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Environmental Fate of Remifentanil in Soil and Relevant Waters

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14. ABSTRACT:
In this study, we assessed the stability and persistence of remifentanil in four water sources, buffered and unbuffered water, four different types of soil in contact with water containing 0.01 M $CaCl_2$, and several soil–water environments. In unbuffered water samples, remifentanil appeared to degrade following first-order kinetics. A slightly acidic citrate buffer appeared to stabilize the remifentanil. Soil samples were spiked with a known amount of remifentanil in solution; the aqueous and solid (soil) layers were separated and extracted after contact times up to 11 weeks to track compound distribution and recovery. The analyte was detected in several of the aqueous-phase samples in contact with different types of soil, which enabled the calculation of distribution coefficient values. Our work shows that remifentanil is strongly bound to all of the soil types that we studied. The primary remifentanil hydrolysis product (R26) was detected in extracts from each soil type. In each case, it appeared that a low level of R26 formed relatively quickly. These results indicate that remifentanil is relatively stable in low pH water and moist soil types and is immobile in a soil environment.

15. SUBJECT TERMS

Soil–water environment	Remifentanil	Transport
Primary remifentanil hydrolysis product (R26)	Stability	Opioids
Distribution coefficient (K_d)	Adsorption coefficient (K_{oc})	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 March 2016 and completed in December 2016. 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 REMIFENTANIL IN SOIL AND RELEVANT WATERS

1. INTRODUCTION

Methyl 1-(3-methoxy-3-oxopropyl)-4-(*N*-phenylpropionamido) piperidine-4-carboxylate is commonly known as remifentanil (Chemical Abstracts Service [CAS] number 132875-61-7). In the United States, remifentanil is classified as a Schedule II narcotic controlled substance. Remifentanil has been studied extensively and is known to have a short half-life in blood and plasma, which is attributed to the presence of endemic esterases.[1–12] Remifentanil half-life has been shown to be significantly extended in the presence of formic and citric acids with an optimum pH balance in the range of 2–3.5.[13] The remifentanil structure and the primary remifentanil hydrolysis product (R26; formed by de-esterification to methanol and the corresponding acid) are presented in Figure 1.

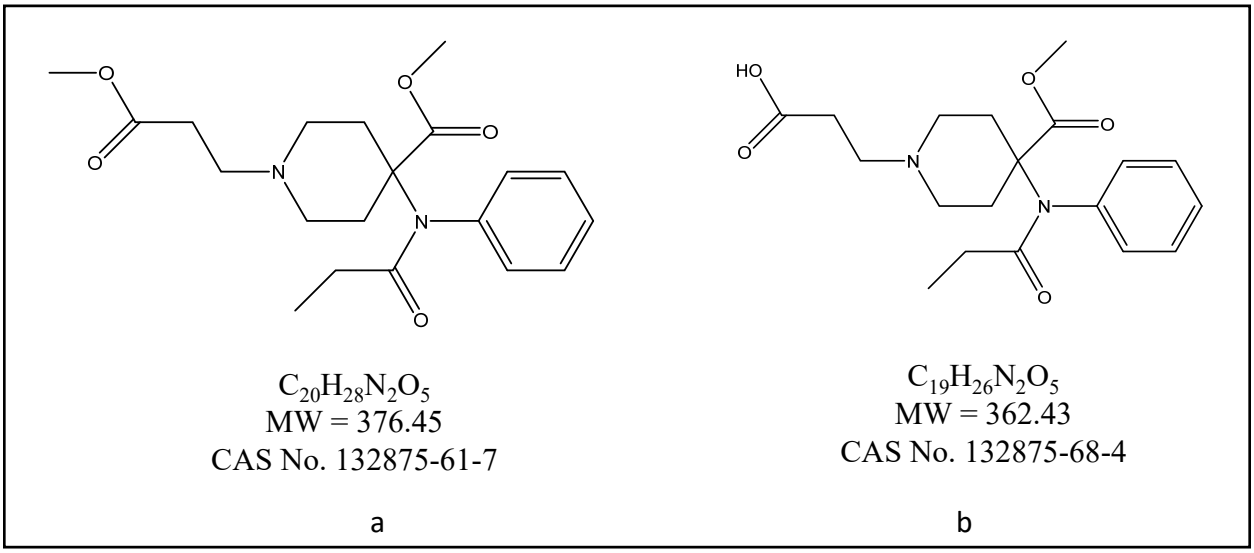

Figure 1. Structures, formulas, and molecular weights (mw) of (a) remifentanil and (b) R26.

