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Environmental Fate of Dexmedetomidine in Soil and Relevant Waters

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

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14. ABSTRACT:
The objectives of this work were to assess the stability and persistence of dexmedetomidine in four soil types and water collected from eight different sources and to determine the distribution of dexmedetomidine when it is in contact with soil and water for as long as 12 weeks. Soil samples in contact with 0.01 M calcium chloride solution were spiked with a known amount of dexmedetomidine in solution and then were extracted after several contact time periods to track compound recovery and distribution. The water samples were spiked with a known amount of dexmedetomidine, and aliquots were taken for analysis after multiple time periods. The results showed that the amount of dexmedetomidine recovered from soil extracts accounted for 28–98% of the spike, and smaller amounts were recovered from the supernatant. More than 80% of dexmedetomidine was recovered from each of the water samples during this 12 week trial. These results indicate that dexmedetomidine is relatively stable in water and moist soils. The current data also suggest that dexmedetomidine is persistent with some mobility into groundwater, depending on soil type.

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PREFACE

The work described in this report was authorized by the Defense Threat Reduction Agency, Joint Science and Technology Office (DTRA JSTO; Fort Belvoir, VA) under project no. CB10789. The work began in June 2021 and was completed in November 2021.

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1. INTRODUCTION

The discharge of pharmaceuticals into the aquatic environment occurs worldwide, as reported by aus der Beek and co-workers.[1] Those authors performed a comprehensive literature review, which showed that pharmaceuticals and their metabolites have been detected in 71 countries. In total, 631 different human and veterinary pharmaceuticals were quantified above the limit of detection. The authors concluded that globally, the major contamination source is urban wastewater discharge; and locally, emissions from the pharmaceutical industry, agriculture, and aquaculture can be very important.

Medetomidine, a white crystalline, solid synthetic drug, is a new alternative maritime antifoulant compound that effectively prevents barnacle settlement.[2] A potent and highly specific α_2-adrenoceptor agonist, medetomidine is marketed as a racemic mixture of two stereoisomers, dextro-medetomidine (referred to as dexmedetomidine in this study) and levo-medetomidine. The levo isomer has minimal pharmacological activity and only shows mild sedative and analgesic properties at high doses. The beneficial effects of dexmedetomidine are the same as those of other α_2-agonists and include reliable sedation, analgesia, muscle relaxation, and anxiolysis, as well as a decrease in the anesthetic requirements of injectable and inhalant agents (anesthetic sparing).[3] Medetomidine is not a controlled substance; therefore, its use does not require extensive record keeping. The structure of dexmedetomidine is provided in Figure 1.

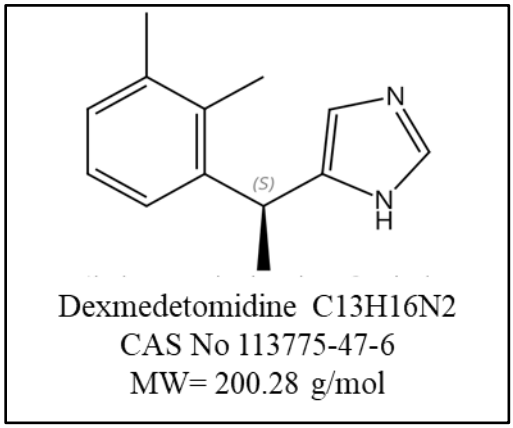

Figure 1. Structure, formula, and molecular weight of dexmedetomidine.
CAS, Chemical Abstracts Service; MW, molecular weight.

Previous work investigated the soil–water distribution behavior of racemic medetomidine.[4] The focus of this work is to elucidate the chemical and physical interactions between dexmedetomidine, soil, and water to advance our understanding of how dexmedetomidine behaves in the environment. This study also explored differences in the stability and soil interactions between the two medetomidine stereoisomers.

The soil partitioning coefficient constant (K_d) is used to describe the distribution of chemicals in contact with soil and water and is typically related to the organic content of the soil. K_d can be calculated from the pesticide–soil organic partition coefficient (K_{oc}).[5] Previous studies have concluded that adsorption of pesticides increases with pH and organic matter content but decreases with ionic strength.[6]

The partitioning behavior of a pesticide or chemical agent determines in which medium it will concentrate: water or soil. Partitioning coefficients are used in predictive models to help researchers understand the behavior of a compound in a specific environment. For the predictive models selected as the most useful (namely, Pearl and GeoPearl), the soil–organic matter partition coefficient (K_{om}) is of particular interest. This value can be accurately estimated from the octanol–water partition coefficient (K_{ow}), which is relatively easy to measure. Depending on the agent, additional variations in the partitioning coefficient or determinations of additional coefficients may be necessary. These include a pH-dependent K_{om} and the Freundlich coefficient. The Freundlich coefficient is necessary when sorption of the agent is dependent on soil components other than organic matter, such as clay or other soil colloids. Determining the Freundlich coefficient is very time-consuming. A screening coefficient can be measured in advance to determine whether the Freundlich coefficient must be included in the parameter list for each compound of interest. The screening coefficient is K_d, which is calculated by measuring the water- and soil-phase concentrations of the analyte in the presence of four different soils. The soils vary in pH, clay content, and organic carbon (oc) content. A high K_d value indicates that an agent is strongly adsorbed to the soil and less likely to leach into the groundwater. The K_d value can also be used to determine the K_{oc} by using the relationship $K_d = K_{oc} \times f_{oc}$, where f_{oc} is the fraction of oc.[7]

In this study, we observed the stability and extractability of dexmedetomidine for periods of up to 12 weeks in 4 different soils and 8 different water sources collected from various sites in the continental United States.

2. SOIL ANALYSIS

2.1 Reagents and Chemicals

All commercial materials were used as received. The following reagents and chemicals were used during testing:

- acetonitrile and methanol (Sigma-Aldrich; St. Louis, MO), high-performance liquid chromatography (HPLC) grade with ≥99.9% purity;
- in-house 16 MΩ water for HPLC mobile phase;
- sodium sulfate, sodium chloride, trisodium citrate dihydrate (TRIS), and disodium hydrogen citrate sesquihydrate, American Chemical Society grade with ≥99% purity (Sigma-Aldrich);
- calcium chloride (Acros Organics; Pittsburgh, PA), ≥99% purity;

- 15 mL centrifuge tubes (Restek; Bellefonte, PA), used for dispersive solid-phase extraction (dSPE) cleanup for 6 mL extract, Q370 for quick, easy, cheap, effective, rugged, safe (QuEChERS) extract cleanup; and
- dexmedetomidine hydrochloride, ≥99% purity (BOC Sciences; Shirley, NY).

