
Methane generation and consumption rates for soils collected along a vadose-zone methane gradient

Unocal-Sponsored EBI Research Project

Guadalupe Restoration Project
September 1, 2004 to August 30, 2005

FINAL REPORT
January 11, 2006

Yarrow M. Nelson, Ph.D., Principal Investigator
Christopher Kitts*, Ph.D., Co- Principal Investigator
C. Kirk Gonzalez, Graduate Research Assistant

Department of Civil and Environmental Engineering
* Department of Biological Sciences

Environmental Biotechnology Institute
California Polytechnic State University
San Luis Obispo, CA 93407

Executive Summary

Methane flux through the vadose zone above source zones at the former Guadalupe Oil Field can be used as an indicator of anaerobic biodegradation of hydrocarbons in non-aqueous phase liquid (NAPL) pools of diluent at the capillary fringe. It is important to understand the kinetics of biological processes that both generate and consume methane in order to accurately quantify and model this biodegradation. To obtain such kinetic data, soil microcosms were prepared using soil samples collected along a vertical transect for which methane concentrations had previously been characterized (near compressor plant site). The rates of methane generation by methanogenic bacteria and methane consumption by methanotrophic bacteria were determined in these microcosms by measuring headspace methane concentrations over time. Methane production was observed under anaerobic conditions in microcosms with soils collected below the water table, and observed methane production rates were $1.8 \pm 0.6 \times 10^{-4}$ ug CH₄/g-soil-hr. Under aerobic conditions methane consumption was rapid. Methane consumption rates for soils collected from the vadose zone were 0.29 ± 0.08 ug CH₄/g-soil-hr. Acetylene controls showed no methane consumption, confirming that observed methane utilization was indeed due to biological methanotrophic activity. On a per-gram soil basis, the methane consumption rates were about 1500 times higher than the methane production rates. Therefore methanotrophic activity is an extremely important consideration in interpretation of methane flux. In a companion study, terminal restriction fragment (TRF) analysis was used to characterize the microbial communities associated with methanogenesis and methanotrophy. A strong correlation of methanogenic vs. methanotrophic bacteria was observed with changes in depth and redox potential, which agreed with the microcosm study results.

Acknowledgements

This project was the brain-child of Dr. Paul Lundegard (Unocal/Chevron), and the authors are grateful for his expert guidance of this work. Don Eley (LFR) also provided valuable geological expertise for soil sampling and experimental design, and Greg Oulette (Inland Empire Analytical) provided gas headspace analyses. The project was also supported by logistical help from Kim Tullidge (Permitting Coordinator, Environmental Management Company) and Guadalupe Restoration Project Manager Gonzalo Garcia (Unocal Corp.).

Introduction

This research was conducted as part of an ongoing investigation into methane flux at the former Guadalupe Oil Field to derive kinetic data for biological methane production and consumption rates and to characterize the microbial communities associated with methanogenesis and methanotrophy using terminal restriction fragment (TRF) analysis. Soil samples were collected from the compressor plant site at the site where methane concentrations have previously been characterized in the vadose zone. The rates of methane generation by methanogenic bacteria and methane consumption by methanotrophic bacteria were determined using soil microcosms with soil collected at the compressor plant site. TRF analyses were conducted for soil samples from a range of depths through the vadose zone to allow observation of shifts in the microbial community structure with changes in depth and redox potential. This research was undertaken to increase our understanding of the observed methane gradients in the vadose zone and to contribute to modeling efforts for estimating natural attenuation rates at the site under methanogenic conditions.

The specific objectives of this work were to address the following research questions:

1. Does microbial community structure reflect the change from methanogenesis near the water table to methanotrophy higher in the vadose zone? Our hypothesis was that anaerobic methanogens found below the water table (where high methane concentrations are observed) will give way to aerobic methanotrophs in the upper vadose zone.
2. What is the rate of methane generation for soils with methanogenic bacteria under anaerobic conditions?
3. What is the rate of methane consumption for soils with methanotrophic bacteria under aerobic conditions?
4. Can the rates of methanogenesis and methanotrophy be used in a model to describe the observed methane profile?

Background

Methane concentration gradients have been determined at the compressor plant site using a gas monitoring well. The methane concentration directly above the water table, at about 120 ft bgs, is 4.5%, and decreases to near zero at 87 ft bgs (Figure 1; see also soil gas data in Appendix 1). The hypothesis is that methane is generated in the anaerobic zones below the water table in conjunction with anaerobic hydrocarbon biodegradation. The decrease in methane concentrations higher in the vadose zone is attributed to methane consumption by methanotrophic bacteria under aerobic conditions.

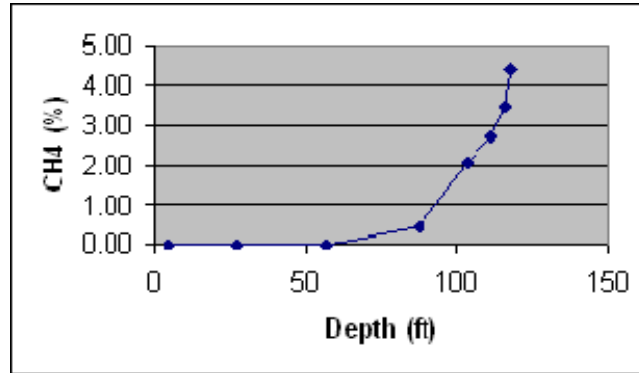


Figure 1. Methane profile in the vadose zone at compressor plant site (data from Paul Lundegard, Unocal Corp.)

The observed methane concentration profile can be used to determine methane flux rates, which can in turn be used to estimate rates of anaerobic hydrocarbon biodegradation. To model this flux rate it is important to consider sources and sinks of methane and the kinetics of these processes. In the present work we determine rates of methanogenesis and methanotrophy using controlled soil microcosms maintained under conditions mimicking subsurface conditions at the site. These rates are expected to be valuable for use in modeling efforts to determine anaerobic natural attenuation rates at the Guadalupe site.

