Plywood LMB | 0.17 | 76% |
| | Stainless Steel Coupon | Stainless Steel Replicate 3 | 0.14 | 62% |

The average and standard deviation for recovered malathion mass for all samples were calculated to compare actual surrogate recoveries to the acceptance range of ± 20% of the spike control average. The average mass was 205 ng, which represented a recovery of 89% compared to the average recovered malathion for all spike controls (230 ng). The surrogate SD was 25 ng, resulting in acceptance limits of 67% - 111% based on ± 2 SD around the average recovery. All but six of the surrogate recoveries shown in Table 48 are within these acceptance limits, indicating that these limits may be more representative of expected surrogate recovery for this procedure. Note that these limits are not necessarily how EPA would establish control limits but are useful for evaluating this data set. While low surrogate recovery was observed for multiple samples, the collected EA 6162 data are believed to be reliable and reflective of the strippable coating technology being tested.

Throughout all testing, temperature and RH in the test environment were monitored but not controlled. Average temperature and RH results are shown in Table 49.

***Table 49. Part 2 Average Environmental Conditions***

| Trial | Average Temperature (°C) | Average RH (%) |
|---|---|---|
| EE 1 | 21 | 38 |
| EE 2 | 21 | 47 |
| Eff 1 | 22 | 56 |
| Eff 2 | 22 | 53 |
| Eff 3 | 21 | 58 |
| Eff 4 | 21 | 58 |

For the extraction efficiency testing, acetone and 3:7 MeOH:Ace provided similar EA 6162 recovery from latex body paint and from DETEX (see Table 50), with effectively all EA 6162 recovered from DETEX (98% for both solvents) and lower recoveries from the latex body paint (79% for 3:7 MeOH:Ace and 76% for acetone). Recovery of EA 6162 from DETEX using methanol (84%) and from latex body paint (65%) were lower than acetone and 3:7 MeOH:Ace recoveries. Note that essentially no EA 6162 remained on the stainless-steel coupons (< 0.1% on average for all tests) indicating that both strippable coatings were effective in removing EA 6162 from the control material surface. Acetone was selected as the solvent to use during the recovery efficacy testing due to its high extraction efficiency and since it provided a somewhat simpler approach to strippable coating and coupon extraction, i.e., no need to mix methanol and acetone.

***Table 50. Coating Extraction Efficiency Average EA 6162 Mass Recovered***

| Solvent | Test Item | EA 6162 Mass (µg) [1] | Recovery vs Spike Control | RSD |
|---|---|---|---|---|
| Methanol | Latex Body Paint | 6.2 | **65%** | 46% |
| | Stainless Steel Coupon | 0.0074 | 0.08% | 54% |
| | DETEX | 7.9 | **84%** | 3.7% |
| | Stainless Steel Coupon | 0.0066 | 0.07% | 27% |
| Acetone | Latex Body Paint | 7.3 | **76%** | 18% |
| | Stainless Steel Coupon | 0.0066 | 0.07% | 34% |
| | DETEX | 9.2 | **98%** | 3.5% |
| | Stainless Steel Coupon | 0.0043 | 0.05% | 13% |
| 3:7 MeOH:Ace | Latex Body Paint | 7.5 | **79%** | 13% |
| | Stainless Steel Coupon | 0.0050 | 0.05% | 17% |
| | DETEX | 9.2 | **98%** | 3.6% |
| | Stainless Steel Coupon | 0.0060 | 0.06% | 24% |

[1] 5 replicates

The average recovery of EA 6162 from each of the four test materials using latex body paint is shown in Table 51. The average EA 6162 recoveries from the stainless steel LFBs for each trial

(Eff 1 and Eff 2) are shown separately. The highest average recovery for the latex body paint was from ceramic tile at 91%, which might be expected due to the smooth and impervious surface of the tile. Ceramic tile also had the lowest coating RSD, indicating that the procedure was reproducible. The high RSD for the ceramic tile coupon (195%) as well as most of the coupons is likely related to low, variable masses of EA 6162 remaining on the coupon.

***Table 51. Latex Body Paint Average EA 6162 Recovery Efficacy***

| Material | Test Item | EA 6162 Mass (µg) [1] | Recovery vs Spike Control | RSD |
|---|---|---|---|---|
| Wood Dimensional Lumber | Coating | 2.5 | **26%** | 16% |
| | Coupon | 2.2 | 23% | 24% |
| Vinyl Tile | Coating | 6.7 | **72%** | 31% |
| | Coupon | 0.035 | 0.37% | 65% |
| Ceramic Tile | Coating | 8.2 | **91%** | 8.6% |
| | Coupon | 0.016 | 0.17% | 195% |
| Plywood | Coating | 2.1 | **23%** | 53% |
| | Coupon | 4.2 | 46% | 13% |
| Stainless Steel (Eff 1) | Coating | 7.5 | **80%** | 25% |
| | Coupon | 0.015 | 0.16% | 121% |
| Stainless Steel (Eff 2) | Coating | 6.6 | **73%** | 25% |
| | Coupon | 0.0060 | 0.066% | 73% |

[1] 5 replicates of test materials and 3 replicates of stainless steel

The average recovery from vinyl tile was 72%, which was not statistically different from EA 6162 recovery from stainless steel (see Appendix D). The somewhat high RSD for the vinyl tile coating (31%) indicated that the ability to recover EA 6162 from vinyl coating with latex body paint may be more variable than the ability to recover EA 6162 from ceramic tile or even wood. Average recovery from both wood dimensional lumber (26%) and plywood (23%) was statistically lower (see Appendix D) than ceramic tile, vinyl tile, and stainless steel. A substantial amount of EA 6162 remained in the plywood coupons (46% on average) and the wood dimensional lumber coupons (23% on average). Lower coupon RSDs were observed when a higher mass of EA 6162 remained on the coupon, particularly for the wood dimensional lumber and plywood coupons.

The average recovery of EA 6162 from each of the four test materials using DETEX is shown in Table 52. The average EA 6162 recovery from the stainless steel LFBs for each trial (Eff 3 and Eff 4) are shown separately. The highest average recoveries were from vinyl tile at 96% and from ceramic tile at 94%, which were not statistically different from recoveries from stainless steel. The average recovery of EA 6162 from wood dimensional lumber using DETEX (46%) was higher than for the latex body paint but still statistically lower than recovery from stainless steel. The average recovery of EA 6162 from plywood was only 24% for DETEX, like latex body paint and statistically lower than recovery from stainless steel. The variance in EA 6162 recovery was fairly low for DETEX, with plywood having the highest RSD at 43%. As observed for the latex body paint samples, a substantial amount of EA 6162 remained in the wood dimensional

lumber and plywood samples after DETEX removal. Refer to Appendix D for statistical analysis results.

***Table 52. DETEX Average EA 6162 Recovery Efficacy***

| Material | Test Item | EA 6162 Mass (µg) [1] | Recovery vs Spike Control | RSD |
|---|---|---|---|---|
| Wood Dimensional Lumber | Coating | 4.3 | **46%** | 11% |
| | Coupon | 3.0 | 32% | 13% |
| Vinyl Tile | Coating | 9.2 | **96%** | 2.3% |
| | Coupon | 0.028 | 0.29% | 26% |
| Ceramic Tile | Coating | 9.3 | **94%** | 7.2% |
| | Coupon | 0.0031 | 0.03% | 33% |
| Plywood | Coating | 2.4 | **24%** | 43% |
| | Coupon | 4.6 | 47% | 10% |
| Stainless Steel (Eff 3) | Coating | 9.4 | **99%** | 0.85% |
| | Coupon | 0.0040 | 0.04% | 21% |
| Stainless Steel (Eff 4) | Coating | 9.4 | **95%** | 3.7% |
| | Coupon | 0.0064 | 0.06% | 25% |

[1] 5 replicates of test materials and 3 replicates of stainless steel

The absorption of EA 6162 solution into the wood dimensional lumber and plywood coupons and the lower LFSM sample recovery for EA 6162 from wood dimensional lumber compared to other materials (Table 45 and Table 46) indicate that quantitative recovery of EA 6162 from wood dimensional lumber and plywood using a strippable coating may be difficult; however, the qualitative result afforded even for lower recoveries may be informative for field operations, although the possibility of false negatives should be considered.

Figure 12 shows a comparison of EA 6162 recovery for both strippable coatings from all four test materials and the stainless steel LFB. The stainless-steel data for Trials Eff1 and Eff 2 were combined, as were the data for Trials Eff 3 and Eff 4. Error bars of ± 1 SD are shown for all data in Figure 12. While the recovery of EA 6162 from wood dimensional lumber and plywood was statistically lower than recovery from vinyl tile and ceramic tile for both strippable coatings, this testing did show that some recovery was possible under the conditions tested.

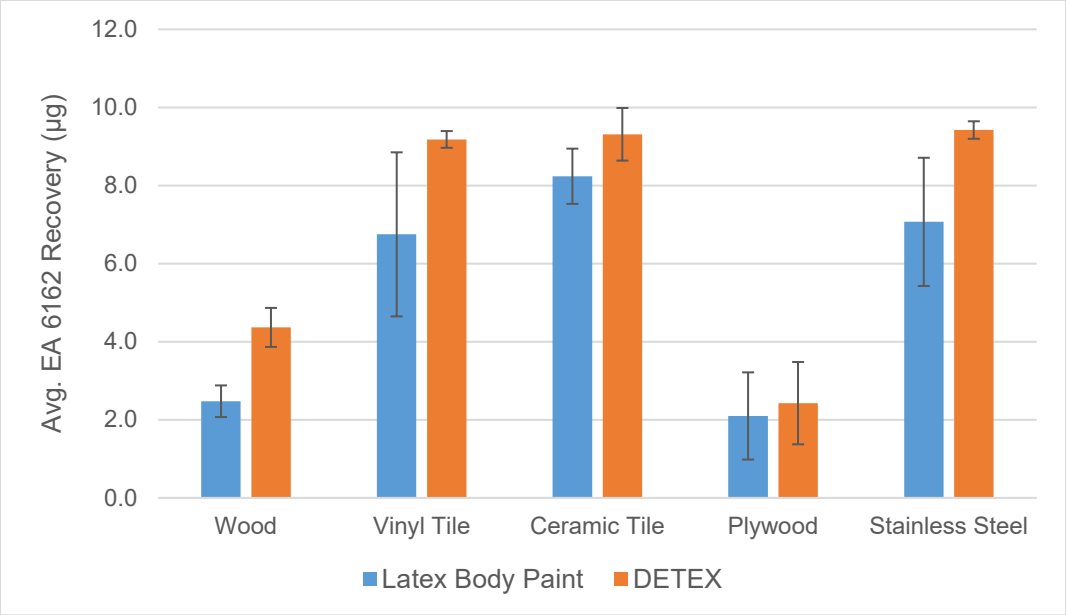



***Figure 12. Average Recovery of EA 6162 for Both Strippable Coatings from All Materials***

While DETEX recovered statistically significantly more EA 6162 only from wood dimensional lumber compared to latex body paint (see Appendix D), DETEX had better and more consistent EA 6162 extraction efficiency and was easier to remove from all surfaces.

### *F.3 Part 3 Water Testing*

The IDC met all testing criteria including that the P&A average was within ± 30% of nominal EA 6162 spike concentration with an RSD ≤ 20%. Additionally, the QL average was within ± 20% of the nominal concentration with an RSD ≤ 30%. The average malathion surrogate recoveries were within ± 20% of the nominal concentration with an RSD ≤ 30%. Finally, both LMBs were below the reporting limit of 0.1 ng/mL. See Table 53 for a summary of all IDC results.

***Table 53. EA 6162 IDC Results in Drinking Water***

| Sample Type | Analyte | Nominal Conc. (ng/mL) | Average Measured Conc. (ng/mL) [2] | Average Recovery | RSD |
|---|---|---|---|---|---|
| P&A | EA 6162 | 5.0 | 4.9 | 97% | 3.6% |
| | Malathion [1] | 5.0 | 4.6 | 92% | 3.0% |
| QL | EA 6162 | 0.50 | 0.44 | 88% | 4.4% |
| | Malathion | 0.50 | 0.48 | 96% | 3.7% |
| HPLC Water LMB | EA 6162 | NA | <0.10 | NA | |
| | Malathion | 5.0 | 4.8 [3] | 96% | |
| Source 1 LMB | EA 6162 | NA | <0.10 | NA | |
| | Malathion | 5.0 | 4.8 [3] | 97% | |

[1] Surrogate [2] Four P&A replicates, Seven QL replicates [3] Single measurement

The recovery efficacy results met all testing criteria as shown in Table 54:

- The average recoveries for all water source samples were within ± 20% of nominal EA 6162 concentration with an RSD less than 30%;
- The average recoveries for all LFBs were also within ± 20% of nominal concentration with an RSD less than 30%;
- The average malathion surrogate recoveries for all water source samples and LFBs were within ± 20% of the nominal concentration with an RSD less than 30%; and
- For all the water source laboratory method blanks, no EA 6162 chromatographic peak was detected.

The average and standard deviation for the malathion recovered from all samples were calculated to compare actual surrogate recoveries to the acceptance range of ± 20% of the nominal concentration. The average mass was 4.8 ng/mL, which represented a recovery of 96% compared to the nominal malathion concentration (5.0 ng/mL). The surrogate SD was 0.25 ng/mL, resulting in acceptance limits of 85% - 106% based on ± 2 SD around the average recovery, narrower recovery limits than the ± 20% limit uses for this testing. Note that these limits are not necessarily how EPA would establish control limits but are useful for evaluating this data set.

***Table 54. Part 3 EA 6162 Recovery Efficacy Results***

| Sample Type | Analyte | Nominal Conc. (ng/mL) | Average Measured Conc. (ng/mL) [2] | Average Recovery | RSD |
|---|---|---|---|---|---|
| Water Source 1 | EA 6162 | 5.0 | 4.8 | 95% | 6.1% |
| | Malathion [1] | 5.0 | 4.8 | 96% | 5.7% |
| LFB | EA 6162 | 5.0 | 4.7 | 95% | 4.8% |
| | Malathion | 5.0 | 4.6 | 92% | 3.6% |
| Water Source 2 | EA 6162 | 5.0 | 5.0 | 100% | 1.1% |
| | Malathion | 5.0 | 4.8 | 97% | 3.8% |
| LFB | EA 6162 | 5.0 | 4.9 | 97% | 2.2% |
| | Malathion | 5.0 | 4.7 | 93% | 2.7% |
| Water Source 3 | EA 6162 | 5.0 | 4.9 | 97% | 2.3% |
| | Malathion | 5.0 | 5.0 | 101% | 3.6% |
| LFB | EA 6162 | 5.0 | 4.8 | 97% | 0.96% |
| | Malathion | 5.0 | 5.0 | 100% | 2.4% |

[1] Surrogate [2] 5 replicates

As discussed in Section E.3, all sample preparation was performed on Day 0. Stability samples were stored in a refrigerator whose temperature ranged from 3 to 6 °C, with an average temperature of 5 °C over the course of all stability testing. To account for possible matrix effects, the percent recoveries of the Day 0 stability samples were calculated against the average recovery of the LFB samples, not against the nominal concentration. The percent recoveries of the Day 7, 14, and 28 samples were calculated against the average recovery of the Day 0 samples. The concentration of EA 6162 at each time point under the test conditions remained unchanged within experimental error, with average recoveries for each water at Days 0, 7, 14, and 28 of:

- Water Source 1: 101%, 100%, 98%, and 102%;
- Water Source 2: 97%, 103%, 101%, and 102%; and
- Water Source 3: 98%, 99%, 98%, and 99%.

