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Enzymatic Decontamination of Chemical Warfare Agent Cyclosarin (GF)

Office of Research and Development
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Enzymatic Decontamination of Chemical Warfare Agent Cyclosarin (GF)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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DISCLAIMER

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ACRONYMS

AMU	atomic mass unit
BBRC	Battelle Biomedical Research Center
°C	Degree(s) Celsius
CAS	Chemical Abstracts Service
CCV	continuing calibration verification
cm	centimeter(s)
CWA	chemical warfare agent
EPA	U.S. Environmental Protection Agency
FPD	flame photometric detector
g	gram(s)
GC	gas chromatography
GC/MS	gas chromatography/mass spectrometry
GD	soman
GF	cyclosarin
HD	sulfur mustard
IS	internal standard
kHz	kilohertz
L	liter(s)
m	meter(s)
MDL	method detection limit
min	minute(s)
mg	milligram(s)
mL	milliliter(s)
mm	millimeter(s)
µg	microgram(s)
µL	microliter(s)
µm	micrometer(s)
NIST	National Institute of Standards and Technology
PE	performance evaluation
QA	quality assurance
QC	quality control
RH	relative humidity
SD	standard deviation
SIM	selected ion monitoring
SRC	surrogate recovery compound
TBP	tributyl phosphate
TGD	thickened soman
TSA	technical systems audit

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) is the primary federal agency responsible for remediation of public areas in the aftermath of a terrorist release of a chemical warfare agent (CWA). The threat of a release in a building or transportation hub drove the EPA to evaluate the effectiveness of DEFENZ™ VX-G, an enzyme-based technology, for decontamination of G-type nerve agents and VX. In previous testing, thickened soman (TGD) was the G-type agent.[1] A thickened agent was used because of the high evaporation rate of soman (GD). However, high variability that was attributed to the inherent difficulty of precise application of small amounts of TGD (1 µL applications) onto coupons was observed with the TGD decontamination study. Here, the efficacy of DEFENZ™ VX-G against the G-type agent cyclosarin (GF) was systematically evaluated. Because of an evaporation rate lower than soman, GF (without thickener) was expected to persist on building materials sufficiently to enable decontamination efficacy testing. Application of small, precise volumes was easier to achieve without the presence of thickener.

Efficacy results, i.e., GF recovered from test coupons after decontamination with the enzyme product relative to GF recovered from positive control coupons, are summarized in Table ES-1. Application of DEFENZ™ VX-G prepared at the manufacturer's recommended concentration ("1X") reduced the amount of GF on the coupon with a 15 minute (min) contact time. A >90% efficacy was observed against GF on non-porous galvanized metal and decorative laminate. Lower efficacy (77%, 80%) was observed against GF on vinyl flooring and industrial carpet, respectively, while the observed efficacy against GF on wood flooring was the lowest (36%).

Table ES-1. Summary of Decontamination Efficacy Results for DEFENZ™ VX-G against GF

Material	Contact Time, min*	Concentration†	Mean Test Coupons Efficacy
Galvanized metal	15	1X	92%
Wood flooring	15	1X	36%
Industrial Carpet	15	1X	80%
Vinyl flooring	15	1X	77%
Decorative laminate	15	1X	94%
Wood flooring	30	1X	47%
Wood flooring	15 + 15	1X	61%
Wood flooring	15	3X	53%
Wood flooring	30	3X	44%
Wood flooring	15 + 15	3X	79%

* Manufacturer recommends 15-min contact time; 15+15 indicates that after an initial application with a 15-min contact time, the enzyme was reapplied for an additional 15-min contact time; *ibid* for 30+30.

† 1X is enzyme diluted with deionized water per manufacturer's recommendation; 3X is enzyme diluted with one-third of the recommended water.

The standard DEFENZ™ VX-G enzyme preparation (1X) was tested on wood flooring at a longer contact time (30 min). The efficacy did not increase significantly with the 30-min contact time compared to a 15-min contact time (Student's t-test p = 0.29). This result was obtained in spite of the potential for evaporative loss during the additional contact period. Reapplication of the 1X enzyme for a second 15-min period resulted in a significant increase in efficacy (p = 0.014), presumably due to the replenishment with fresh enzymes. Given the results from the single 30-min application of the enzyme, the increased efficacy with a second application of the enzyme is unlikely to be explained by increased evaporation.

Concentrations of DEFENZ™ VX-G enzymes higher than the manufacturer's recommendation ("3X") were tested with 15-min and 30-min contact times, and with repeated 15-min applications. Results are shown in Table ES-1. Increasing the concentration to 3X did not significantly increase efficacy at 15-min (p = 0.17) or 30-min (no improvement) contact times compared to decontamination for 15-min using the 1X concentration. With repeated 15-min application of the 3X enzyme, efficacy was significantly higher (p = 0.009) compared to decontamination for 15-min using the 1X concentration. However, the higher (3X) concentration with repeated 15-min application did not result in significantly greater efficacy than the repeated 15-min application using the 1X concentration (p = 0.17). In summary, reapplication of the standard enzyme preparation was demonstrated to increase efficacy. Higher enzyme concentrations and/or longer contact times did not significantly increase efficacy against GF.

A simulated enzyme reactor test was performed for GF in which a neat CWA (here, GF) is added to the enzyme solution in a vial (no coupon surface present) and sonicated for a contact time of 15 min as a simulation of the stirring process during a normal enzyme reactor test. This test simulates conditions generally used by a vendor to claim a product's efficacy against a CWA. The result of the simulated enzyme reactor test is shown in Table ES-2. DEFENZ™ VX-G exhibited higher efficacy against GF when compared to the coupon testing. Ninety-nine percent of GF was decomposed with the 15 min contact time. This more dynamic interaction is apparently important in reaching a higher efficacy against GF. Lower efficacy results obtained during coupon testing can be explained by the more static interaction of the enzyme solution with GF on the test coupon.

Table ES-2. Simulated Enzyme Reactor Results for DEFENZ™ VX-G Enzyme and GF

CWA	Enzyme Used	Blank Solution, µg	Mean Positive Control Total Mass, µg (SD)	Mean Test Total Mass, µg (SD)	Mean Efficacy
GF	DEFENZ™ VX-G	ND*	704 (66)	10 (13)	99%

*ND indicates no GF was detected.

No obvious visual damage resulted from the application of the enzyme solution. Caution should be used in extrapolating from the bench testing to field application of the enzymes. However, given the observed efficacies of the DEFENZ™ enzymes against GF and the lack of visible damage to a range of indoor building materials, the enzymes appear to be useful for decontaminating this CWA on indoor building materials after a terrorist release.

1.0 Introduction

1.1 Background

Protecting human health and the environment is the mission of the U.S. Environmental Protection Agency (EPA). The threat of a chemical warfare agent (CWA) release in a building or transportation hub is driving the EPA to develop a research program that systematically evaluates potential decontaminants for CWAs. The EPA may be tasked to clean-up these agents after a release in a public setting. Information about suitable decontamination technologies is limited and optimal decontaminant concentration and contact times have been determined primarily by vendors with limited third party verification. Effectiveness of available enzymatic decontamination technologies against CWAs on surfaces is generally unknown.

This report describes a systematic investigation to evaluate the efficacy of an enzyme-based technology produced by Genencor®, (a Danisco Division, Palo Alto, CA): DEFENZ™ VX-G (for decontamination of VX and G-type nerve agents). (In May 2011, DuPont acquired a majority stake in Danisco A/S and the Genencor® enzymes are now marketed within DuPont Industrial Biosciences.) In previous testing, the focus was on thickened soman (TGD) as the G-type agent.[1] Thickened agent was used because of the high evaporation rate of soman (GD). However, the high variability that was observed with the TGD decontamination study was attributed to the inherent difficulty of precise application of small amounts of TGD (1 microliter [µL] applications) onto coupons. Here, the efficacy of DEFENZ™ VX-G against the G-type agent cyclosarin (GF) is evaluated systematically. Because GF has a lower evaporation rate than soman, GF (without thickener) was expected to persist sufficiently on building materials to enable decontamination efficacy testing. In comparison, the vapor pressure of GF is ~9 times lower than the value for GD.