In a companion study (Chris Kitts, PI) the microbial community structure along this methane profile has been elucidated using TRF analyses on a series of soil samples collected from the same site at which the methane concentration profile was determined. The TRF analyses showed a definite switch from anaerobic methanogens near the water table to aerobic methanotrophs in the vadose zone above the hydrocarbon contaminated zone.

Methodology

Laboratory microcosms were set up using soil collected from along the profile described above. Methane generation was measured in anaerobic microcosms with soils collected near and within the saturated zone. Methane consumption was measured in aerobic microcosms prepared with soils collected from the aerobic portion of the vadose zone. These aerobic microcosms were spiked with methane. Gas headspaces were monitored for methane concentration over time to determine rates of methane generation and consumption. Details of soil sampling, microcosm set-up and laboratory analyses are described below.

Soil Sampling

Soil for the microcosms was obtained from boring SBCP81 near soil gas monitoring point SVCP-1 at the GOF Compressor Plant site on November 16th, 2004 by LFR Levine-Fricke (Figure 2). Soil was chosen from this site based on data suggesting methanotrophic activity in the vadose zone and methanogenic activity near the capillary fringe and below the water table. Soil-gas analyses indicate the site is methane-free for the first 60 ft bgs and lower concentrations begin to appear at around 87 feet bgs (see Appendix 1).

In each 18-inch core interval the deepest and middle 6-inch sample sleeves were retained while the highest sleeve was discarded. To minimize exposure of soil intended for use in methanogenic soil microcosms to oxygen the borehole was flooded with nitrogen during sampling and sleeves removed from the soil cores were kept under nitrogen during transportation and storage. All sample sleeves were stored at 10°C and were received the day after sampling via couriers for Zymax Envirotechnology Inc. (San Luis Obispo, CA).

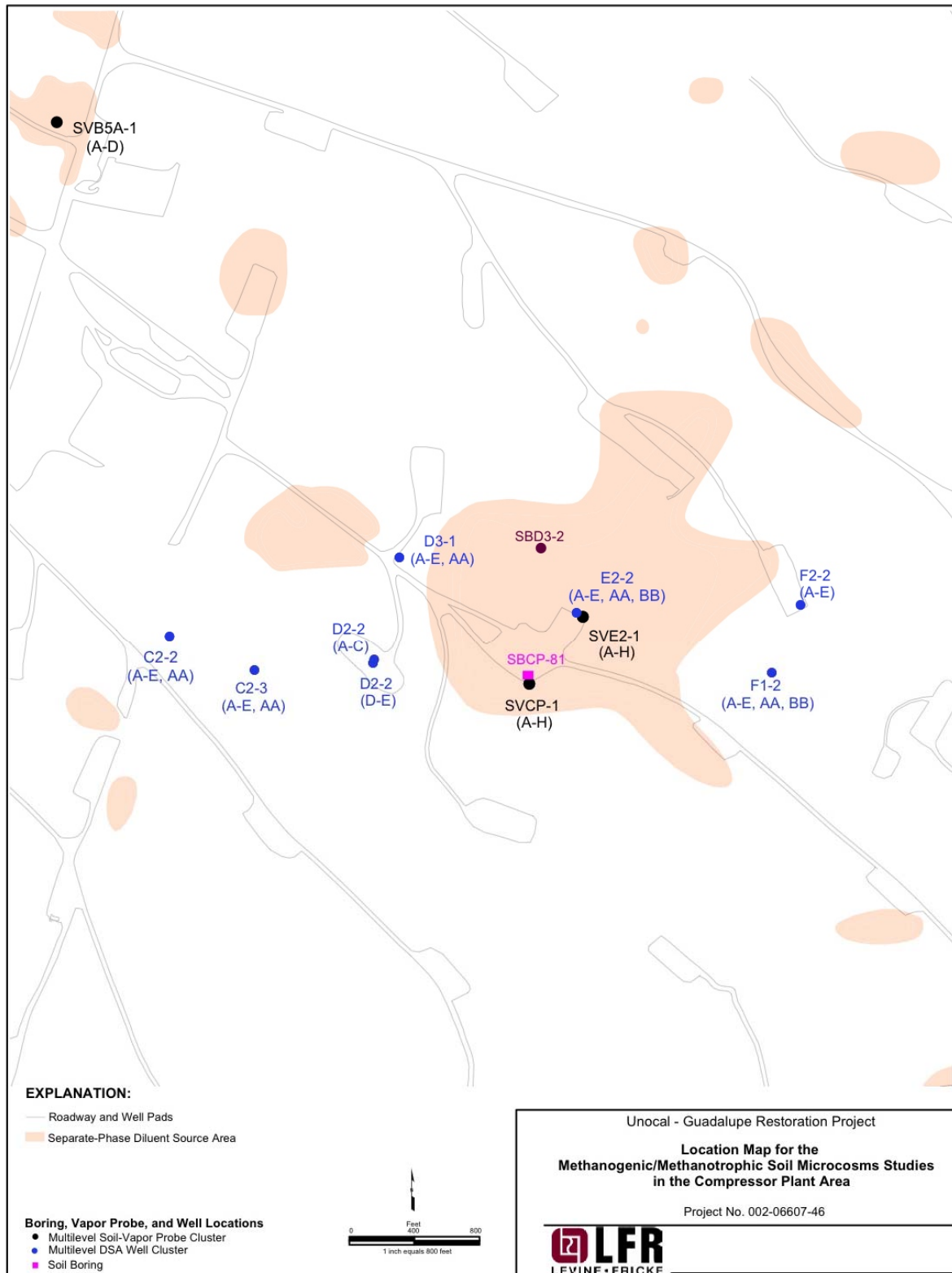


Figure 2: Location of SBCP81 boring at the compressor plant site (LFR, 2005).