Note that the final sample for Water Source 3 was analyzed on Day 33 due to schedule constraints. Average surrogate recovery was ≥ 90% for all sample sets. Refer to Table 55, Table 56, and Table 57 for a summary of average recovery and RSD for each water at each time point.

Graphical representation of EA 6162 concentration for each replicate for each day of analysis is shown in Figure 13, Figure 14, and Figure 15 for Water Source 1, Water Source 2, and Water Source 3, respectively. As can be seen from the graphs, as well as the low RSD values shown in the tables, EA 6162 recovery was very consistent within experimental error for all samples.

***Table 55. Water Source 1 EA 6162 Stability Results***

| Sample Type | Analyte | Analyte Conc. (ng/mL) | Average Measured Conc. (ng/mL) [3] | Average Recovery | RSD |
|---|---|---|---|---|---|
| LFB | EA 6162 | 5.0 | 4.7 | 94% | 1.3% |
| | Malathion | 5.0 | 4.5 | 90% | 3.9% |
| Stability Day 0 | EA 6162 | 4.7[1] | 4.7 | 101% | 0.94% |
| | Malathion | 5.0 | 4.5 | 90% | 5.2% |
| Stability Day 7 | EA 6162 | 4.7[2] | 4.7 | 100% | 2.1% |
| | Malathion | 5.0 | 4.7 | 94% | 3.4% |
| Stability Day 14 | EA 6162 | 4.7[2] | 4.7 | 98% | 2.3% |
| | Malathion | 5.0 | 4.9 | 98% | 4.2% |
| Stability Day 28 | EA 6162 | 4.7[2] | 4.8 | 102% | 3.2% |
| | Malathion | 5.0 | 4.6 | 92% | 5.5% |

[1]Average LFB concentration [2]Average Day 0 concentration [3] 7 replicates

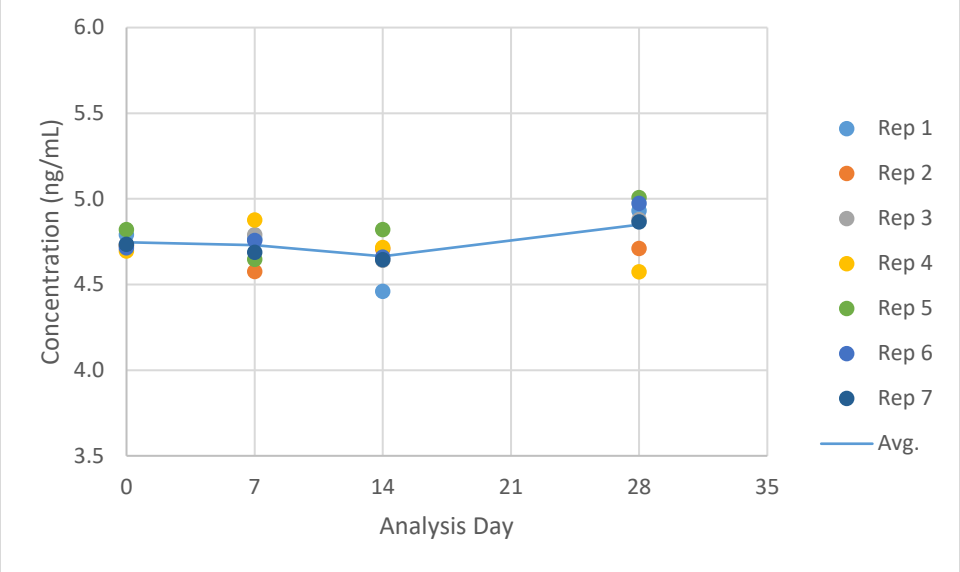



***Figure 13. Water Source 1 EA 6162 Stability Results***

***Table 56. Water Source 2 EA 6162 Stability Results***

| Sample Type | Analyte | Analyte Conc. (ng/mL) | Average Measured Conc. (ng/mL) [3] | Average Recovery | RSD |
|---|---|---|---|---|---|
| LFB | EA 6162 | 5.0 | 4.9 | 98% | 2.2% |
| | Malathion | 5.0 | 4.9 | 97% | 4.8% |
| Stability Day 0 | EA 6162 | 4.9[1] | 4.8 | 97% | 0.97% |
| | Malathion | 5.0 | 5.0 | 100% | 3.7% |
| Stability Day 7 | EA 6162 | 4.8[2] | 4.9 | 103% | 2.0% |
| | Malathion | 5.0 | 4.9 | 98% | 5.2% |
| Stability Day 14 | EA 6162 | 4.8[2] | 4.8 | 101% | 1.4% |
| | Malathion | 5.0 | 4.6 | 92% | 5.2% |
| Stability Day 28 | EA 6162 | 4.8[2] | 4.8 | 102% | 1.2% |
| | Malathion | 5.0 | 5.0 | 101% | 3.0% |

[1]Average LFB concentration [2]Average Day 0 concentration [3] 7 replicates

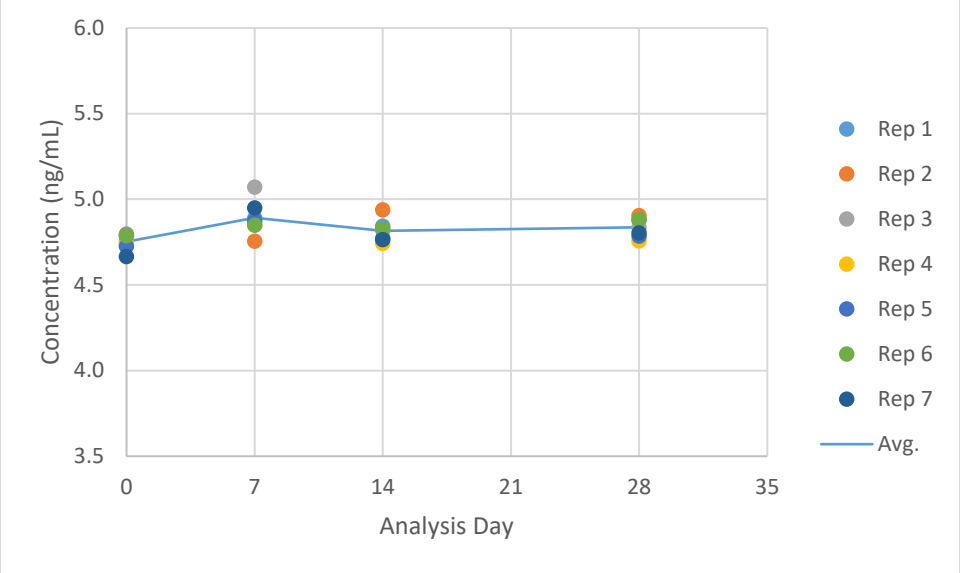



***Figure 14. Water Source 2 EA 6162 Stability Results***

***Table 57. Water Source 3 EA 6162 Stability Results***

| Sample Type | Analyte | Analyte Conc. (ng/mL) | Average Measured Conc. (ng/mL) [3] | Average Recovery | RSD |
|---|---|---|---|---|---|
| LFB | EA 6162 | 5.0 | 5.0 | 100% | 1.9% |
| | Malathion | 5.0 | 4.7 | 95% | 4.1% |
| Stability Day 0 | EA 6162 | 5.0[1] | 4.9 | 98% | 2.5% |
| | Malathion | 5.0 | 4.6 | 93% | 2.7% |
| Stability Day 7 | EA 6162 | 4.9[2] | 4.9 | 99% | 1.0% |
| | Malathion | 5.0 | 4.6 | 91% | 2.9% |
| Stability Day 14 | EA 6162 | 4.9[2] | 4.8 | 98% | 2.2% |
| | Malathion | 5.0 | 5.0 | 99% | 3.4% |
| Stability Day 33 | EA 6162 | 4.9[2] | 4.9 | 99% | 1.2% |
| | Malathion | 5.0 | 5.0 | 100% | 1.3% |

[1]Average LFB concentration [2]Average Day 0 concentration [3] 7 replicates

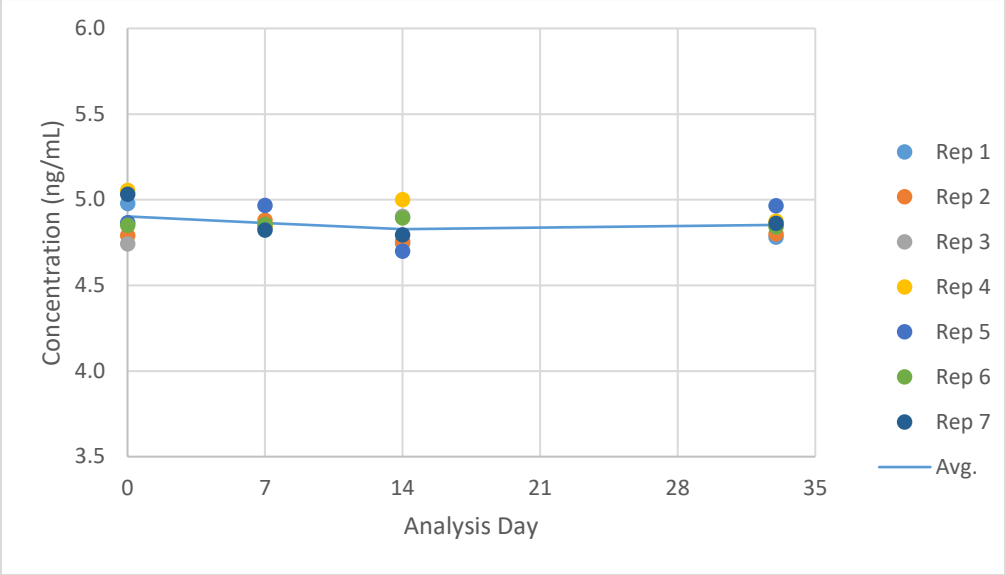



***Figure 15. Water Source 3 EA 6162 Stability Results***

Figure 16 shows the average EA 6162 concentration for each water source over time along with error bars of ± 1 SD. This graph demonstrates the consistent recovery within experimental error of EA 6162 over time for all three tested source waters.

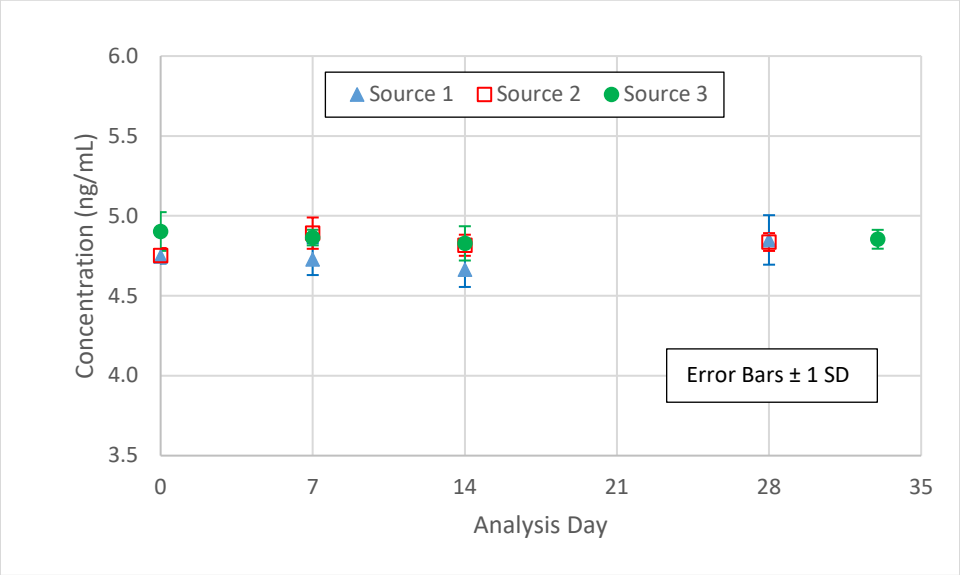



***Figure 16. Average Water Source EA 6162 Stability Results***

As noted in Section E.3, water MDL replicates were analyzed on three separate dates, with the analyses 7 days apart. Table 58 shows the standard deviation (SD) for the eight replicates analyzed for each water sample type. The MDL was calculated by multiplying the SD by 2.998, the Student's single-tailed 99$^{th}$ percentile t-value for eight replicates. The MDL values were very consistent for all three water sources, as well as the LFB. Each MDL value was greater than 10% of the 0.050 ng/mL spike level and below the spike level suggesting that the selected EA 6162 spike concentration was not too high. Earlier versions of 40 CFR Part 136 Appendix B had the requirement that MDLs be below the spike level but above 10% of the spike level to prevent the use of high spike concentrations, which can result in low variance for the results, creating artificially low MDLs. For all the laboratory method blanks, no EA 6162 chromatographic peak was detected. Since EA 6162 was not detected in the LMBs, it is reasonable to conclude that EA 6162 would not be detected in any the required MDL blanks, or to exceed any of the calculated MDLs; thus, limiting the number of LMBs collected during this study would not impact the final reported MDL.

***Table 58. Part 3 EA 6162 MDL Study Results***

| Sample Type | SD (ng/mL) | MDL (ng/mL) | Percent of Spike Level | Average Surrogate Recovery |
|---|---|---|---|---|
| LFB | 0.0056 | **0.017** | 33% | 101% |
| Water Source 1 | 0.0061 | **0.018** | 37% | 103% |
| Water Source 2 | 0.0070 | **0.021** | 42% | 103% |
| Water Source 3 | 0.0070 | **0.021** | 42% | 103% |

The results of Part 3 testing indicated that the developed direct LC-MS/MS analysis of preserved drinking water samples allowed for the quantitation of EA 6162. EA 6162 should be stable in preserved drinking water samples, at least those resembling the waters tested, for up to 28 days when stored at 2-8 °C.

### *F.4 Part 4 Soil Testing*

The average of the spike controls was required to be within ± 20% of the nominal EA 6162 spike mass with an RSD of ≤ 30% for replicates. The average EA 6162 results are shown in Table 59,and all results were within tolerance for each trial.

***Table 59. Part 4 EA 6162 Spike Control Results***

| Trial | Spike Control Nominal Mass (ng) | Average EA 6162 Mass (ng) [1] | Average Recovery | RSD |
|---|---|---|---|---|
| IDC | 50 | 47 | 93% | 2.5% |
| | 10 | 9.9 | 99% | 2.6% |
| 60 Minute Dwell | 50 | 48 | 96% | 0.82% |
| | 5.0 | 4.7 | 93% | 3.1% |
| 24 Hour Dwell | 50 | 48 | 97% | 5.1% |
| | 5.0 | 4.6 | 91% | 2.4% |
| 7 Day Dwell | 50 | 48 | 97% | 5.9% |
| | 5.0 | 4.7 | 93% | 4.9% |
| 14 Day Dwell | 50 | 49 | 98% | 2.7% |
| | 5.0 | 4.8 | 96% | 3.8% |

[1] 3 replicates for 10 ng and 5.0 ng spikes and 5 replicates for 50 ng spikes

The average of all replicate LFSMs was required to be within ± 20% of the spike control average with an RSD of ≤ 30% across replicates. The average LFSM results are shown in Table 60, and all results were within tolerance for each test, indicating that matrix effects did not impact sample analysis. The LFSM spike mass was 5.0 ng. However, average recoveries were calculated against the average spike control mass shown in Table 59.