2.2 Soil Collecting and Processing

The soils used during this study were collected from the A horizon, which is the topmost portion of the soil horizon, also known as the topsoil. Leafy matter was removed from the sample location, and a few inches down into the soil were removed and inspected to confirm absence of boundary horizon change. A circle was then dug outward. If a well-developed O horizon was found, it was incorporated into the sample. The samples were air-dried, crushed, and sieved using a 2 mm standard sieve (ASTM International; West Conshohocken, PA). All sieved samples were stored in plastic-capped containers at room temperature, and remaining moisture levels were measured before each test was started.

2.3 Soil Experiments

The procedures used during this portion of the study were based on Organisation for Economic Co-operation and Development (OECD; Paris, France) Test 106.[7] This guideline contains recommendations for determining the persistence of a chemical in soil and suggests the testing of different naturally occurring soils with varying pH balances, clay contents, and organic matter contents. The following four soils were identified and collected for detailed testing:

- Sassafras sandy loam (SSL),
- Pennsylvania Ernest silt loam (PEL),
- North Dakota loam (NDL), and
- Utah Timpie loam (UTL).

The soils were well mixed, and triplicate subsamples of each selected soil were analyzed by the Pennsylvania State University Agricultural Analytical Services Laboratory (University Park, PA) for texture, pH, and organic content. The soil characterization results are presented in Table 1.

Table 1. Soil Information

Soil Name and Type	Source Location	Content (%)			Textural Class	pH	oc (%)
		Sand	Silt	Clay			
SSL	Maryland	53	30	17	Sandy loam	4.5	1.1
PEL	Pennsylvania	34	45	21	Loam	4.5	3.9
NDL	North Dakota	28	49	22	Loam	7.6	3.1
UTL	Utah	27	47	26	Loam	8.4	1.4

The SSL and NDL soils had been collected previously for other projects. The remaining two soil types were collected by removing their A horizons, which typically consisted of ~13 mm of the topmost portion of the soil horizon. If an O horizon was present, the nonfibrous portion of the O horizon was collected and mixed with the A horizon. The OECD guideline suggests using 2–50 g of soil for testing. Because of the hazardous nature of the compound used in our work and the need to execute experiments safely and efficiently, 2 g of soil were used in each of the 72 sample vials and 28 negative controls during our experiments (the minimum amount specified in the guideline). No soil was used for the 28 positive controls. The 2 g of soil, corrected for remaining moisture content in calculations and reported as dry weight, were reconstituted with 2 mL of 0.01 M calcium chloride solution on the day before the dexmedetomidine spikes were performed. Vials of soil and solution (Figure 2) were left overnight at room temperature to fully moisten the soils.

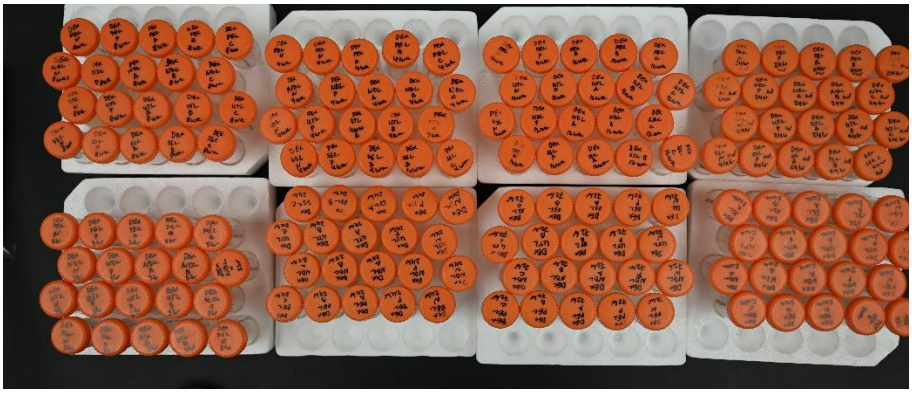

Figure 2. Dexmedetomidine in soil sample sets.

A set of samples was prepared for each soil type and time period. Each sample set was prepared in triplicate and also contained a positive and a negative control. Each negative control contained the selected soil type and 0.01 M calcium chloride solution but no dexmedetomidine. The no-soil positive-control samples were prepared in calcium chloride solution only for each sample set, maintaining the same sacrificial time schedule used for the soil samples. The 0.01 M calcium chloride solutions (2 mL) were spiked with dexmedetomidine by adding 10 µL of a 1000 µg/mL solution, so that the dexmedetomidine concentration was 5 µg/mL for each positive control.

Samples were prepared for sacrificial collection and extraction of the dexmedetomidine at time points of 3, 24, and 72 h and 2, 4, 8, and 12 weeks. A total of 140 vials (5 vials per sample set × 7 exposure times × 4 soil types) were used in this portion of the work. At the time of data measurement, the samples selected for analysis were centrifuged to separate the soil from the supernatant, and the liquid phase was collected, filtered, and analyzed for dexmedetomidine using a Xevo G2-XS quadrupole time-of-flight (QToF) mass spectrometer (Waters; Milford, MA).

Dexmedetomidine was extracted from the soil phase using a QuEChERS method[8] modified to include the addition of a TRIS buffer (pH 8.3) prior to extraction. The buffer

increased the pH of the soil and dexmedetomidine solution to 8.0, thereby optimizing the release of analyte from the organic matter component of the soil so that it could be extracted more efficiently. This approach has been successfully applied in similar soil work with a variety of compounds of concern, including pharmaceutical agents.[9–12]

At each time point, the soil mixtures were centrifuged, and the supernatant was filtered using a 13 mm, 0.45 µm hydrophilic polyvinylidene fluoride membrane syringe filter (Pall Life Sciences; Port Washington, NY; part number 4545). After the supernatant was removed, 9 mL of TRIS buffer at pH 8.3 was added to the soil and vortexed for 30 s. Acetonitrile (10 mL) was then added, and the samples were sonicated for 30 min. Next, 4 g of magnesium sulfate was added with 1 g of sodium chloride, 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate. The mixture was vortexed for 30 s and then centrifuged for 5 min at 3500 rpm in an Eppendorf 5804 centrifuge (Hamburg, Germany). The QuEChERS kit was purchased from VWR International (Radnor, PA). It contained Q-sep QuEChERS dSPE tubes for extract cleanup (Restek original unbuffered; European EN 15662; VWR part number 10057-974). A dSPE cleanup was carried out by adding the supernatant volume (approximately 6 mL) to a 15 mL centrifuge tube containing 1.5 g of magnesium sulfate and 0.250 g of primary–secondary amine and vortexing the tube contents for 30 s. Afterward, the tube was centrifuged at 3500 rpm for 5 min. All data were corrected for dilution, and recovery for each sample was based on the amount of dexmedetomidine found in the extraction samples at each time point.

