EDGEWOOD CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1405

RAIN-INDUCED WASH-OFF OF CHEMICAL WARFARE AGENT (VX) FROM FOLIAR SURFACES OF LIVING PLANTS MAINTAINED IN A SURETY HOOD

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September 2016

U.S. ARMY RDECOM

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)* XX-09-2016	2. REPORT TYPE Final	3. DATES COVERED *(From - To)* May 2014 – Sep 2015

4. TITLE AND SUBTITLE
Rain-Induced Wash-Off of Chemical Warfare Agent (VX) from Foliar Surfaces of Living Plants Maintained in a Surety Hood

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
WBS R.0013813.81.4

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT-M, APG, MD 21010-5424
Excet, Inc.; 6225 Brandon Ave., Suite 360; Springfield, VA 22150-2519

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1405

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency; 8725 John J. Kingman Road, MSC 6201; Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
In separate, replicated experiments, we experimentally established wash-off coefficients (k_w) for the chemical agent VX on grass, utilizing 1 and 3 µL VX droplets at 0.017 (1 min), 1, and 4 h after dissemination. A 10 mm (0.39 in.) rain event at 0.017 h after dissemination washed off 95 and 83% of the 1 and 3 µL VX droplets, respectively. At 1 h after dissemination, a 10 mm rain event washed off 0.03 and 0.5% of the 1 and 3 µL VX droplets, respectively. At 0.017 h after dissemination, the k_w values for 1 and 3 µL droplets were 0.095 and 0.083 mm^{-1}, respectively. At 1 and 4 h after dissemination, the k_w values for VX were approximately 3 orders of magnitude less than those at 0.017 h. Grass contaminated with 3 µL VX droplets was exposed to multiple (10×) 100 µL light rain events, followed by multiple (10×) surface wipes at 0.017, 0.5, 1, 4, and 24 h after dissemination. The cumulative proportions of 3 µL VX droplets washed off by the rain events at 0.017 and 1 h after dissemination were 75.3 and 0.99%, respectively, and the cumulative proportions of VX wiped off after those rain events were 0.8 and 0.3%, respectively. Results from these investigations of agent–plant interactions provide input for predictive models and functional information for "Go/No-Go" decisions that can affect soldiers on agent-contaminated battlefields.

15. SUBJECT TERMS

Wash-off coefficient	*Echinochloa crus-galli*	Foliage
Chemical warfare agent (CWA)	*O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX)	Barnyard grass

16. SECURITY CLASSIFICATION OF:

17. LIMITATION OF ABSTRACT

18. NUMBER OF PAGES

19a. NAME OF RESPONSIBLE PERSON
Renu B. Rastogi

a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	28	(410) 436-7545

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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PREFACE

The work described in this report was authorized under project no. WBS R.0013813.81.4 The work was started in May 2014 and completed in September 2015.

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Acknowledgments

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1.   INTRODUCTION

Defensive capabilities are needed to identify threats in the event of the release of chemical warfare agent (CWA) into the natural environment. Commanders who have soldiers under battlefield conditions must be armed with functional information for "Go/No-Go" decisions related to the exposure of soldiers to CWA on agent-contaminated battlefields. Little scientific information exists that describes the hazards to the solider associated with agent–plant interactions. Without a more-complete understanding of these interactions, it is difficult to predict the presence and persistence of the potential exposure hazard posed by CWA-contaminated foliage.

The dissemination of CWA in the field can be subject to many environmental pathways that affect its fate and may ultimately pose an exposure hazard. A particularly important exposure pathway is the agent–plant interface, where many environmental functionalities occur including CWA absorption into plant tissue, evaporation from the leaf surface, transformation, fixation, photodegradation, and rain-induced wash-off from foliar surfaces (Hulbert et al., 2011; Van Emon et al., 1998). The research presented in this report focuses on rain-induced wash-off of *O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX) from contaminated grass (*Echinochloa crus-galli* [L.[1]] P. Beauv[2]) leaves (also referred to by the common names barnyard grass and Japanese millet).

The fate of chemical compounds in the environment is influenced by their physical and chemical properties as well as ambient meteorological conditions and the environmental material with which the compounds interact (Talmage et al., 2007). Persistence on plant foliage is also a function of the biochemical, physiological, and micromorphological properties of the plant leaves, including leaf epicuticular waxes and cuticle (Sanyal et al., 2006). The extent of persistence, penetration (absorption), or evaporation of agent droplets on foliage will depend on the vapor pressure, the hydrophilic nature of the agent, and the extent of hydrophobicity of the foliar surfaces (Rothamsted, 2013). Therefore, the extent of absorption and persistence of CWAs on foliar surfaces and within plant tissues among different plant species will vary depending on the compound.

