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Environmental Fate of Carfentanil Oxalate in Soil and Relevant Waters

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In this study, our objective was to assess the stability and persistence of carfentanil oxalate (henceforth referred to as carfentanil) in four different soil types and six different water sources. The soil samples were spiked with a known amount of carfentanil solution and then extracted after several contact time points to track compound recovery. The water samples were spiked with a known amount of carfentanil, and aliquots were taken at different time points for analysis. The results showed that the amount of carfentanil in contact with each soil type was nearly constant during the 12 week testing period, and it accounted for 60–80% of the spike amount. Likewise, the water samples were stable for each time point up to 11 weeks, although only about 80% of the original spike could be accounted for. These results indicate that carfentanil is relatively stable in water and moist soils and immobile in the environment.

15. SUBJECT TERMS

Carfentanil oxalate	Soil	Water	Opioids
Adsorption coefficient (K_{oc})	Partition coefficient (K_{ow})	Transport	Recovery

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PREFACE

The work described in this report was authorized by the Defense Threat Reduction Agency (DTRA; Fort Belvoir, VA) Joint Science and Technology Office (JSTO) under project number HDTRA1620640. The work was started in July 2018 and completed in October 2018. At the time the work was conducted, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC; Aberdeen Proving Ground, MD) was known as the U.S. Army Edgewood Chemical Biological Center.

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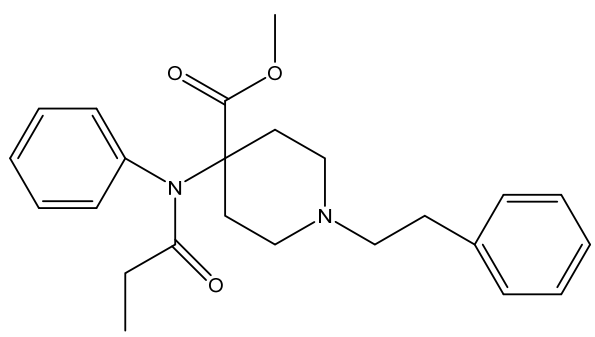
ENVIRONMENTAL FATE OF CARFENTANIL OXALATE IN SOIL AND RELEVANT WATERS

1. INTRODUCTION

Carfentanil oxalate (Chemical Abstracts Service [CAS] number 59708-52-0; henceforth referred to as carfentanil in this study) is a Schedule II controlled substance that is known for its ability to immobilize large animals. It is typically used by veterinarians to facilitate treatment of cervidae (such as deer, elk, and moose). Carfentanil is a high potency synthetic opioid, which is found in several forms, including powder, blotter paper, tablets, and spray. Carfentanil can be absorbed through the skin or inhaled as an airborne powder. Figure 1 shows the structure of carfentanil.



$C_{24}H_{30}N_2O_3$
MW = 394.52
CAS No. 59708-52-0

Figure 1. Structure, formula, and molecular weight of carfentanil.

Janssen and coworkers produced many potent and clinically useful compounds, such as fentanyl, carfentanil, and sufentanil.[1] During the late 1980s, researchers in the United States investigated fentanyl derivatives as potential incapacitating agents; however, the program ended in the early 1990s.

Interest in fentanyl was renewed after the 2002 Moscow theatre siege when a Russian Special Forces unit used a chemical aerosol in the Dubrovka Theatre (Moscow, Russia) to incapacitate Chechen rebels and take control of a hostage situation. Scientists at the Defense Science and Technology Laboratory (Porton Down, UK) determined that the aerosol used by the Russian Special Forces was a mixture of carfentanil and remifentanil.[2] Over 125 people died from aerosol inhalation and inadequate medical care during the Dubrovka Theatre incident. In addition, this incident confirmed Russian interest in chemicals affecting human physiological responses.