2.4 Sample Analysis

Dexmedetomidine samples were analyzed using the Xevo G2-XS QToF spectrometer. Separation of the compound of interest was carried out using an Acquity HPLC system (consisting of a vacuum degasser, autosampler, and binary pump; Waters) equipped with a reverse-phase BEH C18 50 × 2.1 mm column with a particle size of 1.7 µm (Waters). Column temperature was maintained at 40 ºC. Mobile phase A was 0.1% formic acid in acetonitrile. Mobile phase B was 0.01% ammonium formate and 0.01% formic acid in 1 L of water. The pump program was isocratic 40% A and 60% B; the flow rate was kept constant at 0.4 mL/min. The total run time was 2 min, and the injection volume was 2 µL.

The HPLC system was coupled with a Waters Xevo G2-XS QToF equipped with an electrospray ionization (ESI) interface and Mass Lynx software (Version 4.2). The QToF spectrometer was operated in a positive ESI mode. Data acquisition was performed in MS^e scan (60–600 Da) mode. Capillary voltage was 2.0 kV; nitrogen was used as the spray gas. The source temperature was set at 150 ºC. The cone voltage was 40 V.

The analytical system was calibrated prior to each series of measurements using standards prepared from stock solutions on the day of each analysis. Two stock solutions at 1 mg/mL concentration in acetonitrile were prepared and analyzed against each other for accuracy. A six-point calibration curve in the 0.01–0.5 µg/mL range was prepared using one of the stock solutions. A calibration check sample was prepared from the second stock solution. Results obtained from these standards agreed to within 5%. Aqueous-phase samples, positive controls, and extracted soil samples were diluted with acetonitrile as needed to keep the experimental concentrations within the calibration range.

3. WATER ANALYSIS

In addition to determining the stability of dexmedetomidine in soil, we also determined dexmedetomidine stability in eight water sources at two different temperature settings (22 ± 1 and 45 ± 1 °C). Samples are shown in Figure 3.

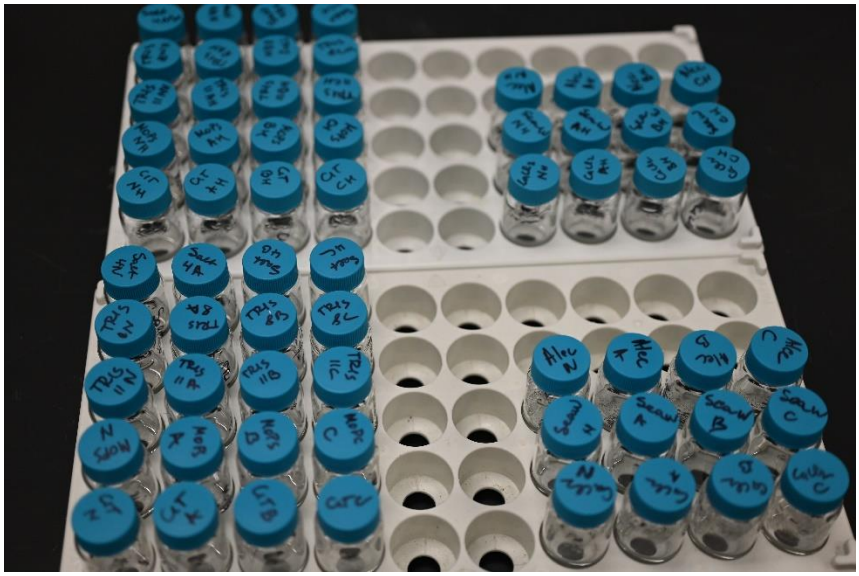

Figure 3. Dexmedetomidine in water sample sets.

3.1 Water Sources

Water samples were obtained from the following locations:

- Groundwater was collected on 15 July 2021 (initial pH 4.5) from the Anita C. Leight Estuary Center (ALEC; Harford County, MD).
- 0.1 M citrate buffer (pH 4.5) was prepared in-house.
- 1 M TRIS buffer (pH 8.6) was prepared in-house.
- 1 M Trizma base (pH 10.9) was prepared in-house (Sigma-Aldrich; lot number SLBX2689).

- 0.2 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0) was prepared in-house.
- Sea salt 4 was prepared in-house by adding 4 g of NaCl to 100 mL of deionized water (pH 6.14). (Note: This concentration was selected to simulate ocean water.)
- 0.01 M calcium chloride solution (initial pH 8.29) was prepared in-house.
- Seawater (pH 8.11) was acquired from Sigma-Aldrich (lot number SLBQ5359V).

3.2 Water Sample Preparation

Samples (20 mL) of each water type were added to separate glass vials. Each vial, except the negative control for each water type, was then spiked with dexmedetomidine by adding 20 µL of a 1000 µg/mL stock solution, so that the starting concentration in each vial was 1 µg/mL. Samples from each water type were prepared in triplicate, and a negative-control sample was prepared for each type. The samples were stored at 22 ± 1 °C over the course of the 12 week experimental period. A replicate set of the aforementioned samples was kept in the incubator at 45 ± 1 °C. After each designated time period, 100 µL of solution from each sample set was removed and diluted to a volume of 1000 µL. The diluted samples were analyzed using the method described in Section 2.4. Samples were analyzed at 24 h; 5 days; and 4, 8, and 12 weeks after preparation.

4. RESULTS AND DISCUSSION

4.1 Dexmedetomidine in Soil

The data describing dexmedetomidine recovery after soil contact are listed in Table 2 and shown graphically in Figure 4. Total recovery varied between about 28 and 98% after 12 weeks of exposure. Those data suggest long-term environmental stability of dexmedetomidine that is almost identical to the stability of medetomidine as described in technical report DEVCOM CBC-TR-1762.[4] Soil pH does not appear to play a role in dexmedetomidine sorption, given that soils with different pH values (i.e., UTL [pH 8.4] and SSL [pH 4.5] or NDL [pH 7.7] and PEL [pH 4.5]) showed similar sorption for dexmedetomidine.