1.2 Test Facility Description

All testing was performed at the Battelle Biomedical Research Center (BBRC) site in West Jefferson, Ohio. Battelle is certified to work with chemical surety material at the BBRC through its contract with the Defense Threat Reduction Agency (Contract Number: W81XWH-05-D-0001/DO 0001).

1.3 Project Objectives

The main objective of this evaluation was to determine the decontamination efficacy of DEFENZ™ VX-G enzyme decontamination technology against GF applied to coupons made from materials consistent with items found in indoor environments. The efficacy was evaluated as a function of material type, time, repeated application, and concentration. The enzyme was initially prepared per manufacturer's directions, stored and used in accordance with the label instructions. Efficacy of the enzyme when appropriately applied against GF was evaluated on each of five different building materials (galvanized metal, decorative laminate, industrial carpet, wood flooring, and vinyl flooring) at one contact time (15 min as specified in the DEFENZ™ VX-G instructions for use). Higher concentrations of DEFENZ™ VX-G, longer contact times, and repeated applications were also evaluated. Specifically, the enzyme solution

prepared according to the manufacturer's recommendations was tested with a 30-min contact time and a 15-min contact time with a reapplication and additional 15-min contact time to decontaminate GF on wood. Wood was selected here as the building material associated with the lowest efficacy against GF in the first round of experiments. In addition, a 3 times higher concentration of recommended enzyme to water was tested with a 15-min contact time, 30-min contact time, and a 15-min contact time with a reapplication and an additional 15-min contact time to decontaminate GF on wood.

As a secondary objective, the effects of the enzyme-based decontamination technologies on the building materials were qualitatively evaluated by visual inspection, identifying changes in color, reflectivity or roughness. Such assessment would indicate whether material incompatibility was observed.

Simulated enzyme reactor testing was performed to determine the decontamination efficacy of enzyme decontamination technologies (DEFENZ™ VX-G against GF). This test simulates conditions generally used by a vendor to claim a product's efficacy against a CWA. Results of this simulation would indicate whether this more dynamic environment is important in reaching a higher efficacy against a CWA. Such test is also without potential confounds arising from application to and extraction from material coupons.

Testing was performed in accord with *Test/Quality Assurance (QA) Plan for Enzymatic Decontamination of Chemical Warfare Agents, Version 2* (July 2010) (available upon request).[2]

2.0 Procedures

2.1 Technology Descriptions

DEFENZ™ VX-G is an enzyme-based technology produced by Genencor® (a Danisco Division, Palo Alto, CA). The details of the technologies are proprietary. Instructions for creating default enzyme solutions are listed and were followed as per vendor's directions.

The DEFENZ™ VX-G product consists of a pouch containing two packets:

- Enzyme packet (110 grams [g]) of granulated powder
- Buffer packet (250 g of powder) containing predominantly sodium hydrogen carbonate ($NaHCO_3$).

The enzyme packet contains two pre-mixed constituent powders: 10 g of "organophosphorous [sic] acid anhydrolase" enzyme (DEFENZ™ 120) and 100 g of "organophosphorous [sic] hydrolase" enzyme (DEFENZ™ 130). The enzyme and buffer dissolve in 10 liters (L) of water.

2.2 Chemical Warfare Agents

The CWA used to evaluate the efficacy of decontamination in this report was GF (cyclohexyl methylphosphonofluoridate, CAS Registry Number 329-99-7), (Table 1). The target purity of the neat agent was expected to be at least 85%. Purity for each ampoule of GF was determined using gas chromatography (GC)-flame photometric detection (FPD) prior to beginning testing. Observed purity was more than the required 85%.

Table 1. Purity of Chemical Warfare Agents Used in Testing

Agent	Manufacturer/Supplier Name	Observed Neat CWA Purity
GF	US Army from EPA stocks*	99%

*EPA-owned stocks of CWAs are stored at Battelle's facilities in West Jefferson, OH.

2.3 Preparation of Enzyme-Based Decontamination Technologies

2.3.1 Preparation Procedure for DEFENZ™ VX-G

The DEFENZ™ VX-G enzyme pouch contained two types of enzymes appropriate for G-type agents (DEFENZ™ 120) and VX (DEFENZ™ 130). Because the enzymes were together in a single pouch but may not be thoroughly mixed, the following method was used to ensure homogeneity among enzyme solutions prepared using only a portion of the enzyme mixture to make less than 10 L of enzyme solution. This method enabled the same proportions as recommended by the vendor to be used to prepare batches smaller than 10 L.

The enzyme packet and buffer packet were opened and the contents were separately weighed. The weight ratio between the enzyme and the buffer (110:250) was the ratio used to create smaller quantities. Laboratory batches of the buffer (sufficient to produce 500 mL of enzyme solution) were prepared by dividing the contents of the buffer packet (nominally 250 g)

into 20 equal portions (12.5 g ± 0.1 g each) in separate, appropriately labeled, scintillation vials (03-337-14/vial; 02-912-068/cap, Fisher Scientific, Pittsburgh, PA). The vials of buffer powder were stored at ambient temperature in a desiccator until needed.

Laboratory batches of enzymes (each sufficient to produce 500 mL of enzyme solution) were prepared, as shown in Table 2 and Figure 1, to ensure product uniformity as much as practical. The enzyme packet contents (DEFENZ™ VX-G enzyme, nominally 110 g) were divided into five equal portions (22.0 g ± 0.1 g each) using an analytical balance (Model AX-205 ID # C21236, Mettler-Toledo, Toledo, OH). Each sample was retained in a weighing pan (08-732-103, Fisher Scientific, Pittsburgh, PA). Five mixed samples (22.0 g ± 0.1 g each) were then produced by transferring an equal amount (4.4 g ± 0.1 g) from each sample into each of five new weighing pans (08-732-103, Fisher Scientific, Pittsburgh, PA). Twenty batches (5.50 g ± 0.25 g each) were then produced by transferring an equal amount (1.1 g ± 0.05 g) from each mixed sample prepared in each of 20 scintillation vials (03-337-14/vial; 02-912-068/cap, Fisher Scientific, Pittsburgh, PA). Each vial, sufficient to prepare 500 mL of enzyme solution, was marked to indicate that the vial contains DEFENZ™ VX-G enzyme (5.5 g) and stored at ambient temperature in a desiccator until needed.

The manufacturer's instructions call for the contents of the enzyme packets to be mixed into 10 L of water. Enzyme solutions were prepared fresh each day of testing in accordance with manufacturer's instructions (1X), but with smaller proportionate amounts of enzyme (5.5 g) and buffer (12.5 g). Deionized water was used to prepare the solutions. The pH of the enzyme solution was measured and documented prior to each day of use using a pH meter (pH meter Model SevenMulti, Mettler Toledo, Columbus, OH). The enzyme solutions used were verified as being pH 8.3 ± 0.3.

For the 3X concentration, the full packet of enzyme would be mixed in 3.3 L of water. Actual 3X mixtures were based on this proportion applied to the amounts of enzyme in the "batch packets" as follows: add the contents intended for 500 mL to 167 mL of deionized water. The preparation of DEFENZ™ VX-G per manufacturer's recommended concentration (1X) and 3X concentration is shown in Table 2.

