U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER

ABERDEEN PROVING GROUND, MD 21010-5424

DEVCOM CBC-TR-1762

Environmental Fate of Medetomidine in Soil and Relevant Waters

Roberta Xega
Bruce E. King
Kenneth B. Sumpter

RESEARCH AND TECHNOLOGY DIRECTORATE

February 2022

Approved for public release: distribution unlimited.

Disclaimer

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.

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 h per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *(DD-MM-YYYY)*
XX-02-2022

2. REPORT TYPE
Final

3. DATES COVERED *(From - To)*
Jul 2020–Nov 2020

4. TITLE AND SUBTITLE
Environmental Fate of Medetomidine in Soil and Relevant Waters

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Xega, Roberta; King, Bruce E.; Sumpter, Kenneth B.

5d. PROJECT NUMBER
CB10789

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, DEVCOM CBC, ATTN: FCDD-CBR-CP, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
DEVCOM CBC-TR-1762

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

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

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

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
The objectives of this work were to assess the stability and persistence of medetomidine in four soil types and six different water sources and to determine the distribution of medetomidine 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 were spiked with a known amount of medetomidine 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 medetomidine, and aliquots were taken for analysis after multiple time periods. The results showed that the amount of medetomidine recovered from soil extracts accounted for 40–90% of the spike. More than 70% of medetomidine was recovered from each of the water samples during this 12 week trial. These results indicate that medetomidine is relatively stable in water and moist soils. The current data also suggest that medetomidine may be mobile in the environment, depending on soil type.

15. SUBJECT TERMS

Medetomidine	Soil	Water	Opioids
Soil partitioning coefficient constant (K_d)	Stability	Transport	Recovery

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON / 19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	28	Renu B. Rastogi (410) 436-7545

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

Blank

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 July 2020 and was completed in November 2020.

The use of either trade or manufacturers' names in this report does not constitute an endorsement of any commercial product. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

The authors acknowledge the following individuals, without whose assistance this work would not have been possible:

- Dr. Frederic Berg and Dr. Michael Ellzy (U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) for technical guidance;
- Mrs. Sara Peacock (DTRA) for programmatic support; and
- Dr. David Tevault (SciTech Services; Havre de Grace, MD) for critical review.

Blank

CONTENTS

PREFACE iii

1. INTRODUCTION 1

2. SOIL ANALYSIS 3

2.1 Reagents and Chemicals 3
2.2 Soil Collecting and Processing 3
2.3 Soil Experiments 3
2.4 Sample Analysis 6

3. WATER ANALYSIS 6

3.1 Water Sources 6
3.2 Water Sample Preparation 7

4. RESULTS AND DISCUSSION 7

4.1 Medetomidine in Soil 7
4.2 Medetomidine in Water 12

5. CONCLUSIONS 14

LITERATURE CITED 15

ACRONYMS AND ABBREVIATIONS 15

FIGURES

1. Structure, formula, and molecular weight of medetomidine 2
2. Medetomidine in soil sample sets 5
3. Medetomidine recovery from soil 8
4. Medetomidine recovery from supernatant 9
5. Medetomidine total recovery from SSL soil 10
6. Medetomidine total recovery from UTL soil 10
7. Medetomidine total recovery from NDL soil 11
8. Medetomidine total recovery from PEL soil 11
9. Medetomidine recovery from six water sources over 12 weeks 13

TABLES

1. Soil Information 4
2. Medetomidine Recovery from Soils 8
3. Medetomidine Recovery from Supernatant 8
4. Total Medetomidine Recovery from Soil and Supernatant 9
5. K_d Values for Medetomidine in Five Soils after 24 h 12
6. Medetomidine Recovery from Water 13

ENVIRONMENTAL FATE OF MEDETOMIDINE IN SOIL AND RELEVANT WATERS

1. INTRODUCTION

The discharge of pharmaceuticals into the aquatic environment occurs worldwide, as reported by aus der Beek and coworkers.[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 is a new alternative maritime antifoulant compound that effectively prevents barnacle settlement. The uptake, elimination, and bioconcentration of medetomidine in *Mytilus edulis*, *Abra nitida*, *Crangon crangon*, and periphyton communities has been investigated to evaluate the risk of bioaccumulation in the marine environment.[2] That study demonstrated that the bioconcentration and bioaccumulation of medetomidine differs among aquatic organisms, and that microalgal communities in the form of periphyton have the highest bioconcentration factor of the organisms tested.