Our objective in investigating agent–plant interactions in this study was to determine whether rainfall reduces the exposure hazard to the Warfighter by decreasing the quantity of CWA that remains available on natural living vegetation. This, in turn, would reduce the potential for contact transfer of agent onto Army uniforms and provide data for models used to predict the fate of CWA and related chemicals in the environment. The pesticide industry uses rainfastness characteristics (a measure of the post-dissemination time that pesticides will remain effective on foliage following a rain event) to help determine the interval for pesticide

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[1]L. indicates that Carl Linnaeus is the authority for the species name.
[2]P. Beauv. indicates that Palisot de Beauvois was the author of this botanical name.

re-application (Wise, 2015). Typically, pesticide studies used to determine rainfastness involve the uniform application of a dilute mixture of pesticide to outdoor test plots or in controlled chambers (Hulbert et al., 2011; Van Emon et al., 1998). In our studies, we could not use outdoor plots or spray into chambers. We used high-purity VX (neat) to simulate battlefield conditions. We applied single droplets of neat VX to the live foliar surfaces of the grass, *E. crus-galli*, which was contained in a chemical surety hood. At predetermined time intervals after agent dissemination, we applied a simulated rainfall directly onto the agent droplet site using ASTM Type I water that was allowed to equilibrate to ambient conditions. The water runoff was collected and analyzed to determine the amount of agent that was found in the wash-off solution. Immediately after the rain event, we wiped the leaf surface to determine the amount of agent remaining that may be available for transfer to Army uniforms by contact.

The data generated from this study were directly applicable for input into the Pesticide Emission Assessment at Regional and Local scales (PEARL) model. The PEARL model is used to predict the fate and transformation of a pesticide in soil–plant systems (Leistra, 2001; Van den Berg and Leistra, 2004). Because VX is an organophosphate (OP) compound, with physical properties similar to OP pesticides, the PEARL model is considered to be one of the models reasonably useful for predicting the fate of VX in the environment.

2.   METHODS

2.1   Plant Selection

We selected the grass species *E. crus-galli* for research investigations described in this report. *E. crus-galli* is one of the most-prevalent natural grass species worldwide, is tolerant of both dry and wet natural habitats, and is used as forage for grazing animals as well as for wildlife food and habitat (USDA, 2015). We used novel methods that were developed to enable and sustain the culture of living, physiologically healthy plants within a chemical agent surety hood, as described by Simini et al. (2016).

2.2   Chemicals

The CWA used in this study was VX at 93% purity, Chemical Agent Standard Analytical Reference Material (CASARM) grade, Chemical Abstracts Service (CAS) no. 50782-69-9), which was stabilized with 5% by weight diisopropylcarbodiimide (CAS no. 693-13-0; Sigma-Aldrich Company; St. Louis, MO). Reagent-grade isopropyl alcohol (IPA; CAS no. 67-63-0; Sigma-Aldrich) was used as an extractant. The simulated rainwater was ASTM Type I water (18 MΩ cm) (ASTM, 2004) that was allowed to equilibrate to ambient conditions. Miracle-Gro Water Soluble All Purpose Plant Food (Scotts Miracle-Gro Company; Marysville, OH) fertilizer (24% total nitrogen [calculated as N], 8% available phosphate [calculated as P_2O_5], 16% soluble potash [calculated as K_2O], 0.02% boron, 0.07% copper [water soluble], 0.15% iron [chelated], 0.05% manganese [chelated], 0.0005% molybdenum, 0.06% zinc [water soluble], and 1.14% ethylenediaminetetraacetic acid chelating agent) was used to prepare the dilute phytonutrient solution (530 mg/L) with ASTM Type I water.

2.3   Dissemination of VX Droplets onto Leaves

Plant stands were constructed to hold the pots in a fixed position. A hole was cut in each Petri dish cover, and each pot was placed through the hole and onto the Petri dish (Figure 1). Each pot was secured to a ring stand with an adjustable ring clamp. Plant leaves were laid horizontally across a ring near the top of the plant canopy and secured to the ring by lengths of clear plastic (cellulose acetate) tape that was folded in half lengthwise (thus preventing sticky contact of acrylate adhesive to leaf surface) and placed across the leaf surface. The ends of the folded tape were then secured to the ring with additional tape, while maintaining slight pressure on the leaf surface (Figure 2). This method of securing individual leaves in a horizontal position prevented any possible leaf surface damage caused by tape removal and ensured that disseminated agent droplets contacted the leaf surface at the point intended and that those locations were easily identified for further investigation. Individual leaves on the living plants remained secured in this horizontal position during and after dissemination of VX to prevent uncontrolled agent deposition throughout testing. Physiologically healthy plants were maintained within the chemical agent surety hood environment (Simini et al., 2016). The temperature within the surety hood was maintained at 22 ± 2 °C, and the relative humidity was maintained at $50 \pm 10\%$. The average airflow through the hood was measured at 1.5 ± 0.09 mph (measured at the face of the hood using an Airdata Multimeter; ADM-870C; Shortridge Instruments, Inc. [Scottsdale, AZ]).
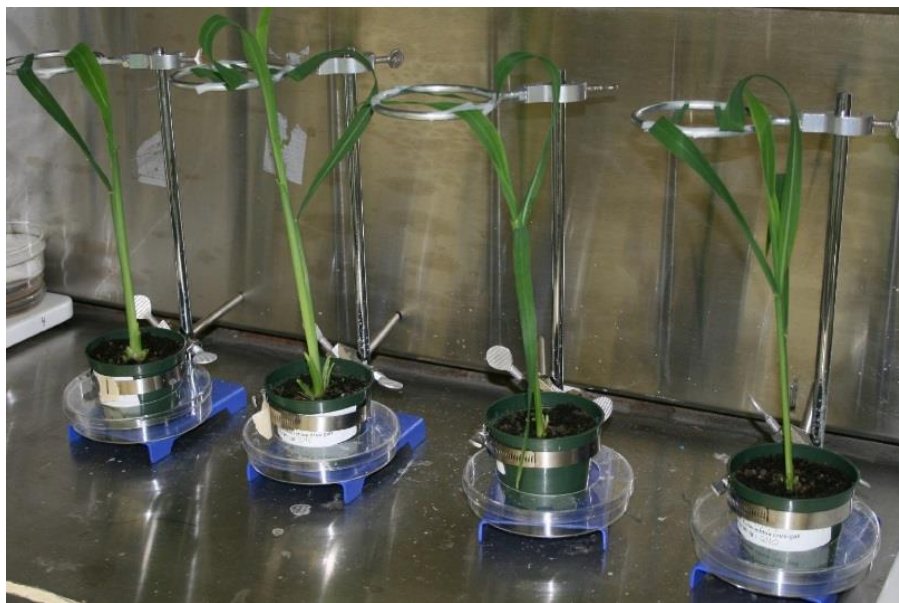