Carfentanil production and use as a radiotracer in positron-emission tomography and as a large animal tranquilizer may result in its release into the environment through various waste streams. If released in the air, the estimated vapor pressure of 2×10^{-10} Torr (or 2.7×10^{-8} Pa) at 25 °C indicates that carfentanil exists almost exclusively in the condensed phase at normal atmospheric temperatures. Particulate-phase carfentanil is removed from the atmosphere by wet and dry deposition. If released in soil, carfentanil is expected to have minimal mobility based on the estimated water-partitioning adsorption coefficient (K_{oc}) of 800 at neutral pH.[3] The log octanol-water partition coefficient (K_{ow}) of carfentanil is 3.52, which indicates that carfentanil will persist in an organic rather than aqueous phase. The acid dissociation constant (p*K*a) of carfentanil is estimated to be 8.05, indicating that this compound will exist partially in a cationic form in the environment. Cations generally adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Volatilization from moist soil surfaces is not expected to be an important fate process based upon the estimated p*K*a and an estimated Henry's Law constant of 4.4×10^{-13} atm-m^3/mole. Because of its estimated vapor pressure, carfentanil is not expected to volatilize from dry soil surfaces. Biodegradation data in soil or water were not available. If released into water, carfentanil is expected to adsorb to suspended solids and sediment based on the estimated K_{oc} value. Volatilization from water surfaces is not expected to be an important fate process based on the estimated p*K*a of this compound and Henry's Law constant. An estimated biological concentration factor (BCF) of 111 suggests that the potential for bio-concentration in aquatic organisms is moderate.[3] Estimated hydrolysis half-lives are 35 and 3.5 years at pH balances of 7 and 8, respectively.[4–8]

In this study, we observed the stability and extractability of carfentanil in four different soil types and six different water sources collected from various continental U.S. sites for time points up to 12 weeks.

2. SOIL ANALYSIS

2.1 Reagents and Chemicals

The following reagents and chemicals were used in this study:

- acetonitrile and methanol (Sigma-Aldrich Corporation; St Louis, MO; high-performance liquid chromatography [HPLC] grade with ≥99.9% purity);
- in-house 16 MΩ of water (used to prepare samples and HPLC mobile phase);
- sodium sulfate, sodium chloride, trisodium citrate dehydrate, and disodium hydrogen citrate sesquihydrate (Sigma-Aldrich; American Chemical Society [ACS] grade with ≥99% purity);
- calcium chloride (ACROS Organics; Pittsburg, PA; ≥99% purity); and

- carfentanil (prepared and purified in-house, and analysis by liquid chromatography–tandem mass spectrometry [HPLC–MS/MS] indicated a purity of 98.3%).

In addition to the reagents and chemicals, 15 mL centrifuge tubes (Restek Corporation; Bellefonte, PA) with dispersive solid-phase extraction (dSPE) clean-up for 6 mL extract (Q370) were used for quick, easy, cheap, effective, rugged, safe (QuEChERS) extract clean-up.

2.2 Soil Collection and Processing

The soil samples used during this study were collected primarily from the A horizon after leafy matter was removed from the area. A few inches were dug into the soil to confirm no boundary horizon change had occurred, and a circle was dug outward from the initial location. If a well-developed O horizon was present, it was incorporated into the sample. Samples were air-dried, crushed, and sieved using a 2 mm ASTM International (West Conshohocken, PA) standard sieve. All sieved samples were stored in plastic-capped containers at room temperature. Remaining moisture levels were measured before testing.

2.3 Soil Experiments

The procedures followed during this portion of the study were based on the Organization for Economic Co-operation and Development (OECD; Paris, France) guideline 106.[9] This guideline presents recommendations for determining the persistence of a chemical in soil. In addition, the guideline suggests testing different naturally occurring soils with varying pH, clay, and organic matter content. Four soil types were identified and collected for testing. Each soil type was well mixed, and triplicate subsamples were analyzed by members of the Pennsylvania State University Agricultural Analytical Services Laboratory (University Park, PA) for texture, pH, and organic content. Results of the soil characterization analyses are presented in Table 1.