The estimated normal boiling point of remifentanil (488 ± 45 °C) suggests that it exists almost exclusively in the condensed phase at atmospheric temperatures.[14] Calculations were performed using Advanced Chemistry Development (Toronto, Canada) Software V11.02 (1994–2019). Particulate-phase remifentanil was removed from the atmosphere by wet or dry deposition. Remifentanil water solubility was 44 mg/mL.

For 11 weeks, we observed remifentanil stability and extractability in four different soils and four different water sources collected from various continental U.S. sites. A partial dataset was also measured for a fifth soil to validate our data and comply with the Organisation for Economic Co-operation and Development (OECD; Paris, France) guidelines[15] for the water–soil distribution coefficient (K_d) calculations.

2. WATER ANALYSES

2.1 Water Sources

Remifentanil and R26 stabilities were monitored in water samples obtained from the following sources:

- ground water from the Anita C. Leight Estuary Center (ALEC; Harford County, MD) was collected on 10 July 2018,
- 16 MΩ of deionized (DI) water was obtained in-house at the U.S. Army Edgewood Chemical Biological Center (now known as the U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD),
- salt water 1 was formed by mixing 4 g of NaCl and 100 mL of DI water (this concentration simulated that of ocean water), and
- salt water 2 was formed by mixing 8 g of NaCl and 100 mL of DI water.

2.2 Water Sample Preparation and Analysis

We used 20 mL of each water type in each experiment. Separate vials were used for each water sample, and the experiments were conducted in triplicate with a negative control. Remifentanil was added by pipetting 100 µL of a 1000 µg/mL solution so that the remifentanil starting concentration was 5 µg/mL for each replicate. The samples were stored at 22 ± 1 °C for 1 week (experimental period).

The freshwater samples were stored for 1 h; 1, 2, and 3 days; and 1 week after preparation. The saltwater samples were stored for 1 h and 4 days after preparation. After each designated time period, 100 µL of experimental replicate was removed and diluted to a final volume of 1000 µL. The diluted samples were analyzed using an ultra-high performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS) system (Waters Corporation; Milford, MA). In this report, this system is referred to as the liquid chromatography (LC)–MS/MS. The instrument was calibrated on the day of analysis with freshly prepared standards.

2.3 Stability in Buffered Water Samples

Over a 28 day period, remifentanil stability data were measured in triplicate using buffered DI water with citrate, 3-(*N*-morpholino)propanesulfonic acid [MOPS]), and tris(hydroxy-methyl)aminomethane (TRIS) at pH values of 4.0, 7.3, and 8.6, respectively.

3. SOIL ANALYSIS

3.1 Reagents and Chemicals

The following reagents and chemicals were used during testing:

- acetonitrile and methanol (high-performance liquid chromatography [HPLC] grade with ≥99.9% purity; Sigma-Aldrich Corporation; St Louis, MO);
- in-house 16 MΩ of water (for sample preparation and HPLC mobile phase);
- sodium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate (American Chemical Society [Washington, DC] grade with ≥99% purity; Sigma-Aldrich);
- calcium chloride (≥99% purity; ACROS Organics; Pittsburgh, PA);
- remifentanil citrate (synthesized and purified in-house; LC–MS/MS analysis indicated 98.3% purity); and
- 100 µg/mL of remifentanil acid (R-026; CAS number 132875-68-4) in acetonitrile certified reference material (Cerilliant Corporation; Round Rock, TX).

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 QuEChERS (quick, easy, cheap, effective, rugged, safe) extract clean-up. [16]

3.2 Soil Collection and Processing

The soils used during this study were collected primarily from the A horizon. At each location, we removed all leafy matter from the sampling area. We dug a hole a few inches deep and then dug outward in a circle. If well-developed O horizon matter was present, it was incorporated into the sample. The 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-lidded containers at room temperature. Remaining moisture levels were measured before testing began, and data were reported as dry soil mass.