Table 2. Formulae for Preparing DEFENZ™ VX-G Solutions

	Enzyme (g)	Buffer (g)	Water (mL)
Manufacturer's Recommended Concentration (1x)	1 vial containing DEFENZ™ VX-G enzyme, 5.5 g ± 0.25 g	1 vial containing DEFENZ™ VX-G buffer (sodium hydrogen carbonate), 12.5 g ± 0.1 g	500
3X Preparation	1 vial containing DEFENZ™ VX-G enzyme, 5.5 g ± 0.25 g	1 vial containing DEFENZ™ VX-G buffer (sodium hydrogen carbonate), 12.5 g ± 0.1 g	167

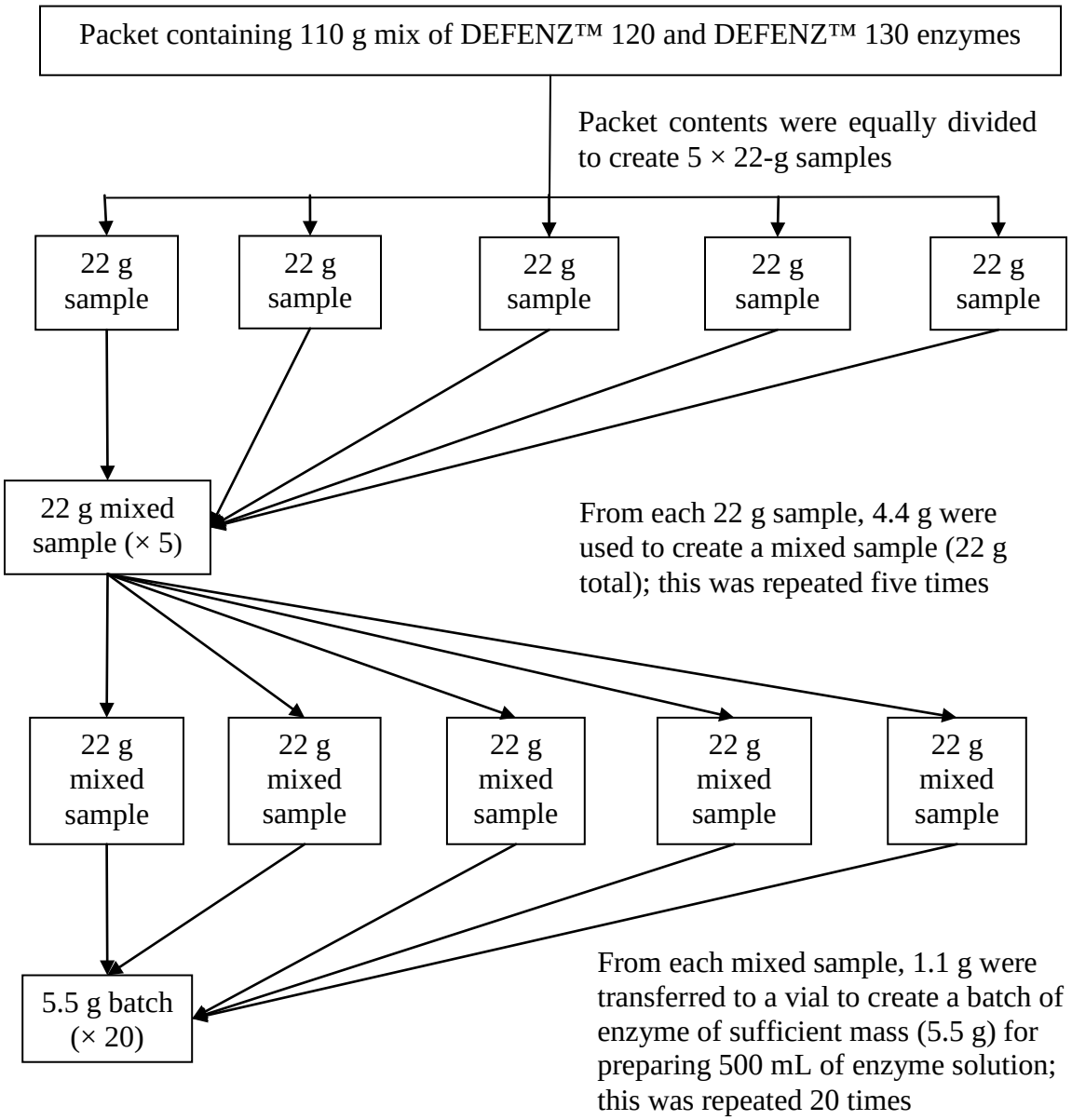

Figure 1. Approach used to ensure homogeneity of DEFENZ™ 120 and DEFENZ™ 130 enzymes in 500 mL enzyme solutions.

2.4 Building Material Coupons

This bench-scale investigation utilized small coupons of interior building materials (presented in Table 3) contaminated with GF.

Table 3. Test Materials

Material	Description	Manufacturer/ Supplier Name	Coupon Surface Size L x W (cm)	Material Preparation
Galvanized metal ductwork	Industry heating, ventilation, and air conditioning standard; 24 gauge galvanized steel; thickness 0.7 mm (Adept Manufacturing)	Adept Products, Inc., West Jefferson, OH	3.5 x 1.5	Clean with acetone
Decorative laminate	Pionite® or Formica® laminate/white matte finish; grade 10; thickness ~1.2 mm	A' Jack Inc., Columbus, OH	3.5 x 1.5	Clean with dry air to remove loose dust
Industrial grade carpet	Shaw Industries Inc. EcoWorx thickness ~0.7 cm	Carpet Corporation of America, Rome, GA	3.5 x 1.5	Clean with dry air to remove loose dust
Wood Flooring material	Fir plywood (bare); thickness 0.9 cm	Lowe's, Columbus, OH	3.5 x 1.5	Clean with dry air to remove loose dust
Vinyl flooring material	Armstrong Excelon	Lowe's, Columbus, OH	3.5 x 1.5	Clean with dry air to remove loose dust

2.5 Coupon Spiking

For each contact time and material combination:

- Five replicate test coupons were spiked with GF with subsequent decontamination;
- Five replicate positive controls were spiked with GF without subsequent decontamination;
- Two procedural blank coupons were not spiked with GF but were decontaminated;
- Two laboratory blank coupons that were not spiked with GF and were not decontaminated.

All test and positive control coupons were spiked with a nominal 1 µL of neat GF, delivering approximately 1.1 milligram (mg) of GF. The contamination level was approximately 2 g/square meter (m^2) (1.1 mg/ [3.5 centimeters (cm) x 1.5 cm] = 0.21 mg/cm^2 = 2.1 g/m^2). GF was dispensed using a calibrated Hamilton syringe (P/N CAL80975 [50 µL] equipped with a 22-gauge needle [P/N 91022] and repeating dispenser [P/N 83700], Hamilton Co., Reno NV).

Polytetrafluoroethylene (Teflon®) spike control coupons (P/N 5Y43BYD, Thomas Scientific, Swedesboro, NJ) were evaluated, one at the beginning, one at the middle, and one at end of each trial (total of three spike control coupons per trial). A day of decontamination and subsequent extraction and analysis is referred to as a "trial". Each spike control coupon was spiked with three 1 µL droplets of neat GF, using the same syringe and repeating dispenser settings as for spiking the test and positive control coupons, then immediately placed in 20 milliliters (mL) of extraction solution, shaken for 15 seconds, and passively extracted for one

hour. The first spike control coupon was prepared at the beginning of the evaluation. The second spike control coupon was prepared midway though application of agent to test coupons and positive controls. The final spike control coupon was prepared after the last test coupon was contaminated. The mass of CWA per spiked droplet applied to test and positive control coupons is assumed to be equal to the mean of the CWA per droplet recovered from the spike control coupons calculated as shown in Equation 1:

$$\alpha = \frac{\sum_1^3 CWA_i}{9 \text{ droplets}} \quad (1)$$

where:

α = Mean mass of CWA per spiked droplet
CWA_i = Mass of CWA recovered from the i^{th} spike control coupon.