Medetomidine is a white, crystalline, solid synthetic drug, usually used as the hydrochloride salt (referred to as medetomidine in this study). Medetomidine is a potent and highly specific α_2-adrenoceptor agonist that is marketed as a racemic mixture of two stereoisomers, dextro- 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 medetomidine 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 medetomidine is provided in Figure 1.

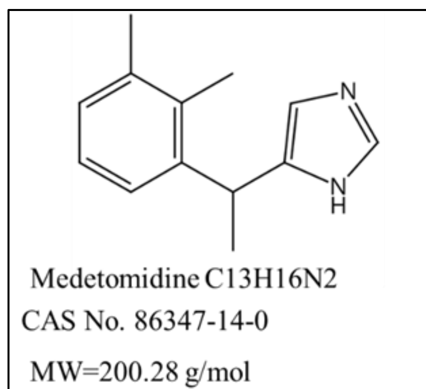

Figure 1. Structure, formula, and molecular weight of medetomidine.
CAS, Chemical Abstracts Service; MW, molecular weight.

The focus of this work was to elucidate chemical and physical interactions between medetomidine, soil, and water to advance our understanding of how medetomidine behaves in the environment. Pesticides have been studied more extensively in association with the soil environment than any other chemical class.[4] Understanding the adsorption of pesticides in soils is important for regulating their use for crops. However, the intention of the chemical warfare defense community is to use similar data to determine how materials of concern interact with the environment to inform warfighter decisions regarding the hazards, persistence, and environmental transport of these materials after dissemination. For example, if a chemical is soluble in water and does not adsorb to soil, it would be expected to migrate through the soil and leach, which would likely contaminate the groundwater.

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 being 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 medetomidine for periods of up to 12 weeks in 4 different soils and 6 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) for dispersive solid-phase extraction (dSPE) cleanup for 6 mL extract, Q370 for quick, easy, cheap, effective, rugged, safe (QuEChERS) extract cleanup; and
- medetomidine 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).

A fifth soil, Nunn clay loam (CO) from Colorado, was used to determine K_d values after 24 h of contact with the analyte.

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
CO*	Colorado	45	23	32	Clay loam	7.6	1.2

*CO data were measured and used only for K_d calculations.

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 24 negative controls during our experiments (the minimum amount specified in the guideline). No soil was used for the 24 positive controls. The 2 g of soil, corrected for remaining moisture content in calculations and reported as dry weight, was reconstituted with 2 mL of 0.01 M calcium chloride solution on the day before the medetomidine spikes were performed. Vials of soil and solution (Figure 2) were left overnight at room temperature to fully moisten the soils.

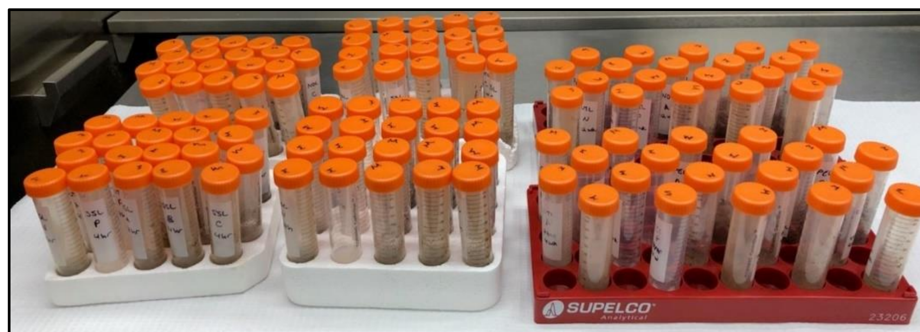

Figure 2. Medetomidine in soil sample sets.

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 medetomidine. 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 medetomidine by adding 10 µL of a 1000 µg/mL solution, so that the medetomidine concentration was 5 µg/mL for each positive control.

Samples were prepared for sacrificial collection and extraction of the medetomidine at time points of 4 and 24 h and 1, 4, 8, and 12 weeks. A total of 120 vials (5 vials per sample set × 6 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 medetomidine using a Xevo G2-XS quadrupole time-of-flight (QToF) mass spectrometer (Waters; Milford, MA).