Soil microcosm preparation

For each soil depth interval chosen for these experiments, three microcosms were prepared from two homogenized sample sleeves (Table 1). Sample sleeves containing soil to be used in anaerobic microcosms (collected below the water table at the compressor plant site) were transferred into an anaerobic glove box with a UHP nitrogen-only atmosphere. Inside the glove box the inner inch of six-inch sleeve of soil sample (two inches in diameter) corresponding to each depth interval was extracted manually with a 1" ID stainless steel tube-corer. Approximately 380 grams of soil was recovered for each interval from two sample sleeves. Soil for each interval was homogenized by manual mixing in a glass beaker. For anaerobic microcosms (CP81-119.0-1,2,3 and CP81-125.0-1,2,3) three 160-mL serum bottles, each containing 100 gram aliquots of homogenized soil were prepared for each soil interval (119.5' to 120.5' and 125.0' to 126.0'). The six serum bottles containing methanogenic samples were sealed with Teflon® -lined septa while inside the glove box to maintain an anaerobic environment. Anaerobic microcosms were prepared before aerobic microcosms to avoid exposure to oxygen from samples collected in the unsaturated zone. Excess soil was placed in 15-mL Falcon® tubes and stored for TRF analysis.

Following preparation of anaerobic soil microcosms, eight sample sleeves containing soil from the unsaturated zone below the compressor plant site (for aerobic microcosms) were transferred into the anaerobic glove box. Three aerobic microcosms were prepared for each soil interval in the same manner as anaerobic microcosms, summarized above. After preparation, the six anaerobic and twelve aerobic soil microcosms (CP81-80.5-1,2,3; CP81-87.5-1,2,3; CP81-88.0-1,2,3 and CP81-90.0-1,2,3) were removed from the anaerobic glove box and stored at 19°C. Headspace amendments of oxygen and methane were made the aerobic microcosms the following day.

Table 1: Soil microcosm sample depth intervals and labels.

Soil Depth Interval	Sample sleeves	Microcosm Label*	Aquifer Zone
80.5' - 81.5'	80.5, 81.0	CP81-80.5	Unsaturated
87.0' - 88.0'	87.0, 87.5	CP81-87.0	Unsaturated
90.0' - 91.0'	90.0, 90.5	CP81-90.0	Unsaturated
88.0' - 89.0'	88.0, 88.5	CP81-88.0	Unsaturated
119.5' - 120.5'	119.5, 120.0	CP81-119.5	Unsaturated
125.0' - 126.0'	125.0, 125.5	CP81-125.0	Saturated

*Three microcosms were prepared from each soil depth interval.

Aerobic soil microcosm experiments

Two sets of aerobic microcosm experiments were run using soil from the unsaturated zone below the compressor plant site. For the first experiment gas headspaces were sampled at 7 and 14 days after set-up, but all of the methane had been consumed within 7 days by methanotrophic metabolism. Thus a second aerobic microcosm experiment was run with much shorter sampling intervals to allow a more precise examination of methane utilization in the microcosms over time. Details of the methods of both sets of experiments are described in this section.

Soil for the aerobic microcosms was obtained from three depths: 80.5, 87, and 90 feet below ground surface (ft bgs). Controls were run with soil collected from 88 ft bgs. For control microcosms, 10 mL of headspace was removed to accommodate 10 mL acetylene (methanotrophic inhibitor) added by injection with a syringe before other headspace amendments were made. The final concentration of acetylene was about 7.5%. Headspaces of the microcosms were amended to initially contain 1.0% oxygen, 0.5% methane, and 2% helium. Approximately 9.75 mL of the 130 mL headspace was drawn from each aerobic microcosm with a syringe. Methane (0.65 mL) and helium (2.6 mL) were added to each headspace. Helium served as a tracer for leak detection. 6.5 mL of air was added in a similar manner to achieve a headspace oxygen concentration of approximately 1%.

Immediately after amending each aerobic microcosm headspace, headspace gas composition was determined to confirm adequate concentrations of methane and oxygen, and to accurately determine initial gas concentrations. Microcosms were incubated at 19°C in an incubator and gas headspaces were analyzed at 7 and 14 days after initial headspace amendments were made.

Gas headspaces were analyzed using gas chromatography with a thermal conductivity detector (TCD) by Greg Ouellette (Inland Empire Analytical, Norco, CA) for methane, hydrogen, oxygen, carbon dioxide, helium and nitrogen. Gas aliquots of 2 mL were drawn from each microcosm headspace for each analysis. Following analysis, 2 mL of helium was injected back into each microcosm to replace the volume of headspace sampled.

The second phase of aerobic microcosm experiments was conducted using much shorter sampling intervals. For this phase, initial headspace methane and oxygen concentrations were doubled. Approximately 14.8 mL of headspace was drawn from the headspace of each microcosm and each microcosm was amended with 1.3 mL methane and 13.5 mL. Headspace analyses on all non-control microcosms for methane, hydrogen, oxygen, carbon dioxide, helium and nitrogen were conducted at time-zero (immediately after amendment), and 12, 24, 48, 72, and 120 hours after set up. Controls were not repeated during this second phase because the controls for the first experiment sufficiently demonstrated that observed methane losses were due to biological methanotrophy.

Anaerobic microcosm experiments

Anaerobic microcosms were prepared with soil from below the water table at the compressor plant site. Following headspace amendments and initial analyses of

headspace compositions, methane accumulation in microcosm headspaces was observed over an experimental period of 60 days. Soil from the saturated zone (historically methanogenic) was added to the microcosm bottles in an anaerobic glove-box to avoid any exposure to oxygen and ensure anaerobic conditions in the microcosms. Helium was added to the gas headspaces of microcosms to a concentration of 2% helium to serve as a tracer to indicate microcosm gas integrity.