3.3 Soil Experiments

The procedures followed during this portion of the study were based on the OECD Guideline 106[16] and were modified in accordance with the method of Stein and coworkers.[17] This guideline presents recommendations for determining the durability of a chemical in soil. The guideline recommends testing different naturally occurring soils with varying pH, clay content, and organic matter content. Four soils were identified and collected for testing. These 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. Analyses results are shown in Table 1 and identified in this report as Sassafras sandy loam (SSL), Pennsylvania Ernest silt loam (PEL), North Dakota loam (NDL), and Utah Timpie loam (UTL). As mentioned in Section 1, a fifth soil, Nunn clay loam (CO), was also analyzed to validate our data.

Table 1. Soil Information

Soil Name and Type	Location	Sand Content (%)	Silt Content (%)	Clay Content (%)	Textural Class	pH	Organic Carbon (%)
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	Timpie loam	8.4	1.4
Nunn Clay Loam	Colorado	45	23	32	Clay loam	7.6	1.2

The OECD guidelines[16] suggest using large quantities (e.g., 2–50 g) of soil for testing. Because of the toxicity of the remifentanil used in the current work and the need to execute experiments safely and efficiently, only 2 g (the minimum amount specified in the guidelines) of soil was used in each of the (108) sample vials and (36) negative controls during our experiments. No soil was used for the (36) positive controls. The 2 g of soil, corrected for remaining moisture content in our calculations, was reconstituted with 2 mL of 0.01 M $CaCl_2$ solution on the day before the remifentanil spike was performed. Vials of soil and solution were left overnight to ensure the soil was fully moistened.

A set of samples was prepared in triplicate for each soil type and time period, and each set contained negative- and positive-control samples. Each negative-control sample contained a soil type and 0.01 M $CaCl_2$ of solution but no remifentanil. The positive-control samples were prepared in $CaCl_2$ solution only for each sample set that maintained the same sacrificial time schedule as used for the soil samples. Otherwise, 2 mL of the 0.01 M calcium chloride solution was spiked with remifentanil by adding 10 µL of a 1000 µg/mL solution so that the concentration of remifentanil was 5 µg/mL for each positive control.

Soils were prepared for sacrificially collecting and extracting the remifentanil at time points of 4, 24, 48, and 72 h and 1, 2, 4, 8, and 11 weeks. The total number of vials used in this portion of the work was 180. On the day of data collection, the tubes selected for analysis

were centrifuged to separate the soil from the solution, and the liquid phase was collected, filtered, and analyzed for remifentanil using the LC–MS/MS system. Remifentanil was then extracted from the soil phase using a modified QuEChERS method. The modification included the addition of a TRIS buffer (pH 8.6) prior to extracting. The buffer increases the pH of the soil and remifentanil solution to 8.0, thus optimizing the release of analyte from the organic matter of the soil, so that it is more efficiently extracted. The modified QuEChERS method was selected after the results were compared with several extraction methods found in the literature and technical reports. All data were corrected for dilution, and recovery for each sample was determined based on the amount of remifentanil found in the extraction samples at each time point. We were able to detect low levels of remifentanil in several of these samples; therefore, we could determine the K_d value for those samples. In all the cases, the vast majority of recoverable analyte was found in the soil environment.

3.4 Analyses of Samples

Analysis of remifentanil samples was carried out using the Waters Acquity UHPLC system, consisting of a vacuum degasser, autosampler, and binary pump. This system was equipped with a reversed-phase pinnacle DB intrinsically base-deactivated biphenyl column 100 × 2.1 mm with particle size 1.9 µm (Restek Corporation). The LC column temperature was maintained at 40 ºC. Mobile phases A and B consisted principally of 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) or methanol (B), respectively. The flow rate was kept constant at 0.35 mL/min. The LC system was run in isocratic mode with the water/methanol ratio at 10:90 for the duration of the run. Total run time was 5 min. The analyte injection volume was 0.5 µL.

The LC system was coupled with a Waters Quattro Premier triple-quadrupole mass spectrometer (TQMS) equipped with an electrospray ionization (ESI) interface and Mass Lynx software (Version 4.1). The TQMS system was operated in positive ESI mode.