Medetomidine 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 medetomidine 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 medetomidine found in the extraction samples at each time point.

2.4 Sample Analysis

Medetomidine 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 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. Column temperature was maintained at 40 °C. Mobile phase A was 0.01% 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 Xevo G2-XS QToF spectrometer equipped with an electrospray ionization (ESI) interface and MassLynx 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 °C. 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 medetomidine in soil, we also determined medetomidine stability in six water sources, as described in this section.

3.1 Water Sources

Water samples were obtained from the following locations:

- Groundwater was collected on 24 August 2020 (initial pH 4.9) from the Anita C. Leight Estuary Center (ALEC; Harford County, MD).
- 0.1 M citrate buffer (pH 3.6) was prepared in-house.

- 1 M TRIS buffer (pH 8.3) was prepared in-house.
- 0.2 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.1) was prepared in-house.
- Sea salt 4 was prepared in-house by adding 4 g of NaCl to 100 mL of deionized water (pH 5.4). (Note: This concentration was selected to simulate ocean water.)
- 0.01 M calcium chloride solution (initial pH 5.1) was prepared in-house.

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 medetomidine 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. After each designated time period, 100 µL of solution 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 2 and 24 h and 1, 4, 8, and 12 weeks after preparation.

4. RESULTS AND DISCUSSION

4.1 Medetomidine in Soil

The data describing medetomidine recovery after soil contact are listed in Table 2 and shown graphically in Figure 3. Total recovery varied between about 39 and 96% after 12 weeks of exposure. Those data suggest long-term environmental stability of medetomidine. Soil pH does not appear to play a role in the medetomidine 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 medetomidine.

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

Table 2. Medetomidine Recovery from Soils*

Time (weeks)	Recovery (%)							
	SSL	**SD**	**UTL**	**SD**	**NDL**	**SD**	**PEL**	**SD**
0.02	73	4	78	7	82	1	65	6
0.14	85	1	87	2	70	8	60	5
1	87	3	88	5	67	7	56	2
4	80	4	83	4	50	1	55	4
8	62	9	62	9	40	1	38	1
12	60	5	56	6	39	1	41	2

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

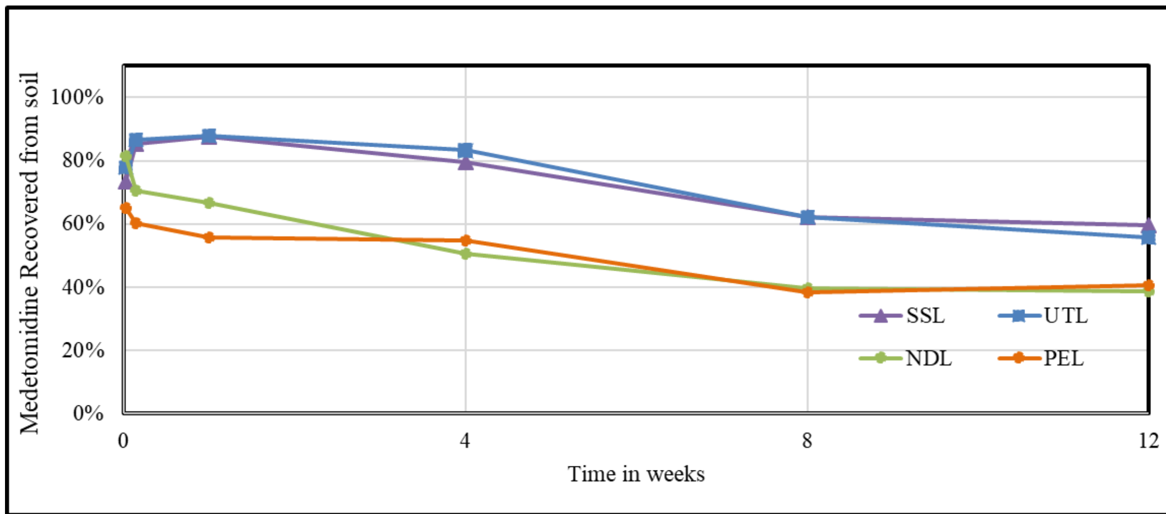

Figure 3. Medetomidine recovery from soil.