Table 1. Soil Information

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

The OECD guideline recommends using large quantities of soil for testing (e.g., 2–50 g of soil). Because of the hazardous nature of the compound used in our work, and the need to execute experiments safely and efficiently, only 2 g of soil was used in each of the 96 sample tubes and 32 negative controls during our experiments. This was the minimum amount specified in the guideline. The 2 g of soil, corrected for remaining moisture content in our calculations and reported as dry weight, was reconstituted with 2 mL of 0.01 M calcium chloride solution on the day before the carfentanil spike was performed. Tubes of each soil type and solution were left overnight at room temperature to fully moisten the soil. A set of samples was prepared for each soil type and for each time point. Each set of soil samples was prepared in triplicate, and each set contained positive and negative controls. The negative-control samples contained each soil type and 0.01 M of calcium chloride solution but were not spiked with carfentanil. The positive-control samples were prepared for each sample set in the absence of soil on the same time schedule as used for the soil samples. This was done by spiking 2 mL of the 0.01 M calcium chloride solution with carfentanil and by adding 10 µL of a 1000 µg/mL solution so that the concentration of carfentanil was 5 µg/mL, which was the same as the final concentration in each experimental sample.

Tubes were prepared for sacrificial collection and extraction of the carfentanil at time points of 4, 24, and 48 h and 1, 2, 4, 8, and 12 weeks. A total of 160 vials was used in this portion of the work. At the time of data extraction, the tubes selected for analysis were centrifuged to separate the soil from the solution, and the liquid phase was collected, filtered, and analyzed for carfentanil using the Waters Corporation (Milford, MA) ultra-high performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS) system.

Carfentanil was extracted from the soil phase using a modified QuEChERS method.[10] The modification included the addition of tris(hydroxy-methyl)aminomethane (TRIS) to ensure that the pH level was 8.3 before extraction was performed. The TRIS buffer increased the pH of the soil and carfentanil solution to about 8.0; therefore, release of analyte from the organic matter component of the soil was optimized so that it could be extracted more efficiently. The modified QuEChERS method was selected after comparing results from several different extraction methods found in the literature and technical reports.

At each time point, the soil mixture was centrifuged, and the supernatant was filtered using a 13 mm, 0.45 µm polyvinylidene fluoride membrane syringe filter (PALL Life Sciences Corporation; Port Washington, NY; part number [PN] 4452T). After removal of the supernatant, 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, 1 g of sodium chloride, 1 g of dehydrated trisodium citrate, and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The mixture was vortexed for 30 s and then centrifuged for 5 min at 3500 rpm in a 5804 centrifuge from Eppendorf (Hamburg, Germany). The QuEChERS kit (Restek Q-sep QuEChERS dSPE tubes for extract cleanup; original unbuffered, European EN 15662 method; VWR PN 10057-974) was purchased from VWR International (Radnor, PA). A dSPE clean-up step was performed 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. The solution was vortexed for 30 s. Finally, centrifugation was carried out at 3500 rpm for 5 min.

All data were corrected for dilution, and recovery for each sample was determined based on the amount of carfentanil found in the extraction samples at each time point.

2.4 Sample Analysis

Analysis of carfentanil samples was carried out using the UHPLC system, which consisted of a vacuum degasser, autosampler, and binary pump. This system was equipped with a reversed-phase pinnacle DB intrinsically base-deactivated biphenyl column of 100 × 2.1 mm with a particle size of 1.9 µm (Restek no. 9409212). The liquid chromatography (LC) column temperature was maintained at 40 ºC. Mobile phases A and B were water and methanol. The mobile phase was prepared by adding 2 mL of 1 M ammonium formate and 2 mL of 1 M formic acid to 1 L of water (A) and methanol (B), respectively. The flow rate was kept constant at 0.35 mL/min. HPLC was run in isocratic mode. The water/methanol ratio was 10:90 for the duration of the run. The total run time was 5 min. The sample injection volume was 0.5 µL.