Gas headspace samples were taken immediately after amending microcosm headspaces and 30 and 60 days later for analysis of methane, hydrogen, oxygen, carbon dioxide, helium and nitrogen, as described above. For each headspace analysis 2 mL were drawn from each microcosm, and following analysis 2 mL of helium was injected back into each microcosm to replace the volume of headspace sampled. Microcosms were incubated in the dark at 19°C to mimic subsurface conditions at GOF.

During injection of headspace gases, methane was inadvertently added to one of the two sets of microcosms (CP81-119.5-1,2,3). No methane was added to CP81-125.0 microcosms 1, 2, and 3. Addition of methane to CP81-119.5 microcosms was expected to have little or no effect on methane production by anaerobic microorganisms, so the experiment was continued with the expectation that methane concentrations would increase. However, the initial methane concentrations in these microcosms turned out to be much higher than the methane production (see Results section), precluding the measurement of methane production in this set of microcosms.

Results

Methane consumption by methanotrophs in aerobic microcosms: Phase I

During the first phase of the methanotrophic experiments, the microcosms were incubated 14 days and gas headspaces were sampled at 7 and 14 days. The majority of the methane injected into the biologically active microcosms was consumed within one week (Table 2). By Day 7, methane in microcosms containing soil from 87 and 90 feet which was initially between 4000 and 5000 ppmv was depleted to below the method detection limit (5 ppmv). Methane in all microcosms with soil from 80.5 feet was depleted to 3% or less of the initial concentration by Day 7 and had fallen below the detection limit by Day 14. In contrast, methane concentrations in control microcosms (amended with acetylene for methanotrophic inhibition) did not change significantly over 14 days, confirming that the observed methane losses in the non-control microcosms were due to biological activity.

Oxygen concentrations decreased to below 0.5% v/v in all microcosms by Day 14 (Table 2). Helium concentrations in microcosm headspaces changed very little over 14 days of incubation indicating no leakage (Table 2).

Methane consumption by methanotrophs in aerobic microcosms: Phase II

More frequent headspace sampling was used in the second phase of aerobic soil microcosm experiments to allow for accurate measurement of methane consumption rates by methanotrophs. For these microcosms the amount of methane and oxygen added to microcosms was also doubled, bringing initial headspace concentrations to between 10,000 and 11,500 ppmv methane and approximately 20,000 ppmv oxygen. Headspace sampling took place immediately after amendments of methane and oxygen were made, and at 12, 24, 48, 72, and 120 hours. Results indicate methane was significantly depleted in all aerobic microcosms within 12 hours of incubation and after 48 hours almost all methane had been consumed (Table 3). Methane concentrations are also shown graphically in Figure 3. Acetylene controls were not repeated for Phase II because results of Phase I experimental controls showed little or no loss of methane over a longer period of time. Methane concentrations fell below the detection level after 48 hours for the CP81-80.5 and CP81-90.0 samples. Low concentrations of methane remained in all CP81-87.0 microcosms and remained stable toward the end of the incubation period (Table 3).

Methane utilization rates in each microcosm over intervals between headspace sampling events were very rapid (Table 4). On average, microcosms demonstrated the most methane utilization between 24 and 42 hours (Figure 4). Little change in methane concentration was observed in CP81-87.0 microcosms after 48 hours of incubation (Figure 3). Oxygen availability likely prevented further methane utilization in CP81-87.0 microcosms (Table 5). All oxygen was depleted in 87.0-ft depth microcosms after 48 hours (Table 5).

Table2: Aerobic soil microcosm Phase I headspace gas concentrations.

Microcosm	Initial			Day 7			Day 14		
	CH ₄ , ppmv	O ₂ , %V	He, %V	CH ₄ , ppmv	O ₂ , %V	He, %V	CH ₄ , ppmv	O ₂ , %V	He, %V
80.5-01	4926	1.5	2.7	37	0.5	2.6	<5	0.3	2.5
80.5-02	4918	1.5	2.7	28	0.5	2.6	<5	0.4	2.5
80.5-03	4894	1.5	2.7	150	0.5	2.5	<5	0.4	2.5
87.0-01	4082	1.1	2.4	<5	<0.01	2.4	<5	0.0	2.2
87.0-02	4160	1.2	2.5	<5	0.0	2.6	<5	0.0	2.2
87.0-03	4278	1.2	2.5	<5	0.0	2.5	<5	0.0	2.3
90.0-01	4710	1.4	2.6	<5	0.0	2.6	<5	0.0	2.2
90.0-02	4692	1.5	2.6	<5	0.0	2.6	<5	0.0	2.2
90.0-03	4645	1.5	2.6	<5	0.1	2.6	<5	0.0	2.3
88.0-01 (control)	4452	1.3	2.4	4340	0.2	2.4	4001	0.0	2.4
88.0-02 (control)	4489	1.3	2.4	4418	0.3	2.4	4037	0.0	2.5
88.0-03 (control)	4437	1.2	2.4	4447	0.2	2.4	4136	0.0	2.4

Table 3: Methane detected in Phase II of aerobic soil microcosm headspaces.

Microcosm Depth, ft	CH ₄ , ppm					
	t = 0 hrs	12 hrs	24 hrs	48 hrs	72 hrs	120 hrs
80.5-01	10340	8787	5800	39	<5	
80.5-02	11391	9233	6317	83	<5	
80.5-03	11327	9311	6545	80	<5	
87.0-01	10124	5364	70	46	34	30
87.0-02	10565	6148	492	228	215	197
87.0-03	10174	7177	2448	508	479	441
90.0-01	11448	6763	2678	<5		
90.0-02	10862	6426	2604	9	<5	
90.0-03	11197	5915	1691	<5		

* Method detection limit = 5 ppmv, blank indicates no data.