Medetomidine was recovered from the aqueous phase throughout the experimental period, including the week 12 samples. Recovery data are listed in Table 3 and shown graphically in Figure 4. Medetomidine recovery from the supernatant (Table 3) was in the 1–2% range for all soils except SSL, which had a higher recovery rate.

Table 3. Medetomidine Recovery from Supernatant*

Time (weeks)	Recovery (%)							
	SSL	**SD**	**UTL**	**SD**	**NDL**	**SD**	**PEL**	**SD**
0.02	10	1	1	0	1	0	3	0
0.14	8	0	2	0	1	0	2	0
1	9	1	1	0	1	0	2	0
4	6	0	1	0	1	0	1	0
8	3	0	1	0	0	0	0	0
12	3	0	0	0	0	0	0	0

*Values are averages of three measurements.

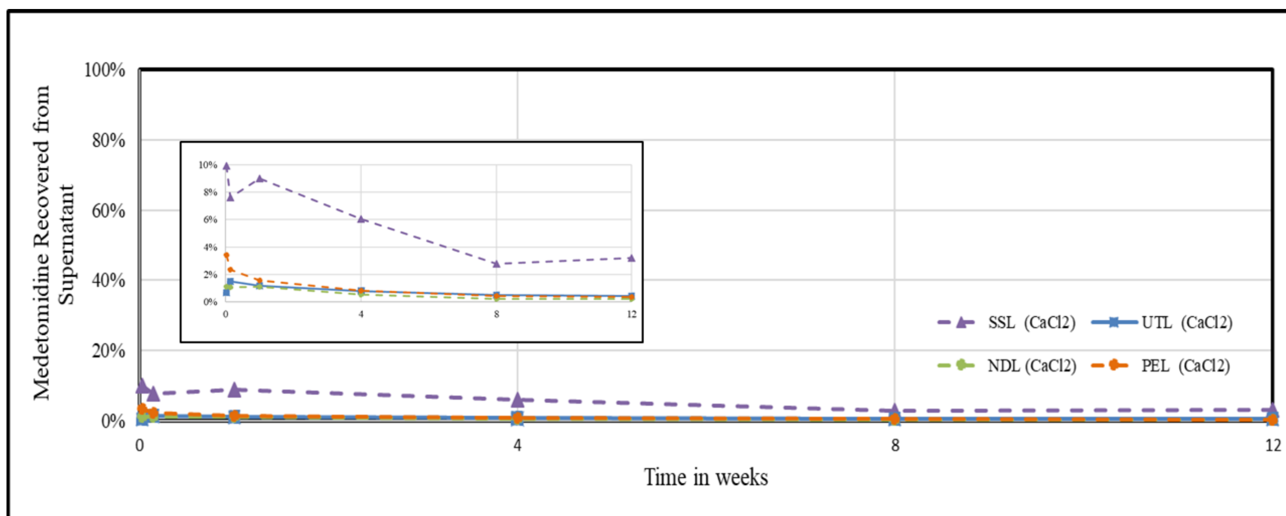

Figure 4. Medetomidine recovery from supernatant.

Table 4 presents the total medetomidine recovery from the soil and the aqueous supernatant. Figures 5, 6, 7, and 8 demonstrate the ratio of the medetomidine recovered from soil to that recovered from the aqueous calcium chloride supernatant for SSL, UTL, NDL, and PEL, respectively. Total medetomidine recovery varied between 38 and 96%, emphasizing the point that medetomidine is recoverable from the soil for much longer than 12 weeks.

Table 4. Total Medetomidine Recovery from Soil and Supernatant*

Time (weeks)	Total Recovery (%)			
	SSL	UTL	NDL	PEL
0.02	83	79	83	69
0.14	93	88	71	63
1	96	89	68	57
4	86	84	51	56
8	65	63	40	39
12	63	56	39	41

*Values are averages of three measurements.