***Table 60. Part 4 EA 6162 LFSM Results***

| Trial | Test Material | Average EA 6162 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|
| 60 Minute Dwell | Nebraska Soil | 4.8 | 103% | 4.1% |
| | Georgia Soil | 5.1 | 110% | 4.4% |
| | Clean Loam | 4.8 | 104% | 4.4% |
| 24 Hour Dwell | Nebraska Soil | 4.6 | 101% | 1.1% |
| | Georgia Soil | 4.8 | 106% | 2.4% |
| | Clean Loam | 4.6 | 100% | 0.92% |
| 7 Day Dwell | Nebraska Soil | 5.3 | 113% | 12% |
| | Georgia Soil | 4.9 | 105% | 2.2% |
| | Clean Loam | 5.1 | 110% | 6.2% |
| 14 Day Dwell | Nebraska Soil | 4.7 | 98% | 1.3% |
| | Georgia Soil | 4.9 | 101% | 2.2% |
| | Clean Loam | 5.0 | 103% | 4.2% |

[1] 3 replicates

LMBs were required to have no quantifiable EA 6162 results above the reporting limit of 1.0 ng. All LMB results were non-detect for each test. Average malathion surrogate recoveries were required to be within ± 20% of the spike control surrogate average with an RSD of ≤ 30% across replicates. All surrogate results were within tolerance for all trials. The average and standard deviation for the recovered malathion 50 ng spike mass for all samples were calculated to compare actual surrogate recoveries to the acceptance range of ± 20% of the spike control average. The average mass was 42 ng, which represented a recovery of 87% compared to the average recovered malathion for all spike controls (48 ng). The surrogate SD was 2.8 ng, resulting in acceptance limits of 75% - 99% based on ± 2 SD around the average recovery. Note that these limits are not necessarily how EPA would establish control limits but are useful for evaluating this data set.

Throughout all Part 4 soil testing, temperature and RH in the test environment were monitored, but not controlled. Average temperature and RH results are shown in Table 61. Both temperature and RH were quite similar across all trials and did not exhibit any impact on the data across all tested matrix types.

***Table 61. Part 4 Environmental Conditions***

| Trial | Average Temperature (°C) | Average RH (%) |
|---|---|---|
| IDC | 21 | 12 |
| 60 Minute Dwell | 21 | 29 |
| 24 Hour Dwell | 22 | 19 |
| 7 Day Dwell | 22 | 11 |
| 14 Day Dwell | 21 | 24 |
| MDL Day 1 | 21 | 25 |
| MDL Day 2 | 21 | 26 |
| MDL Day 3 | 21 | 18 |

For the IDC tests, the EA 6162 average recovery for P&A samples was required to be within ±30% of the spike control average with an RSD of ≤ 20% across replicates. The EA 6162 average recovery for QL samples was required to be within ± 20% of the spike control average with an RSD of ≤ 30% across replicates. The average EA 6162 mass recovered for the IDC tests are shown in Table 62. Results for the P&A and QL samples met the acceptance criteria. Average recoveries were calculated against the average spike control mass shown in Table 59.

***Table 62. Part 4 EA 6162 IDC Results***

| Trial | Test Material | Spike Mass (ng) | Average EA 6162 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|---|
| P&A | Sand | 50 | 46 | 99% | 11% |
| QL | Sand | 10 | 9.2 | 93% | 10% |

[1] 4 P&A replicates, 7 QL replicates

The average EA 6162 mass recovered for the four efficacy testing trials is shown in Table 63. The spike mass was 50 ng; however, average recoveries were calculated against the average spike control results shown in Table 59. The average of the LFBs (sand samples) were required to be within ± 20% of the spike control average with an RSD of ≤ 30% across replicates. The average sand results are shown in Table 63, all results were within tolerance for each test.

***Table 63. EA 6162 Recovery Efficacy Results in Tested Soils***

| Trial | Test Material | Average EA 6612 Mass (ng) [1] | Average Recovery vs Spike Control | RSD |
|---|---|---|---|---|
| 60 Minute Dwell | Nebraska Soil | 37 | 77% | 2.6% |
| | Georgia Soil | 42 | 87% | 8.1% |
| | Clean Loam | 40 | 83% | 11% |
| | Sand | 44 | 91% | 0.87% |
| 24 Hour Dwell | Nebraska Soil | 34 | 70% | 4.7% |
| | Georgia Soil | 36 | 75% | 1.6% |
| | Clean Loam | 38 | 78% | 9.2% |
| | Sand | 40 | 83% | 7.3% |
| 7 Day Dwell | Nebraska Soil | 24 | 50% | 8.1% |
| | Georgia Soil | 14 | 30% | 3.8% |
| | Clean Loam | 33 | 68% | 4.3% |
| | Sand | 44 | 90% | 1.1% |
| 14 Day Dwell | Nebraska Soil | 11 | 22% | 5.1% |
| | Georgia Soil | 5.2 | 11% | 9.6% |
| | Clean Loam | 19 | 38% | 12% |
| | Sand | 43 | 87% | 2.3% |

[1]5 replicates of soils and 3 replicates of sand

The percent recovery for each sample replicate (vs the spike control average) from each recovery efficacy trial is shown in Figure 17. This figure demonstrates that EA 6162 recovery from the sand was consistent regardless of dwell time, compared to each of the test soils, in which EA 6162 recovery decreased with increasing dwell time. An ANOVA model was fitted to the natural log-transformed EA 6162 recovery data with effects for soil type, hold time, and the interaction between soil type and hold time; see Appendix E. The decrease in recovered EA 6162 over time was statistically significant for Nebraska soil, Georgia soil, and clean loam. Some differences in recovered EA 6162 between soil types at the same time point were also statistically significant, indicating that the soil type impacted the ability to recover EA 6162. The relative difference in EA 6162 recovery from the Nebraska soil, Georgia soil, and clean loam did not appear to correlate with the overall texture of the soils indicated on the texture triangle shown in Figure 10. For example, the higher sand content of the Georgia soil relative to the Nebraska soil might correlate to higher EA 6162 recovery for the Georgia soil but the Nebraska soil had significantly higher recoveries for the 7-day and 14-day timepoints.

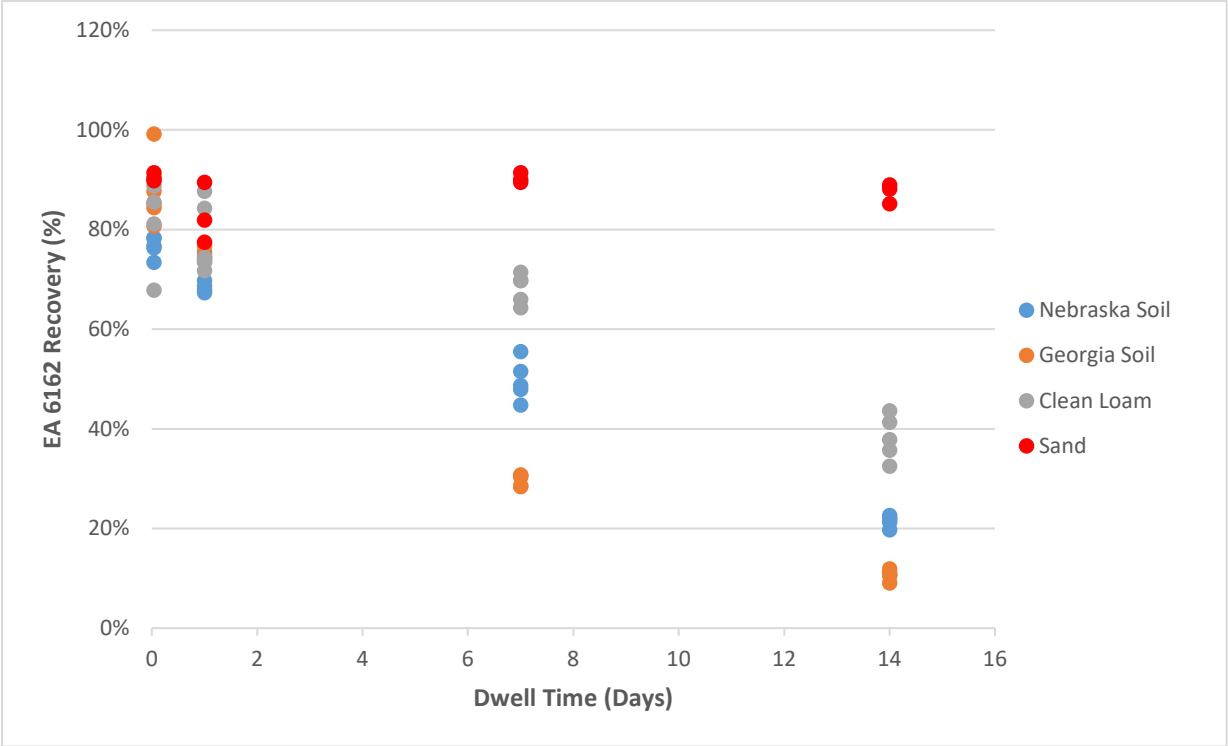



***Figure 17. Soil EA 6162 Replicate Percent Recovery***

The average mass recoveries for the four efficacy testing trials are shown in Figure 18. Error bars represent ± 1 SD. Regression lines were fit to the data. A linear regression provided a fit with coefficient of determination ($R^2$) > 0.90 for the Nebraska soil and the clean loam. A second-order polynomial regression provided a better fit to the Georgia soil compared to a linear regression. These regression lines provide an empirical relationship between recoverable EA 6162 mass from soils and time. Because of the variation between soils, the performance of the method for any specific soil that is not 100% sand may need to be verified, since a predictive model of recovery vs time may never be comprehensive. That being said, few soils are 100% sand, and it is not known if different types of sand behave differently with regard to whatever (current unstudied) processes result in declining method performance over time for soils.

For the ANOVA comparison of hold time within soil type, for 100% sand there was no significant difference in geometric mean mass recovery among any of the hold times. By contrast, for the clean loam, Georgia soil, and Nebraska soil, the average mass recovery at 14 days was significantly lower than the average mass recovery at 60 minutes, 1 day, and 7 days; and the average mass recovery at 7 days was significantly lower than the average mass recovery at 1 day and 60 minutes. Additionally for the Georgia soil, the average mass recovery at 1 day was significantly lower than the average mass recovery at 60 minutes. These times may be particularly impactful for field operations, where realistic hold times between sample collection and analysis may be days or weeks, providing additional reason to verify method performance for site-specific soils.

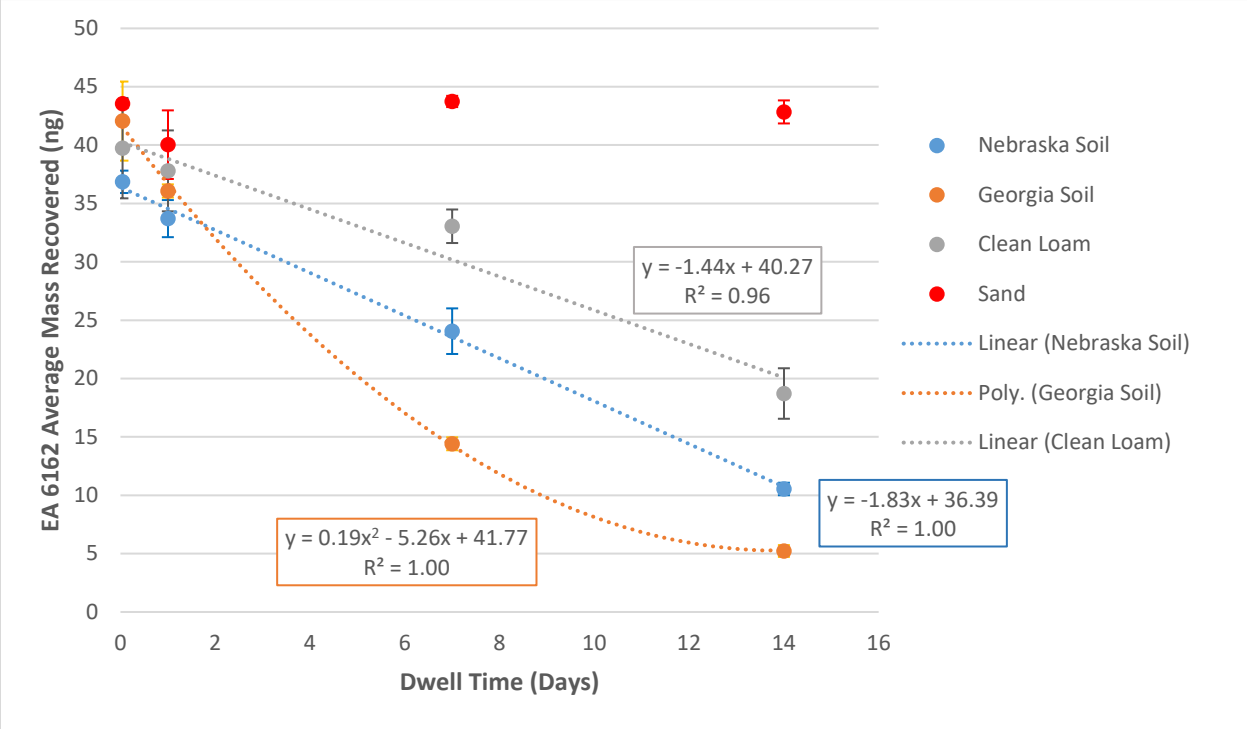



***Figure 18. Soil EA 6162 Average Mass Recovered***

As noted above, MDL replicates were prepared on three separate dates, with the analyses six days apart. Table 64 shows the standard deviation for the eight replicates analyzed for each soil sample type. Note that the concentration was calculated based on the recovered mass of EA 6162 divided by the nominal soil weight of 5 g. The MDL was calculated by multiplying the standard deviation by 2.998, the Student's single-tailed 99$^{th}$ percentile t-value for eight replicates. The MDL values were consistent for all three soil types and sand. Except for the sand, each MDL value was greater than or equal to 10% of the 0.40 ng/g spike level and below the spike level, indicating that the selected EA 6162 spike concentration was not too high for the tested soil types. Soil matrix types can be difficult to work with given matrix interferents associated with the various soils that were tested, which is why a higher spike level may be needed. For all the laboratory method blanks, no EA 6162 chromatographic peak was detected. Since EA 6162 was not detected in the LMBs, it is reasonable to conclude that EA 6162 would not be detected in any the required MDL blanks, or to exceed any of the calculated MDLs; thus, limiting the number of LMBs collected during this study would not impact the final reported MDL.

***Table 64. Part 4 EA 6162 MDL Results***

| Sample Type | SD (ng/g) | MDL (ng/g) | Percent of Spike Level | Average Surrogate Recovery |
|---|---|---|---|---|
| Nebraska Soil | 0.053 | **0.16** | 40% | 79% |
| Georgia Soil | 0.014 | **0.041** | 10% | 82% |
| Clean Loam | 0.029 | **0.087** | 22% | 83% |
| Sand | 0.010 | **0.031** | 7.8% | 88% |

The results of Part 4 testing indicated that the developed methanol extraction method with analysis by LC-MS/MS may allow recovery of EA 6162 from soil at the low levels studied. Observed reduced recovery of EA 6162 from soils over time indicates the potential need for timely collection and extraction of soil samples to ensure detection of EA 6162. In addition, method verification for a site-specific soil and/or method modification may be warranted.