The UHPLC system was coupled with a Quattro Premier triple-quadrupole mass spectrometer (Waters) equipped with an electrospray-ionization (ESI) interface and Mass Lynx software (Version 4.1; Waters). The tandem mass spectrometer was operated in positive ESI mode. This technology is referred to as the liquid chromatography–tandem mass spectrometry (LC–MS/MS) analytical system.

Data acquisition was performed by working in selective ion-recording mode. Capillary voltage was 2.0 kV; nitrogen was used as the spray gas. Source temperature was set at 120 ºC. The optimized setting for cone voltage was 30 V.

The LC–MS/MS analytical system was calibrated before each series of measurements using standard solutions prepared from stock solutions on the day of each analysis. Two stock solutions at 1 mg/mL concentration in methanol were prepared, analyzed, and compared for accuracy. An eight-point calibration curve in the range of 0.01 to 1 µg/mL was determined from dilutions prepared using one of the stock solutions. A good signal-to-noise ratio was observed at the lowest calibration concentration. A calibration-check sample was prepared from the second stock solution. Responses from these standards agreed to within 5%. Positive-control samples were diluted by a factor of 10 for liquid-phase analysis. Aqueous-phase samples were not diluted because the results were below the lowest point in the calibration curve (0.01 µg/mL). Positive-control and extracted-soil samples were diluted by a factor of 4 with acetonitrile to keep the experimental concentrations in the calibration range. Carfentanil solubility in water was 2.5 mg/mL.

3. WATER ANALYSIS

In addition to determining carfentanil stability in soil, we determined carfentanil stability in six distinct water sources.

3.1 Water Sources

The water sources were obtained in the following manner:

- ground water was collected from the Anita C. Leight Estuary Center (ALEC; Bel Air, MD) on 10 July 2018; the experiment was started the day after the ALEC water collection;
- 16 MΩ of deionized (DI) water was obtained in-house;
- salt water (SW) 1 was prepared in-house by adding 4 g of sodium chloride to 100 mL of DI water (this concentration simulates ocean water);
- SW 2 was prepared in-house by adding 8 g of sodium chloride to 100 mL of DI water;
- rain water was collected in Bel Air (MD) on 30 July 2018; the experiment was started the day after the rain water collection; and
- in-house 0.01 M calcium chloride solution was used.

3.2 Water Sample Preparation

Twenty milliliters of each water type was added to a glass vial. Each vial, minus the negative-control samples for each water type, was then spiked with carfentanil by adding 100 µL of a 1000 µg/mL solution so that the starting concentration was 5 µg/mL. Samples from each water type were prepared in triplicate together with a negative-control sample. The samples were stored at 22 ± 1 °C over the course of the 11 week experimental period. After each designated time point, 100 µL were removed and diluted to a final volume of 1000 µL. The diluted samples were analyzed using the LC–MS/MS system. Observation time points were 1 h and 1, 2, and 6 days and 3 and 11 weeks after preparation.

4. RESULTS AND DISCUSSION

4.1 Soil-Testing Results

Carfentanil was not detectable in any supenatant sample, although the samples were run prior to dilution, which indicated that the majority of the carfentanil was in solid phase. Recovery of carfentanil from the soil using the modified QuEChERS method ranged from 50–89% during our testing, and the recovered amount appeared to decrease with time. The majority of carfentanil adsorbed into the soil, most likely into the organic matter, during the experiment. The data shown graphically in Figure 2 and listed in Table 2 indicate that the soil-extraction efficiency was high, even in higher organic carbon containing soils such as NDL, which has an organic content of 3.1%. PEL (high carbon content) and UTL (high clay content) showed lower recoveries of carfentanil. This confirmed our assumption that because of the

estimated p*K*a for carfentanil (8.05), this compound exists partially in a cationic form in the environment. Cations generally adsorb more strongly to soils containing organic carbon and clay as opposed to neutral compounds.