2.6 Test Matrices

2.6.1 Enzyme Application Rate

A backpack type sprayer would be the most likely method for application of an enzyme solution in the field setting. However, for this laboratory study, in order to reduce variability in amounts of enzyme solution applied to the small coupons, the enzyme solution was delivered to coupon surfaces as measured amounts from pipettes. In a previous study, a spray application was used to determine the mass of enzyme solution (DEFENZ™ VX-G) that would be applied to a surface in a typical spray application.[1] These data provided material-specific target values for the amount of enzyme solution to be applied to coupons to evaluate decontamination efficacy. The applied enzyme solution amounts are shown in Table 4. Enzyme solutions were applied to coupons using a positive displacement pipette ((P/N M-250 [250 µL] and D-200 [2-200 µL] tip, Gilson Inc., Middleton, WI).

Table 4. Enzyme Application Amounts for Bench-Scale Coupon Testing

Material	Enzyme Solution Application (mL)
Galvanized metal	0.06
Decorative laminate	0.06
Wood flooring	0.09
Industrial carpet	0.12
Vinyl flooring	0.06

2.6.2 Simulated Enzyme Reactor Efficacy Testing

A simulated enzyme reactor test was performed for GF utilizing DEFENZ™ VX-G. The simulated enzyme reactor test involves combining neat GF with the enzyme solution in a vial

(no coupon surface present) with sonication of the vial at 50-60 kiloHertz (kHz) during a contact time of 15 min as a simulation of stirring during an enzyme reactor test. The test matrix is shown in Table 5.

Neat agent (1 µL) was delivered using a calibrated Hamilton syringe (P/N CAL80975 [50 µL] equipped with a 22-gauge needle [P/N 91022] and repeating dispenser [P/N 83700], Hamilton Co., Reno NV) into each vial designated as a test sample or positive control. The enzyme decontaminant (60 µL) was added to each test sample. This amount was selected because it is consistent with the application to nonporous surfaces in coupon testing. The GF and enzyme solution were always in contact during sonication. Positive control samples for the simulated enzyme reactor testing were vials spiked with GF to which 60 µL of DI water was added (i.e., no enzymatic decontamination). Procedural blanks are defined as vials with only the 60 µL enzyme solution and no GF.

GF was extracted individually by transferring the solution from each test, positive control, and blank vial each into a separate 40 mL glass bottle (S236-0040, Fisher Scientific, Pittsburgh, PA) that contained 10 mL of hexane/internal standard (IS), (naphthalene-d_8), then sonicating at 50-60 kHz for 10 min. The GF amount present in the vials was determined by the gas chromatography/mass spectrometry (GC/MS) analysis method in use for analysis of the coupon extracts. Samples that were not analyzed the same day were stored at -20 °C ± 3 °C or colder.

GC/MS results were reviewed to identify by-products from GF decontamination.

Table 5. Test Matrix for Simulated Enzyme Reactor Testing

Agent	Enzyme Product	Number of Test Samples	Number of Positive Controls	Number of Blanks
GF	DEFENZ™ VX-G	3	3	1

2.6.3 Test Matrices for DEFENZ™ VX-G against GF on Various Building Materials

The DEFENZ™ VX-G enzyme-based decontamination technology was evaluated against GF using a 15-min contact time and manufacturer-specified enzyme concentration for the material combinations as shown in Table 6. The test coupons were spiked with GF and allowed to weather for 30 minutes; then the (60 µL) DEFENZ™ VX-G enzyme was added for the specified contact time for the decontamination test. The positive control coupons were spiked with GF and allowed to weather for 30 minutes plus the contact time for the corresponding decontamination test. When the appropriate time had been reached (equivalent to contact time), all coupons were spiked with surrogate recovery compound (SRC), tributyl phosphate (TBP), and placed into separate vials containing 10 mL of hexane (GC Resolv grade, Fisher Scientific, Pittsburg, PA) containing the IS (naphthalene-d_8), and the coupons were extracted, and analyzed as described in Section 2.8. This SRC was added as a check for possible matrix effects.

Table 6. Test Matrix for Decontamination of GF with DEFENZ™ VX-G Prepared per Manufacturer's Recommendations and 15-Min Contact Time

Agent	Material	Test Coupons*	Positive Controls†	Procedural Blanks‡	Laboratory Blanks§
GF	Galvanized Metal	5	5	2	2
GF	Decorative Laminate	5	5	2	2
GF	Industrial Carpet	5	5	2	2
GF	Wood Flooring	5	5	2	2
GF	Vinyl Flooring	5	5	2	2

* Test coupons are spiked with GF and undergo decontamination.
† Positive controls are spiked with GF but do not undergo decontamination.
‡ Procedural blanks are not spiked with GF but undergo decontamination; one of the three procedural blanks was extracted and analyzed, the second procedural blank was not extracted but was held for 48 hours (or longer if over a weekend) and examined for visually-obvious changes. See Section 2.7.
§ Laboratory blanks were not spiked with GF and did not undergo decontamination.

DEFENZ™ VX-G efficacy against GF on wood was evaluated with a repeated application (a total of two applications of 15 min contact time each), a longer contact time (30 min), and at a higher enzyme concentrations (3X) at 15 min, two applications of 15 min each, and a 30 min application. Wood flooring was selected because this material exhibited the least efficacy observed with a 15-min contact time against GF. The question being answered was whether a longer contact time, reapplication, or higher enzyme concentrations would increase efficacy for decontaminating materials on which the vendor-recommended enzyme concentrations and contact time had the least efficacy. The test matrix for the systematic evaluation of enzyme efficacy against GF is shown in Table 7.

Table 7. Test Matrix for Systematic Evaluation of DEFENZ™ VX-G against GF

Material	Contact Time	Concentration	Test Coupons	Positive Controls	Procedural Blanks	Laboratory Blanks
Wood flooring	15+15	1X	5	5	2	2
	30	1X	5			
	15	3X	5	5	2	2
	15+15	3X	5	5	2	2
	30	3X	5			

2.7 Observation of Surface Damage

Procedural blanks were visually inspected and compared to coupons not exposed to the decontamination treatment to look for obvious changes in appearance of the procedural blanks (for example, in the color, reflectivity, or apparent roughness of the coupon surfaces). Observations were recorded in the evaluation records.

2.8 Extraction and Analysis

After the appropriate contact time the test, positive control, procedural blank, and laboratory blank coupons were transferred to individual extraction bottles (S236-0040, Fisher Scientific, Pittsburgh, PA) containing 10 mL of hexane with naphthalene-d_8 as an IS. The extraction bottles were sealed, shaken by hand for about 5-10 seconds, and placed into a sonicator. After all bottles containing coupons to be extracted for a given time were placed in the sonicator, they were sonicated at 50 - 60 kHz for 10 min. Within 30 min after the completion of sonication, a 1.0 mL aliquot was transferred to a GC vial (P/N 06-718-439 and 06-719-003, Fisher Scientific [Restek Corp], Hanover Park, IL) and sealed. This process was repeated for all samples until each test, positive control, solution control, procedural blank, and laboratory blank coupon had been shaken, sonicated, and aliquoted for analysis.