**Table 4: Calculated methane utilization rates by methanotrophic bacteria
Phase II aerobic microcosms**

Microcosm Depth, ft	CH ₄ utilization rate, ug/ hr				
	t = 0 - 12 hrs	12 - 24 hrs	24 - 48 hrs	48 - 72 hrs	72 - 120 hrs
80.5-01	8.81	20.05	20.89	0.14	NA
80.5-02	13.08	19.39	22.60	0.30	NA
80.5-03	12.02	18.24	23.43	0.29	NA
87.0-01	33.03	38.38	0.08	0.04	0.01
87.0-02	30.32	40.88	0.93	0.02	0.02
87.0-03	19.73	33.62	6.96	0.04	0.04
90.0-01	32.09	28.88	9.71	NA	NA
90.0-02	30.38	26.99	9.41	0.03	NA
90.0-03	36.66	30.16	6.13	NA	NA

Calculation assumes observed change in CH₄ conc. occurred over 24 hrs.

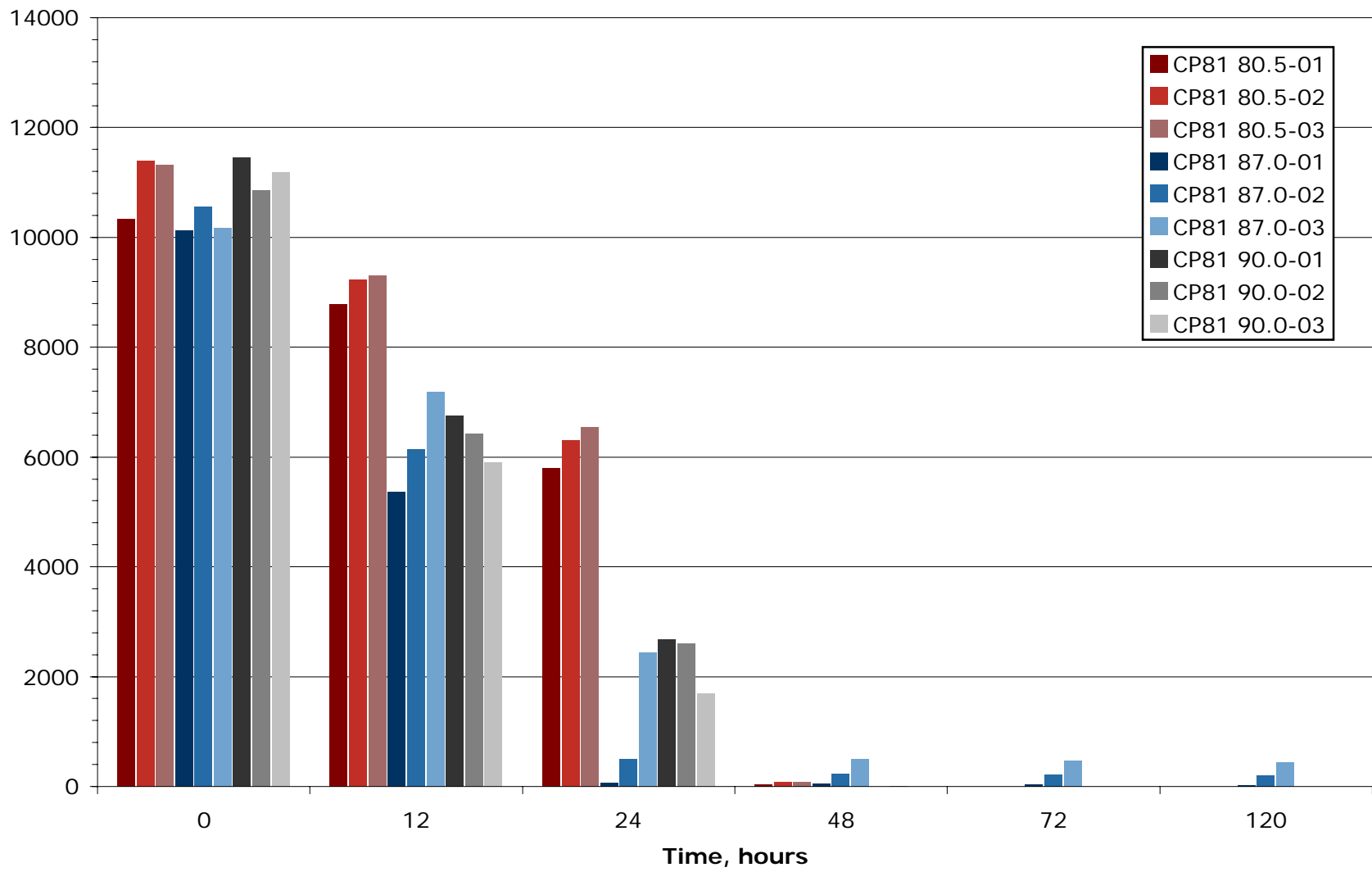


Figure 3: Methane concentrations in aerobic microcosms over time.