There appears to be a correlation between oc content and dexmedetomidine sorption. Soils with higher organic contents (i.e., for NDL, oc was 3.1% and for PEL, oc was 3.9%) had lower recoveries compared with soils with lower organic contents (i.e., for UTL, oc was 1.4% and for SSL, oc was 1.1%).

Table 2. Dexmedetomidine Recovery from Soils*

Time (weeks)	Recovery (%)							
	SSL	SD	UTL	SD	NDL	SD	PEL	SD
0.02	86	3	94	0	80	4	75	1
0.14	83	1	87	2	80	1	73	2
0.43	96	3	98	2	81	2	83	1
2	83	2	89	1	61	5	53	3
4	75	1	76	3	52	3	51	2
8	61	1	61	1	45	1	46	4
12	51	2	50	7	28	4	37	3

*Values are averages of three measurements.
SD, standard deviation.

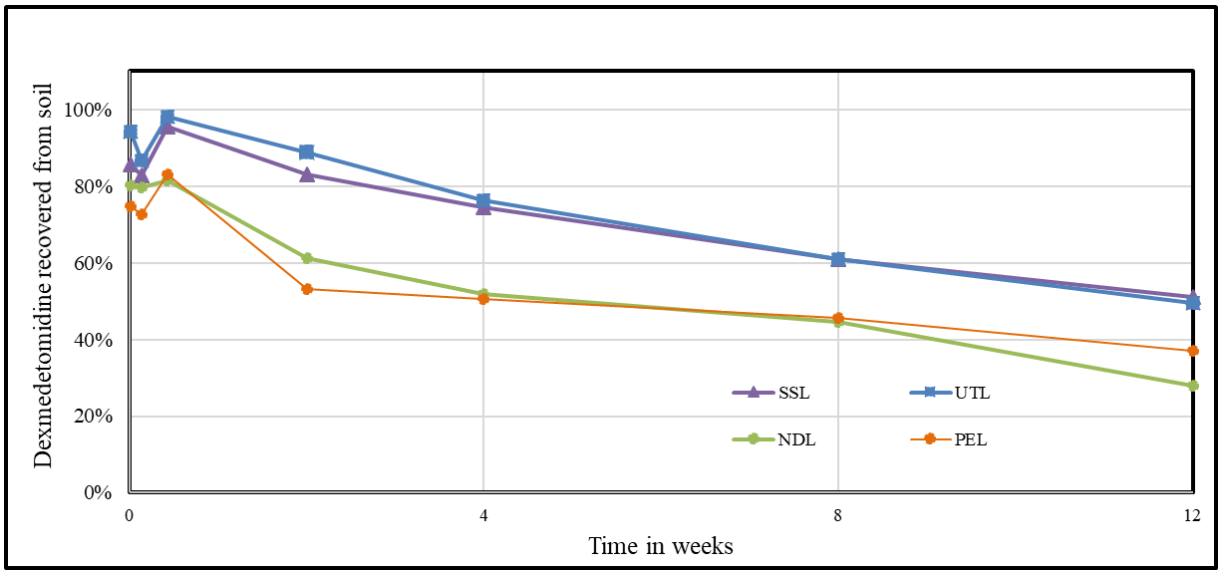

Figure 4. Dexmedetomidine recovery from soil.

Dexmedetomidine was recovered from the supernatant throughout the experimental period, including the week 12 samples. Recovery data are listed in Table 3 and shown graphically in Figure 5.

With the exception of the SSL soil, dexmedetomidine recovery from the supernatant was generally in the 0–3% range for all exposure times. SSL recovery was 16% immediately after exposure and decreased gradually to 3% during the 12 week exposure.

Table 3. Dexmedetomidine Recovery from Supernatant*

Time (weeks)	**Recovery (%)**							
	SSL	**SD**	**UTL**	**SD**	**NDL**	**SD**	**PEL**	**SD**
0.02	16	0	2	0	1	0	5	0
0.14	9	1	1	1	0	0	3	0
0.43	9	1	2	0	1	0	3	0
2	6	0	1	0	0	0	1	0
4	5	0	0	0	0	0	1	0
8	3	0	0	0	0	0	0	0
12	3	1	0	0	0	0	1	1

*Values are averages of three measurements.

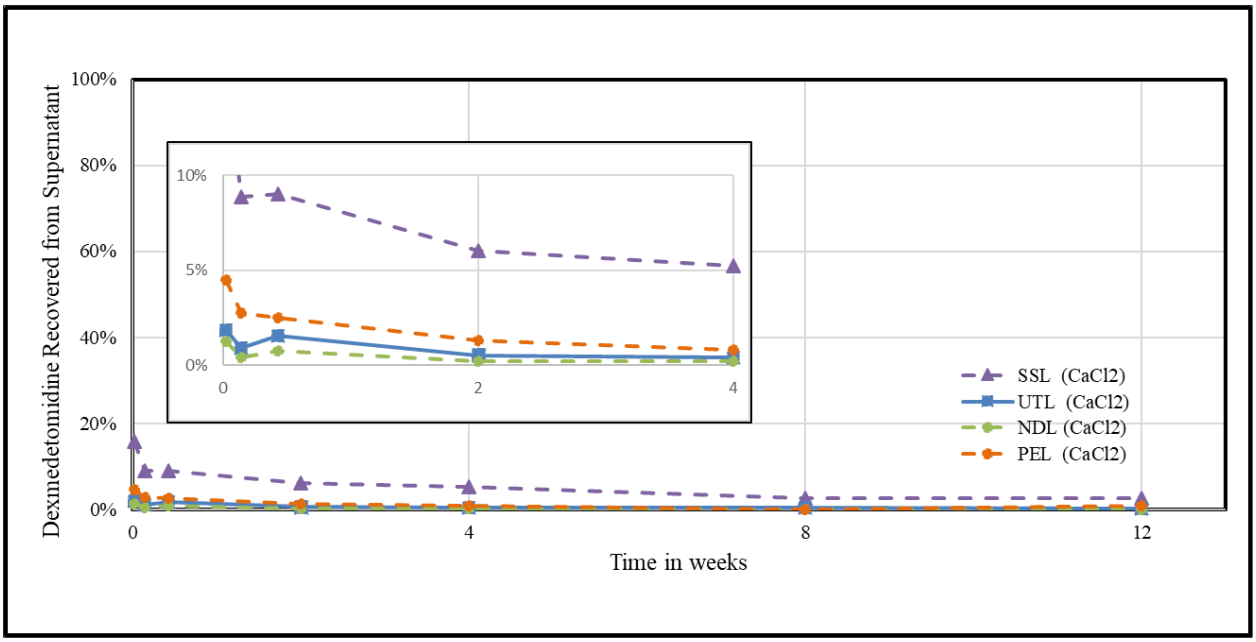

Figure 5. Dexmedetomidine recovery from supernatant.