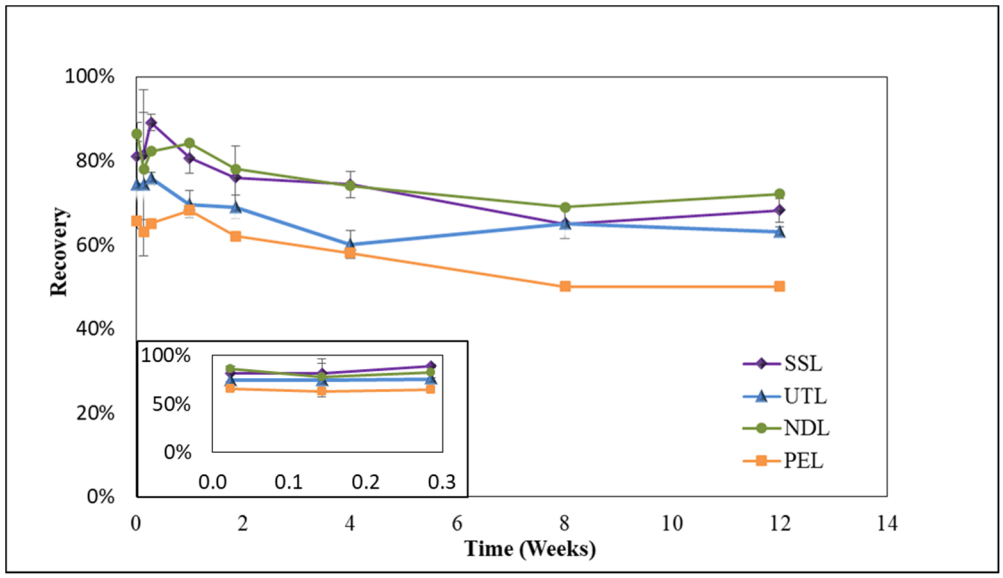


Figure 2. Carfentanil recovery from soil samples.

Table 2. Carfentanil Recovery from Soil Samples

Weeks	SSL	SD	UTL	SD	NDL	SD	PEL	SD
	(%)							
0.024	81	4	74	2	86	4	66	1
0.14	81	1	74	7	78	2	63	3
0.27	89	1	76	1	82	3	65	1
1.00	81	3	70	6	84	2	68	2
1.86	76	1	69	2	78	2	62	4
4.0	74	1	60	9	74	1	58	1
8.0	65	2	65	5	69	7	50	0
12.0	68	2	63	2	72	2	50	1

After an initial loss of ~15–35%, the amount of extractable carfentanil decreased gradually over the 12 week testing period in all the soils. PEL had the lowest initial and 12 week recoveries. PEL also had the lowest pH (4.5) and highest organic carbon content (3.9%). SSL had the second highest 12 week recovery, with the same pH as PEL but a lower organic carbon content. UTL had a low organic carbon content and the highest pH; UTL can be characterized as having close to the mean 12 week recovery. NDL and UTL (the soils with the lowest sand content) had the second and third highest recoveries.

Carfentanil loss at the initial time point (4 h) appears to be outside the ascribed experimental uncertainty based on comparisons with the positive-control samples. The loss could be attributed to sample-handling, strong physical interactions with the soil substrates, or even chemical reactions with reactive sites within the soil samples. It could also be ascribed to the difficulty of extracting carfentanil from organic content. The overall extractable amount of carfentanil decreased very little in each soil type over the 12 week period, indicating that little chemical change had occurred. The carfentanil was probably still present in its original form in the soil samples even after 12 weeks, and it could likely continue to persist in the soils over a long period of time. Slower decreases were observed after the initial loss. Further work will be needed to confirm these observations.

4.2 Water-Testing Results

Carfentanil stability in six different water sources was monitored for 11 weeks. The pH of the water samples was 5–8 at the beginning of the trial, and a slight increase was noted during the course of this work, with the pH values rising to the 6–9 range. Data describing recovery of carfentanil from water samples at predetermined time points are presented in Table 3 and illustrated in Figure 3. There was a small decrease in the concentration of carfentanil recovered in the water during the early time points, but that decrease was most likely due to experimental error such as machine or sample variance.