Figure 1. *E. crus-galli* grass plants in surety hood with plant stands.

Figure 2. *E. crus-galli* leaf secured in horizontal position using tape folded in half lengthwise (to prevent leaf contact with the adhesive).

In separate experiments, single 1 or 3 µL droplets of CASARM-grade VX (0.8278 ± 0.0386 mg or 3.010 ± 0.137 mg, respectively) that represent the range of droplet sizes expected from CWA dissemination under field conditions (TOP, 2011) were individually dispensed onto plant leaves using a calibrated 10 µL Hamilton (Reno, NV) gastight syringe. A single droplet was applied onto a single live leaf that was still attached to the plant; this prevented the droplets from merging on the foliage (for the purpose of subsequent measurements). The disseminated VX droplets were allowed to equilibrate on the foliage for 0.017, 1, and 4 h before the treated leaf was removed from the plant. The treated leaf was then subjected to a single 10 mm rain event, as required when developing data for PEARL model input. Each of these experiments was conducted using quadruplicate replication ($n = 4$).

The PEARL model is used to predict the fate and transformation of a pesticide in a soil plant system (Leistra et al., 2001; Van den Berg and Leistra, 2004). Because VX is an OP compound, with properties similar to OP pesticides, the PEARL model can be used to reasonably predict the fate of VX in the natural environment. PEARL model input requires a wash-off coefficient (k_w), which is based on the percentage of pesticide washed off the foliage from a 10 mm rain event. The PEARL model user's guide (Van den Berg and Leistra, 2004) identifies five wash-off classifications on the basis of the percentage of wash-off from a 10 mm rainfall (Table 1) and suggests interpolation to more accurately determine k_w values from generated experimental data. Analysis of the PEARL model wash-off coefficient classes (Table 1), by performing a linear regression of these percent compound wash-off versus k_w values, yielded a coefficient of determination of unity ($r^2 = 1.00$). We used linear interpolation with our wash-off data to determine the values of k_w for VX on grass at time points after dissemination.

Table 1. PEARL Model Wash-Off Coefficient Classes

Compound Wash-Off (%)	Wash-Off Coefficient (mm^{-1})
90	0.09
70	0.07
50	0.05
30	0.03
10	0.01

2.4   **Simulated Rainfall and Leaf Surface Wipe Events**

In separate simulated rainfall experiments, the k_w values required for input into the PEARL model were determined for 1 and 3 µL droplets of VX on foliage. The rain application used in these separate studies was a single, 10 mm rain event that was required by the PEARL model to represent a moderate rainfall (Llasat, 2001; Hunsche et al., 2007) onto VX-contaminated foliage.

After the disseminated VX had equilibrated with the leaf surface for a predetermined amount of time, the contaminated leaf was removed from the plant by holding the leaf with a forceps at a position approximately 3 in. (7.5 cm) from the end closest to the stem of the leaf containing the VX droplet spread. The leaf was then cut next to the forceps (on the side of the forceps nearer the stem). While still holding the cut leaf with the forceps, the end of the leaf containing the absorbed VX droplet was inserted into a 50 mL collection vial. The collection vial and the leaf were tilted to approximately 45 degrees, then the simulated raindrops (cumulatively, 10 mm ± 1.09%) were applied onto the leaf above the site of the agent. This caused the applied raindrops to run down the leaf surface and across the target area of agent droplet spread, which simulated wash-off that can occur during a natural rain event. The simulated rain was applied using a calibrated 5 mL Gilson Pipetman (Middleton, WI) pipette (raindrop size of 45.6 ± 2 µL).

The vials used to collect the wash-off from the 10 mm rain events were prefilled with a volume of IPA equal to the volume of raindrops applied. This was done to slow the degradation of VX in water (see the VX stability study in Section 2.6), which resulted in a 1:1 dilution of rainwater with IPA. The volume of raindrops applied was calculated based on the area of agent droplet spread at predetermined time points after dissemination (Simini et al., 2016). During a 4 h period, the VX droplet spread increased in area from 132 to 163 mm^2 for the 1 µL droplets and from 166 to 303 mm^2 for the 3 µL droplets. The volumes of rain applied for the 1 and 3 µL agent droplets to yield 10 mm (0.39 in.) of rainfall were calculated based on the target area of the agent droplet spread at the specific sampling times after dissemination (Table 2).