# G. QUALITY ASSURANCE/QUALITY CONTROL

Data quality indicators, quality control (QC) elements, and QA audits described in the sections below provide the requirements for determining the adequacy of data generated during this project. Data were considered valid if the data quality indicators for the test measurements were met, and the performance evaluation, and data quality audits demonstrated acceptable results.

## *G.1 Data Quality Indicators*

Data quality indicators and results are provided in Table 65 and throughout this report as referenced in Table 65. Most data quality indicator results were acceptable, including checks of the measurement methods for time, temperature, RH, volume, and EA 6162 levels in blank samples, post-spiked samples, and spike controls, except as noted in Table 65.

***Table 65. Data Quality Indicators and Results***

| Parameter | Measurement Method | Data Quality Indicators | Results |
|---|---|---|---|
| Time (seconds) | Timer/data logger | Compare to time provided at NIST.time.gov once before testing; agree ± 2 seconds/hour. | No difference was observed between the timers and NIST.time.gov after one hour. |
| Temperature (°C) | National Institute of Standards and Testing (NIST)-traceable thermometer | Compare against calibrated thermometer once before testing; agree ± 1 °C through 60 min. | The HOBO UX100 datalogger remained within 0.12°C of the calibrated reference through 60 min. |
| Relative Humidity (%) | NIST-traceable hygrometer | Compare against calibrated hygrometer once before testing; agree ± 10% through 60 min. | The HOBO UX100 datalogger remained within 0.50% of the calibrated reference through 60 min. |
| Volume (µL) | Syringe with repeating dispenser or calibrated pipette (EA 6162 solution application) | Syringe and pipette were checked for accuracy and repeatability once before use by determining the mass of water delivered onto a calibrated balance. The syringe and pipette were acceptable if the average mass of five replicate droplets was ± 10% of expected (percent error). | The syringe used for EA 6162 solution application and pipette used for pesticide application were checked. Percent error was calculated using the following equation: % Error = $\frac{\lvert Expected\ Weight - Mean\ Weight \rvert}{Expected\ Weight}\ x\ 100$ Percent error results for each are provided below: • 50 µL syringe: 4.1% (1 µL droplet) • 3-25 µL pipette: 2.6% (13 µL droplet) |
| Volume (µL) | Calibrated pipettes (LC-MS/MS sample dilution) | Pipettes were checked for accuracy and repeatability before use by the manufacturer. The pipette was acceptable if the percent error was ± 10% of expected. | Pipettes used for LC-MS/MS sample dilution were checked. Systematic error for each is provided below: • MR-10 at 5 µL: 0.39% • MR-50 at 35 µL: 1.3% • MR-250 at 100 µL: 0.42% • MR-1000 at 500 µL: -0.13% |
| EA 6162 in LMB | LC-MS/MS | No quantifiable results above the reporting limit. | Some low-level EA 6162 detections were measured. Refer to Table 32, Table 39, and Table 47 and the associated discussion. |
| EA 6162 in LFSM and LFB | LC-MS/MS | Average of all replicate post-spiked control samples within 80% to 120% of the spike control average with an RSD of ≤ 30%. | High LFB recoveries observed for some Part 1A 3.0-ng spikes, refer to Table 31 and the associated discussion. Low LFSM recoveries observed for some Part 2 coupons, refer to Table 45 and Table 46 and the associated discussions. |

| Parameter | Measurement Method | Data Quality Indicators | Results |
|---|---|---|---|
| Malathion surrogate recovery | LC-MS/MS | Average of all replicates within 80% to 120% of the spike control surrogate average with an RSD of ≤ 30%. | High surrogate recoveries observed for some Part 1A 3.0-ng spikes; refer to Table 33 and the associated discussion. High surrogate recovery observed for Part 2 spike controls; refer to Table 44 and the associated discussion. Low surrogate recoveries observed for some Part 2 samples; refer to Table 48 and the associated discussion. |
| EA 6162 in spike controls | LC-MS/MS | Average of spike controls within 80% to 120% of the nominal spike mass with an RSD of ≤ 30%. | High spike control recoveries observed for four Part 1 tests; refer to Table 28 and Table 35 and associated discussion. |

## *G.2 Quality Control Elements*

Data accuracy was ensured by the calibration of all instruments. Instrumentation used during this project was maintained and operated according to the quality requirements and standard operating procedures for instrument use. Except for the LC-MS/MS, all instruments utilized during the project were calibrated as stipulated by the manufacturer or, at a minimum, annually. The LC-MS/MS was calibrated as described in Section D.6. Table 66 provides calibration frequency for instruments that were used during this project.

***Table 66. Instrument Calibration Frequency***

| Instrument | Frequency |
|---|---|
| Timer | Prior to testing, performed by the manufacturer. After the manufacturer-provided calibration expired, use of the expired unit was discontinued, and the unit was discarded. A new manufacturer-calibrated unit was obtained for use. |
| Calibrated UX100 HOBO Thermometer/Hygrometer | Prior to testing, performed by the manufacturer. After the manufacturer-provided calibration expired, use of the expired unit was discontinued, and the unit was discarded. A new manufacturer-calibrated unit was obtained for use. |
| Calibrated Pipettes | Prior to testing by the vendor and annually thereafter. Calibration/accuracy was also verified as described in Table 65. |
| LC-MS/MS | Calibrated prior to analysis of each set of test samples (calibration curve) and a calibration verification standard was analyzed after every ten samples and at the end of a set of samples (see detailed discussion in Section D.6). |

At all times during the project, standard operating procedures were followed in the movement and use of EA 6162 within the test facility. Chain of Custody (CoC) forms were used to ensure that test samples generated during the work were traceable throughout all parts of testing. Test measurements and information were recorded on Test Parameter Control Sheets (TPCSs) or in a laboratory record book (LRB). Monitoring of test conditions, parameters, and times was performed by technical staff familiar with quality requirements, and testing was documented on the TPCS. The results of each test set were provided to the client electronically in the form of Microsoft Excel™ files. Each Excel file included the CoC, LC-MS/MS analytical results, final results for each sample showing all calculations, and a summary of the results. Each sample was traceable from the CoC, to the analytical results, to the final results.

### *G.3 Quality Assurance*

Performance evaluation audits were essentially conducted continuously and addressed those reference measurements that factored into the data used in quantitative analysis during the evaluation, including volume, mass, and time measurements and LC-MS/MS calibration and performance; see results provided in Table 65. The volume of EA 6162 solution dispensed and solution concentration correlated directly to the mass of EA 6162 applied to test samples. Daily calibration of the LC-MS/MS, CCVs, and internal standard recovery provided confidence that the analysis system was providing accurate data.

While temperature and RH were measured and recorded for all testing using a calibrated device, these parameters were not controlled, therefore, no performance evaluation audit could be performed.

An independent QA audit for this testing was performed on June 9, 2021. The purpose of the audit was to ensure that testing was performed in accordance with the planned testing. The QA Officer reviewed the investigation methods, compared test procedures to those specified in the written test plan, and reviewed data acquisition and handling procedures. The QA Officer did not identify any findings that required corrective action.

A data quality audit provided validation of the data, including verification of the completeness of the data, compliance with the acceptance criteria in the test plan, recalculation checks, and tracing of the data from instrument outputs through the final report. One hundred percent (100%) of the data was reviewed prior to use in calculations or any data manipulation, and review was completed before the data were provided to QA for the data quality audit.

The QA Officer, operating independently of the laboratory testing effort, audited at least 10% of the data generated during testing. Data were traced from initial acquisition through reduction and to final reporting. All calculations were checked. The QA Officer did not identify any findings that required corrective action.

Through the data quality audit and review of reports, the QA Officer ensured that data generated during testing were valid, meeting the requirements of the test plan.

## H. SUMMARY

Multiple methods were developed for the recovery of EA 6162 from surfaces, water, and soil with analysis by LC-MS/MS. EA 6162 recovery efficacy and MDL studies were performed for most of these methods. The below observations can be made based on IDC, recovery efficacy, and MDL studies performed during each part of testing.

For Part 1, surface wipe testing, three wipe types (rayon/polyester gauze, cotton gauze, and cotton balls) using two wipe-wetting solvents (methanol and IPA) were evaluated for EA 6162 recovery from the surface of three materials (galvanized steel, vinyl floor tile, and ceramic floor tile). The test materials were 23 cm x 23 cm in size. The EA 6162 solution was applied to surfaces as 1-µL droplets in a 4 x 4 pattern (10 µg total mass loading) and allowed to dwell on the surface at different times: 1 minute, 30 minutes, or 60 minutes. IPA solutions of EA 6162 resulted in low recovery results at the 30- or 60-minute dwell times whereas an EA 6162 application in a water solution to the test material followed by a 30-minute dwell time resulted in much higher recoveries and was deemed an appropriate approach for test sample preparation. Two wipe samples were collected in series for each test item. All wipe samples were extracted with methanol and the extracts analyzed by LC-MS/MS. Cotton ball wipes presented a logistical challenge in having sufficient surface area to sample a surface and required more handling during extraction to ensure adequate solvent recovery. These factors might contribute to more variability in the data and recovery results. Sampling with the rayon/polyester gauze and with cotton gauze wipes, both with methanol wetting solvent, resulted in significantly better recoveries of EA 6162 from all three surface materials than when using IPA as a wetting solvent. There was no significant difference between the performance of rayon/polyester gauze wipes and cotton gauze wipes when methanol was used as a wipe wetting solvent. Cotton gauze wipes wetted with methanol provided some of the highest average recovery efficacies for each surface material (≥ 95% recovery for all materials with low relative RSDs), as shown in Table 67. The low RSDs indicated good method reproducibility. Surface wipe MDL values for each material ranged 0.014 to 0.026 ng/cm$^2$ based on a 529 cm$^2$ sample size. The surface wipe sample results indicated that the developed wipe collection, solvent extraction, and analysis methodology enabled sufficient recovery of EA 6162 from the tested surfaces.

For Part 2 testing, two strippable coatings (latex body paint and DETEX) were evaluated for EA 6162 recovery from the surface of four materials (dimensional wood lumber, vinyl floor tile, ceramic floor tile, and plywood sheathing). Test material coupons were 2.5 cm x 4.0 cm in size. The aqueous EA 6162 solution was applied to the coupon surface as 1-µL droplets in a 4 x 4 pattern for a total applied mass of 10 µg and allowed to dwell on the surface for 30 minutes. Each strippable coating type was allowed to cure for 2 hours prior to removal. Once removed, the strippable coating and the material coupon (minus coating) were extracted separately with acetone, and the extracts were analyzed by LC-MS/MS. EA 6162 recovery from vinyl tile (96%) and ceramic tile (94%) using the DETEX coating was high. A significantly lower amount of EA

6162 was recovered from wood dimensional lumber (46%) and plywood (24; refer to Table 67 (EA 6162 mass remaining on the coupons not shown). Absorption of EA 6162 into the wood dimensional lumber and plywood during the 30-minute dwell time may have limited the ability of the DETEX coating to remove EA 6162. Compared to DETEX, latex body paint was more difficult to remove and yielded lower and more variable recoveries of EA 6162.

***Table 67. Summary of EA 6162 Recovery Efficacy and MDLs***

| Testing | Sample Collection Approach | Material | Average EA 6162 Recovery | Recovery RSD | MDL |
|---|---|---|---|---|---|
| Part 1-Surface Wipe | Cotton Gauze Wipe with Methanol | Galvanized Steel | 96% | 3.9% | 0.019 ng/cm$^2$ |
| | | Vinyl Tile | 99% | 5.0% | 0.014 ng/cm$^2$ |
| | | Ceramic Tile | 95% | 1.9% | 0.026 ng/cm$^2$ |
| Part 2-Strippable Coating | DETEX Strippable Coating | Wood Dimensional Lumber | 46% | 11% | Not Determined |
| | | Vinyl Tile | 96% | 2.3% | |
| | | Ceramic Tile | 94% | 7.2% | |
| | | Plywood | 24% | 43% | |
| Part 3-Drinking water | Direct Sample Analysis | Water Source 1 | 95% | 6.1% | 0.018 ng/mL |
| | | Water Source 2 | 100% | 1.1% | 0.021 ng/mL |
| | | Water Source 3 | 97% | 2.3% | 0.021 ng/mL |
| Part 4-Soils | Methanol Extraction | Nebraska Soil | 77% | 2.6% | 0.16 ng/g |
| | | Georgia Soil | 87% | 8.1% | 0.041 ng/g |
| | | Clean Loam | 83% | 11% | 0.087 ng/g |
| | | Sand | 91% | 0.87% | 0.031 ng/g |

For Part 3 testing, three domestic drinking water sources were evaluated for EA 6162 recovery. Water samples spiked at a 5.0 ng/mL EA 6162 concentration were directly analyzed by LC-MS/MS with no additional preparation required. The average recovery was 95% to 100% as shown in Table 67 with low RSDs. The measured Part 3 MDL values for each water source ranged from 0.018 to 0.021 ng/mL based on a 40-mL water sample. EA 6162 was also demonstrated to be stable in preserved drinking water samples when refrigerated for up to 28 days with recoveries > 95% for all measured time points. These results indicated that the developed direct analysis methodology enabled recovery of EA 6162 from the tested drinking source waters.

For Part 4 testing, three soil types were evaluated for EA 6162 recovery; quartz sand was also used as a control. An EA 6162 mass of 50 ng was applied to soil samples in an IPA solution and allowed to dwell on the soil for four time periods: 60 minutes, 24 hours, 7 days, and 14 days, prior to extraction with methanol and analysis by LC-MS/MS. As shown in Table 67, EA 6162 recoveries after a 60-minute dwell time were variable, with recoveries from Nebraska soil (77%)

statistically significantly lower than Georgia soil (87% recovery) and sand (91% recovery). EA 6162 recoveries decreased for each of the three soil types over time, with the lowest average recovery (11%) observed for Georgia soil after 14 days, while recovery from sand did not decrease. The cause for the difference in EA 6162 recovery between soil types and over time was not identified. The measured Part 4 MDL values ranged from 0.041 ng/g to 0.16 ng/g based on a nominal 5 g soil sample but did differ 4-fold between the lowest and highest value. The developed soil extraction and analysis methodology resulted in recovery of EA 6162 from the tested soils.

As a more general note, laboratory cross contamination may limit the quantitation limit for application of these methods. While this type of limitation is a general possibility for all types of contaminants of interest at ultra-trace levels, the results of this study suggest it is particularly true for surface and soil samples. Laboratories may wish to pay special attention to analysis of these matrices. Further, although analytical technology may in principle push down quantitation limits, the benefits of newer technology may not necessarily result in lowering of any concentration-derived health-based goals for site cleanup.