All test, positive control, solution control, procedural blank, and laboratory blank coupons were individually extracted and the amount of GF in the extraction solution was determined using a GC/MS, (Model 6890, Agilent Technologies, Santa Clara, CA) interfaced with a 5973 network quadrupole mass-selective detector. Chromatographic separation of the analytes was conducted using an RTX-5MS (cross-linked methyl silicone) fused silica capillary column, 30.0 meter (m) length x 0.25 millimeter (mm) diameter x 0.25 micrometer (µm) coating thickness. The GC/MS parameters for GF analysis are shown in Table 8.

Table 8. Gas Chromatographic/Mass Spectrometric Parameters for GF Analysis

Parameters	
Analysis Method	GC/MS (Scan)
Model & SN	HP6890N GC (CN10331014) & 5973N MSD (US30985853)
Data System	MSD ChemStation
Liner Type	4 mm Split/Splitless
Column	RTX-5MS, 30 m length, 0.25 mm diameter, 0.25 µm film coating thickness
Mode	Constant Pressure
Inlet (Injector) Temperature	250 °C
Transfer Line Temperature	280 °C
Sample Size	1 µL
Oven Program for Analysis	40 °C (1.0 min hold) to 100 °C @ 30 °C/min to 150 °C @ 5 °C/min to 325 °C (1.0 min hold) @ 15 °C/min*

* The final temperature (325 °C) is used to ensure all compounds eluted and carryover between runs was avoided.

The mass selective detector was operated in the full-scan mode for compounds ranging from 40 to 400 atomic mass units (AMUs). The GC/MS measurements were used to compare and evaluate co-extractive sample components and GF response. Table 9 outlines the selected ion monitoring (SIM) masses that were used to quantify GF.

Table 9. Pertinent Parameters for Target Chemical

Analyte	SIM Ions
GF	99, 67, 54, 81

2.9 Method Demonstration

2.9.1 Recovery of GF from Test Coupons

Method demonstration was conducted, consistent with previous testing[1], to establish that extraction efficiencies (recoveries) from test coupons were sufficiently high and to establish method detection limits (MDL[s]) for GF from the five materials included in the testing. The extraction efficiency was determined as a percent of the GF recovered from the spiked coupon relative to the amount spiked. The extraction method was acceptable if the extraction efficiency was 40% - 120% with a coefficient of variance between samples not exceeding 30%.

Recovery efficiencies were determined by spiking each of three coupons of each material type with 1.0 µL of neat GF. Hexane [GC Resolv grade, Fisher Scientific, Pittsburg, PA] was selected to extract GF from the aqueous (enzyme containing) phase. The SRC was also applied to the coupon surface (1.0 µL). Sufficient hexane to cover the coupons (10 mL) was used for each extraction. The coupons were transferred into hexane within 0.5 min of spiking with GF. Immediately after transfer, the vial was capped and shaken by hand for 5-10 seconds and placed into a sonicator. After all vials containing the coupons to be extracted were placed into the sonicator, the samples were sonicated at 50-60 kHz for approximately 10 min. Within 30 min after the completion of sonication, an aliquot of extract was transferred to a GC vial (P/N 06-718-439 and 06-719-003, Fisher Scientific [Restek Corp], Hanover Park, IL) and sealed. The amount of spiked GF was confirmed using control samples where the GF was spiked directly into hexane and analyzed.

The aliquots of hexane extracts of coupons spiked with GF (1 µL) and aliquots of hexane containing the same spike amount as applied to the coupons were analyzed for GF as described in Section 2.8.

Extraction efficiency was calculated using a series of equations. The GF concentration in a coupon extract or spiked hexane sample was determined by Equation 2:

$$\frac{A_s}{A_{is}} = M\,\frac{C_s}{C_{is}} + W \qquad (2)$$

where:

A_s = Area of the target analyte peak in the sample

A_{is} = Area of the internal standard peak

C_s = Concentration of the target analyte in the sample (µg/mL)

C_{is} = Concentration of the internal standard (µg/mL)

M = Slope of the GC calibration line

W = Y intercept of the GC calibration line

GC concentration results (μg/mL) were converted to total mass by multiplying by extract volume as shown in Equation 3:

$$M_m = C \times E_v \quad (3)$$

where:

M_m = Measured mass of CWA (μg)

C = GC concentration (μg/mL)

E_v = Volume of extract (mL)

Extraction efficiency was then defined by Equation 4 as:

$$Extraction\ Efficiency = \left(\frac{M_m\ of\ CWA\ on\ Test\ Coupon}{M_m\ of\ CWA\ in\ Hexane} \right) \times 100\% \quad (4)$$

where:

M_m = Measured mass of CWA (μg) recovered from an individual test coupon or recovered from hexane spiked with CWA.

2.9.2 MDL for GF Extracted from Coupon Materials

In addition to determining extraction efficiencies, the MDL was determined for analysis of the GF from each of the five building materials included in the testing by following the EPA guidelines (40 Code of Federal Regulations, Part 136, Appendix B).[3] To achieve the low spike levels for this testing required the use of dilute solutions of GF. Eight replicate coupons of each material type were laid out in the hood on a clean surface. The coupons were spiked with 10 μL of ~1,000 microgram (μg)/mL of GF in hexane (~10 μg of agent). The actual spike mass was recorded. The coupons were also spiked with 1 μL of SRC. Within 5 min of initiation of spiking, the coupons were placed, spiked side down, into separate bottles containing 10 mL of hexane/IS. The bottles were immediately placed into a sonicator and sonicated for 10 min at 50- 60 kHz. At the completion of sonication, the bottles were removed from the sonicator and within 30 min an aliquot of each sample was pulled using a Pasteur pipette and placed into a GC vial for analysis.

The MDLs were calculated as shown in Equation 5:

$$MDL = t(n-1,1-\alpha = 0.99) \times SD \quad (5)$$

where:

$t(n-1,1-\alpha = 0.99)$ = the Students' t value for a 99% confidence level and standard deviation estimate with n-1 degrees of freedom

SD = standard deviation of the replicate analyses.

2.9.3 Quench of Decontamination Reaction

Hexane extraction was expected to remove the GF (reactant) from the aqueous phase in which the enzyme is active thereby halting (quenching) the decontamination reaction. Enzymes are not expected to be functional in the non-polar phase so other additives are not expected to be needed to quench the reaction. The neutralization method was assumed not to be impacted by the coupon material. Quench methods were therefore evaluated using solution tests.

The use of hexane extraction as a quench method was assessed as follows:

1. Enzyme (60 µL) was added (using a positive displacement pipette (P/N M-250 [250 µL] and D-200 [2-200 µL] tip, Gilson Inc, Middleton, WI) to a vial containing 10 mL of hexane and IS (naphthalene-d_8) and 1 µL of GF, shaken for 15 seconds, and allowed to stand for 10 min.
2. Distilled water, equivalent to the amount of enzyme solution in Step 1, was added (using a positive displacement pipette), to a vial containing 10 mL of hexane/IS and 1 µL of GF, shaken for 15 seconds, and allowed to stand for 10 min.
3. The extracts from Steps 1 and 2 were analyzed using GC/MS. Extraction alone, without additional neutralization, was acceptable if the amount of GF recovered in Step 1 (enzyme present) was at least 70% of the amount of GF recovered in Step 2 (no enzyme present).

All GF recoveries with hexane exceeded the required 70% (Table 10).

Table 10. Recovery of GF Using Hexane Extraction as Quench

Agent	Recovery with Water, µg (SD) n = 3	Recovery with Enzyme, µg (SD) n = 3	Mean % Recovery "with Quenched Enzyme" Compared to "with Water"
GF	1008 (109)	826 (11)	82

2.10 Efficacy Determination

The decontamination efficacy was determined by measuring the amount of residual GF on test coupons and comparing this amount with positive controls (spiked with GF, not decontaminated and analyzed after the same "contact time" as the test coupons). Aliquots of extracts from blanks, positive controls, and decontaminated coupons were analyzed for GF according to methods described in Section 2.8. Decontamination efficacy was calculated as follows:

1. Concentration of GF (or SRC) in a coupon extract sample is determined by Equation 2

2. GC concentration results (µg/mL) are converted to total mass by multiplying by the extract volume shown in Equation 3.