Data acquisition was performed 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. On the day of each analysis, the LC–MS/MS analytical system was calibrated before each series of measurements using standard solutions prepared from stock solutions. Two stock solutions at 1 mg/mL concentration in methanol were prepared and analyzed against each other 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 were within 5% agreement. 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 controls and extracted soil samples were diluted by a factor of 4 with acetonitrile to keep the experimental concentrations in the calibration range.

4. RESULTS AND DISCUSSION

4.1 Water Stability

The stability of remifentanil and R26 was monitored in water taken from four different (unbuffered) sources over a period of 7 days. It was found that the most stable environment for the analyte was DI water, although ground water from ALEC was nearly as stable. However, the results for both fresh water sources showed that the remifentanil was nearly completely degraded by the end of the 7 day test period. Based on an exponential decay curve, the analyte appeared to decompose in the DI and ALEC water samples. It is possible that similar behavior would have been seen for the analytes in the saltwater, but no data are available to support this hypothesis because of the rapid decomposition of the analytes.

The data describing the recovery of remifentanil from water during several time intervals (up to one week) are presented in Table 2 and illustrated in Figure 2. The remifentanil was stable enough in the DI and ALEC ground water samples that remifentanil concentrations could be measured at several intermediate times before it became undetectable (within about a week). The exponential decay is consistent with first-order decomposition reaction kinetics. Correlation equations corresponding to the experimental data are provided in Figure 2. The analyte concentrations in both simulated seawater samples decreased significantly after 1 h and were below the detection limit after 4 days. As a result, we were unable to estimate the decomposition rates in those media.

Table 2. Remifentanil Percentage Recovery from Water

Time (days)	ALEC Ground Water	DI Water	Saltwater 1 (4 g NaCl/100 mL)	Saltwater 2 (8 g NaCl/100 mL)
0.04	100	100	59	49
1	40	73	*	*
2	20	40	*	*
3	6	26	*	*
4	*	*	ND	ND
7	0	4	ND	ND

*No data.
ND < 0.01 µg/mL.