After the 10 mm rain event was completed, the leaf was removed from the vial and placed onto a clean plastic Petri dish. Using forceps, a 2.5 × 2.5 cm swatch of VectraR QuanTex TX1080 wipe (Texwipe; Kernersville, NC), folded in half, was used to apply a one-pass wipe to the surface of the leaf. The wiping motion started at the stem end of the leaf

segment above the VX droplet application site, wiped through the VX application site, and ended at the tip of the leaf. A force of $16g$ ($\pm 2.9g$) was applied to the wipe during this process. The wipe was then immediately placed into 1000 µL of IPA for a minimum of 1 h to extract VX from the wipe.

Table 2. Volume of Rain Used To Produce a 10 mm Rain Event[a]

Time after Dissemination (h)	Area of 1 µL[b] Droplet Spread (mm^2)	Area of 3 µL[b] Droplet Spread (mm^2)	Rain Applied to 1 µL VX Droplet Target Area (mL)	Rain Applied to 3 µL VX Droplet Target Area (mL)
0.017	132	166	1.32	1.66
1	163	301	1.63	3.01
4	135	303	1.35	3.03

[a] Data from Simini et al., 2016.
[b] Based on the area of the VX droplet spread after dissemination.

Additional rainfall studies were conducted separately using 3 µL VX droplets that were equilibrated on the leaf for 0.017, 0.5, 1, 4, and 24 h. The individual contaminated leaves were removed from living plants, and each of these leaves was then subjected to multiple (10×) consecutive rain events of 100 µL (101.7 ± 0.3 µL) each, which represented individual light rainfalls that cumulatively produced a moderate rainfall (Hunsche et al., 2007; Llasat, 2001). The cumulative rainfall from each series of multiple 100 µL rain events was less than a single 10 mm (0.39 in.) rainfall, and their equivalency ranged from 30 to 60% of 10 mm. A calibrated 200 µL Gilson Pipetman pipette was used to apply the 100 µL rain events (raindrop size, 17.2 ± 1.8 µL). After the consecutive 100 µL rain events were completed, the leaf was removed from the collection vial, placed into a clean plastic Petri dish, and wiped in the manner described previously. However, after the conclusion of the multiple rainfalls onto these contaminated leaves, each leaf received 10 separate single-pass wipes, using a fresh, single layer for each wipe. Each wipe was immediately placed into a vial containing 1000 µL of IPA and extracted for a minimum of 1 h. These additional simulated rainfall experiments were conducted with a minimum of three and maximum of six replications.

2.5   Extractability of VX from Wipes

To determine the extractability of VX from wipes, a 2.5×2.5 cm^2 piece of wipe was placed into a 50 mL collection vial, and then a 3 µL droplet of neat VX was placed on the wipe. Within 15 s, 1000 µL of IPA was added to the vial, and it was capped. The wipe was extracted for a minimum of 1 h before subsamples of the IPA were removed for analysis. To determine the VX-absorbing efficiency of the wipe from a glass surface, we conducted comparative studies. A 3 µL droplet of VX was placed onto a clean glass disk (3.68 cm diameter $\times$ 0.071 cm thick) and allowed to equilibrate for 1 min. Using forceps, a wipe was drawn across the VX droplet (one pass) in the manner described in Section 2.4. The wipe was then placed into 1000 µL of IPA to extract the VX. This was followed with four additional separate, single-pass wipes, and then each of these wipes was also extracted in IPA. The glass disk was then placed in IPA and extracted for a minimum of 1 h before the IPA was analyzed.

Positive-control samples were obtained by placing a 3 µL VX droplet directly onto the glass disk, immediately placing this disk into IPA, and extracting the contaminated glass disk for a minimum of 1 h before analyzing the IPA. All extractability experiments were replicated in quadruplicate ($n = 4$).

2.6   VX Stability Studies

In this study, a simulated rain consisting of ASTM Type I water was allowed to equilibrate with the atmosphere, similar to natural rainwater, and was then used to displace VX from plant leaves. Results of previous reports have shown that the persistence of VX in aqueous solution varies depending on the properties of the solution, temperature, and concentration (Safety Data Sheet, 2015; Epstein et al., 1974).

In our studies, the wash-off from a leaf exposed to a single rain event (10 mm of rain) was collected into a vial containing enough IPA to produce a 1:1 mixture of IPA and rainwater. We hypothesized that if the wash-off samples were diluted 1:1 with IPA and analyzed within 24 h, the amount of degradation of VX by hydrolysis subsequent to sampling would be insignificant. To test our hypothesis, we conducted VX stability studies by transferring neat VX into a 1:1 mixture of IPA and rainwater leaf rinse (rainwater after rinsing a non-contaminated leaf) to yield a final VX stock concentration of 929.6 ng/µL. The stock was then immediately diluted using the 1:1 mixture of IPA and rainwater leaf rinse to yield additional VX concentrations of 93.9 and 9.3 ng/µL. Samples were removed from each VX concentration, placed into separate sample vials, and capped. This resulted in three 1 mL samples for each concentration to allow the analysis of replicates at each of four time intervals. The concentrations of VX in solution were analytically determined at 4, 24, 48, and 168 h after preparation.

