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Environmental Fate of Fentanyl in Soil and Relevant Waters

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and aliquots we	re taken for analy	vsis at different ti	me periods. The	results show	ed that the amount of fentanyl recovered from			
soil extracts acc	counted for 20–50)% of the spike. I	Fentanyl was fully	v recovered f	from water samples for at least 7 weeks. These			
results indicate	that fentanyl is re	elatively stable in	water and moist	soils. The cu	irrent data also suggest that fentanyl is			
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PREFACE

The work described in this report was authorized by the Defense Threat Reduction Agency (DTRA) Joint Science and Technology Office (JSTO; Fort Belvoir, VA) under project no. HDTRA1620640. The work began in November 2015 and was completed in November 2016. At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center (CCDC CBC) was known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

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ENVIRONMENTAL FATE OF FENTANYL IN SOIL AND RELEVANT WATERS

1. INTRODUCTION

Pharmaceuticals represent an important class of emerging organic micropollutants. More than 100 pharmaceuticals of different therapeutic classes have been detected in rivers and streams. These compounds are introduced to surface waters mainly by discharge from wastewater treatment plants and by runoff from agricultural fields.¹ Fentanyl citrate (referred to as fentanyl in this study) is a synthetic opioid that is 80–100 times more potent as a narcotic than morphine. Fentanyl was originally developed for pain management of cancer patients. Because of its powerful narcotic properties, it is also diverted for abuse. Fentanyl is commonly added to heroin to increase the heroin's potency, which often results in overdose by users. The structure of fentanyl is provided in Figure 1.



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

The focus of the current work is to elucidate chemical and physical interactions between fentanyl, soil, and water to advance our understanding of how fentanyl behaves in the environment. Pesticides have been studied more extensively in association with the soil environment than any other chemical class.² 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 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}) .³ Previous studies have concluded that adsorption of pesticides increases with pH and organic matter content but decreases with ionic strength.⁴

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 better understand the behavior of a compound in a specific environment. For the models selected as the most useful for predictive modeling (namely, Pearl and GeoPearl), the soilorganic 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. It is timeconsuming to determine, so a screening coefficient can be measured in advance to determine whether the Freundlich coefficient must be included in the parameter list of the agent. The screening coefficient is the soil distribution coefficient (K_d), which is calculated by measuring the water- and soil-phase concentrations of the agent in the presence of four different soils. The soils vary in pH, clay content, and organic carbon content. A high K_d value indicates that an agent is strongly adsorbed to the soil and less likely to leach to the groundwater. The K_d value can also be used to determine the organic carbon distribution coefficient (K_{oc}) by using the relationship $K_d = K_{oc} \times f_{oc}$, where K_{oc} is the partitioning coefficient for organic carbon and f_{oc} is the fraction of organic carbon.⁵

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

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 were obtained from Sigma-Aldrich Corp. (St. Louis, MO); they were high-performance liquid chromatography (HPLC grade) with ≥99.9% purity;
- in-house 16 M Ω water was used to prepare samples as well as the HPLC mobile phase;
- sodium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate were American Chemical Society grade with ≥99% purity (Sigma-Aldrich);

- calcium chloride (Acros Organics; Pittsburgh, PA) had \geq 99% purity;
- 15 mL centrifuge tubes (Restek Corp.; Bellefonte, PA) used for dispersive solid-phase extraction (dSPE) cleanup for 6 mL extract, Q370, were used for QuEChERS (quick, easy, cheap, effective, rugged, safe) extract cleanup; and
- fentanyl citrate, ≥96% purity by liquid chromatography–mass spectrometry (LC/MS), was synthesized and purified in-house.

2.2 Soil Collecting and Processing

The soils used during this study were collected from the A horizon. 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 airdried, 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, 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) Guideline 106.⁵ 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 content, and organic matter content. 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 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.

Soil Name	Source	Content (%)			Textural Class	pН	Organic Carbon
und Type	Location	Sand	Silt	Clay	01055		(%)
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

Table 1. Soil Information

The SSL and NDL soils had previously been collected 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, also known as the topsoil. 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 large quantities of soil for testing (2–50 g). 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 96 sample vials and 32 negative controls during our experiments (the minimum amount specified in the guideline). No soil was used for the 32 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 fentanyl spike was performed. Vials of soil and solution were left overnight at room temperature to fully moisten the soils.