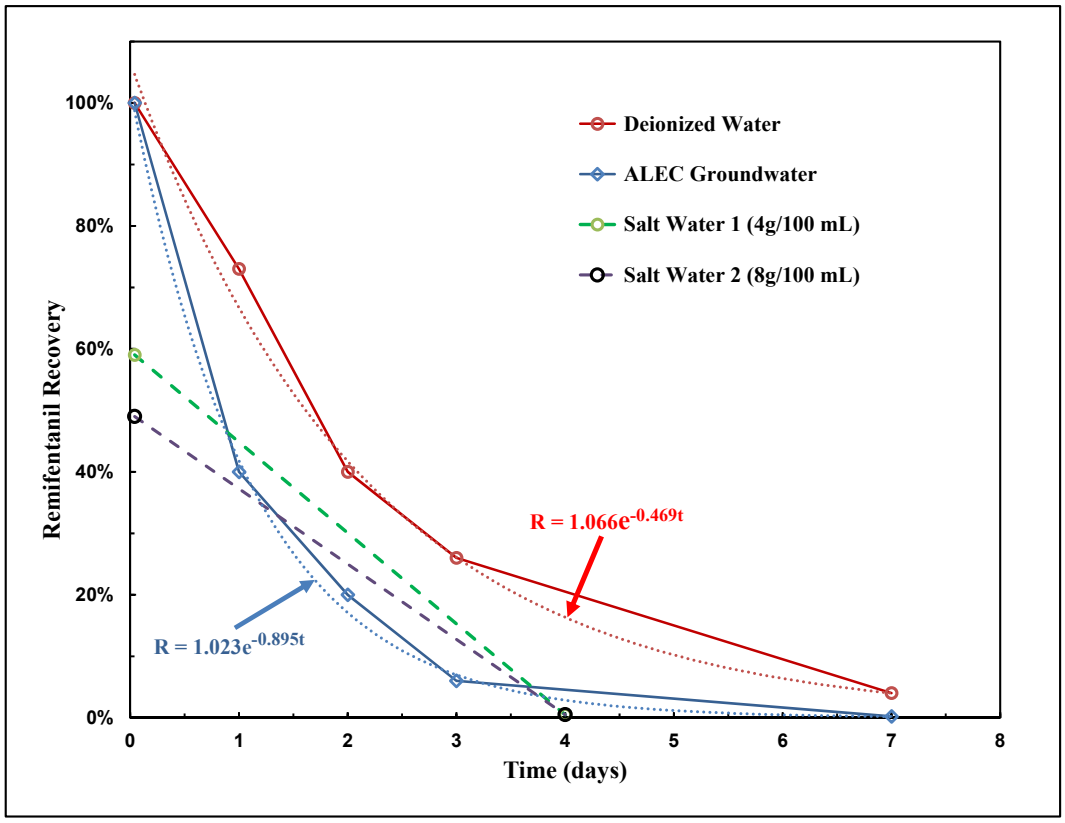

Figure 2. Remifentanil recovery from four different water sources.

The results of the buffered water tests are listed in Table 3 and illustrated in Figure 3. These data clearly demonstrate the stability of remifentanil in the acidic medium for at least 28 days. Remifentanil degraded rapidly in neutral and slightly basic media and was significantly reduced in the MOPS and TRIS buffers after 1 day. The buffer pH was unchanged.

Table 3. Remifentanil Percentage Recoveries and Standard Deviations (SDs) from Buffered DI Water Samples

Time (days)	Citrate Buffer pH	Citrate Buffer	SD	MOPS Buffer pH	MOPS Buffer	SD	TRIS Buffer pH	TRIS Buffer	SD
0.04	4.0	78.30	1.53	7.3	69.30	1.15	8.6	64.31	1.53
0.2	–	78.63	1.15	–	55.31	1.15	–	43.98	0.0
1	3.9	78.97	2.64	7.3	13.33	1.15	8.5	4.00	0.0
4	4.0	89.30	1.15	7.3	ND	ND	8.6	ND	ND
7	4.0	92.29	2.08	–	–	–	–	–	–
14	4.0	78.30	0.58	–	–	–	–	–	–
28	4.0	79.63	0.58	–	–	–	–	–	–

ND < 0.01 µg/mL.
–, not available.

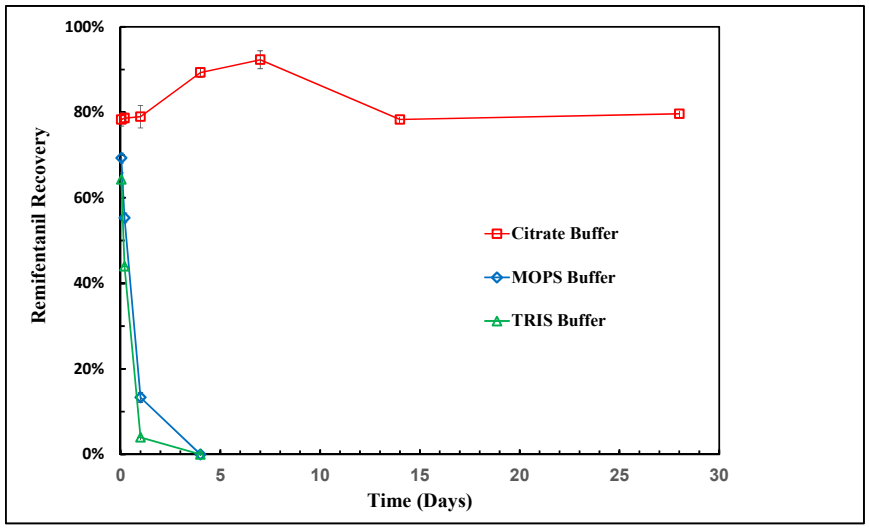

Figure 3. Remifentanil recovery from buffered water sources.

Complementary data were measured for R26. These data, listed in Table 4 and illustrated in Figure 4, show a relatively rapid formation and degradation of R26 in the MOPS and TRIS buffers. This behavior is consistent with the formation and further reaction of the R26 intermediate. On the other hand, during the observation period, the R26 concentration in the citrate buffer showed a slow increase for the citrate buffer, which is consistent with the slow remifentanil degradation as well as stability of R26 in an acidic medium. The results for all three buffers are consistent with the remifentanil stability data discussed above.