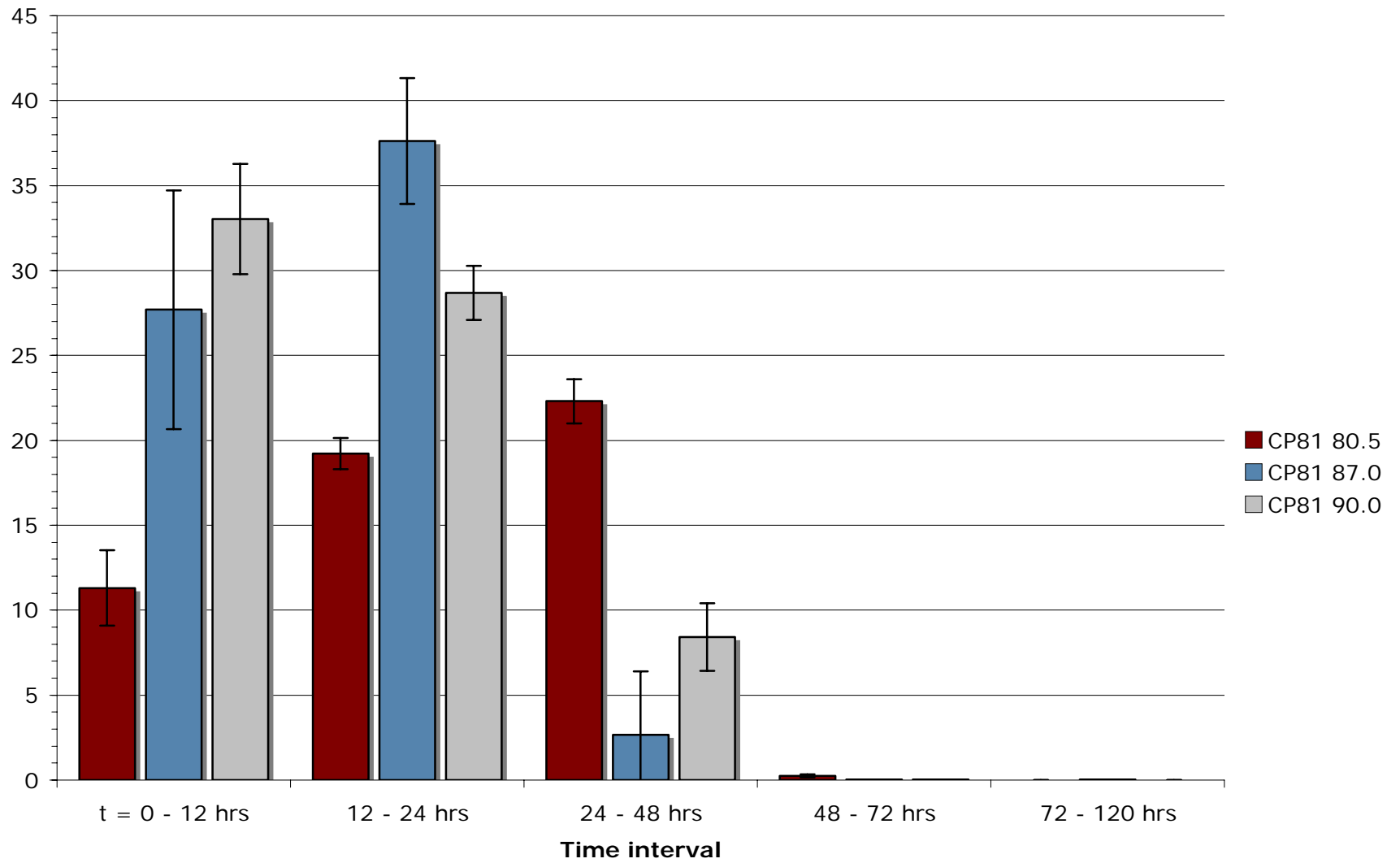


Figure 4: Average methane utilization rates by methanotrophs during Phase II aerobic microcosm experiment.

Table 5: Oxygen detected in Phase II of soil microcosm headspace analyses.

Microcosm Depth, ft	O ₂ , %V					
	t = 0 hrs	12 hrs	24 hrs	48 hrs	72 hrs	120 hrs
80.5-01	3.54	3.24	2.59	1.57	1.42	0
80.5-02	2.78	2.42	1.85	0.77	0.69	0
80.5-03	2.75	2.42	1.86	0.77	0.7	0
87.0-01	2.34	1.2	0.04	0.02	0.02	0.02
87.0-02	2.35	1.3	0.1	0.02	0.02	0.05
87.0-03	2.36	1.61	0.53	0.02	0.01	0.01
90.0-01	2.54	1.68	0.86	0.24	0	0
90.0-02	2.51	1.68	0.93	0.34	0.24	0
90.0-03	2.52	1.54	0.72	0.29	1.42	0

* Zero value indicates value below detection limit

Anaerobic soil microcosm results - 125 ft depth

Following 30 days of incubation, methane was detected in all the headspaces of anaerobic microcosms prepared with soil sampled from compressor plant boring CP81 between 125.0 and 126.0 feet bgs (Table 6 and Figure 5). No significant oxygen (>0.04%) was observed in any CP81-125.0 microcosm over the experimental period confirming anaerobic conditions were maintained. Carbon dioxide and helium content of headspaces changed very little confirming the integrity of the microcosms (Table 6). Average initial methane concentration (measured at time 0) was 52 ppm with a standard deviation of 9 ppm. The average methane concentration at 30 days was 201 ppm with a standard deviation of 55 ppm. Thus methane concentration increased an average of 149 ppm over the first 30 days. A decrease in headspace methane concentration was observed in all microcosms between Day 30 and Day 60. Average final headspace methane concentration at Day 60 was 163 ppm with a standard deviation of 49 ppm, a decrease of 32 ppm methane from Day 30 on average.

Table 6: CP81 125.0 anaerobic soil microcosm headspace composition.