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

Samples were prepared for sacrificial collection and extraction of the fentanyl at time points of 1, 4, 24, and 72 h, and 1, 2, 4, 8, and 12 weeks. A total of 172 vials 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 fentanyl using an Agilent (Santa Clara, CA) LC–tandem MS system.

Fentanyl was extracted from the soil phase using a modified QuEChERS method.⁶ Modification included the addition of a TRIS buffer (pH 8.3) prior to extracting. The buffer increased the pH of the soil and fentanyl solution to 8.0, thus optimizing the release of analyte from the organic matter component of the soil, so that it could be extracted more efficiently. The modified QuEChERS method was selected after results from several extraction methods found in the literature and technical reports were compared.

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 Corp.; Port Washington, NY; part number 4545). 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 after the Tris buffer, 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 a 5804 centrifuge from Eppendorf (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 (PSA), followed by vortexing for 30 s. Afterwards, centrifugation was carried out at 3500 rpm for 5 min. All data were corrected for dilution, and recovery for each sample was based on the amount of fentanyl found in the extraction samples at each time point.

2.4 Sample Analysis

Analysis of fentanyl samples was carried out using an Agilent 1100 LC/MS system (consisting of vacuum degasser, autosampler, binary pump, and MS detector) equipped with a reversed-phase Waters HSS (high-strength silica) C18 column of 50×4.6 mm with particle size of 3.5 µm (Waters Corp.; Milford, MA). Column temperature was maintained at 30 °C. Mobile phases A and B were water and methanol, respectively. 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) or methanol (B). The flow rate was kept constant at 1.5 mL/min. Total run time was 3 min, and injection volume was 1.0 µL. The mass spectrometer was operated at positive electrospray ionization mode. Data acquisition was performed in selected ion monitoring mode.

The LC/MS analytical system was calibrated prior to 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 acetonitrile were prepared and analyzed against each other for accuracy. An eight-point calibration curve in the range $0.01-1 \ \mu g/mL$ 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 the liquid phase of the analysis. Aqueous-phase samples were not diluted because the results were below the lowest point in the calibration curve (0.01 $\mu g/mL$). Positive controls and extracted soil samples were diluted by a factor of 4 with acetonitrile to keep the experimental concentrations in the calibration range.

3. WATER ANALYSIS

In addition to determining the stability of fentanyl in soil, we also determined fentanyl stability in four water sources, as described in this section.

3.1 Water Sources

Water samples were obtained from the following locations:

- Ground water was collected on 10 April 2016 (initial pH 5.1) from the Anita C. Leight Estuary Center (ALEC; Harford County, MD).
- Sea salt 4 was prepared in-house by adding 4 g of NaCl to 100 mL of deionized (DI) water. (Note: This concentration was selected to simulate that of ocean water.)
- Sea salt 8 was prepared in-house by adding 8 g of NaCl to 100 mL of DI water.
- $16 \text{ M}\Omega$ water was produced using an in-house system.

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 fentanyl by adding 100 μ L of a 1000 μ g/mL stock solution, so that the starting concentration was 5 μ g/mL for each. 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 7 week experimental period. Samples were stored for 1, 24, 48, and 72 h, and 2, 4, and 7 weeks after preparation. After each designated time period, 100 μ L of solution was removed and diluted to a volume of 1000 μ L. The diluted samples were analyzed using LC/MS technology.

4. **RESULTS AND DISCUSSION**

4.1 Fentanyl in Soil

The data describing recovery of fentanyl following soil contact are listed in Table 2 and shown graphically in Figure 2. Recovery varied between about 20 and 50% after 12 weeks of exposure. Those data suggest long-term environmental stability of fentanyl. It is significant to note that the initial losses of about 60–80% observed for all soils tested over the first 24 h of exposure were followed by very slow degradation over the length of the experiment.