3. Decontamination efficacy (percent removal achieved during decontamination) is then defined in Equation 6 as:

$$E = \left(1 - \frac{M\ of\ CWA\ on\ Test\ Coupon}{M_m\ of\ CWA\ on\ Positive\ Control\ Coupon}\right) \times 100\% \quad (6)$$

where:

M = Measured mass of CWA (μg) on an individual test coupon

M_m = Mean of measured mass of CWA (μg) from five positive control coupons.

The mean efficacy is the average efficacy from five test coupons included in a given decontamination test (i.e., enzyme type, enzyme concentration, and contact time).

2.11 Statistical Analysis

The standard deviation is calculated as shown in Equation 7:

$$\sigma = \frac{1}{N}\sqrt{\sum_{i=1}^{N}(x_i - \mu)^2} \quad (7)$$

where:

σ = standard deviation

μ = mean

x_i = i^{th} value of the variable being evaluated, e.g., control coupon

N = total number of elements in the population.

A two-tailed Student's t-test is used to compare the means of the positive control coupon and the test coupon recoveries. Unequal variance between the populations is assumed. A p-value is the result of the comparison. Results are considered significant if $p < 0.05$.

A two-tailed Student's t-test is also used to compare the means of the test coupons subjected to alternative treatment (longer contact time, repeated applications, and/or higher enzyme concentrations) to the standard treatment (1X concentration applied once for 15 min). Unequal variance between the populations is assumed. A p-value is the result of comparison. Results are considered significant if $p < 0.05$.

2.12 Analysis of By-products

The GC/MS instrumentation was operated in the full scan mode to detect (toxic) GF decontamination by-products in the extracts of the simulated enzyme reactor tests. A National Institute of Standards and Technology (NIST) 2002 mass spectral library was used to tentatively identify compounds in the mass spectra. Reports were generated using ChemStation software (Version D.01.02.16 [15 June 2004], Agilent, Santa Clara, CA).

3.0 Quality Assurance/Quality Control

3.1 Control of Monitoring and Measuring Devices

QC requirements and results are shown in Table 11.

Table 11. Data Quality Objectives and Results for Test Measurements

Parameter	Measurement Method	QC Requirement	Results
Time	Timer/data logger	Two seconds/hour; check once before beginning testing	Passed requirement
Mass	Balance with daily calibration check using standard weights	Balance precision at least 0.1x lowest measured value	Daily balance calibration check passed QC requirement
pH	pH meter	Calibrate with two standard buffer solutions spanning range of interest	Daily calibration check passed QC requirement
Background Contaminants	Analyze blank solvent using GC/MS	<MDL for analyte; include with each batch of samples	No background contamination detected
Mass of CWA (in extraction solvent)	Extract in solvent and analyze using GC/MS	>70% of GF spike is recovered; determine once during method demonstration	GF recoveries met the QC requirement
Mass of CWA (in neutralized enzyme solution)	Extract in solvent and analyze using GC/MS	>70% of GF, spike is recovered; determine once during method demonstration	GF recoveries met the QC requirement
Mass of SRC (test and positive control coupons and laboratory and procedural blanks)	Extract in solvent and analyze using GC/MS	>70% recovery of SRC (which provides a check for matrix effects)	All SRC recoveries met the QC requirement
Mass of CWA (on positive controls)	Extraction/ chromatographic quantitation	Result were considered an outlier if the recovery value for analyte from a coupon falls outside of three standard deviations of the mean. Criterion applies only if concentration of analyte is ≥5 times the MDL	No outliers were noted
Mass of CWA (on spike controls)	Extraction/ chromatographic quantitation	≥85% of GF spike target	All spike control recoveries met the QC requirement
Mass of CWA (on laboratory blank)	Extraction/ chromatographic quantitation	<MDL for GF	No GF was detected on any laboratory blank coupon

Quality checks on the prepared DEFENZ™ VX-G solutions were obtained through pH measurement of the solution. All prepared solutions were pH = 8.0

3.2 Chemical Analysis Equipment Calibration

A six-point calibration for GF and the SRC was generally used with a lower calibration level of 0.5 µg/mL and an upper range of approximately 50 µg/mL. Naphthalene-d_8 was used as the IS for quantitation of GF and TBP. An average response (relative standard deviation <15%) and quadratic regression curve fit were applied to the calibration data. Samples exceeding the upper calibration limit were diluted to a concentration within the calibration range and reanalyzed.

Continuing calibration verification (CCV) standards were included prior to sample analysis, following every fifth sample and at the end of each batch of samples. Two CCV concentrations were used (0.5 µg/mL and 25 µg/mL), one of which was equal to the low calibration standard. A CCV response within 25% of nominal concentration was acceptable. One CCV was low, so the analysis was repeated for that batch of test samples.

For GC/MS, the neat GF was diluted with hexane to prepare standard solutions that were analyzed to construct a standard curve within an appropriate range. The standard solutions were included each day that an analysis was performed. The GC/MS calibration curves met the following performance requirements:

- r^2 greater than 0.98;
- % bias for the lowest standard less than 25%;
- % bias for the remaining standards less than 15%;
- % bias for the lowest calibration check standard less than 35%;
- % bias for the remaining calibration check standard less than 20%; and
- difference between replicate samples less than 20%.

The calibration curve r^2 was >0.99 and the % bias for all standards was ±7%.

3.3 Technical Systems Audit

The QA Manager performed a Technical Systems Audit (TSA) during the performance of the decontamination testing. The purpose of the TSA was to ensure that testing was performed in accordance with the test/quality assurance (QA) plan. In the audit a QA Officer reviewed the sampling and analysis methods used, compared actual test procedures to those specified in the test/QA plan, and reviewed data acquisition and handling procedures. The QA Manager prepared a report, the findings of which were addressed either by modifications to the test procedures or by documentation in the test records.

The TSA addressed the systematic decontamination of GF. The TSA report noted that all work followed written procedures. No issues were noted.

3.4 Performance Evaluation Audits

A performance evaluation (PE) audit was conducted for each performance parameter shown in Table 12 to assess the quality of the measurements made during testing. The audits

for mass, chemical mass, pH, and time were performed once during testing by analyzing a standard(s) that is independent of standards used during the testing.

Table 12. Performance Parameters Audited

Parameter	Audit Procedure	Expected Tolerance	PE Audit Results
Time	Compare time to independent clock or watch value	±2 seconds/hour	Both timers used during testing were compared and found to be within 2-second requirement
Chemical Mass	Use GC/MS to measure SRC from secondary source and compare to primary source	±10%	Primary and secondary sources were found to be within ±10% tolerance requirement
	Determine mass of agent delivered to Teflon® spike control coupons and compare to target application level	≥85% of spike target	Spike controls were at 87% of spike target
Mass	Use balance to determine the mass of a reference weight	±0.1 g	Balance used was within annual calibration and calibration checks performed regularly to ±0.1 g criterion
pH	Use pH meter to determine pH of a standard solution	±0.1 pH units	pH meter was found to be within ±0.1 pH units

3.5 Data Quality Audit

The QA Manager audited at least 10% of the investigation data and traced the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All data analysis calculations were checked.

3.6 Amendments

Nine amendments were incorporated into the test/QA plan. It included enzymatic decontamination tests against TGD, HD and VX.[1] A brief summary of the amendment related to decontamination of GF follows:

- Amendment 8: A required deliverable of Amendment 1 to contract EP-C-10-001 Work Assignment 2-04, provided test/QA details necessary to apply the plan to testing of DEFENZ™ VX-G against GF.