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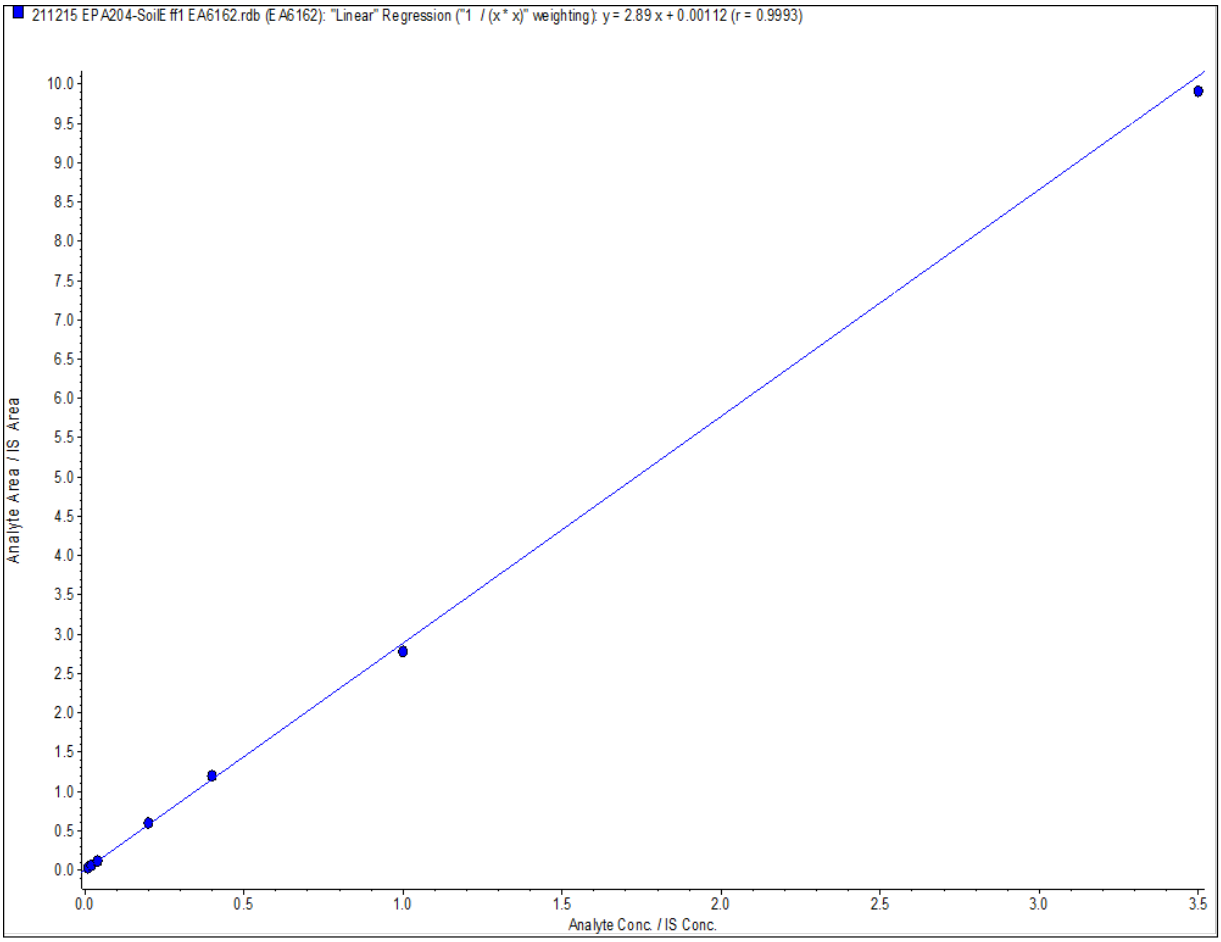
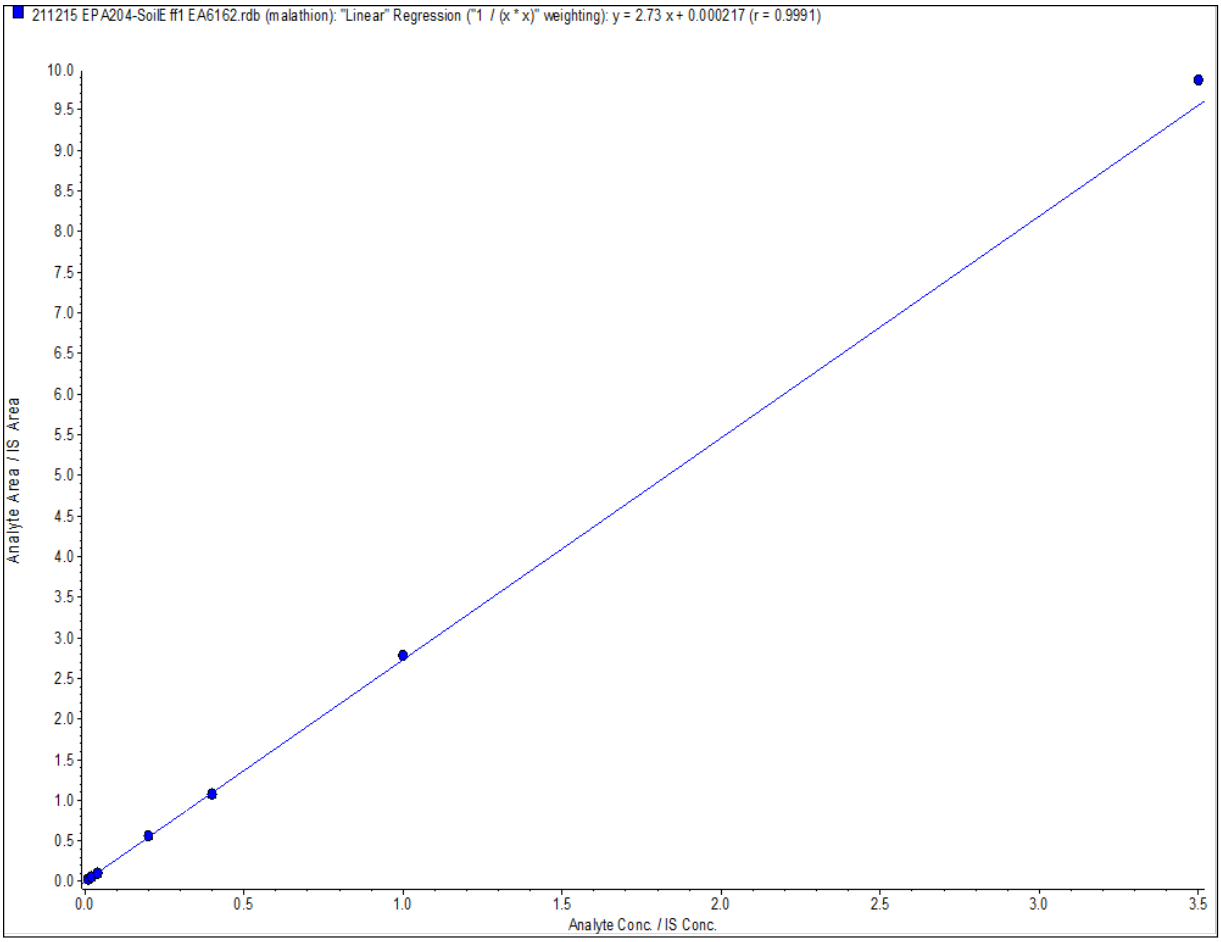
# APPENDIX A

## Example Calibration Curves




**Example 7-Point Calibration Curve for EA 6162**




**Example 7-Point Calibration Curve for Malathion**

# APPENDIX B

## Part 1A ANOVA

The objective of the following analysis of Part 1A testing was to compare total EA 6162 mass recovered from a galvanized steel using three different wipe types: cotton balls (CB), cotton gauze (CG), and rayon/polyester gauze (RPG), with each of two different wipe wetting solvents: isopropyl alcohol (IPA) and methanol (MeOH). Each of these six wipe-type and solvent combinations was tested in each of three different testing iterations, where 10 µg of EA 6162 was spiked onto the galvanized steel surface using the following iterative approaches:

1. Spiking with an IPA solution followed by a 60-minute wait time before wiping,
2. Spiking with an IPA solution followed by a 1-minute wait time before wiping, and
3. Spiking with a water solution followed by a 30-minute wait time before wiping.

Two wipes were collected in series and their collection masses summed to produce one total mass for each replicate sample. Five replicates were tested in each study condition. Table B 1 summarizes the study design.

***Table B 1. Study Design for Testing Wipe Type and Solvent Over Three Iterations***

| Iteration | Wipe | Solvent | Number of Replicates |
|---|---|---|---|
| 1 | CB | IPA | 5 |
| 1 | CB | MeOH | 5 |
| 1 | CG | IPA | 5 |
| 1 | CG | MeOH | 5 |
| 1 | RPG | IPA | 5 |
| 1 | RPG | MeOH | 5 |
| 2 | CB | IPA | 5 |
| 2 | CB | MeOH | 5 |
| 2 | CG | IPA | 5 |
| 2 | CG | MeOH | 5 |
| 2 | RPG | IPA | 5 |
| 2 | RPG | MeOH | 5 |
| 3 | CB | IPA | 5 |
| 3 | CB | MeOH | 5 |
| 3 | CG | IPA | 5 |
| 3 | CG | MeOH | 5 |
| 3 | RPG | IPA | 5 |
| 3 | RPG | MeOH | 5 |

A fixed effects ANOVA model was fitted separately to the total mass recovery data for each iteration. The models contained effects for condition (the combination of wipe type and wipe wetting solvent) and a residual error term. Data were found to adequately meet assumptions of normality and equal variance when no transformation was applied, and thus data were left

untransformed for analysis. No random effect of trial was fitted due to only one trial being run for each condition.

The models were fitted using SAS (version 9.4, 64-bit). The form of the model is presented in Equation B1 below.

$$Mass\ (\mu g) = \beta_0 + \beta_{ij} + \varepsilon_{ijk} \qquad \text{Equation B1}$$

where:

- $\beta_0$ = intercept or mean total mass collected
- $\beta_{ij}$ = the fixed effect for the $i^{th}$ wipe type and the $j^{th}$ solvent
- $\varepsilon_{ijk}$ = random error for the $k^{th}$ replicate from the $j^{th}$ solvent and the $i^{th}$ wipe type. The random error is assumed to be N(0, $\sigma_\epsilon^2$).

Using the models fitted separately to each iteration, arithmetic means and 95% confidence intervals were calculated for the total mass for each wipe type and solvent condition. Pairwise comparisons were also conducted to test for significant differences between each pair of conditions within an iteration. Tukey's multiple comparisons procedure was performed to adjust the p-values of the pairwise comparisons so that a familywise error rate of 0.05 was maintained per each ANOVA model over the 15 comparisons performed within an iteration.

Potential outliers were determined by calculating the deleted studentized residuals. If the absolute value of the standardized residual was greater than 3, then the observation was considered a potential outlier. If potential outliers were found, the results were checked to determine the validity of the outlying data and probable causes for the outliers. If no probable cause was found, the outlier was included in the subsequent analysis. Table B 2 presents a potential outlier identified from deleted studentized residuals. Probable cause was not identified for any of the listed outliers. Thus, all replicates were included in the final analysis.

***Table B 2. Potential Outliers***

| Iteration | Wipe | Solvent | Replicate | Total Mass (µg) | Deleted Studentized Residual |
|---|---|---|---|---|---|
| 1 | CB | MeOH | Test Sample 10 | 6.0 | 3.9 |

Figure B 1, Figure B 2, and Figure B 3 display the total EA 6162 mass recoveries for the five replicates in each wipe type and wetting solvent condition for the three iterations. Statistical summaries including arithmetic means and 95% confidence intervals are presented in Table B 3, Table B 4, and Table B 5. These tables also display the results of the Tukey's pairwise comparisons between wipe type and solvent conditions that were statistically significant. Of the 45 pairwise comparisons between wipe type and wipe-wetting solvent conditions, 22 were statistically significant. Table B 6, Table B 7, and Table B 8 display the full set of p-values for all pairwise comparisons within each iteration.