Table 4. Percentage Recovery of R26 from Buffered Water Samples

Time (days)	Citrate Buffer	SD	MOPS Buffer	SD	TRIS Buffer	SD
0.04	8.0	0.0	8.0	0.0	4.7	2.31
0.2	8.0	0.0	12.0	0.0	23.3	2.31
1	8.7	1.15	22.0	0.0	34.3	1.53
4	6.7	1.15	1.6	0.0	7.6	0.40
7	6.0	0.00	0.4	0.0	8.3	0.21
14	7.7	0.58	ND	–	ND	–
28	10.0	0.0	ND	–	ND	–

ND < 0.01 µg/mL.
–, Not available.

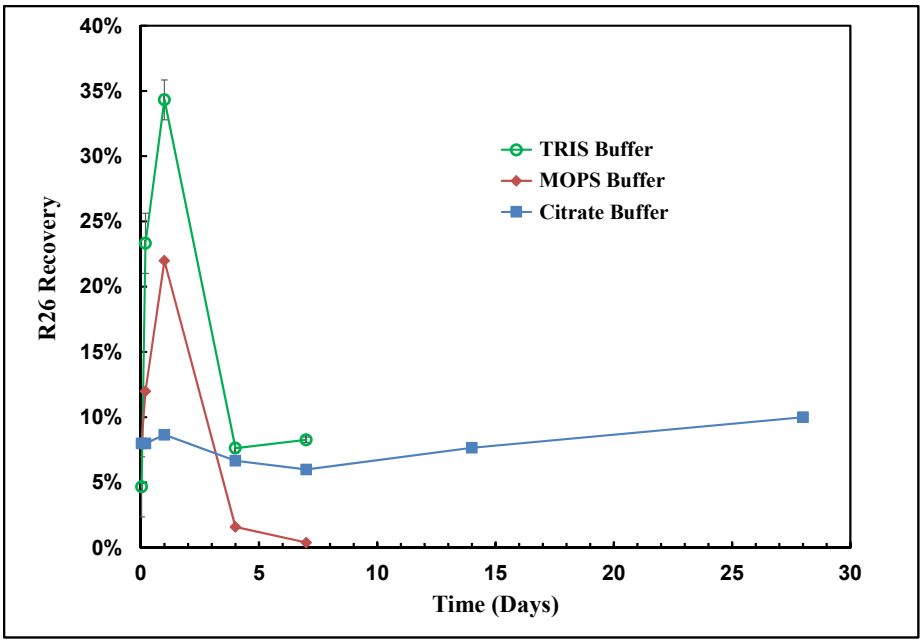

Figure 4. R26 recovery from buffered water sources.

4.2 Soil

Recovery of remifentanil from the various soil types in contact with 0.01 M $CaCl_2$ water was quantified over an 11 week period using a modified QuEChERS method. The majority of remifentanil adsorbed to the soil, most likely to the organic matter, for the soil types that were tested. The data are listed in Table 5 and illustrated in Figure 5. These data indicate an initial decrease in remifentanil concentration at neutral pH before stabilization occurred.

Table 5. Percent Recoveries of Remifentanil and SDs for UTL, NDL, PEL, and SSL over 11 Weeks*

T (weeks)	UTL	SD	NDL	SD	PEL	SD	SSL	SD
0.024	86.1	1.41	92.4	2.49	65.9	4.18	74.7	9.94
0.14	75.7	1.95	73.2	3.12	52.6	2.89	71.2	2.89
0.29	74.8	1.18	77.5	1.69	66.0	2.04	73.3	0.46
0.43	50.1	7.04	85.5	6.16	61.3	5.90	83.8	3.33
1	69.6	2.97	76.2	5.07	62.3	7.16	101.6	2.59
2	51.3	2.70	63.3	8.49	50.6	6.27	86.0	21.42
4	36.1	3.31	26.4	23.67	46.1	3.11	71.1	1.51
8	46.2	10.28	11.7	–	39.2	4.62	68.9	2.75
11	45.9	3.10	0.0	0.0	28.1	1.80	71.0	9.68

*Data and SDs resulted from triplicate measurements.