Table 4 presents the total dexmedetomidine recovery from the soil and the aqueous supernatant. Figures 6, 7, 8, and 9 demonstrate the ratio of the dexmedetomidine recovered from soil to that recovered from the aqueous calcium chloride supernatant for SSL, UTL, NDL, and PEL, respectively. Total dexmedetomidine recovery varied between 28 and 100%, emphasizing the point that dexmedetomidine is recoverable from the soil for much longer than 12 weeks.

Table 4. Total Dexmedetomidine Recovery from Soil and Supernatant*

Time (weeks)	**Total Recovery (%)**			
	SSL	**UTL**	**NDL**	**PEL**
0.02	101	96	81	79
0.14	92	88	80	75
0.43	105	100	82	86
2	89	89	62	54
4	80	77	52	51
8	63	61	45	46
12	54	50	28	38

*Values are averages of three measurements.

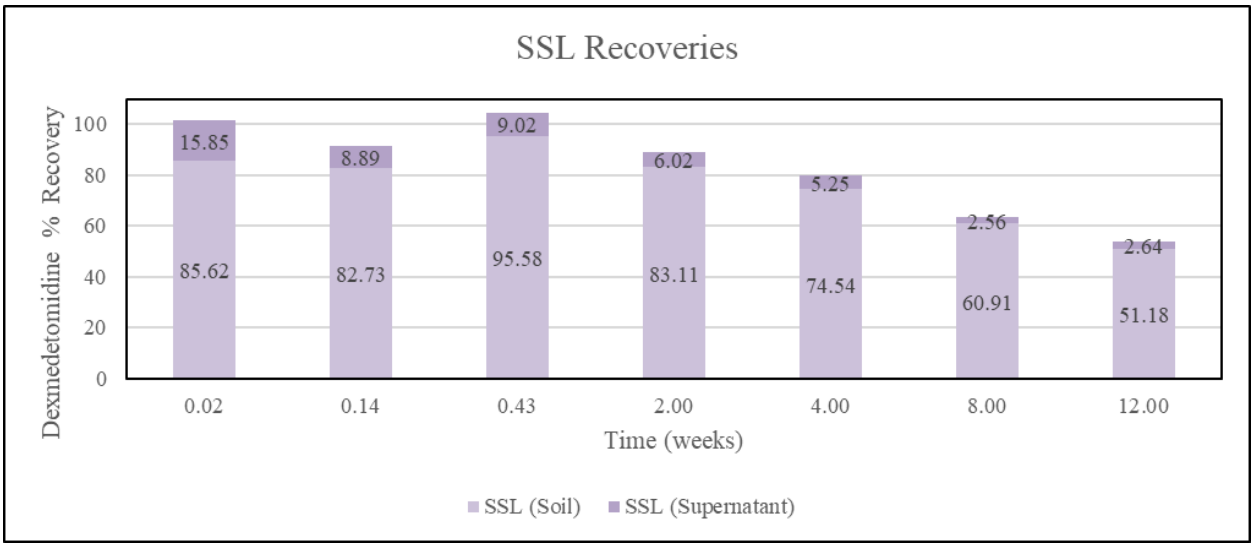

Figure 6. Dexmedetomidine total recovery from SSL soil.

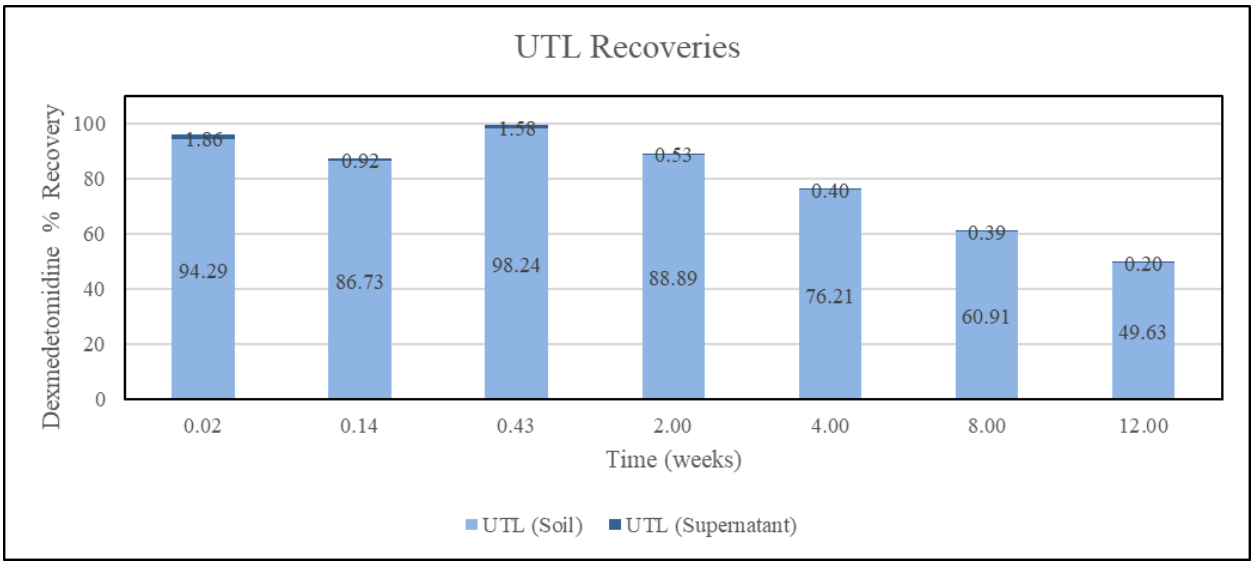

Figure 7. Dexmedetomidine total recovery from UTL soil.