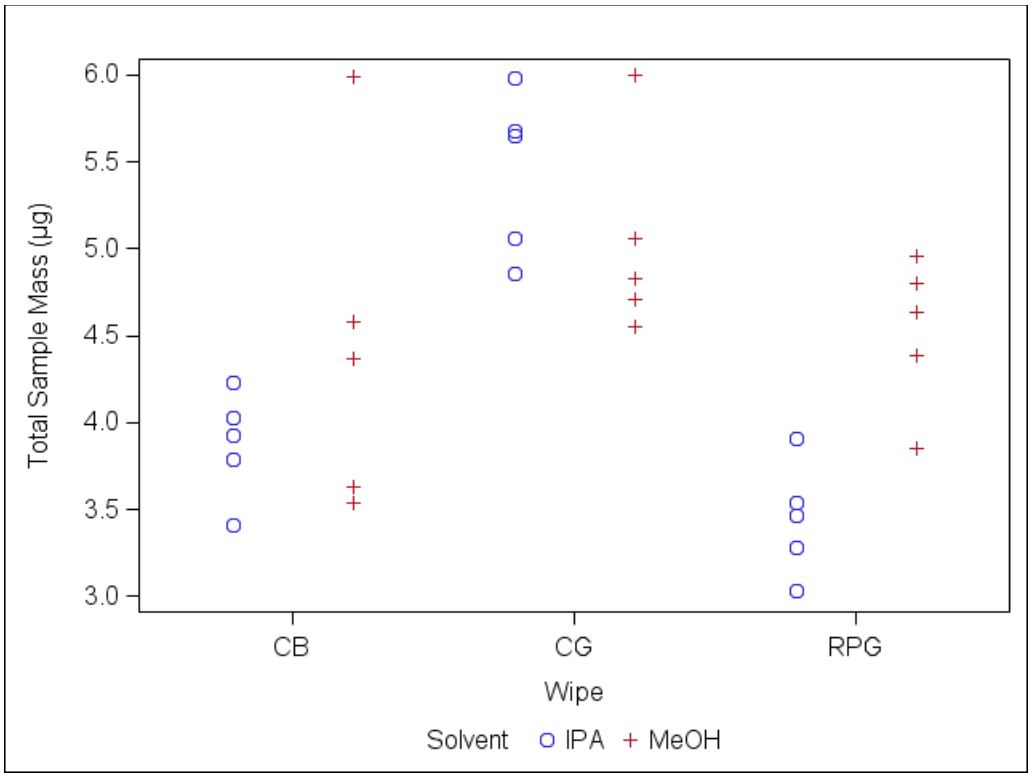



***Figure B 1. Total EA 6162 Recovery Mass – Iteration 1***

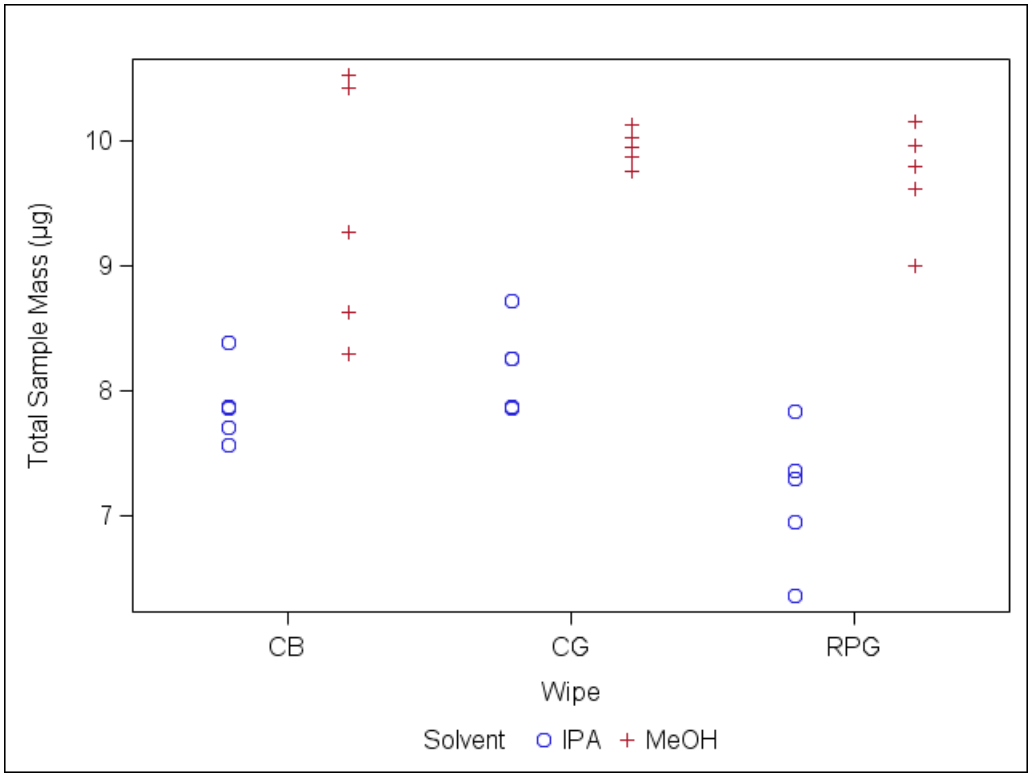



***Figure B 2. Total EA 6162 Recovery Mass – Iteration 2***

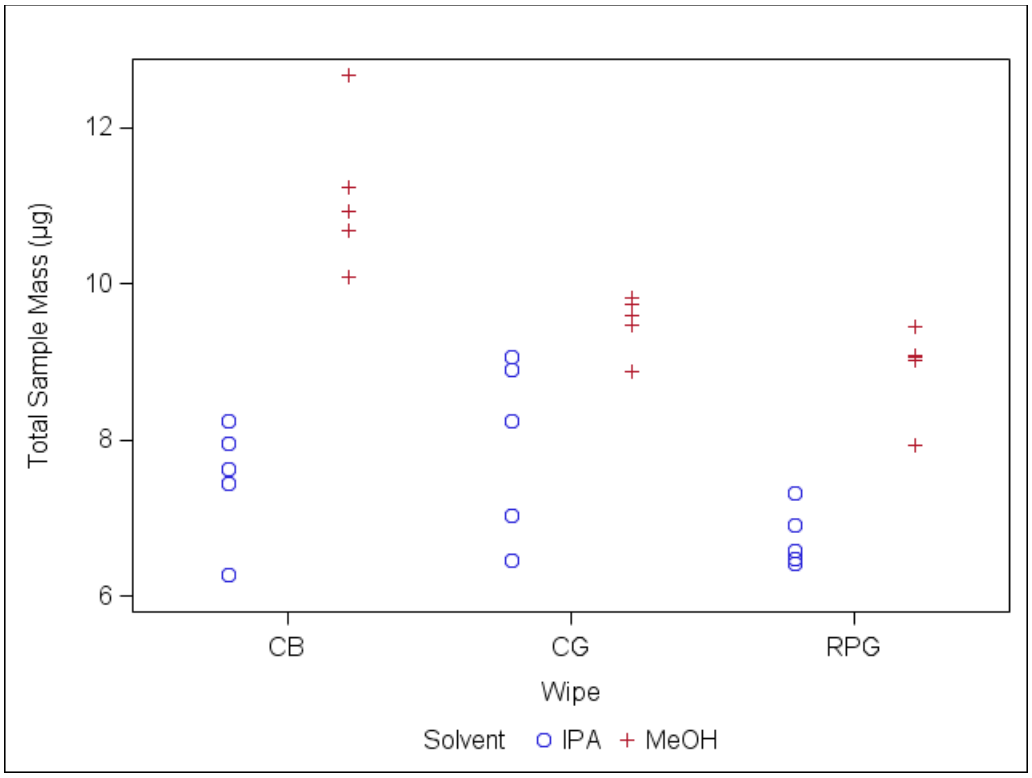



***Figure B 3. Total EA 6162 Recovery Mass – Iteration 3***

The capital letters in the "Similarity Designation" column of Table B 3, Table B 4, and Table B 5 indicate the statistical similarity of the mean total mass of a given condition to that of all other conditions tested. All rows with the same similarity designation value are not statistically significantly different from each other, while rows that did not share any similarity designation values are significantly different. For example, in Table B 3, the RPG wipe with the IPA solvent has similarity designation A. This indicates that it is similar to other conditions with the A designation, including the CB wipe with the IPA solvent and the CB wipe with the MeOH solvent, but it is different from conditions without this designation, such as the CG wipe with the MeOH solvent.

For Iteration 1, the highest mean total masses recovered were observed for the CG wipe type when it was used with the IPA wipe-wetting solvent or with the MeOH wipe-wetting solvent. The CG wipe type used with either of the wetting solvents significantly differed from both the RPG and CB wipe types when they were used with the IPA wetting solvent. No other conditions differed significantly.

For Iteration 2, the mean total mass recovery for the MeOH wetting solvent was significantly higher than the mean total mass recovery of the IPA solvent for all wipe types. There were no significant differences detected between conditions with different wipe types when the wetting solvents were the same.

For Iteration 3, the CB wipe with the MeOH solvent had the highest mean total mass recovery and was statistically significantly different from all five other conditions. The CG wipe with the MeOH wetting solvent had the second highest mean total mass recovery and was significantly

different from all wipe types with the IPA wetting solvent. The RPG wipe with the MeOH wetting solvent had the third highest mean total mass recovery and was significantly different from the RPG with the IPA wetting solvent, which had the lowest mean total mass recovery.

***Table B 3. ANOVA Results for Iteration 1***

| Wipe | Solvent | Similarity Designation | Mean Total Mass Recovery (µg) | Lower 95% Confidence Bound | Upper 95% Confidence Bound | Significant Difference |
|---|---|---|---|---|---|---|
| RPG | IPA | A | 3.4 | 2.9 | 4.0 | RPG-IPA < CG-MeOH<br>CB-IPA < CG-MeOH<br><br>RPG-IPA < CG-IPA<br>CB-IPA < CG-IPA |
| CB | IPA | A | 3.9 | 3.4 | 4.4 | |
| CB | MeOH | AB | 4.4 | 3.9 | 4.9 | |
| RPG | MeOH | AB | 4.5 | 4.0 | 5.0 | |
| CG | MeOH | B | 5.0 | 4.5 | 5.6 | |
| CG | IPA | B | 5.4 | 4.9 | 6.0 | |

***Table B 4. ANOVA Results for Iteration 2***

| Wipe | Solvent | Similarity Designation | Mean Total Mass Recovery (µg) | Lower 95% Confidence Bound | Upper 95% Confidence Bound | Significant Difference |
|---|---|---|---|---|---|---|
| RPG | IPA | A | 7.2 | 6.7 | 7.7 | RPG-IPA < CB-MeOH<br>CB-IPA < CB-MeOH<br>CG-IPA < CB-MeOH<br><br>RPG-IPA < RPG-MeOH<br>CB-IPA < RPG-MeOH<br>CG-IPA < RPG-MeOH<br><br>RPG-IPA < CG-MeOH<br>CB-IPA < CG-MeOH<br>CG-IPA < CG-MeOH |
| CB | IPA | A | 7.9 | 7.4 | 8.4 | |
| CG | IPA | A | 8.2 | 7.7 | 8.7 | |
| CB | MeOH | B | 9.4 | 8.9 | 9.9 | |
| RPG | MeOH | B | 9.7 | 9.2 | 10 | |
| CG | MeOH | B | 9.9 | 9.4 | 10 | |

***Table B 5. ANOVA Results for Iteration 3***

| Wipe | Solvent | Similarity Designation | Mean Total Mass Recovery (µg) | Lower 95% Confidence Bound | Upper 95% Confidence Bound | Significant Difference |
|---|---|---|---|---|---|---|
| RPG | IPA | A | 6.7 | 6.0 | 7.4 | RPG-IPA < RPG-MeOH<br><br>RPG-IPA < CG-MeOH<br>CB-IPA < CG-MeOH<br>CG-IPA < CG-MeOH<br><br>RPG-IPA < CB-MeOH<br>CB-IPA < CB-MeOH<br>CG-IPA < CB-MeOH<br>RPG-MeOH < CB-MeOH<br>0CG-MeOH < CB-MeOH |
| CB | IPA | A | 7.5 | 6.8 | 8.2 | |
| CG | IPA | A | 7.9 | 7.2 | 8.6 | |
| RPG | MeOH | B | 8.9 | 8.2 | 9.6 | |
| CG | MeOH | B | 9.5 | 8.8 | 10 | |
| CB | MeOH | C | 11 | 10 | 12 | |

***Table B 6. Pairwise Comparison Tukey Adjusted p-Values for Iteration 1***

| Wipe | Solvent | CB | CG | | RPG | |
|---|---|---|---|---|---|---|
| | | MeOH | IPA | MeOH | IPA | MeOH |
| CB | IPA | 0.6492 | 0.0023 | 0.0359 | 0.8302 | 0.4662 |
| CB | MeOH | | 0.0788 | 0.5367 | 0.1046 | 0.9996 |
| CG | IPA | | | 0.8498 | 0.0001 | 0.1423 |
| CG | MeOH | | | | 0.0021 | 0.7192 |
| RPG | IPA | | | | | 0.0564 |

***Table B 7. Pairwise Comparison Tukey Adjusted p-Values for Iteration 2***

| Wipe | Solvent | CB | CG | | RPG | |
|---|---|---|---|---|---|---|
| | | MeOH | IPA | MeOH | IPA | MeOH |
| CB | IPA | 0.0018 | 0.9398 | <0.0001 | 0.3167 | 0.0002 |
| CB | MeOH | | 0.0157 | 0.6680 | <0.0001 | 0.9653 |
| CG | IPA | | | 0.0004 | 0.0584 | 0.0023 |
| CG | MeOH | | | | <0.0001 | 0.9799 |
| RPG | IPA | | | | | <0.0001 |

***Table B 8. Pairwise Comparison Tukey Adjusted p-Values for Iteration 3***

| Wipe | | CB | CG | | RPG | |
|---|---|---|---|---|---|---|
| | | MeOH | IPA | MeOH | IPA | MeOH |
| CB | IPA | <0.0001 | 0.9411 | 0.0040 | 0.6067 | 0.0687 |
| CB | MeOH | | <0.0001 | 0.0258 | <0.0001 | 0.0013 |
| CG | IPA | | | 0.0335 | 0.1620 | 0.3525 |
| CG | MeOH | | | | <0.0001 | 0.8138 |
| RPG | IPA | | | | | 0.0017 |

# APPENDIX C

## Part 1B and 1C ANOVA

The objective of the following analysis of Part 1B and 1C testing was to compare total EA 6162 mass recovered from each of three different surface materials: galvanized steel, vinyl tile, and ceramic tile using two different wipe types: cotton gauze sponges (CG) and rayon/polyester gauze sponges/RPG, with each of two different wipe wetting solvents: isopropyl alcohol (IPA) and methanol (MeOH). Each of these twelve material, wipe, and solvent combinations was tested using the same approach (iteration number 3 from Part 1A), where 10 µg of EA 6162 in a water solution was spiked onto the test material surface with a 30-minute wait time before wiping. Two wipes were collected from each surface in series and their collection masses were summed to produce one total mass for each replicate sample. Five replicates were tested for each combination. Table C 1 summarizes the study design. Note that galvanized steel data from Part 1A, Iteration 3 was included as part of this study design.

***Table C 1. Study Design for Testing Wipe Type and Solvent for Three Materials***

| Testing Part | Material | Wipe | Solvent | Number of Replicates |
|---|---|---|---|---|
| Part 1A | Galvanized Steel | CG | IPA | 5 |
| Part 1A | Galvanized Steel | CG | MeOH | 5 |
| Part 1A | Galvanized Steel | RPG | IPA | 5 |
| Part 1A | Galvanized Steel | RPG | MeOH | 5 |
| Part 1B | Vinyl Tile | CG | IPA | 5 |
| Part 1B | Vinyl Tile | CG | MeOH | 5 |
| Part 1B | Vinyl Tile | RPG | IPA | 5 |
| Part 1B | Vinyl Tile | RPG | MeOH | 5 |
| Part 1C | Ceramic Tile | CG | IPA | 5 |
| Part 1C | Ceramic Tile | CG | MeOH | 5 |
| Part 1C | Ceramic Tile | RPG | IPA | 5 |
| Part 1C | Ceramic Tile | RPG | MeOH | 5 |

A fixed effects ANOVA model was fitted to the total mass recovery data over all materials, wipes, and solvents. The models contained an effect for the combination of material, wipe type and wipe wetting solvent tested and a residual error term. Data were found to adequately meet assumptions of normality and equal variance when no transformation was applied, and thus data were left untransformed for analysis. No random effect of trial was fitted due to only one trial being run for each material/wipe/solvent combination.

The models were fitted using SAS (version 9.4, 64-bit). The form of the model is presented in Equation 1C below.

$$Mass\ (\mu g) = \beta_0 + \beta_{ijk} + \varepsilon_{ijkl} \qquad \text{Equation 1C}$$

where:

- $\beta_0$ = intercept or overall mean total mass collected

- $\beta_{ij}$ = the fixed effect for the $i^{th}$ material, $j^{th}$ wipe type, and the $k^{th}$ solvent
- $\varepsilon_{ijk}$ = random error for the $l^{th}$ replicate from the $k^{th}$ solvent, $j^{th}$ wipe type, and $i^{th}$ material. The random error is assumed to be N(0, $\sigma_{\epsilon}^2$).

Using the model fitted to all total mass recovery data, arithmetic means and 95% confidence intervals were calculated for the total mass recovered for each material, wipe type, and solvent combination. Pairwise comparisons were conducted to test for significant differences between pairs of combinations. There were 66 possible pairwise comparisons between the 12 material/wipe/solvent combinations, but not all such comparisons were of interest. Instead, the pairwise comparisons performed were restricted to include:

1. The 18 comparisons between different wipe/solvent combinations tested on the same material (e.g., RPG with MeOH on vinyl tile *vs* CG with IPA on vinyl tile).
2. The 12 comparisons between different materials tested using the same wipe/solvent combination (e.g., RPG with MeOH on vinyl tile *vs* RPG with MeOH on ceramic tile).

This set of data amounted to 30 total comparisons. The Bonferroni-Holm multiple comparisons procedure was performed to adjust the p-values of the pairwise comparisons so that a familywise error rate of 0.05 was maintained over all 30 comparisons of interest. The Bonferroni-Holm procedure was selected over the Tukey procedure applied in Part 1A because the Bonferroni-Holm procedure may be more powerful in detecting true differences when performing a restricted number of pairwise comparisons, as compared to the Tukey procedure.

Potential outliers were determined by calculating the deleted studentized residuals. If the absolute value of the standardized residual was greater than 3, then the observation was considered a potential outlier. If potential outliers were found, the results were checked to determine the validity of the outlying data and probable causes for the outliers. If no probable cause was found, the outlier was included in the subsequent analysis. No outliers were identified among the replicates from deleted studentized residuals. Thus, all replicates were included in the final analysis.