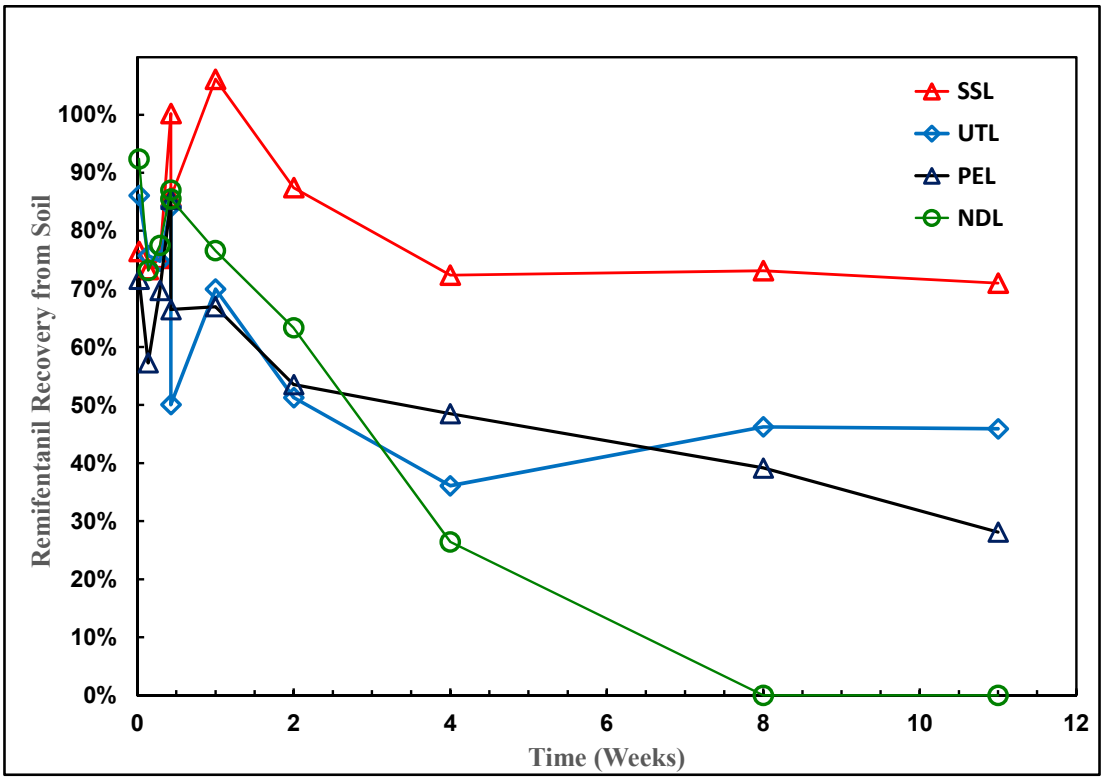

Figure 5. Recovery of remifentanil from four soil types over 11 weeks.

The concentration of remifentanil in the aqueous phase of these experiments was low in all the cases and below our current detection limits in several instances. Only the SSL and PEL soils had consistently measureable quantities of remifentanil in the aqueous phase. These data show that remifentanil binds strongly to each soil type.

It is important to note that the recovery of remifentanil from the aqueous phase and soil extraction was less than quantitative and tended to decrease with time. The consistent decrease with time suggests that some of the analyte was not extracted from the soil or it decomposed with other products that were not identified by the current analysis. This observation is consistent with the earlier observation that remifentanil decomposes rapidly in basic or neutral aqueous solutions.

The K_d, values were typically measured at the 24 h time point when it was expected that the agent had reached equilibrium between the soil and liquid phases. Because of the decreasing trend of remifentanil concentration with time, the K_d value was calculated from the 4 h measurement to provide insight into the behavior of the chemical in the soil. For that reason, it should be noted here that our values were not necessarily consistent with similar measurements done at the 24 h mark. The extraction method was the same as that described in Section 3.3, with the exception that 10 mL of 0.01 M $CaCl_2$ was added instead of 2 mL. The data for the K_d values of the five soil types are presented in Table 6. The K_d value for each was determined using the equation

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

where $C_s^{ads}(eq)$ is the remifentanil concentration in the solid phase at equilibrium, and $C_{aq}^{ads}(eq)$ is the concentration of the liquid phase at equilibrium.