The multiple rain events (100 µL per event) were collected into vials containing 1000 µL of IPA (final sample contained approximately 90% IPA). Stability studies were also conducted in triplicate using a mixture of 10% leaf water rinse and 90% IPA that contained a VX concentration of 46.5 ng/µL. The VX concentrations were subsequently determined at 0, 24, and 96 h.

A one-way analysis of variance (ANOVA) was used to determine whether there were significant differences (probability [p] ≤ 0.05) among VX concentrations across the time intervals used in these stability studies of VX in IPA–water mixtures. The Fisher's multiple comparison test was used to determine significant differences ($p \leq 0.05$) in VX concentrations between the individual time intervals.

2.7   Analytical Determination of VX

Quantitative analysis of VX was conducted using Agilent 6890 gas chromatography (GC; Agilent Technologies; Santa Clara, CA) equipped with a flame photometric detector. Quantification was achieved using an Agilent DB-5 fused silica column (30 m × 0.32 mm, 0.5 mm film thickness). The sample volumes of 1 µL were injected into the GC using an Agilent (7683B series) autosampler. Sample-inlet temperature was maintained at 225 °C, in splitless mode. The initial oven temperature was 80 °C with a temperature ramp rate of 45 to 300 °C. A nine-point calibration curve (0.014, 0.072, 0.14, 0.73, 1.45, 3.91, 5.81, 11.62,

and 23.82 ng/µL) was used to determine the VX concentration in the wash-off solution. The r^2 for the linear regression of the standard curve throughout these studies was 0.9995 ± 0.0003. The instrument limit of detection was 0.005 ng/µL, based on peak-to-peak background noise for this method.

Quantitative analytical determinations for low levels of VX and confirmation of GC results were conducted using high-performance liquid chromatography (HPLC) linked with tandem mass spectrometry (MS/MS) (Agilent 1260 liquid chromatograph triple-quadrupole mass spectrometer with MassHunter data acquisition and analysis software). The HPLC system was fitted with an Agilent Eclipse XDB-C_{18} column (5 µm, 4.6×150 mm). Sample injections were 1 µL. A 13 min separation method was used; the composition of mobile phase A was 0.1% formic acid (v/v) in H_2O, and mobile phase B was 0.1% formic acid (v/v) in methanol (MeOH). The gradient conditions used for HPLC separation are shown in Table 3.

Table 3. HPLC Gradient Table for VX Quantitation

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	99.9	0.1
2	99.9	0.1
7	5.0	95.0
8	5.0	95.0
11	99.9	0.1
13	99.9	0.1

The HPLC column eluent was delivered to an electrospray ionization source that was maintained in positive ion mode. MS/MS discrimination was performed via the multiple reaction monitoring (MRM) technique that incorporated an isotope dilution (VX-d_5) and used the following three mass transitions: VX quantitation, VX confirmation, and VX-d_5 internal standard (Table 4).

Table 4. MRM Mass Transitions

Analyte	Precursor Mass (Da)	Product Mass (Da)
VX-d_5 internal standard	273	128
VX quantitation	268	128
VX qualifier	268	86

Calibration was conducted by plotting the relative responses of VX and VX-d_5 as a function of concentration. An 11-point calibration curve (5.0–5000 pg/µL of VX, each with 50 pg/µL of VX-d_5) was used to construct a linear calibration curve ($1/x$ weighting). All analyzed samples were prepared to contain 50 pg/µL of VX-d_5 as the internal standard, and reported VX concentrations were calculated by applying the equation of fit and dilution factors, as applicable. The instrument limit of detection was 0.5 pg/µL, as based on the peak-to-peak background noise for this method.

3. RESULTS

3.1 Extractability of VX from Wipes and Glass

In the wipe extractability experiments, we applied VX to the wipe and then allowed the VX to equilibrate for 15 s before extracting the contaminated wipe in IPA. After extracting the wipe for 1 h in IPA, we recovered 99.5% ($\pm$4.7%) of the VX that had been added to the wipe.

Compared with IPA positive controls, we recovered 96.1% ($\pm$3.1%) of the VX applied to glass from the first wipe. We recovered a cumulative average of an additional 1.4% ($\pm$0.4%) of the VX from the additional four wipes that were used in subsequent consecutive wipes of glass. Following the wiping procedures, an additional 0.8% ($\pm$0.1%) residual amount of the initial VX was recovered from the wiped glass disks by direct extraction with IPA. In total, 98.3% of the initial VX that was placed on the glass disks was recovered.

3.2 VX Stability Studies

When significant differences ($p \leq 0.05$) were found in the VX concentrations among the respective time intervals used in the stability studies of VX in IPA–water mixtures using ANOVA, then the Fisher's multiple comparison test was used to determine the significant differences ($p \leq 0.05$) in VX concentrations between time intervals.