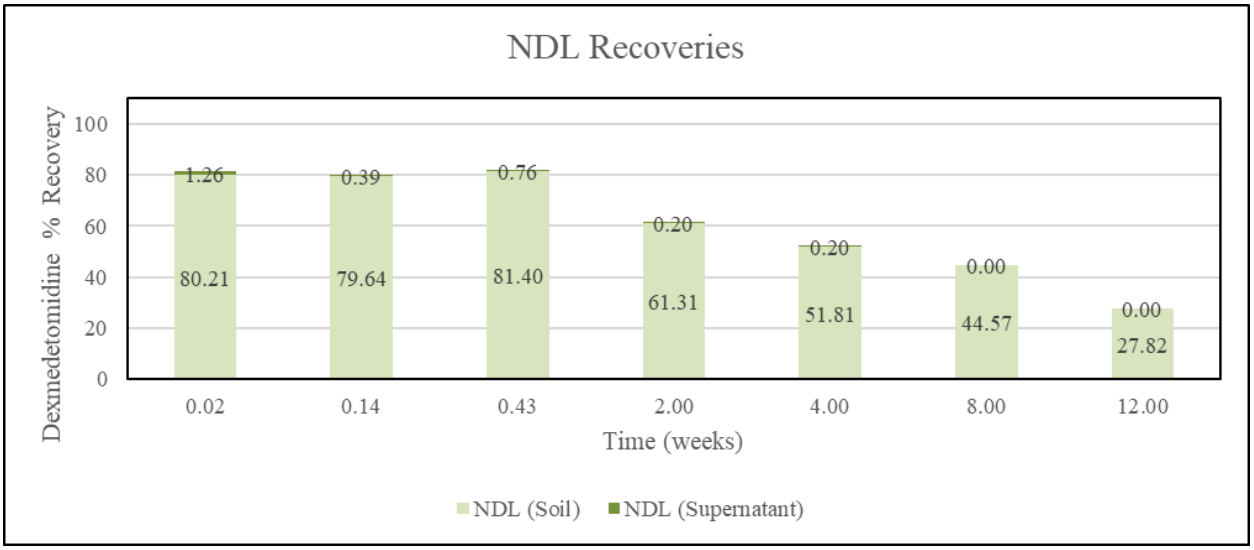

Figure 8. Dexmedetomidine total recovery from NDL soil.

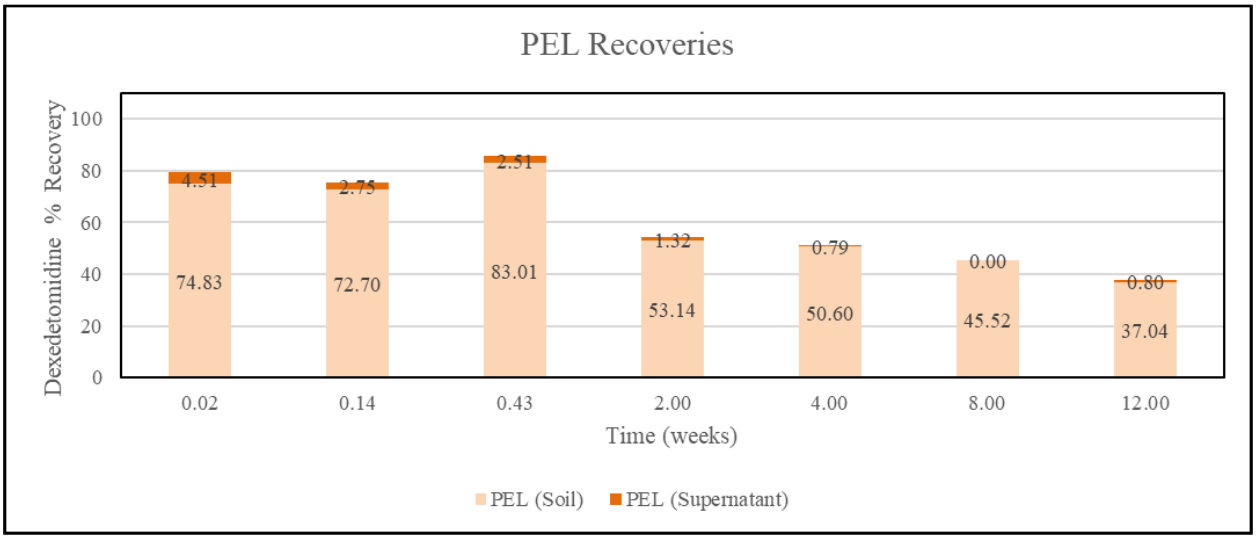

Figure 9. Dexmedetomidine total recovery from PEL soil.

Kd values are typically measured at the 24 h time point, when the analyte is expected to reach equilibrium in the soil and liquid phases. The extraction method described in Section 2.3 was used with only one difference: 10 mL of 0.01 M calcium chloride was added, as recommended by the OECD guidelines.

The K_d values were determined using

$$K_d = \frac{C_s^{ads}(\text{eq})}{C_{aq}^{ads}(\text{eq})}$$

where

- C_s^{ads} is the mass in the solid phase at equilibrium,
- C_{aq}^{ads} is the mass in the liquid phase at equilibrium, and
- K_{oc} is the K_d value normalized by the amount of oc present in the soil.

The K_d values listed in Table 5 indicate a moderate to strong preference for dexmedetomidine to adhere to the soil as opposed to remaining in the aqueous phase. This preference was less pronounced for the SSL and PEL soils. Both had lower silt content than the other soil types that were tested. Both were also characterized by lower pH values.

Table 5. K_d Values for Dexmedetomidine in Four Soils after 24 h

Soil Type	K_d Value at 24 h	Soil Texture (Clay Content, %)	pH	oc (%)	K_{oc}
SSL	8	17	4.5	1.14	688
UTL	56	25	8.4	1.42	3914
NDL	100	22	7.7	3.07	3246
PEL	11	21	4.5	3.97	272

4.2 Dexmedetomidine in Water

Dexmedetomidine stability was monitored in water collected from eight different sources for 12 weeks at 22 and 45 °C. Water samples were not sterilized before the experiments were started, but the samples were also not collected with the intent to preserve microbial communities. No degradation was observed during the experimental period. Data describing recovery of dexmedetomidine from water after several time periods in two temperatures are presented in Table 6 and illustrated in Figure 10.