Figure C 1 displays the total mass recoveries for the five replicates in each material, wipe type, and wetting solvent combination. Statistical summaries including arithmetic means and 95% confidence intervals are presented in Table C 2, and are sorted in order of estimated mean total mass recovery. Confidence bounds were not adjusted for multiple comparisons between material/wipe/solvent combinations, and thus should not be used to indicate significant differences between test conditions. Table C 3 through Table C 5 display the results of the Bonferroni-Holm-adjusted pairwise comparisons between wipe/solvent combinations tested on the same material, and Table C 6 through Table C 9 display the results of the comparisons between each material tested using the same wipe/solvent combination. Of the 30 pairwise comparisons between material/wipe type/wipe wetting solvent combinations, 15 were statistically significant. These significant differences are highlighted in red text in Table C 3 through Table C 9.

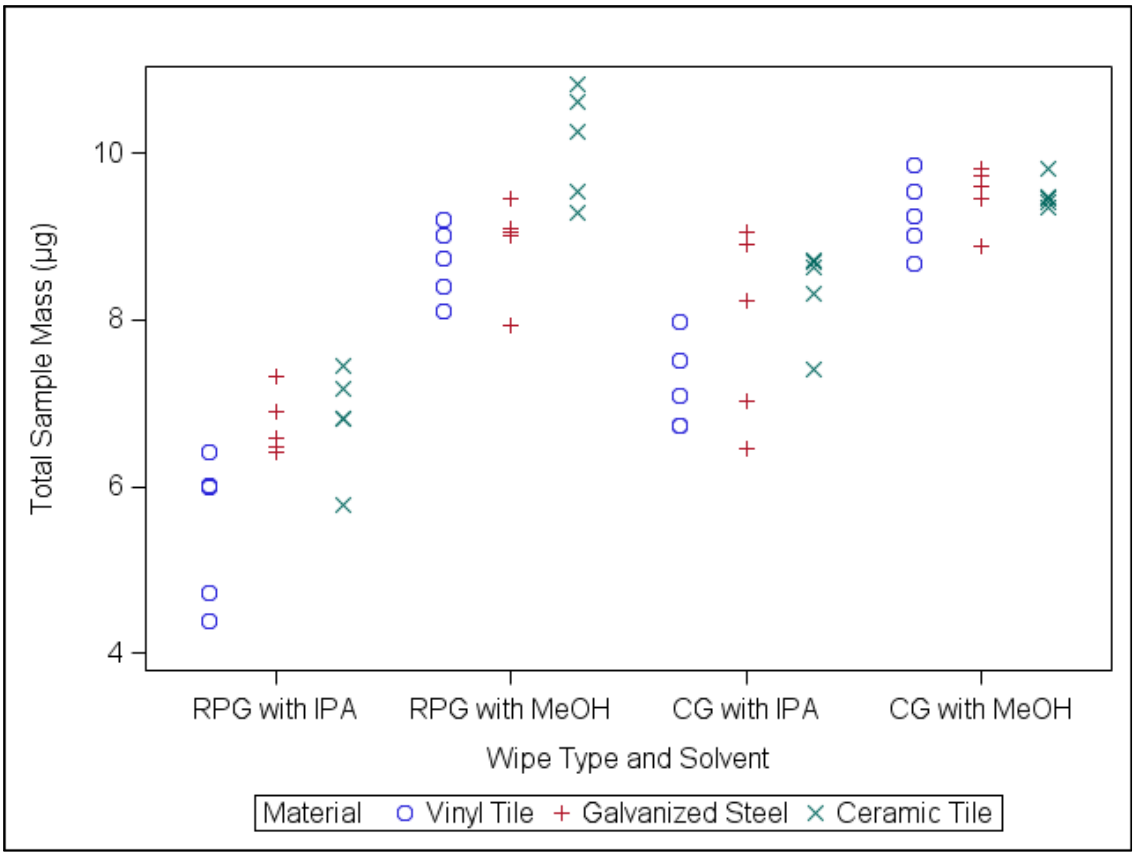



***Figure C 1. Total FGA Recovery Mass of all Material, Wipe, and Solvent Combinations***

***Table C 2. Sorted Arithmetic Means and Unadjusted 95% Confidence Intervals***

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Lower 95% Confidence Bound | Upper 95% Confidence Bound |
|---|---|---|---|---|---|
| Vinyl Tile | RPG | IPA | 5.5 | 4.9 | 6.1 |
| Galv LC Steel | RPG | IPA | 6.7 | 6.2 | 7.3 |
| Ceramic Tile | RPG | IPA | 6.8 | 6.2 | 7.4 |
| Vinyl Tile | CG | IPA | 7.2 | 6.6 | 7.8 |
| Galv LC Steel | CG | IPA | 7.9 | 7.4 | 8.5 |
| Ceramic Tile | CG | IPA | 8.4 | 7.8 | 8.9 |
| Vinyl Tile | RPG | MeOH | 8.7 | 8.1 | 9.2 |
| Galv LC Steel | RPG | MeOH | 8.9 | 8.4 | 9.5 |
| Vinyl Tile | CG | MeOH | 9.3 | 8.7 | 9.8 |
| Galv LC Steel | CG | MeOH | 9.5 | 8.9 | 10 |
| Ceramic Tile | CG | MeOH | 9.5 | 8.9 | 10 |
| Ceramic Tile | RPG | MeOH | 10 | 9.6 | 11 |

The capital letters in the “Similarity Designation” column of Table C 3 through Table C 9 indicate the statistical similarity of the mean total mass of a given material/wipe/solvent combination to the mean total mass of all other combinations tested. All rows with the same similarity designation value are not statistically significantly different from each other, while rows that did not share any similarity designation values are significantly different. For example, in Table C 4, the RPG wipe with the IPA solvent has similarity designation A, indicating that is it similar to other combinations with the A designation, including the CG wipe with the IPA solvent (designation AB), but it is different from combinations without an A in the designation, such as the CG wipe with the MeOH solvent (designation C).

Differences between wipe and solvent combinations tested on the same material were first evaluated. On the vinyl tile material (Table C 3), the mean total mass recoveries of both RPG and CG wipes with the MeOH solvent were significantly higher than the mean total mass recoveries of both the RPG wipe and the CG wipe with the IPA solvent but were not found to differ significantly from each other. The mean total mass recovery of the CG wipe with the IPA solvent was also significantly higher than that of the RPG wipe with the IPA solvent.

On the galvanized steel material (Table C 4), the mean total mass recoveries of the RPG and CG wipes with the MeOH solvent were again the highest of the four wipe/solvent combinations and did not differ significantly from each other. Only the CG wipe with the MeOH solvent had significantly higher mean total mass recovery than the CG wipe with the IPA solvent, but both the RPG and CG wipes with the MeOH solvent had higher mean total mass recovery than the RPG wipe with the IPA solvent. The mean total mass recovery of the CG wipe with the IPA solvent was not found to significantly differ from the mean total mass recovery of the RPG wipe with the IPA solvent. Note that the conclusions for the galvanized steel are equivalent to the conclusions presented for Part 2A, but the *p*-values differ due to inclusion of additional material/wipe/solvent combinations in the calculation for the estimated residual variance, as well as different numbers of pairwise comparisons that were adjusted for.

On the ceramic tile material (Table C 5), the mean total mass recoveries of the RPG and CG wipes with the MeOH solvent were again the highest of all four wipe/solvent combinations and again were not found to differ significantly from each other. The mean total mass recovery of the RPG wipe with the MeOH solvent was significantly higher than the mean total mass recoveries of the RPG and CG wipes with the IPA solvent. The mean total mass recovery of the CG wipe with the MeOH solvent did not significantly differ from the mean total mass recovery of the CG wipe with the IPA solvent but was significantly higher than the mean total mass recovery of the RPG wipe with the IPA solvent. The mean total mass recovery of the CG wipe with the IPA solvent was also significantly higher than the mean total mass recovery of the RPG wipe with the IPA solvent.

Differences between wipe and solvent combinations tested on the same material were also evaluated. For the RPG wipe with the IPA solvent (Table C 6), the mean total mass recoveries on the ceramic tile and the galvanized steel were the highest and were not found to differ significantly from each other. The mean mass recoveries on both the ceramic tile and

galvanized steel were found to be significantly higher than the mean total collection mass on the vinyl tile.

For the RPG wipe with the MeOH solvent (Table C 7), the mean total mass recovery on the ceramic tile was the highest of the three materials and was significantly higher than the mean total mass recovery on the vinyl tile but not significantly different from the mean total mass recovery on the galvanized steel. The mean total mass recovery on the galvanized steel was not found to differ from the mean total mass recovery on the vinyl tile.

For the CG wipe with either the IPA or the MeOH solvents (Table C 8 and Table C 9), no significant differences in mean total collection masses were found between any pairs of the three materials.

In summary, the RPG and CG wipes tested with MeOH solvent consistently had the highest mean total mass recoveries on all three materials. The RPG versus CG wipes with the MeOH solvent were not found to differ significantly from each other on any material. The mean total mass recoveries of the RPG and CG wipes with MeOH solvent were each significantly higher than the mean total mass recoveries of the RPG wipe with the IPA solvent on each of the three materials, and at least one of the wipe types with the MeOH solvent was significantly higher than the mean total mass recoveries of the CG wipe with the IPA solvent on all materials. The CG wipe with the IPA solvent had the second-lowest mean total mass recovered across the three materials and was significantly higher than the mean total mass recovery of the RPG wipe with the IPA solvent on the vinyl tile and the ceramic tile. The RPG wipe with the IPA solvent consistently had the lowest mean total mass recovered of the various wipe/solvent combinations across all three materials tested.

For all four wipe/solvent combinations, the mean total masses recovered on the galvanized steel and the ceramic tile were either not significantly different from or significantly higher than the mean total mass recovery on vinyl tile. Significant differences were not found between the galvanized steel and the ceramic tile for any wipe/solvent combination.

***Table C 3. Pairwise Multiple Comparison Adjusted p-Values for Vinyl Tile***

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | RPG-MeOH | CG-IPA | CG-MeOH | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|---|
| Vinyl Tile | RPG | IPA | 5.5 | A | <0.0001 | 0.0016 | <0.0001 | RPG-IPA < RPG-MeOH<br>CG-IPA < RPG-MeOH<br>RPG-IPA < CG-IPA<br>RPG-IPA < CG-MeOH<br>CG-IPA < CG-MeOH |
| Vinyl Tile | RPG | MeOH | 8.7 | C | | 0.0080 | 1.0000 | |
| Vinyl Tile | CG | IPA | 7.2 | B | | | 0.0001 | |
| Vinyl Tile | CG | MeOH | 9.3 | C | | | | |

***Table C 4. Pairwise Multiple Comparison Adjusted p-Values for Galvanized Steel***

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | RPG-MeOH | CG-IPA | CG-MeOH | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|---|
| Galvanized Steel | RPG | IPA | 6.7 | A | <0.0001 | 0.0533 | <0.0001 | RPG-IPA < RPG-MeOH<br>RPG-IPA < CG-MeOH<br>CG-IPA < CG-MeOH |
| Galvanized Steel | RPG | MeOH | 8.9 | BC | | 0.1826 | 1.0000 | |
| Galvanized Steel | CG | IPA | 7.9 | AB | | | 0.0047 | |
| Galvanized Steel | CG | MeOH | 9.5 | C | | | | |

***Table C 5. Pairwise Multiple Comparison Adjusted p-Values for Ceramic Tile***

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | RPG-MeOH | CG-IPA | CG-MeOH | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|---|
| Ceramic Tile | RPG | IPA | 6.8 | A | <0.0001 | 0.0050 | <0.0001 | RPG-IPA < RPG-MeOH<br>CG-IPA < RPG-MeOH<br>RPG-IPA < CG-IPA<br>RPG-IPA < CG-MeOH |
| Ceramic Tile | RPG | MeOH | 10 | C | | 0.0011 | 1.0000 | |
| Ceramic Tile | CG | IPA | 8.4 | B | | | 0.0668 | |
| Ceramic Tile | CG | MeOH | 9.5 | BC | | | | |

*Table C 6. Pairwise Multiple Comparison Adjusted p-Values for RPG Wipe with IPA Solvent*

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | Galvanized Steel | Ceramic Tile | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|
| Vinyl Tile | RPG | IPA | 5.5 | A | 0.0449 | 0.0293 | Vinyl Tile < Galvanized Steel<br>Vinyl Tile < Ceramic Tile |
| Galvanized Steel | RPG | IPA | 6.7 | B | | 1.0000 | |
| Ceramic Tile | RPG | IPA | 6.8 | B | | | |

*Table C 7. Pairwise Multiple Comparison Adjusted p-Values for RPG Wipe with MeOH Solvent*

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | Galvanized Steel | Ceramic Tile | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|
| Vinyl Tile | RPG | MeOH | 8.7 | A | 1.0000 | 0.0128 | Vinyl Tile < Ceramic Tile |
| Galvanized Steel | RPG | MeOH | 8.9 | AB | | 0.0533 | |
| Ceramic Tile | RPG | MeOH | 10 | B | | | |

*Table C 8. Pairwise Multiple Comparison Adjusted p-Values for CG Wipe with IPA Solvent*

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | Galvanized Steel | Ceramic Tile | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|
| Vinyl Tile | CG | IPA | 7.2 | A | 0.6889 | 0.0668 | No significant differences |
| Galvanized Steel | CG | IPA | 7.9 | A | | 1.0000 | |
| Ceramic Tile | CG | IPA | 8.4 | A | | | |

*Table C 9. Pairwise Multiple Comparison Adjusted p-Values for CG Wipe with MeOH Solvent*

| Material | Wipe | Solvent | Mean Total Mass Recovery (µg) | Similarity Designation | Galvanized Steel | Ceramic Tile | Summary of Significant Bonferroni-Holm Differences |
|---|---|---|---|---|---|---|---|
| Vinyl Tile | CG | MeOH | 9.3 | A | 1.0000 | 1.0000 | No significant differences |
| Galvanized Steel | CG | MeOH | 9.5 | A | | 1.0000 | |
| Ceramic Tile | CG | MeOH | 9.5 | A | | | |

# APPENDIX D

## Part 2 ANOVA

The objective of this statistical analysis was to compare recovered EA 6162 mass from various materials with using two different strippable coatings. Testing involved four trials applying EA 6162 to five materials (wood, plywood, ceramic tile, vinyl tile, and stainless steel used as a control), covering the material with one of two strippable coatings (latex body paint or DETEX), extracting the coating with acetone, and analyzing the extract for recovered EA 6162. Four separate trials were performed:

1. Latex body paint with wood dimensional lumber (wood) and vinyl tile;
2. Latex body paint with ceramic tile and plywood;
3. DETEX with wood dimensional lumber and vinyl tile; and
4. DETEX with ceramic tile and plywood

For each of the strippable coatings, there were five replicates of each material; additionally, each trial had three replicates of the stainless-steel controls. Two separate trials with five replicates each were also run to measure EA 6162 recovery from the strippable coating using the stainless-steel control.