When VX was placed in a 1:1 mixture of IPA and rainwater leaf rinse, there was no significant decline in VX concentration up to 48 h after preparation ($p > 0.05$). However, after 168 h, there was an average decline of $27.1 \pm 11\%$ in VX concentration in the 1:1 IPA-to-rainwater leaf rinse, which was a significantly different concentration when compared with the VX concentration after 4 h ($p \leq 0.05$). When VX was in a mixture consisting of 10% rainwater leaf rinse and 90% IPA, there was no significant decline in VX concentration up to 96 h ($p > 0.05$). These results confirmed that analytical concentrations of VX determined within 48 h of producing rainwater wash-off samples (in corresponding mixtures containing IPA) are accurate well within statistical boundaries. All rainwater wash-off samples containing IPA and VX were analyzed within 24 h of being produced for all definitive experiments described here.

3.3 Wash-Off Coefficients

The k_w values from 10 mm rainfall events were determined in separate experiments using 1 and 3 μL VX droplets at 0.0172, 1, and 4 h after dissemination. A single 10 mm rain event at 0.017 h after dissemination washed off 95% of the 1 μL VX droplet and 83% of the 3 μL VX droplet from the contaminated grass leaf. After 1 h, only 0.03 to 0.5% of the respective 1 and 3 μL VX droplets were washed off the leaf by a 10 mm rainfall (Tables 5 and 6). The k_w values for the 1 and 3 μL droplets at 0.017 h after dissemination were 0.095 and 0.083 mm^{-1}, respectively. The k_w values for the 1 and 3 μL VX droplets at 1 and 4 h after dissemination were approximately 3 orders of magnitude less than these respective values at 0.017 h (Table 5). A single, one-pass wipe was conducted after the 10 mm rain event to determine the amount of dislodgeable residual agent remaining on grass leaves after rainfall. The

amounts of residual VX recoverable by one single-pass wipe of the leaf after a 10 mm rainfall at 0.017 h after dissemination were 2.7 and 1.7% for the 1 and 3 µL droplets, respectively. After a 10 mm rainfall at 1 and 4 h after dissemination, the amounts of VX recovered from a single wipe were orders of magnitude less than those at 0.017 h after dissemination for both the 1 and 3 µL VX droplets (Tables 5 and 6).

Table 5. Single 10 mm Rain Event Applied to
a Grass Leaf Contaminated with 1 µL of VX ($n = 3$)

Contact Time on Leaf (h)	Agent Spread Area (mm^2)[a]	Volume of Rain Applied (mL)[b]	Recovery		k_w (mm^{-1})
			% by Rain (±SD)	% by Wipe[c] (±SD)	
0.017	132	1.32	95.2 (8.7)	2.7 (2.6)	0.0952
1	163	1.63	0.03 (0.03)	0.02 (0.01)	3×10^{-5}
4	135	1.35	0.02 (0.002)	0.006 (0.003)	2×10^{-5}

[a]Data from Simini et al. (2016).
[b]Volume of 10 mm rain applied was based on surface area of the drop spread at reference time after dissemination.
[c]Wipe recovery was conducted after the 10 mm rain event.
SD: standard deviation (shown in parentheses).

Table 6. Single 10 mm Rain Event Applied to
a Grass Leaf Contaminated with 3 µL of VX ($n = 3$)

Contact Time on Leaf (h)	Agent Spread Area (mm^2)[a]	Volume of Rain Applied (mL)[b]	Recovery		k_w (mm^{-1})
			% by Rain (±SD)	% by Wipe[c] (±SD)	
0.017	166	1.66	83.0 (5.1)	1.7 (0.9)	0.083
1	301	3.01	0.5 (0.7)	0.08 (0.07)	5×10^{-4}
4	303	3.03	0.4 (0.2)	0.3 (0.2)	4×10^{-4}

[a]Data from Simini et al., 2016.
[b]Volume of 10 mm rain applied was based on surface area of the drop spread at reference time after dissemination.
[c]Wipe recovery was conducted after the 10 mm rain event.
SD: standard deviation (shown in parentheses).

3.4   Multiple Rain and Leaf Wipe Events

Grass leaves, each contaminated with a single 3 µL droplet of VX, were subjected to multiple (10×) 100 µL rain events at 0.017, 0.5, 1, 4, and 24 h after dissemination. The total cumulative amount of VX washed from the leaf surface at 0.017 h after dissemination was 2.266 mg (Table 7), which represents approximately 75.3% of the VX originally added to the surface of the leaf. At 0.5 h after dissemination, the amount of VX removed from the leaf surface by the consecutive rainfall events was approximately 1 order of magnitude less than that removed at 0.017 h after dissemination (Table 7). The cumulative amount of VX recovered from rain events at 0.017 h after dissemination was significantly greater ($p < 0.05$) than the corresponding recoveries at 0.5, 1, 4, and 24 h after dissemination. There was no significant difference ($p < 0.05$) in the cumulative amount of VX recovered from rain events across the 0.5, 1, 4, and 24 h post-dissemination time points. The total cumulative amount of VX recovered from surface wipes after 10 consecutive 100 µL rain events at 0.5 h after dissemination was

significantly greater ($p < 0.05$) than the recovery from surface wipes at 0.017, 1, 4, and 24 h after dissemination. There were no significant differences ($p > 0.05$) in the cumulative amounts of VX recovered from surface wipes across the 0.017, 1, 4, and 24 h post-dissemination time points. In Figure 3, the cumulative mass of VX recovered from rain events was plotted to show incremental recovery in the wash-off of VX from multiple rain events. The estimated human percutaneous lethal dose for 50% of the population (LD_{50}) level of 3 mg/70 kg soldier (Safety Data Sheet, 2015) is included as a reference point. The cumulative amount of VX recovered from rainwater after consecutive 100 µL rain events on a grass leaf contaminated with a single 3 µL VX droplet was within the same order of magnitude as the estimated human percutaneous LD_{50}.