Table 6. Dexmedetomidine Recovery from Water Sources*

Water Source	Measurement	Time (weeks)				
		0.14	**0.71**	**4**	**8**	**12**
Room Temperature (22 ± 1 °C)						
Citrate buffer (0.1 M)	Recovery (%)	101	94	111	92	94
	SD (%)	0	3	0	3	3
	pH	5	4	4	4	4
MOPS buffer (0.2 M)	Recovery (%)	96	96	106	82	87
	SD (%)	0	6	0	5	0
	pH	7	7	7	7	7
Trizma base (1 M)	Recovery (%)	99	99	112	90	96
	SD (%)	3	3	5	3	3
	pH	11	11	11	11	11
Adjusted TRIS buffer (1 M)	Recovery (%)	98	97	111	90	93
	SD (%)	0	3	3	3	0
	pH	9	9	9	9	9
Sea salt 4 (4 g/100 mL)	Recovery (%)	102	102	112	92	94
	SD (%)	0	0	1	0	2
	pH	6	7	7	7	8
$CaCl_2$ (0.01 M)	Recovery (%)	106	96	112	88	91
	SD (%)	3	3	3	3	3
	pH	8	8	8	8	8
Seawater	Recovery (%)	99	96	112	89	94
	SD (%)	3	3	5	3	3
	pH	8	8	8	8	8
ALEC GW	Recovery (%)	98	95	107	93	95
	SD (%)	3	1	2	2	3
	pH	6	7	7	8	8
Heated (45 ± 1 °C)						
Citrate buffer (0.1 M)	Recovery (%)	101	101	113	94	99
	SD (%)	0	0	3	3	3
	pH	4	4	4	4	4
MOPS buffer (0.2 M)	Recovery (%)	96	99	111	89	94
	SD (%)	0	3	0	10	3
	pH	6	7	7	7	8
Trizma base (1 M)	Recovery (%)	99	101	112	91	98
	SD (%)	3	3	0	3	5
	pH	11	11	11	11	11
Adjusted TRIS buffer (1 M)	Recovery (%)	103	103	114	92	98
	SD (%)	0	0	3	3	0
	pH	8	8	8	8	8

(continued)

Table 7. Dexmedetomidine Recovery from Water Sources (continued)

Water Source	Measurement	Time (weeks)				
		0.14	**0.71**	**4**	**8**	**12**
Salt 4 (4 g/100 mL)	Recovery (%)	100	102	111	94	95
	SD (%)	3	0	5	3	3
	pH	6	7	8	8	8
$CaCl_2$ (0.01 M)	Recovery (%)	106	99	117	90	95
	SD (%)	3	0	3	0	0
	pH	8	8	8	8	8
Seawater	Recovery (%)	107	102	118	92	97
	SD (%)	0	0	3	0	0
	pH	8	8	8	8	8
ALEC GW	Recovery (%)	99	99	114	93	95
	SD (%)	0	0	0	3	0
	pH	6	8	8	8	8

*Values are averages of three measurements.

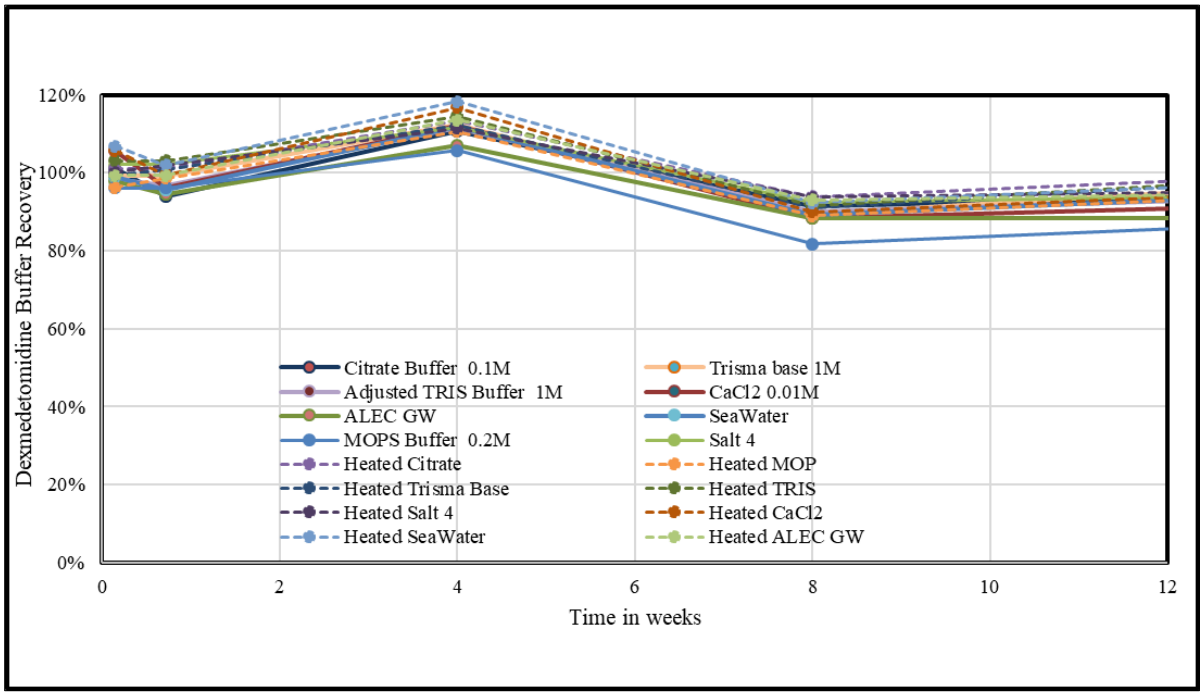

Figure 10. Dexmedetomidine recovery from eight water sources at two temperatures over 12 weeks.

Dexmedetomidine's persistence in water over the 12 week test period indicates that it does not hydrolyze in the environment over time when exposed to water. The behavior of dexmedetomidine under the current protocol shows that dexmedetomidine favors the solid phase of soil (most likely in the organics) and persists over time. As such, dexmedetomidine is unlikely to readily leach into the groundwater.

5. MEDETOMIDINE–DEXMEDETOMIDINE COMPARISON

Results from this study were compared with results from the racemic mixture medetomidine study described in technical report DEVCOM CBC-TR-1762.[4] Total recovery results for the pure stereoisomer and the racemic mixture over the same time period and using the same soil types are shown in Table 8. The $K_{d\ and}$ K_{oc} values for racemic medetomidine and dexmedetomidine are shown in Table 9.