To determineif the mean EA 6162 recovery was significantly different among the coatings and materials, a mixed effect Analysis of Variance (ANOVA) model with fixed effects for coating and material and a random effect for trial was fit with the form below:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_k + \epsilon_{ijkl}$$

where:

- $y$ is the observed log mass recovery for the $l^{th}$ replicate of the $j^{th}$ coating and material $j$ in trial $k$;
- $\mu$ is an overall constant;
- $\alpha_i$ is the effect of coating $j$ ($j$ = latex body paint or DETEX);
- $_j$ is the effect of material $j$ ($j$ = wood, ceramic tile, vinyl tile, plywood, stainless steel controls);
- $\gamma$ is the interaction effect between coating and material for the level combination $j, j$;
- $\epsilon_j$ is the random effect for the $k^{th}$ trial ($k$ = 1 to 4), where the trials are assumed to be independent of each other and of the residual error $N(0, \sigma\sigma^2_{\text{trial}})$; and
- $\epsilon$ is the residual error unexplained by the model; residual errors are assumed to be independent $N(0, \sigma^2)$

For the analysis, the recovered EA 6162 mass data was natural log-transformed to better satisfy the model assumptions of normality and equal variance. Estimated marginal geometric means, with unbalanced replicate numbers accounted for, were calculated and the Tukey-Kramer multiple comparisons procedure was performed to determine if the mean recovered EA 6162

mass differed significantly between each pair of materials within each strippable coating and also between the two coatings for each material. The Tukey-Kramer procedure was used to maintain a family-wise probability of falsely declaring significant differences of 0.05 across the multiple pairwise comparisons performed. The null hypotheses that all sets of material means were equal within a given coating type and that all sets of coating means were equal within a given material were rejected if the adjusted p-values were less than or equal to $0.05$.

Separately, a fixed effects model was fit to compare the stainless-steel controls and recovery efficacy results across trials and within coating types. This model contained fixed effects of coating, trial, and recovery efficacy vs stainless steel control status. Estimated marginal means were calculated, and the Tukey-Kramer procedure was again performed to compare trial and coating conditions.

Potential outliers were identified using deleted studentized residuals, which are the standardized residuals from the model fitted to all data except the current observation. If the absolute value of the deleted studentized residual was greater than three, the observation was considered a potential outlier. No observations were identified as potential outliers.

Table D 1 shows the geometric mean recovered EA 6162 mass for each material within each coating as well as any significant differences between the strippable coatings. In Table D 2, the Tukey-Kramer-adjusted p-value is given for all pairwise comparisons among the materials for each strippable coating; significant differences are highlighted in red.

For both strippable coatings, the geometric mean recovered mass from plywood and wood dimensional lumber was significantly lower than the geometric mean recovered mass for all the other materials, including stainless steel controls. For the DETEX coating, the geometric mean recovered mass from plywood is also significantly lower than the geometric mean recovered mass for wood dimensional lumber for all materials, although there is no significant difference between wood dimensional lumber and plywood for the latex body paint. There were no other significant differences between the test materials and stainless-steel controls for each strippable coating.

***Table D 1. ANOVA Results for Material within Strippable Coating***

| Coating | Material | Geometric Mean EA 6162 Recovery (µg) | Significant Differences |
|---|---|---|---|
| DETEX | Ceramic Tile | 9.3 | Plywood < Wood < Ceramic Tile = Vinyl Tile = Stainless Steel (Trial 3) = Stainless Steel (Trial 4) |
| | Plywood | 2.2 | |
| | Stainless Steel (Trial 3) | 9.4 | |
| | Stainless Steel (Trial 4) | 9.4 | |
| | Vinyl Tile | 9.2 | |
| | Wood | 4.3 | |
| Latex Body Paint | Ceramic Tile | 8.2 | Plywood = Wood < Ceramic Tile = Vinyl Tile = Stainless Steel (Trial 1) = Stainless Steel (Trial 2) |
| | Plywood | 1.9 | |
| | Stainless Steel (Trial 1) | 7.3 | |
| | Stainless Steel (Trial 2) | 6.5 | |
| | Vinyl Tile | 6.4 | |
| | Wood | 2.4 | |

***Table D 2. Pairwise Comparisons for Material within Coating***

| Coating | Material | Tukey-Kramer Adjusted p-Value | | | | |
|---|---|---|---|---|---|---|
| | | Plywood | Stainless Steel (Trial 3) | Stainless Steel (Trial 4) | Vinyl Tile | Wood |
| DETEX | Ceramic Tile | <0.0001 | 1.0000 | 1.0000 | 1.0000 | 0.0066 |
| | Plywood | | <0.0001 | <0.0001 | <0.0001 | 0.0219 |
| | Stainless Steel (Trial 3) | | | | 1.0000 | 0.0063 |
| | Stainless Steel (Trial 4) | | | | 1.0000 | 0.0177 |
| | Vinyl Tile | | | | | 0.0018 |
| **Coating** | **Material** | **Plywood** | **Stainless Steel (Trial 1)** | **Stainless Steel (Trial 2)** | **Vinyl Tile** | **Wood** |
| Latex Body Paint | Ceramic Tile | <0.0001 | 0.9964 | 0.8633 | 0.8423 | <0.0001 |
| | Plywood | | <0.0001 | <0.0001 | <0.0001 | 0.8259 |
| | Stainless Steel (Trial 1) | | | | 0.9854 | <0.0001 |
| | Stainless Steel (Trial 2) | | | | 1.0000 | 0.0011 |
| | Vinyl Tile | | | | | <0.0001 |

Table D 3 shows the geometric mean EA 6162 mass recovered from each coating type within each material as well as the associated Tukey-Kramer adjusted p-value; significant differences are highlighted in red. Wood was the only material with a significant difference between coatings, where the DETEX had higher geometric mean EA 6162 mass recovered than the latex body paint.

***Table D 3. ANOVA Results for Coating Type within Material***

| Material | Coating | Geometric Mean EA 6162 Recovery (µg) | Tukey-Kramer Adjusted Paint p-Value |
|---|---|---|---|
| Ceramic Tile | DETEX | 9.3 | 0.5570 |
| | Latex Body Paint | 8.2 | |
| Plywood | DETEX | 2.2 | 0.4657 |
| | Latex Body Paint | 1.9 | |
| Vinyl Tile | DETEX | 9.2 | 0.0900 |
| | Latex Body Paint | 6.4 | |
| Wood | DETEX | 4.3 | 0.0076 |
| | Latex Body Paint | 2.4 | |

Table D 4 shows the geometric mean EA 6162 mass recovered from the stainless steel for the extraction efficiency testing compared to stainless-steel controls for each trial within each coating type along with the associated Tukey-Kramer adjusted p-values. There were no significant differences among trials within a coating type, indicating that the stainless-steel controls for each trial performed the same as for the extraction efficiency study.

***Table D 4. ANOVA Results with Pairwise Comparisons for Stainless Steel Within Coating***

| Coating | Material | Geometric Mean EA 6162 Recovery (µg) | Tukey-Kramer Adjusted p-Value | |
|---|---|---|---|---|
| | | | Stainless Steel (Trial 3) | Stainless Steel (Trial 4) |
| DETEX | Stainless Steel (Extraction Efficiency) | 9.2 | 0.9831 | 0.9836 |
| | Stainless Steel (Trial 3) | 9.4 | | 1.0000 |
| | Stainless Steel (Trial 4) | 9.4 | | |
| **Coating** | **Material** | **Geometric Mean EA 6162 Recovery (µg)** | **Stainless Steel (Trial 1)** | **Stainless Steel (Trial 2)** |
| Latex Body Paint | Stainless Steel (Extraction Efficiency) | 7.2 | 0.9897 | 0.6532 |
| | Stainless Steel (Trial 1) | 7.3 | | 0.6359 |
| | Stainless Steel (Trial 2) | 6.5 | | |

Table D 5 shows the geometric mean EA 6162 mass recovered across each coating within the stainless-steel extraction efficiency and stainless-steel control for each trial along with the associated Tukey-Kramer adjusted p-value; significant differences are highlighted in red. There was a significant difference between the two coatings for the extraction efficiency and for the stainless-steel controls from Trials 2 and 4. The results suggest that EA 6162 can be extracted more efficiently from DETEX than from latex body paint using acetone. Note that the Tukey-Kramer adjusted p-value for the comparison of stainless-steel controls from Trials 1 and 3 is only slightly above the probability of 0.05, below which a significant difference between the two coating types would be indicated.

***Table D 5. ANOVA Results for Coating Within Stainless Steel Trials***

| Material | Coating | Geometric Mean EA 6162 Recovery (µg) | Tukey-Kramer Adjusted p-Value |
|---|---|---|---|
| Stainless Steel (Extraction Efficiency) | DETEX | 9.2 | 0.0252 |
| | Latex Body Paint | 7.2 | |
| Stainless Steel (Trial 1/3) | DETEX | 9.4 | 0.0692 |
| | Latex Body Paint | 7.3 | |
| Stainless Steel (Trial 2/4) | DETEX | 9.4 | 0.0112 |
| | Latex Body Paint | 6.5 | |

# APPENDIX E

## Part 4 ANOVA

ANOVA models were fitted to the untransformed and natural log-transformed Part 4 data with effects for soil type, dwell time, and the interaction between soil type and dwell time to assess the model assumptions of normality and equal variance. If the assumptions were more reasonable (based on the standardized residuals from the ANOVA models) for the log-transformed data than for the untransformed data, then the log-transformed data were used for the analysis.

To determine if the mean mass recoveries were significantly different among the soil types and dwell times, a two-way ANOVA model was fit with the form shown in Equation E1:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk} \qquad \text{Equation E1}$$

where:

- $y_{ijk}$ = the observed mass recovered for the $k^{th}$ replicate of the $i^{th}$ soil type ($i$ = sand, clean loam, Georgia, Nebraska) at time $j$ ($j$ = 60 minutes, 1 day, 7 days, 14 days);
- $\mu$ = an overall constant;
- $\alpha_i$ = the effect of soil type at level $i$;
- $\beta_j$ = the effect of dwell time at time $j$;
- $\gamma_{ij}$ = the interaction effect between soil type and dwell time for the level combination $i, j$; and
- $\epsilon_{ijk}$ = random error for the $k^{th}$ replicate from the $j^{th}$ time point and the $i^{th}$ soil type. The random error is assumed to be N(0, $\sigma_\epsilon^2$).

Estimated marginal geometric means, so that unbalanced replicate numbers have been accounted for, were calculated, and Tukey-Kramer's multiple comparisons procedure was performed to determine if the geometric mean mass recovery differed significantly between each pair of soil types at each dwell time and also between each pair of dwell times for each soil type. The Tukey-Kramer procedure extends the Tukey procedure in the case of unequal sample sizes, which is the case for the three replicates used for the sand and the five replicates used for all other soil types. The null hypotheses, that each pair of soil type geometric means is equal and that all sets of dwell time geometric means are equal, were rejected if the Tukey-Kramer adjusted p-values were $\leq 0.05$.

The model assumptions of normality and equal variance were more reasonable for the log-transformed data than the untransformed data, so the log-transformed data were used for the analysis.

Comparison of Soil Type Within Dwell Time

Table E 1 shows the geometric mean mass recovery for each soil type at each of the four dwell times as well as any significant differences between the soils. Also, in Table E 1, the Tukey-Kramer adjusted p-value is given for all pairwise comparisons between the soils at each time; significant differences are shown in red.

At 60 minutes, the geometric mean mass recovery of the Nebraska soil is significantly lower than the geometric mean mass recovery of both the sand and the Georgia soil. At 1 day, the mass recovery of the Nebraska soil is significantly lower than the mass recovery of the sand. At 7 and 14 days, all six pairs of soil mass recoveries are significantly different: the Georgia and Nebraska soils are lower than the sand; the Georgia and Nebraska soils are lower than the clean loam; and the Georgia soil is lower than the Nebraska soil.

Comparison of Dwell Times Within Soil Type

Table E 2 shows the geometric mean mass recovery for each dwell time for each soil type as well as any significant differences between the dwell times. Also, in Table E 2, the Tukey-Kramer adjusted p-value is given for all pairwise comparisons between the dwell times for each soil; significant differences are shown in red.

For the sand, there is no significant difference in geometric mean mass recovery among any of the dwell times. For the clean loam, Georgia, and Nebraska soils, the mean mass recovery at 14 days is significantly lower than the mean mass recovery at 60 minutes, 1 day, and 7 days; and the mean mass recovery at 7 days is significantly lower than the mean mass recovery at 1 day and 60 minutes. Additionally for the Georgia soil, the mean mass recovery at 1 day is significantly lower than the mean mass recovery at 60 minutes.

***Table E 1. ANOVA Results for Soil Type within Dwell Time and Pairwise Comparison p-Values***

| Dwell Time | Soil Type | Geometric Mean Mass Recovery (ng) | Tukey-Kramer Adjusted p-Value | | | Significant Differences |
|---|---|---|---|---|---|---|
| | | | Clean Loam | Georgia Soil | Nebraska Soil | |
| 60 Minute | Sand | 43.6 | 0.2486 | 0.8859 | 0.0102 | Nebraska < Sand<br>Nebraska < Georgia |
| | Clean Loam | 39.5 | | 0.5478 | 0.3952 | |
| | Georgia | 42.0 | | | 0.0255 | |
| | Nebraska | 36.8 | | | | |
| 1 Day | Sand | 40.0 | 0.6603 | 0.2023 | 0.0081 | Nebraska < Sand |
| | Clean Loam | 37.7 | | 0.7641 | 0.0680 | |
| | Georgia | 36.1 | | | 0.4194 | |
| | Nebraska | 33.7 | | | | |
| 7 Day | Sand | 43.7 | <0.0001 | <0.0001 | <0.0001 | Georgia < Nebraska < Clean Loam < Sand |
| | Clean Loam | 33.0 | | <0.0001 | <0.0001 | |
| | Georgia | 14.4 | | | <0.0001 | |
| | Nebraska | 24.0 | | | | |
| 14 Day | Sand | 42.8 | <0.0001 | <0.0001 | <0.0001 | Georgia < Nebraska < Clean Loam < Sand |
| | Clean Loam | 18.6 | | <0.0001 | <0.0001 | |
| | Georgia | 5.20 | | | <0.0001 | |
| | Nebraska | 10.5 | | | | |

***Table E 2. ANOVA Results for Dwell Time within Soil Type and Pairwise Comparison p-Values***

| Soil Type | Dwell Time | Geometric Mean Mass Recovery (ng) | Tukey-Kramer Adjusted p-Value | | | Significant Differences |
|---|---|---|---|---|---|---|
| | | | 1 Day | 7 Days | 14 Days | |
| Sand | 60 Minute | 43.6 | 0.4476 | 0.9999 | 0.9915 | No Significant Differences |
| | 1 Day | 40.0 | | 0.4046 | 0.6256 | |
| | 7 Day | 43.7 | | | 0.9835 | |
| | 14 Day | 42.8 | | | | |
| Clean Loam | 60 Minute | 39.5 | 0.6985 | 0.0009 | <0.0001 | 14 Day < 7 Day < 60 Minute<br>14 Day < 7 Day < 1 Day |
| | 1 Day | 37.7 | | 0.0230 | <0.0001 | |
| | 7 Day | 33.0 | | | <0.0007 | |
| | 14 Day | 18.6 | | | | |
| Georgia Soil | 60 Minute | 41.95 | 0.0068 | <0.0001 | <0.0001 | 14 Day < 7 Day < 1 Day < 60 Minute |
| | 1 Day | 36.07 | | <0.0001 | <0.0001 | |
| | 7 Day | 14.4 | | | <0.0001 | |
| | 14 Day | 5.21 | | | | |
| Nebraska Soil | 60 Minute | 36.8 | 0.1929 | <0.0001 | <0.0001 | 14 Day < 7 Day < 60 Minute<br>14 Day < 7 Day < 1 Day |
| | 1 Day | 33.7 | | <0.0001 | <0.0001 | |
| | 7 Day | 24.0 | | | <0.0001 | |
| | 14 Day | 10.5 | | | | |

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