Microcosm	CH ₄ , ppm			He, percent volume			CO ₂ , percent volume		
	0 days	30 days	60 days	0 days	30 days	60 days	0 days	30 days	60 days
CP81 125.0-01	44	140	109	2.60	2.45	2.45	0.34	0.39	0.36
CP81 125.0-02	52	217	176	2.63	2.62	2.62	0.46	0.53	0.46
CP81 125.0-03	61	246	204	2.62	2.63	2.63	0.55	0.62	0.53
Average	52	201	163	2.61	2.57	2.57	0.45	0.51	0.45
Standard Dev.	9	55	49	0.02	0.10	0.10	0.10	0.11	0.09

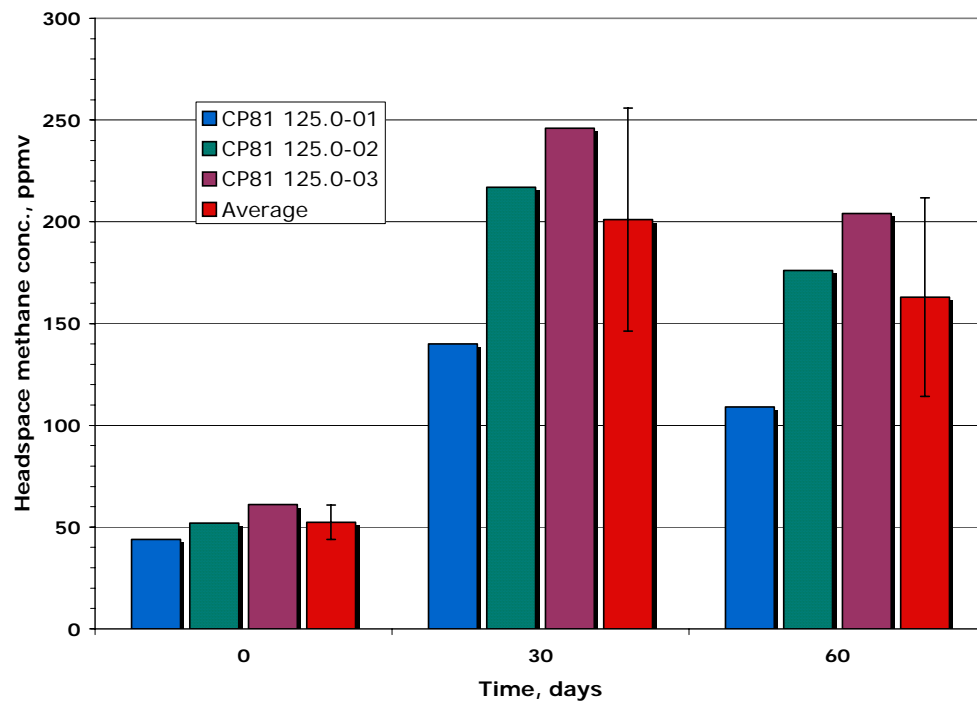


Figure 5: Methane concentrations in CP81 125.0 anaerobic soil microcosms.

Anaerobic soil microcosm results – 119.0 ft depth

Methane was inadvertently added to all of the microcosms with soil from boring CP81 between 119.5 and 120.5 feet bgs, as described above. In all of these microcosms initial methane concentrations were more than an order of magnitude greater than the methane generated in the anaerobic microcosms without added methane (Table 7). The methane concentrations decreased slightly over the 60-day experimental period (Table 7). Average initial methane concentration (measured at time 0) was 4924 ppm. The average methane concentration at 30 days was 4307 ppm, and 3873 ppm on Day 60. Significant concentrations of oxygen were also observed in all CP81-119.5 microcosms during the initial headspace sampling event (0.3% average for time 0). Carbon dioxide in all headspaces increased between the start of incubation and Day 30 and decreased between Day 30 and Day 60. Helium concentration changed very little over the experimental period indicating good gas integrity.

Table 7: CP81 119.0 anaerobic soil microcosm headspace composition (methane was inadvertently added to these microcosms at start-up).

Microcosm	CH ₄ , ppm			He, percent volume			CO ₂ , percent volume		
	0 days	30 days	60 days	0 days	30 days	60 days	0 days	30 days	60 days
CP81 119.5-01	4865	4282	3945	2.64	2.54	2.54	0.49	0.92	0.87
CP81 119.5-02	4860	4155	3525	2.62	2.62	2.62	0.45	0.94	0.87
CP81 119.5-03	5047	4483	4150	2.71	2.63	2.63	0.44	0.89	0.86
Average	4924	4307	3873	2.66	2.60	2.60	0.46	0.92	0.87
Standard Dev.	107	165	319	0.04	0.05	0.05	0.03	0.03	0.01

Conclusions

Methane production was observed in anaerobic microcosms prepared from soil from the compressor plant site from between 125.0 and 126.0 feet bgs and is likely to have resulted from methanogenic activity. Such methane production was expected because historical soil gas analyses show methane is consistently present in the vadose zone below the compressor plant site. Observed methane production rates under anaerobic laboratory conditions averaged $1.8 \pm 0.6 \times 10^{-4}$ ug CH₄/g-soil-hr.

Under aerobic conditions methane consumption was rapid. In fact, methane consumption rates were so rapid the experiment had to be repeated with shorter sampling intervals. Acetylene controls showed no methane consumption, confirming that observed methane utilization was indeed due to biological methanotrophic activity. Helium concentrations did not change significantly in any of the soil microcosms, further demonstrating microcosms were leak-free. Methane consumption rates for soils collected from the vadose zone were 0.29 ± 0.08 ug CH₄/g-soil-hr.

On a per-gram soil basis, the methane consumption rates were about 1500 times higher than the methane production rates. Therefore methanotrophic activity is an extremely important consideration in interpretation of methane flux. Current techniques used to estimate source zone natural attenuation associated with vapor transport related processes rely on estimation of gas flux across a chosen horizontal plane. The activity of methanotrophic bacteria in the subsurface must be accounted for to accurately interpret oxygen and methane gradients in the subsurface. The rates of methane utilization by methanotrophs determined in this study could be helpful for modeling methane in the subsurface. These observations demonstrate the importance of modeling specific source zones independently with soil gas sampling at several depths where data is indicative of methanogenic and methanotrophic activity.

In a companion study, terminal restriction fragment (TRF) analysis was used to characterize the microbial communities associated with methanogenesis and methanotrophy. A strong correlation of methanogenic vs. methanotrophic bacteria was observed with changes in depth and redox potential, which agreed with the microcosm study results.