Table 7. Total VX Recovery from Multiple Rain Events (10×100 µL)
and Surface Wipes from Contaminated Grass Leaves

Time after Dissemination (h)	VX Recovery from Multiple Rain Events (mg) ($\pm$SD)	VX Recovery from Multiple Surface Wipes (mg) ($\pm$SD)	Total VX Recovery (%)
0.017	2.266 (0.493)	0.024 (0.013)	76.1
0.5	0.385 (0.268)	0.094 (0.050)	15.9
1	0.030 (0.039)	0.009 (0.006)	1.29
4	0.012 (0.008)	0.002 (0.0003)	0.46
24	0.065 (0.039)	0.019 (0.005)	2.79

SD: standard deviation (shown in parentheses).

The cumulative mass of VX recovered from wipes after multiple rain events is shown in Figure 4. The cumulative recovery of VX from wipes at 0.5 h after dissemination was significantly greater ($p < 0.05$) than that at the 0.017, 1, 4, and 24 h post-dissemination time points. The cumulative amount of VX recovered from surface wipes applied to a leaf that was contaminated with a single 3 µL droplet was orders of magnitude below the estimated human percutaneous LD_{50} of 3 mg.

*The amount of agent washed off the leaf at 0.017 h was significantly greater ($p < 0.05$) than that at any of the 0.5, 1, 4, and 24 h time points after dissemination.
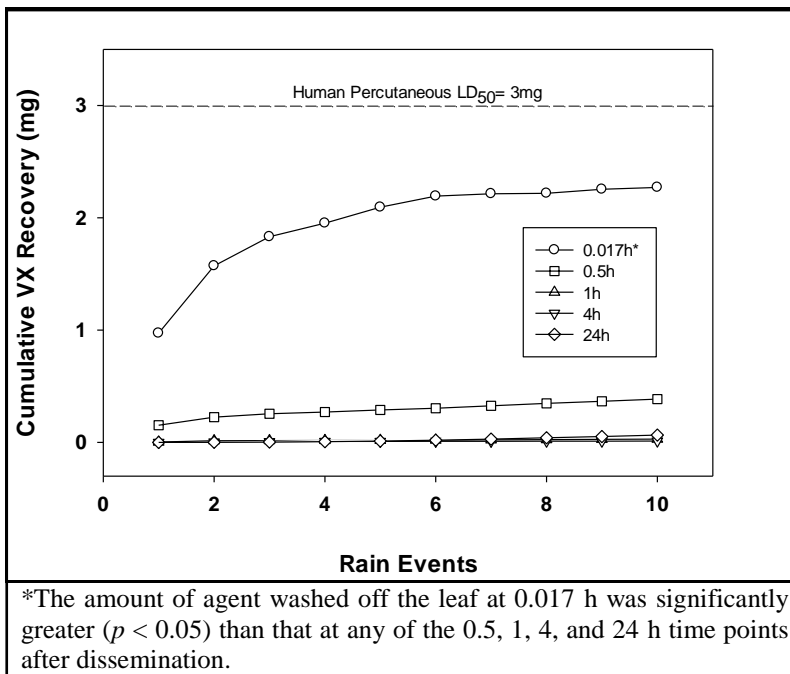
Figure 3. Rain induced wash-off from VX-contaminated grass leaves. Mass of VX recovered after multiple (10×) rain events (100 µL each) from leaves that were contaminated with a single 3 µL droplet of VX. The amount of VX in this 3 µL droplet, disseminated onto each leaf, corresponded to the estimated human percutaneous LD_{50} (Safety Data Sheet, 2015).

*The total amount of agent recovered from wipes at time point 0.5 h after dissemination was significantly greater ($p < 0.05$) than the respective mass of VX wiped from leaves at each of the 0.017, 1, 4, and 24 h post-dissemination time points.
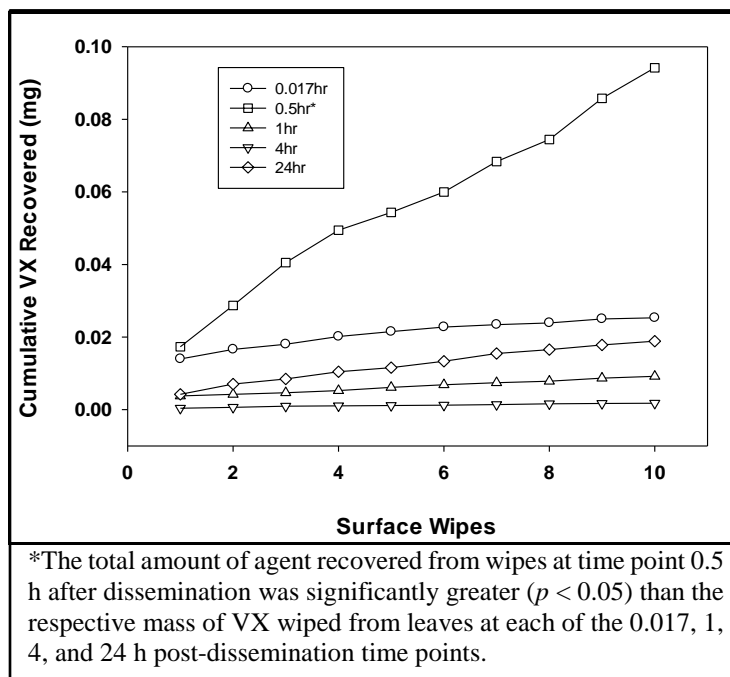
Figure 4. Residual VX recovery from surface wipes. Cumulative mass of VX recovered from multiple wipes (10 separate, consecutive wipe events) after VX-contaminated leaves were subjected to 10 consecutive 100 µL rain events.