Similar to its performance in the soil experiments, carfentanil continued to persist in water over the 11 week testing period, indicating that it does not change chemically in the environment over time. The overall behavior of carfentanil in the experimental setup agrees with our initial assumption that carfentanil would mostly be found in the solid phase of soil, probably in the organic part, and would persist over time. The soil and water samples were not sterilized prior to the experiments because they were not collected with the intent to preserve microbial communities. Subsequent experiments can include active microbial samples to confirm that carfentanil is not degraded by microbial activity or that it impacts the overall ecological community in soil.

Table 3. Carfentanil Recovery from Water Samples

Time (day)	ALEC	SD	DI Water	SD	SW 1 (4g/100 mL)	SD	SW 2 (8g/100 mL)	SD	Rain Water	SD	0.01 M $CaCl_2$ Solution	SD
	(%)											
0.04	85	5	88	3	84	4	89	1	78	2	82	1
1	78	4	73	4	70	2	69	2	76	3	74	2
2	78	3	73	2	69	3	69	2	76	3	78	1
6	80	3	75	2	78	4	82	1	78	3	80	1
21	85	6	71	2	71	1	86	4	85	2	86	4
77	74	3	69	2	69	3	74	2	75	1	73	1

SD, standard deviation.

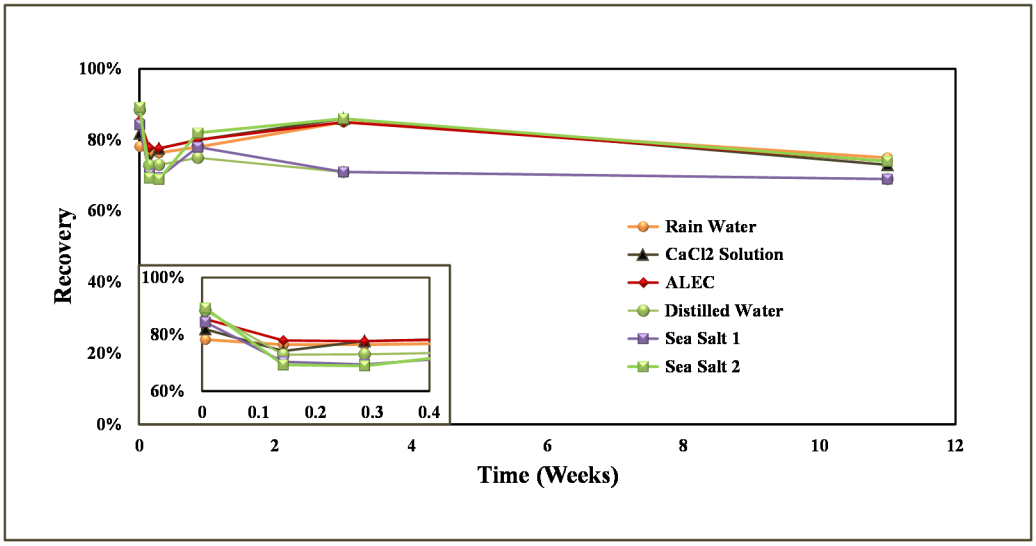


Figure 3. Carfentanil recovery from different water sources.

4.3 Carfentanil in 0.01 M Calcium Chloride Recoveries

A difference was observed between the calcium chloride recoveries in the positive-control samples and those of the calcium chloride spiked samples for the water experiments. The positive-control samples were prepared using the same procedures as the ones used for the soil samples but in polypropylene centrifuge tubes, whereas the water experiments were performed in glass vials. Data describing recovery of carfentanil from the water samples after predetermined time points are presented in Table 4 and illustrated in Figure 4.