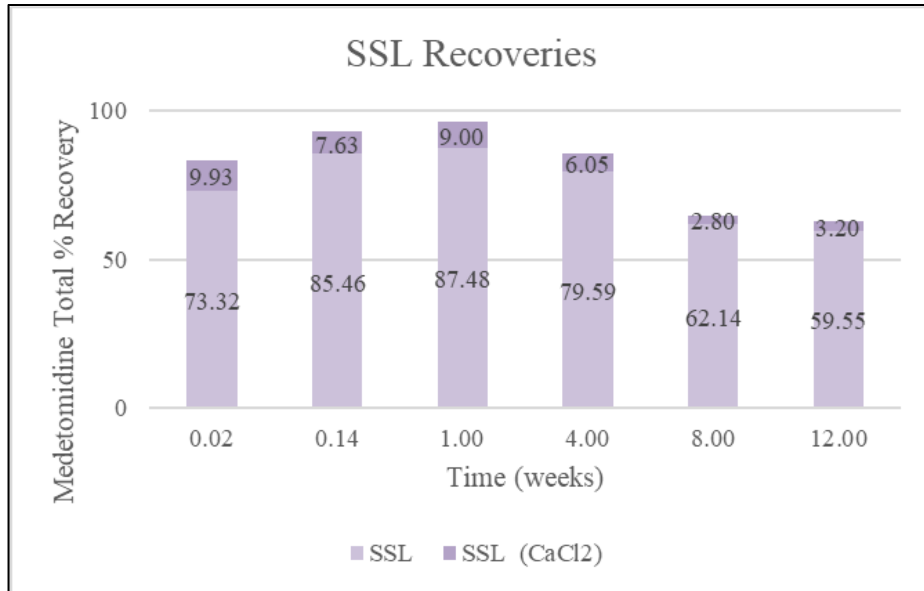

Figure 5. Medetomidine total recovery from SSL soil.

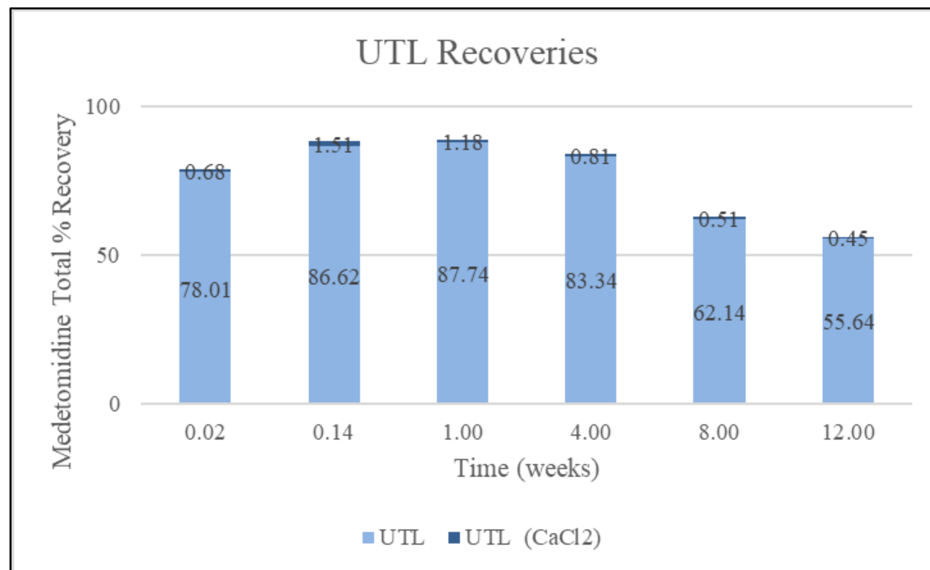

Figure 6. Medetomidine total recovery from UTL soil.

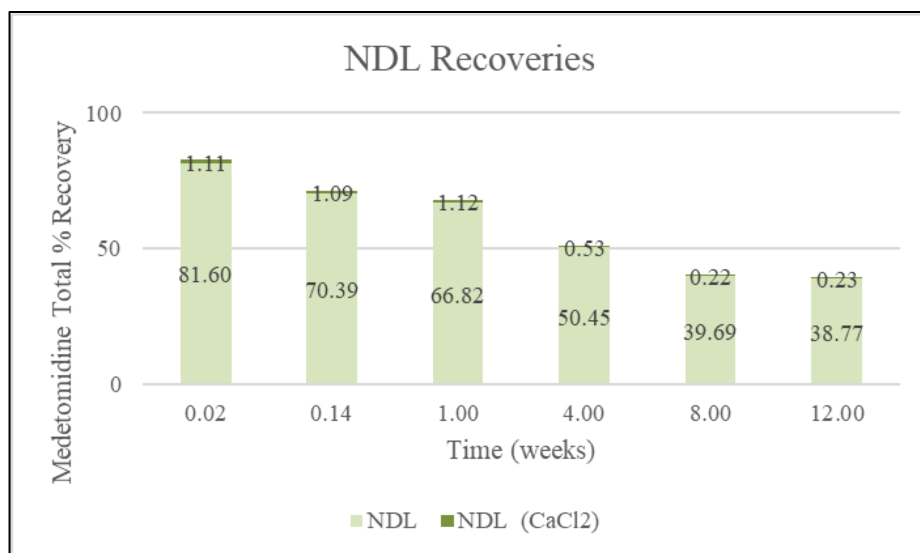

Figure 7. Medetomidine total recovery from NDL soil.