4.   DISCUSSION

We determined the wash-off coefficient (k_w) in separate experiments for 1 and 3 µL droplets of VX on the grass species *E. crus-galli* using one 10 mm moderate rain event (Hunsche et al., 2007; Llasat, 2001) at several time points after dissemination. The k_w results are critical data points needed for the PEARL model to predict the fate and transformation of compounds in a plant and soil system. Incorporation of the data collected in this study into the PEARL model will be discussed in subsequent reports. However, in producing k_w values for the model, we observed several key characteristics that may be helpful for commanders of Warfighters in battlefield conditions when Go/No-Go decisions are critical under stressful situations.

At 1 h after dissemination onto E. *crus-galli* leaves, VX becomes highly rainfast. (The designations are as follows: H is highly rainfast with ≤30% washoff, M is moderately rainfast with ≤50% washoff, and L is low rainfast with ≤70% washoff [Wise, 2015].) In other words, 83% (3 µL droplets) to 95% (1 µL droplets) of the VX was washed off grass leaves when a single 10 mm (0.39 in.) moderate rain event was applied at 0.017 h (1 min) after VX dissemination. At 1 h after dissemination, <1% of VX (1 or 3 µL droplets) was removed from grass leaves by rain displacement that was followed by a single surface wipe.

We also conducted experiments on grass leaves that were each contaminated with a single 3 µL droplet of VX using multiple 100 µL rain events. Individually, these rain events represented light rainfalls, and cumulatively, they produced a moderate rainfall that ranged from 30 to 60% of 10 mm (Llasat, 2001; Hunsche et al., 2007). The cumulative amount of VX washed off a grass leaf from 10 rain events at 0.017 h after dissemination was approximately 75.3% (2.266 mg of VX). The cumulative amount of CWA recovered from 10 consecutive surface wipes after the series of rain events was approximately 0.8% (0.024 mg of VX), which resulted in 76.1% VX recovery of the 3 µL droplets. The total amount of VX recovered from both the rain and wipe events at 1 h after dissemination was 1.3% (0.039 mg of VX). In our research, the average proportion of 3 µL VX droplets on grass removed by moderate rainfall (single and multiple rain events) at 0.017 h (1 min) after dissemination of VX was approximately 79.2%, which corresponds with field studies conducted decades ago, wherein grass sod was contaminated with VX then rinsed with water within minutes after dissemination to remove approximately 66% of the VX applied.

These results indicate that moderate rainfall, occurring within 1 min after dissemination, should reduce the potential amount of VX immediately available for direct contact transfer of VX from grass leaf surfaces to the soldier. However, the wash-off from that foliage would contain high concentrations of VX, which poses additional types of hazards. Depending upon the rainwater pH and buffering capacity of the soil, the potential contact hazard could remain with contaminated rainwater and reside within soil.

5.   CONCLUSION

The time after dissemination of VX on grass-like species (e.g., *E. crus-galli*) can be a decisive factor for reducing the threat of hazard to the Warfighter. Within minutes after dissemination of VX onto grass, the majority of the VX (approximately 75–95%) can be removed by wash-off from a moderate rainfall ($\geq$0.6 mm) or with excess water. Based on these results, wash-off can reduce the potential amount of VX immediately available for direct exposure of the soldier to VX on the surface of contaminated foliage; however, the hazard is then transferred to water and soil.

When the time interval after dissemination of VX is known to be at least 1 h, the proportion of VX remaining immediately accessible on the external surface of grass (i.e., as estimated by measured VX wash-off) is greatly reduced to approximately 1% of that disseminated (based on results for 1 or 3 µL droplets of VX with recoveries of 0.8278 ± 0.0386 mg or 3.010 ± 0.137 mg of VX, respectively).

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ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance
CAS	Chemical Abstract Service
CASARM	Chemical Agent Standard Analytical Reference Material
CWA	chemical warfare agent
GC	gas chromatography
HPLC	high-performance liquid chromatography
IPA	isopropyl alcohol
k_w	wash-off coefficient
LD_{50}	lethal dose for 50% of the population
MRM	multiple reaction monitoring
MS/MS	tandem mass spectrometry
OP	organophosphate
p	probability
PEARL	Pesticide Emission Assessment at Regional and Local scales
r^2	coefficient of determination
SD	standard deviation
VX	O-ethyl-S-(2-diisopropylaminoethyl) methyl phosphonothiolate
VX-d_5	VX isotope internal standard

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