3.7 Deviations

No deviations from the test/QA plan were noted for the work described in this report.

4.0 Results/Discussion

4.1 *Method Demonstration Results*

The extraction methods accepted for use met the acceptance criterion (see Section 2.9.1) of being in the range of 40% - 120% recovery with a coefficient of variance between samples not exceeding 30%. GF recoveries were 85% to 103% and the coefficients of variance for triplicate samples were 1.3% to 8.7% and, therefore, acceptable (Table 13).

Table 13. Extraction Efficiencies for Neat GF from Various Types of Coupons

Material	Mean Extraction Efficiency	Coefficient of Variance
Teflon	88% (n=4)	3.0%
Galvanized metal	93% (n=3)	6.3%
Wood flooring	84% (n=3)	8.5%
Industrial carpet	94% (n=3)	1.3%
Vinyl flooring	101% (n=3)	4.6%
Decorative laminate	102% (n=3)	8.7%

The MDLs for GF extracted from various types of test coupons using 10 mL of hexane are shown in Table 14.

Table 14. MDL Values for GF Extracted from Various Types of Coupons Using Hexane

Material	GF MDL, µg (10 mL Extract)
Galvanized metal	0.11
Wood flooring	0.35
Industrial carpet	0.45
Vinyl flooring	0.25
Decorative laminate	0.20

4.2 Simulated Enzyme Reactor Results

The results of the simulated enzyme reactor results are summarized in Table 15. DEFENZ™ VX-G (mixed with water at the ratio recommended by the manufacturer) demonstrated efficacy against GF. Mean efficacy against GF after the 15-min contact time was 99%. DEFENZ™ VX-G exhibited here a higher efficacy against GF when compared to the coupon testing. Ninety-nine percent of GF was decomposed with the 15 min contact time. This more dynamic interaction is apparently important in reaching a higher efficacy against GF. Lower efficacy results obtained during coupon testing can be explained by the more static interaction of the enzyme solution with GF on the test coupon.

The temperature profile for a fifteen min sonication resulted in a rise of about 7 °C from 17.4 °C when sonication began to 24.2 °C at 15 min.

Table 15. Simulated Enzyme Reactor Results for DEFENZ™ VX-G Enzyme and GF

CWA	Enzyme Used	Blank Solution, µg	Mean Positive Control Total Mass, µg (SD)	Mean Test Total Mass, µg (SD)	Mean Efficacy
GF	DEFENZ™ VX-G	ND*	704 (66)	10 (13)	99%

*ND indicates no GF was detected, <0.5 µg/mL (lower calibration limit).

No GF was detected in the blank solution that was part of the simulated enzyme reactor testing.

4.3 By-Product Analysis

The simulated enzyme reactor GC/MS data were examined in full scan mode for qualitative differences between control and test samples. No substantial differences were observed in chromatographic peaks between the test and control samples.

4.4 Coupon Decontamination Results

Decontamination efficacy results (mean and SD) using DEFENZ™ VX-G enzymes prepared in accordance with the manufacturer's instructions are shown in Table 16. Efficacy was observed for all materials ranging from a low of 36% for wood to 94% for decorative laminate. A graphical representation of the amounts recovered and associated decontamination efficacy is provided in Figure 2. Amounts recovered from the positive control coupons were lower than observed during extraction efficiency demonstration. Lower recoveries observed here maybe indicative of some evaporative loss or degradation of GF during the 45-min time between spiking and extraction of the positive control coupons.

Table 16. GF Decontamination Results Using DEFENZ™ VX-G (1X)

Material	Contact Time, min	Mean Positive Control Coupons, µg (SD)	Mean Test Coupons, µg (SD)	Mean* Test Coupons Efficacy, %
Galvanized metal	15	780 (80)	60 (7)	92
Wood flooring	15	580 (56)	370 (25)	36
Industrial carpet	15	920 (61)	180 (93)	80
Vinyl flooring	15	850 (77)	200 (27)	77
Decorative laminate	15	660 (40)	40 (3)	94

*Calculation of mean efficacy based on analytical results before rounding of mean positive control and mean test coupon

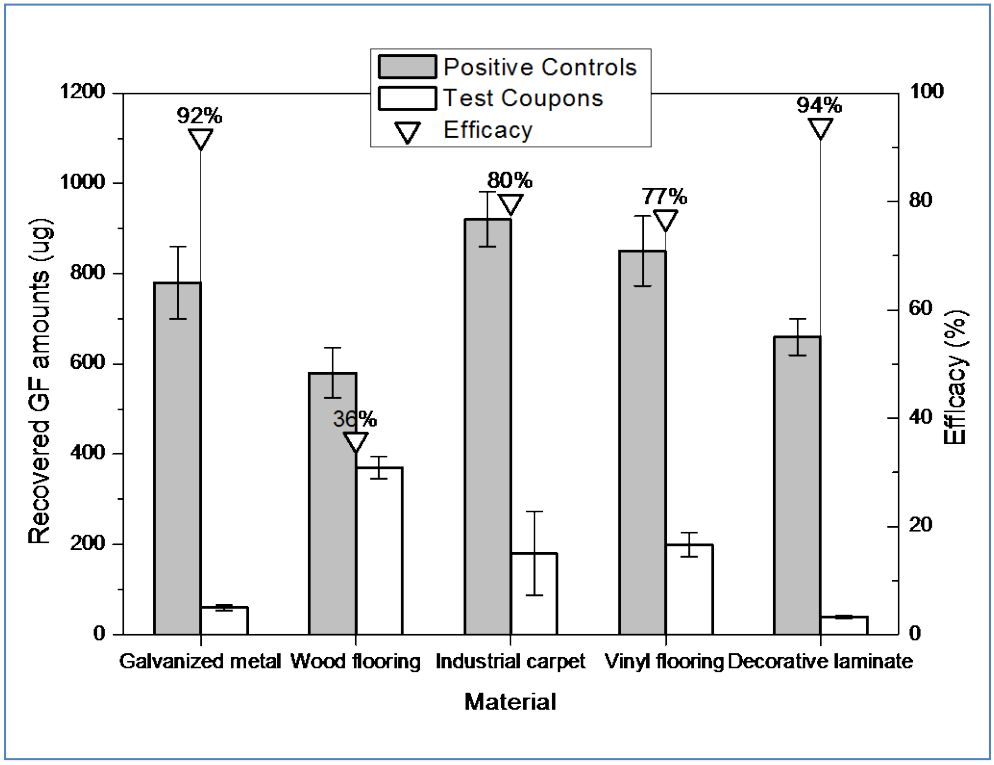

Figure 2. Recovered amounts of GF from materials and associated decontamination efficacy following DEFENZ™ VX-G application (15 minute contact time).

The standard DEFENZ™ VX-G enzyme preparation (1X) was tested at a longer contact time (30 min) and with two sequential 15-min applications to evaluate whether efficacy would increase. Wood flooring material was used as the coupon material because of the lower efficacy observed for GF on wood with a 15-min contact time. Results for decontamination of GF using the longer contact time and repeated applications are shown in Table 17 and visualized in Figure 3. Efficacy did not significantly increase with a 30-min contact time compared to a 15-min contact time ($p = 0.29$). Reapplication of the 1X enzyme for two sequential 15 min periods resulted in a significant increase in efficacy ($p = 0.014$). Given the results from the single 30-min application of the enzyme, the increased efficacy with a second application of the enzyme is unlikely to be explained by increased evaporation.