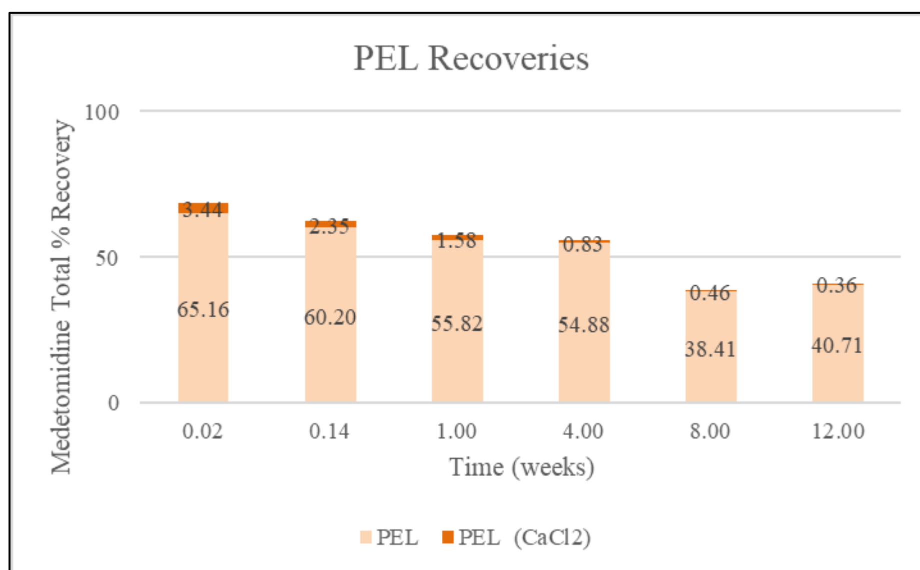

Figure 8. Medetomidine total recovery from PEL soil.

K_d 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 medetomidine 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 Medetomidine in Five Soils after 24 h

Soil Type	**K_d Value at 24 h**	**Soil Texture (Clay Content, %)**	**pH**	**oc (%)**	**K_{oc}**
SSL	6.58	17	4.5	1.14	576
UTL	43.93	25	8.4	1.42	3091
NDL	110.81	22	7.7	3.07	3605
PEL	12.95	21	4.5	3.97	326
CO	38.76	32	7.6	1.17	3324

4.2 Medetomidine in Water

Medetomidine stability was monitored in six different water sources for 12 weeks. 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 medetomidine from water after several time periods are presented in Table 6 and illustrated in Figure 9.

Table 6. Medetomidine Recovery from Water*

Water Source	Measurement	Time (weeks)					
		0.01	0.14	1	4	8	12
Citrate buffer (0.1 M)	Recovery (%)	100	95	100	94	89	81
	SD (%)	3	1	3	3	3	3
	pH	3.55	3.55	3.87	3.79	3.76	3.69
TRIS buffer (1 M)	Recovery (%)	97	98	99	94	87	77
	SD (%)	4	4	1	1	1	2
	pH	8.39	8.39	8.20	8.31	8.38	8.16
MOPS buffer (0.2 M)	Recovery (%)	93	94	95	89	83	76
	SD (%)	4	7	1	1	3	1
	pH	7.12	7.12	7.06	7.08	7.11	6.95
ALEC GW	Recovery (%)	87	94	93	88	85	75
	SD (%)	5	4	1	0	2	1
	pH	4.97	4.97	6.76	7.32	7.33	7.34
$CaCl_2$ (0.01 M)	Recovery (%)	84	86	92	88	85	72
	SD (%)	3	1	0	2	1	2
	pH	5.14	5.14	7.25	7.06	6.43	6.58
Sea salt 4 (4 g/100 mL)	Recovery (%)	96	96	95	92	88	78
	SD (%)	7	4	2	2	3	1
	pH	5.38	5.38	6.64	7.01	6.81	6.72

*Values are averages of three measurements.
GW, groundwater.