Table 4. Carfentanil Recovery from Glass Vials vs Polypropylene Tubes

Recovery in Glass Vials			**Recovery in Polypropylene Tubes**		
Time (weeks)	**0.01 M $CaCl_2$ (%)**	**SD (%)**	**Time (weeks)**	**0.01 M $CaCl_2$ (%)**	**SD (%)**
0.01	82	1	0.02	77	1
0.1	74	2	0.1	75	5
0.3	78	1	0.3	78	4
0.9	80	1	1.0	76	2
3	86	4	4	67	4
11	73	1	12	63	1

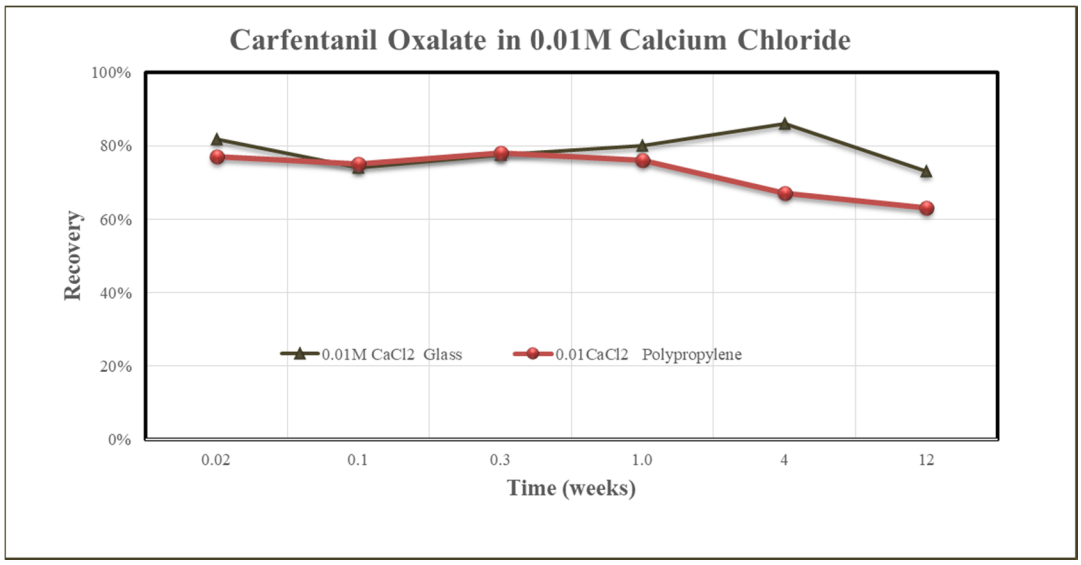


Figure 4. Carfentanil recovery from glass vials vs polypropylene tubes.

The difference in the results could be the higher affinity of carfentanil for polypropylene or just experimental, sampling, or analytical error. However, the reader should be aware of this difference, which may be compound-dependent.

5. CONCLUSIONS

The current work has shown that carfentanil is stable in water at neutral to slightly acidic pH levels and at ambient temperature for several months (at the least). In addition, the equilibrium distribution of carfentanil between the soil and water samples was found to lie strongly in favor of the soils. These data indicate that carfentanil will continue to persist in environmental soil despite exposure to rain water.

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ACRONYMS AND ABBREVIATIONS

ALEC	Anita C. Leight Estuary Center
BCF	biological concentration factor
CAS	Chemical Abstracts Service
CCDC CBC	U.S. Army Combat Capabilities Development Center Chemical Biological Center
DI	deionized
dSPE	dispersive solid-phase extraction
ESI	electrospray ionization
HPLC	high-performance liquid chromatography
K_{oc}	adsorption coefficient
K_{ow}	partition coefficient
LC	liquid chromatography
MS/MS	tandem mass spectrometry
NDL	North Dakota loam
OECD	Organization for Economic Co-operation and Development
PEL	Pennsylvania Ernest silt loam
p*K*a	acid dissociation constant
PN	part number
QuEChERS	quick, easy, cheap, effective, rugged, safe
SD	standard deviation
SSL	Sassafras sandy loam
SW	salt water
TRIS	tris(hydroxy-methyl)aminomethane
UHPLC	ultra-high performance liquid chromatography
UTL	Utah Timpie loam

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