Table 6. K_d Values for Five Soil Types

Soil Type	K_d Value ± SD*	Soil clay content (%)	pH	Organic Carbon (%)	K_{oc}
SSL (MD)	45 ± 1	17	4.5	1.14	3908
UTL (UT)	439 ± 79	25	8.4	1.42	30906
NDL (ND)	565 ± 93	22	7.6	3.07	18376
PEL (PA)	18 ± 1	21	4.5	3.97	444
Nunn Clay Loam (CO)	187 ± 17	32	7.6	1.17	16077

*K_d values calculated after 4 h equilibration.
K_{oc}, adsorption coefficient.

The K_d and K_{oc} values help determine whether soil is one of the factors that plays a role in sorption of the compound and if the more experimentally intensive Freundlich coefficient should be determined. The Freundlich coefficient is necessary when sorption of compound in soil is dependent on components other than organic matter, such as clay or other soil colloids.

The organic carbon-normalized K_{oc} value relates the K_d value to the content of organic carbon in the soil sample: $K_{oc} = K_d \times \frac{100}{\%OC}$ (cm³/g), where $\%oc$ is the percentage of organic carbon in the soil sample (g/g).

The K_{oc} coefficient represents a single value, which characterizes the partitioning of nonpolar organic chemicals between the organic carbon in the soil or sediment and water. The adsorption of these compounds is correlated with the organic content of the sorbing solid; therefore, K_{oc} values depend on the specific characteristics of the humic fractions, which differ considerably in sorption capacity due to differences in origin, genesis, and so forth.[16]

Adsorption of remifentanil tended to be pH dependent; the more basic types of soil had higher K_d values. The relationship between organic carbon content and adsorption strength is less clear. These results indicate a strong binding interaction between remifentanil and the types of soil studied in this work, which is entirely consistent with similar work performed at our laboratory in which we investigated the binding of carfentanil to different soil types.[18] The stabilizing effect of the acidic citrate buffer illustrated in Figure 3 is consistent with the soil data and literature.[13] It is also apparent that the more basic types of soil have some stabilizing effect on the analyte, possibly because remifentanil was sequestered in the soil.

A second set of soil samples was investigated for 2 weeks to validate our findings. It was again found that the vast majority or, in many cases, all of the recoverable remifentanil was found in the soil; little or none was found in the (aqueous) supernatant.

R26 formation was detected in all of the latter studies. There was significant data scatter, and the amount detected was generally in the 5–10% range, and no single value exceeded 11.3%. No obvious trends were noted for these data due to the scatter of the data, although it did appear that the intermediate formed within the first 12–18 h and did not change significantly afterwards.

5. CONCLUSIONS

This study has shown that remifentanil is stable in water at slightly acidic pH values and ambient temperatures for over 3 months. In addition, the equilibrium distribution of remifentanil between water and the types of soil that we tested was found to lie strongly in favor of the soils. In most of the cases, there appeared to be a rapid initial degradation of the remifentanil before a period of relative stability. Further studies are necessary to determine whether the initial loss was caused by the extraction and analysis procedure, or if this procedure is responsible for a limited environmental capacity to denature small amounts of remifentanil. The stability of remifentanil was found to increase when it was in contact with wet soil, as opposed to a neutral aqueous solution. These data indicate that remifentanil can be persistent in environmental soil for many months without significant degradation or transport, even during rainy weather.

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ACRONYMS AND ABBREVIATIONS

ALEC	Anita C. Leight Estuary Center
CAS	Chemical Abstracts Service
DI	deionized
dSPE	dispersive solid-phase extraction
ESI	electrospray ionization
HPLC	high-performance liquid chromatography
K_d	distribution coefficient
K_{oc}	adsorption coefficient
LC	liquid chromatography
MOPS	3-(*N*-morpholino)propanesulfonic acid
MS/MS	tandem mass spectrometry
NDL	North Dakota loam
OECD	Organisation for Economic Co-operation and Development
PEL	Pennsylvania Ernest silt loam
QuEChERS	quick, easy, cheap, effective, rugged, safe
SD	standard deviation
SSL	Sassafras sandy loam
TQMS	triple-quadrupole mass spectrometer
TRIS	tris(hydroxy-methyl)aminomethane
UHPLC	ultra-high performance liquid chromatography
UTL	Utah Timpie loam

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