Table 17. GF Decontamination on Wood Flooring Using Alternative Contact Times, Repeated Application, and/or 3X Enzyme Concentration

Contact Time, min	Concentration	Mean Positive Control Coupons, µg (SD)	Mean Test Coupons, µg (SD)	Mean* Test Coupons Efficacy, %
30	1X	580 (46)	310 (51)	47
15 + 15	1X		230 (45)	61
15	3X	700 (147)	330 (64)	53
30	3X	730 (68)	410 (61)	44
15+15	3X		150 (83)	79

*Calculation of mean efficacy based on analytical results before rounding of mean positive control and mean test coupon

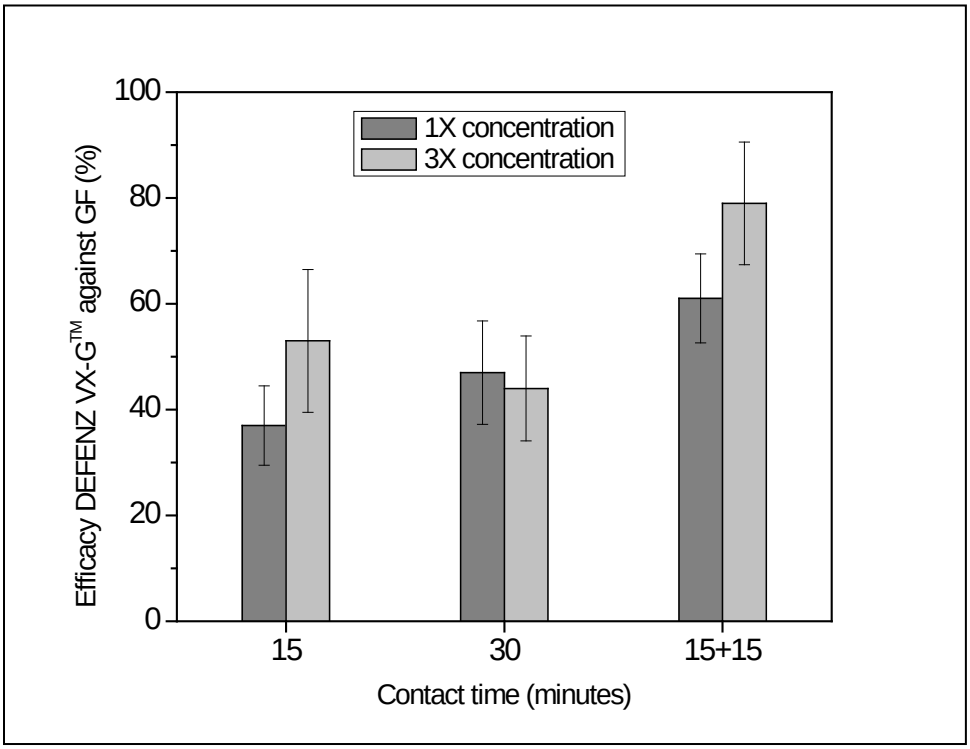

Figure 3. Decontamination efficacy for 15-min, 30-min, and repeated 15-min contact time of DEFENZ™ VX-G against GF

Concentrations of DEFENZ™ VX-G enzymes higher than the manufacturer's recommendation (3X) were tested with 15-min and 30-min contact times, and with a repeated 15-min application of the 3X enzyme to evaluate whether efficacy against GF would increase. Results for decontamination of GF using higher concentrations than the manufacturer's recommendation with a 30-min contact time and sequential 15-min applications are shown in Figure 3. Increasing the concentration to 3X did not significantly increase efficacy at 15-min ($p = 0.17$) or 30-min (no improvement) contact times compared to decontamination for 15-min using the 1X concentration. With a repeated 15-min application of the 3X enzyme efficacy was

significantly higher (p = 0.009) compared to decontamination for 15-min using the 1X concentration. However, the higher (3X) concentration with repeated 15-min applications did not result in significantly greater efficacy than the repeated 15-min applications using the 1X concentration (p = 0.17).

The GF recovered from wood positive control coupons varied from 580 µg to 730 µg across the four sets of test coupons. The differences were not attributable to longer periods of evaporation. Non-homogeneity of the wood, differences in wood moisture content, or other factors associated with the material may account for these differences in recovery. However, no testing has been done to determine the cause of this difference. The observed improved efficacies with longer interaction times and repeated application cannot be explained solely by the wide range in recovered amounts from positive controls.

Quality control (QC) measurements included laboratory blanks, procedural blanks, dose confirmation, and Teflon spike control measurements. GF was not found on any laboratory blank coupon. GF was not found on galvanized metal procedural blank coupons. GF was detected at low levels on procedural control coupons of other material types (≤0.50 µg/mL extract). Dose confirmation (measured concentration in direct spike of 1 µL into 10.0 mL of solvent) was 111% of the target concentration of 110 µg/mL. Recoveries from Teflon spike controls were 86% (standard deviation [SD] = 7%) and 91% (SD = 3%) of the expected concentration acquired as part of the default contact time tests and longer interaction time tests, respectively.

4.5 Observations of Damage to Coupons

DEFENZ™ VX-G treatment resulted in no obvious visible damage to any of the coupons either immediately after decontamination or two days after the decontamination. No detailed examination or testing for structural damage was included in this evaluation. Damage, if any occurred that is not readily visible, would likely not be detected in this evaluation.

5.0 Conclusions

Simulated enzyme reactor testing demonstrated significant efficacy of DEFENZ™ VX-G against GF with a 15 min contact time. GF was reduced by 99%. The impact on the efficacy of an observed temperature increase (about 7 °C) associated with sonication (15 minutes) as part of the simulated enzyme reactor tests was not further evaluated. Temperature typically impacts enzyme performance. However, data using the CWA simulant paraoxon suggests that no appreciable effect on enzyme activity occurs in the 5 – 35 °C range.[4]

The application of DEFENZ™ VX-G resulted in less GF recovered from all materials tested: galvanized metal, wood flooring, industrial carpet, vinyl flooring, and decorative laminate with a 15-min contact time. Tests on wood flooring showed efficacy increased with repeated 15-min applications. Increasing the single application contact time to 30 min or increasing the enzyme concentration to 3X did not significantly increase efficacy.

A comparison of the simulated enzyme reactor testing efficacy (99% reduction in GF amount recovered) versus the surface decontamination (36-94% reduction in GF amount recovered) suggests that the more static interaction of the surface decontamination tests reduces the enzymatic reactivity. Reactor based testing efficacy results may therefore only be considered as an upper limit to the reduction of a CWA (here, GF) from a surface.

Given the observed efficacies and the lack of visible damage to a range of indoor building materials, DEFENZ™ VX-G enzyme appears to be a technology useful for removing GF from building materials after a terrorist release.

Caution should be used in extrapolating from the bench testing to field application of the enzymes. A full-scale test, using spray equipment and larger surfaces, is warranted to ensure that the laboratory results are scalable.

6.0 References

1. U.S. EPA Report, Enzymatic Decontamination of Chemical Warfare Agents, U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-12/033, 2012

2. Test/Quality Assurance (QA) Plan for Enzymatic Decontamination of Chemical Warfare Agents, Version 2 (July 2010). Available upon request from EPA

3. Code of Federal Regulations Title 40: Protection of Environment Part 136 – Guidelines establishing test procedures for the analysis of pollutants. Appendix B - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11 (June 30, 1986)

4. Impact of Environmental Conditions on the Enzymatic Decontamination of a Material Surface Contaminated with Chemical Warfare Agent Simulants. Lukas Oudejans, Barbara Wyrzykowska-Ceradini, Craig Williams, Dennis Tabor, and Jeanelle Martinez. Accepted for publication in Industrial, Chemical and Engineering Research, June 2013

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