Time	UTL	SD	NDL	SD	PEL	SD	SSL	SD
(weeks)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
0.006 (1 h)	57	8	80	6	51	10	74	2
0.024 (4 h)	67	15	79	7	51	17	65	11
0.143 (24 h)	41	2	55	4	19	1	40	1
0.429 (72 h)	40	4	50	3	22	3	44	2
1	46	8	57	11	26	3	46	3
2	46	3	63	3	26	3	49	9
4	28	0	45	1	25	3	40	4
8	35	3	29	22	20	1	41	6
12	24	4	48	1	19	4	37	5

Table 2. Fentanyl Recovery from Soil

SD, standard deviation.



Figure 2. Fentanyl recovery from soil.

In this work, we were unable to detect fentanyl in the aqueous supernatant. As a result, the fentanyl–soil partitioning constant (K_d) could not be calculated. The current results suggest that fentanyl is most likely immobile in soils, particularly in the event of runoff due to heavy rains. It is likely that the initial loss of analyte is attributable to chemical degradation at reactive soil sites or irreversible adsorption of the fentanyl at active sites that prevented extraction. More work, in particular, variation of analyte-to-soil ratios, is needed to explore this hypothesis. Most importantly, if the initial losses observed in our work increase with soil-to-chemical ratio, mobility of the fentanyl in the soil could be insignificant.

4.2 Fentanyl in Water

Fentanyl stability was monitored in four different water sources for 7 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. Future experiments could include microbially active samples to confirm that fentanyl is not degraded by microbial activity or other environmental conditions. Data describing recovery of fentanyl from water after several time periods are presented in Table 3 and illustrated in Figure 3.

Time (days)	ALEC (%)	SD (%)	DI Water (%)	SD (%)	Sea Salt 1 (4 g/100 mL)	SD (%)	Sea Salt 2 (8 g/100 mL)	SD (%)
0.04	108	0	105	2	95	7	99	10
1	114	2	109.3	0	98	0	95	3
2	87	1	90	0	87	1	82	5
3	107	0	94	6	56	26	55	1
7	99	0	100	1	98	12	93	10
14	90	1	93	1	91	6	89	3
28	96	0	94	1	89	5	78	0
49	79	2	81	2	92	23	96	1

Table 3. Fentanyl Recovery from Water

SD, standard deviation.



Figure 3. Fentanyl recovery from four water sources over 7 weeks.

Fentanyl's persistence in water over the 7 week period indicates that it does not hydrolyze in the environment over time when exposed to water. The overall behavior of fentanyl is in agreement with our initial assumption that fentanyl is mostly found in the solid phase of soil (most likely in the organics) and persists over time. A concentration decrease was noted at 48 h for saltwater samples. This decrease was likely due to the analytical techniques that were used, given that the results remained stable in later data.

5. CONCLUSIONS

Results from this study indicate that fentanyl is likely to persist in soil environments for months to years. We also determined that fentanyl is stable in water at ambient temperatures and for several months. In addition, the equilibrium distribution of fentanyl between the several soil and water types tested was found to be in favor of the soils. The amount of fentanyl in contact with the soils was nearly constant for up to 12 weeks, and it accounted for 20–50% of the amount of the spike. Likewise, the water samples were shown to be stable for up to 7 weeks. These results indicate that fentanyl is relatively stable in water and moist soils. The current data also suggest that fentanyl is immobile in the environment. The fentanyl remaining in the soil is likely protected from degradation and could possibly become a secondary hazard. Blank

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ACRONYMS AND ABBREVIATIONS

ALEC	Anita C. Leight Estuary Center
DI	deionized
dSPE	dispersive solid-phase extraction
foc	fraction of organic carbon
HPLC	high-performance liquid chromatography
Kd	soil partitioning coefficient constant
Koc	soil organic partition coefficient
Kom	soil-organic matter partition coefficient
$K_{ m ow}$	octanol-water partition coefficient
LC	liquid chromatography
MS	mass spectrometry
NDL	North Dakota loam
OECD	Organisation for Economic Co-operation and Development
PEL	Pennsylvania Ernest silt loam
PSA	primary-secondary amine
QuEChERS	quick, easy, cheap, effective, rugged, safe (analytical method)
SD	standard deviation
SSL	Sassafras sandy loam
UTL	Utah Timpie loam

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