Table 8. Medetomidine and Dexmedetomidine Total Recovery from Soil

Time (weeks)	Total Recovery From Soil and Supernatant (%)							
	Med	Dex	Med	Dex	Med	Dex	Med	Dex
	SSL		UTL		NDL		PEL	
0.02	83	101	79	96	83	81	69	79
0.14	93	92	88	88	71	80	63	75
4	86	80	84	77	51	52	56	51
8	65	63	63	61	40	45	39	46
12	63	54	56	50	39	28	41	38

Med, racemic medetomidine; Dex, dexmedetomidine.

Table 9. Medetomidine and Dexmedetomidine K_d and K_{oc} Values

Soil Type	K_d Value (24 h)		K_{oc}	
	Medetomidine	Dexmedetomidine	Medetomidine	Dexmedetomidine
SSL	7	8	576	688
UTL	44	56	3091	3914
NDL	111	100	3605	3246
PEL	13	11	326	272

Total recovery results for both compounds at the same time period and using the same water source are shown in Table 10.

Table 10. Medetomidine and Dexmedetomidine Total Recovery from Water Sources

Time (weeks)	Total Recovery from Room Temperature Water Sources (%)											
	Med	Dex	Med	Dex	Med	Dex	Med	Dex	Med	Dex	Med	Dex
	Citrate Buffer (0.1 M)		TRIS Buffer (1 M)		MOPS Buffer (0.2 M)		ALEC GW		$CaCl_2$ (0.01 M)		Sea Salt 4 (4 g/10.0 mL)	
0.14	95	101	98	98	94	96	94	98	86	106	96	102
4	94	111	94	111	89	106	88	107	88	112	92	112
8	89	92	87	90	83	82	85	93	85	89	88	92
12	81	94	77	93	76	87	75	95	72	94	78	94

Med, racemic medetomidine; Dex, dexmedetomidine.

Comparisons of medetomidine and dexmedetomidine recovery results as well as the calculated K_d and K_{oc} values show there was no significant difference between the two compounds' behavior in soil and water.

6. CONCLUSIONS

Results from this study indicate that dexmedetomidine is likely to persist in soil environments for months to years. It was also determined that dexmedetomidine is stable in water, at ambient temperatures and when heated to 45 °C, for several months. The equilibrium distribution of dexmedetomidine between the soil and water types tested was found to be in favor of the soils. The amount of dexmedetomidine recovered from soils accounted for 28–98% of the amount of the spike for up to 12 weeks. Likewise, the water samples were shown to be stable for up to 12 weeks at ambient and elevated temperatures. These results indicate that dexmedetomidine is relatively stable in water and moist soils and would leach slowly into the groundwater. The dexmedetomidine remaining in the soil is likely protected from degradation and could persist as a secondary hazard. In addition, the current results indicate that the behavior of medetomidine is very similar to that of dexmedetomidine when in contact with soil and water over the time and temperature ranges studied.

LITERATURE CITED

1. aus der Beek, T.; Weber, F.A.; Bergmann, A.; Hickmann, S.; Ebert, I.; Hein, A.; Küster, A. Pharmaceuticals in the Environment—Global Occurrences and Perspectives. *Environ. Toxicol. Chem.* **2016,** *35* (4), 823–835. DOI: 10.1002/etc.3339.

2. Hilvarsson, A.; Ohlauson, C.; Blanck, H.; Granmo, Å. Bioaccumulation of the New Antifoulant Medetomidine in Marine Organisms. *Mar. Environ. Res.* **2009,** *68* (1) 19–24. DOI: 10.1016/j.marenvres.2009.03.007.

3. Sinclair, M.D. A Review of the Physiological Effects of α_2-Agonists Related to the Clinical Use of Medetomidine in Small Animal Practice. *Can. Vet. J.* **2003,** *44* (11) 885–897.

4. Xega, R.; King, B.; Sumpter, K. *Environmental Fate of Medetomidine in Selected Soil and Water Sources*; DEVCOM CBC-TR-1762; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2022; UNCLASSIFIED Report.

5. Lohninger, H. Estimation of Soil Partition Coefficients of Pesticides from Their Chemical Structure. *Chemosphere* **1994,** *29* (8), 1611–1626.

6. Weil, R.R.; Brady, N.C. *The Nature and Properties of Soils*, 15th ed.; Pearson Education Ltd.: New York, 2017.

7. Test No. 106: Adsorption–Desorption Using a Batch Equilibrium Method. In *OECD Guideline for the Testing of Chemicals*; Organisation for Economic Co-operation and Development: Paris, France, 2000.

8. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* **2003,** *86* (2), 412–431.

9. Xega, R.; King, B.; Sohrabi, A.; Minyard, M. *Environmental Fate of Remifentanil in Soil and Relevant Waters*; CCDC CBC-TR-1577; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2019; UNCLASSIFIED Report (AD1078500).

10. Xega, R.; King, B.; Sohrabi, A.; Minyard, M. *Environmental Fate of Carfentanil Oxalate in Soil and Relevant Waters*; CCDC CBC-TR-1578; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2019; UNCLASSIFIED Report (AD1078505).

11. Xega, R.; King, B.; Sumpter, K.; Minyard, M. *Environmental Fate of Ketamine in Soil and Relevant Waters*; CCDC CBC-TR-1588; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2019; UNCLASSIFIED Report (AD1083999).

12. Xega, R.; King, B.; Henderson, V.; Minyard, M. *Environmental Fate of Fentanyl in Soil and Relevant Waters*; CCDC CBC-TR-1645; U.S. Army Combat Capabilities Development Command Chemical Biological Center: Aberdeen Proving Ground, MD, 2020; UNCLASSIFIED Report (AD1099736).

ACRONYMS AND ABBREVIATIONS

ALEC	Anita C. Leight Estuary Center
CAS	Chemical Abstracts Service
Da	Dalton
dSPE	dispersive solid-phase extraction
f_{oc}	fraction of organic carbon
HPLC	high-performance liquid chromatography
K_d	soil partitioning coefficient constant
K_{oc}	pesticide–soil organic partition coefficient
K_{om}	soil–organic matter partition coefficient
K_{ow}	octanol–water partition coefficient
MOPS	3-(*N*-morpholino)propanesulfonic acid
MW	molecular weight
NDL	North Dakota loam
oc	organic carbon
OECD	Organisation for Economic Co-operation and Development
PEL	Pennsylvania Ernest silt loam
QToF	quadrupole time-of-flight
QuEChERS	quick, easy, cheap, effective, rugged, safe
SD	standard deviation
SSL	Sassafras sandy loam
TRIS	trisodium citrate dihydrate
UTL	Utah Timpie loam

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