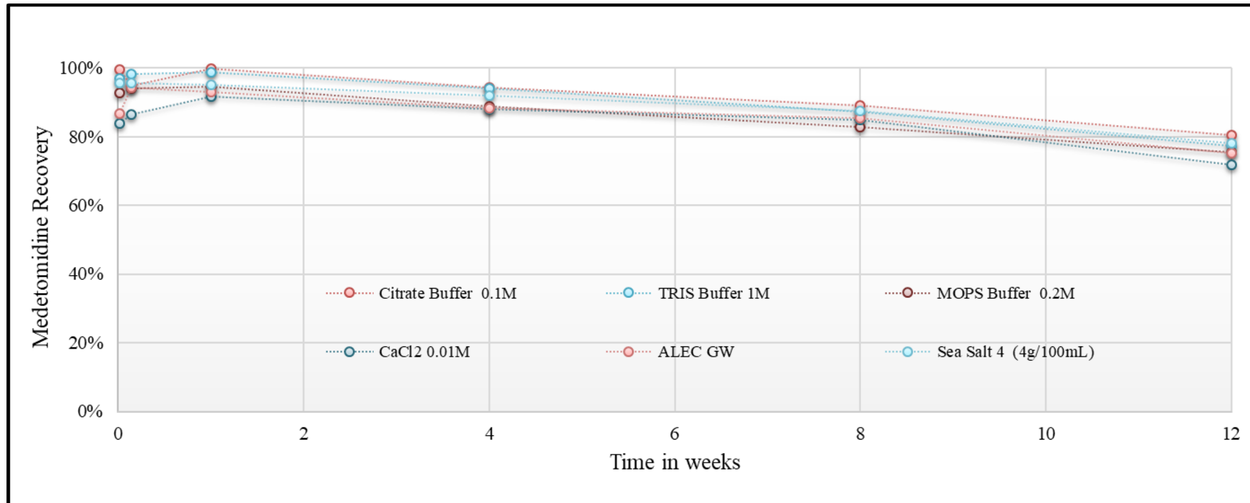

Figure 9. Medetomidine recovery from six water sources over 12 weeks.

Medetomidine'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 medetomidine under the current protocol shows that medetomidine favors the solid phase of soil (most likely in the organics) and persists over time. As such, medetomidine is unlikely to readily leach into the groundwater.

5. CONCLUSIONS

Results from this study indicate that medetomidine is likely to persist in soil environments for months to years. We also determined that medetomidine is stable in water at ambient temperatures for several months. The equilibrium distribution of medetomidine between the soil and water types tested was found to be in favor of the soils. The amount of medetomidine recovered from soils accounted for 38–93% 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. These results indicate that medetomidine is relatively stable in water and moist soils. The medetomidine remaining in the soil is likely protected from degradation and could persist as a secondary hazard.

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. Andrade, S.R.B.; Silva, A.A.; Queiroz, M.E.L.R.; Lima, C.F.; D'Antonino, L. Ametryn Sorption and Desorption in Red-Yellow Latosol and Red-Yellow Ultisol with Different pH Values. *Planta Daninha* **2010,** *28* (1), 177–184.

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
CO	Colorado Nunn Clay Loam
Da	Dalton
dSPE	dispersive solid-phase extraction
ESI	electrospray ionization
f_{oc}	fraction of organic carbon
GW	groundwater
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

DISTRIBUTION LIST

The following individuals and organizations were provided with one electronic version of this report:

U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC)
FCDD-CBR-CP
ATTN: Xega, R.
Ellzy, M.
King, B.
Sumpter, K.
Berg, F.
Kristovich, R.

Defense Threat Reduction Agency
DTRA-RD-IAR
ATTN: Peacock, S.
Wallace, L.

DEVCOM CBC Technical Library
FCDD-CBR-L
ATTN: Foppiano, S.
Stein, J.

Defense Technical Information Center
ATTN: DTIC OA

U.S. ARMY COMBAT CAPABILITIES DEVELOPMENT COMMAND
CHEMICAL BIOLOGICAL CENTER