Appendix 1. Gas concentrations along the compressor-plant vadose-zone profile (courtesy LFR).

Appendix 1
Summary of Soil Gas Analytical Results
Natural Attenuation Studies
Multilevel Vapor Probe Clusters
Third Quarter of 2004
Guadalupe Restoration Project
 LFR 002-06607-05

Sample ID	Depth (feet)	Date Sampled	Methane	Hydrogen	Oxygen	Nitrogen	Carbon Dioxide	Nitrous Oxide	TPH
			(ppm)	(ppm)	(%)	(%)	(%)	(ppm)	(ppmv)
SOURCE ZONE NATURAL ATTENUATION STUDY									
Compressor Plant Area									
Ambient Air	--	06/01/01	<5	<0.5	20.65	80.78	0.032	<10	--
SVCP-1H	5	06/01/01	<5	<0.5	18.21	82.20	2.468	<10	--
SVCP-1H-092801	5	09/28/01	<5	1.6	17.05	76.83	2.134	<10	--
SVCP-1H-121101	5	12/11/01	<5	<0.5	18.84	76.09	2.188	<10	<0.2
SVCP-1H-022702	5	02/27/02	<5	<0.5	18.32	80.51	2.490	<10	<0.2
SVCP-1H-091002	5	09/10/02	<5	<0.5	19.52	81.38	1.875	<10	1.3
SVCP-1H-032603	5	03/26/03	<5	<0.5	18.51	80.59	2.686	<10	<0.2
SVCP-1G	27.5	06/01/01	<5	<0.5	11.32	84.09	7.843	<10	--
SVCP-1G-092801	27.5	09/28/01	<5	0.6	11.78	77.57	6.025	<10	--
SVCP-1G-121101	27.5	12/11/01	<5	<0.5	12.79	77.96	6.252	<10	--
SVCP-1G-022702	27.5	02/27/02	<5	<0.5	11.96	82.07	6.993	<10	--
SVCP-1G-091002	27.5	09/10/02	<5	<0.5	13.28	82.63	6.677	<10	--
SVCP-1G-032603	27.5	03/26/03	<5	<0.5	11.99	80.24	7.079	<10	--
SVCP-1F	57.5	06/01/01	<5	<0.5	5.93	85.53	11.659	<10	--
SVCP-1F-092801	57.5	09/28/01	<5	0.9	6.50	79.80	9.666	<10	--
SVCP-1F-121101	57.5	12/11/01	<5	<0.5	6.80	79.43	10.049	<10	--
SVCP-1F-022702	57.5	02/27/02	<5	<0.5	6.21	83.42	10.885	<10	--
SVCP-1F-091002	57.5	09/10/02	<5	<0.5	6.80	84.72	11.126	<10	--
SVCP-1F-091002-0	57.5	09/10/02	<5	<0.5	6.69	83.74	11.071	<10	--
SVCP-1F-032603	57.5	03/26/03	<5	<0.5	6.37	81.16	11.037	<10	--
SVCP-1E	87.5	06/01/01	308	<0.5	0.37	86.74	16.069	<10	--
SVCP-1E-092801	87.5	09/28/01	3,876	<0.5	0.77	81.21	13.721	<10	--
SVCP-1E-121101	87.5	12/11/01	3,850	<0.5	0.24	81.22	14.328	<10	--
SVCP-1E-022702	87.5	02/27/02	4,354	<0.5	0.23	84.66	15.201	<10	--
SVCP-1E-091002	87.5	09/10/02	4,916	<0.5	0.20	85.52	16.065	<10	--
SVCP-1E-032603	87.5	03/26/03	4,330	<0.5	0.48	82.58	15.728	<10	--

SVCP-1D	103.5	06/01/01	20,957	<0.5	0.34	85.02	16.113	<10	--
SVCP-1D-092801	103.5	09/28/01	24,894	4.1	1.06	79.13	13.612	<10	--
SVCP-1D-121101	103.5	12/11/01	25,252	<0.5	0.19	79.17	14.335	<10	--
SVCP-1D-121101§	103.5	12/11/01	23,545	<0.5	1.85	79.38	13.322	<10	--
SVCP-1D-022702	103.5	02/27/02	25,910	<0.5	0.18	83.15	15.637	<10	--
SVCP-1D-091002	103.5	09/10/02	25,646	<0.5	0.20	83.96	16.015	<10	--
SVCP-1D-032603	103.5	03/26/03	20,414	0.5	0.56	81.74	15.820	<10	--
SVCP-1D-032603-0	103.5	03/26/03	24,704	<0.5	0.29	80.69	16.110	<10	--
SVCP-1C	111.5	06/01/01	25,352	<0.5	0.33	84.62	16.156	<10	--
SVCP-1C-092801	111.5	09/28/01	37,158	0.8	0.70	78.49	14.101	<10	--
SVCP-1C-121101	111.5	12/11/01	36,679	<0.5	0.22	78.87	14.383	<10	--
SVCP-1C-121101§	111.5	12/11/01	33,165	<0.5	2.18	77.90	13.072	<10	--
SVCP-1C-022702	111.5	02/27/02	36,922	<0.5	0.18	81.27	15.562	<10	--
SVCP-1C-091002	111.5	09/10/02	35,555	0.6	0.16	81.69	15.761	<10	--
SVCP-1C-032603	111.5	03/26/03	27,070	0.6	0.38	80.54	16.092	<10	--
SVCP-1B	115.5	06/01/01	38,676	<0.5	0.33	83.09	16.104	<10	--
SVCP-1B-092801	115.5	09/28/01	42,856	1.2	0.72	77.22	13.969	<10	--
SVCP-1B-121101	115.5	12/11/01	42,848	<0.5	0.21	78.39	14.380	<10	--
SVCP-1B-121101-0	115.5	12/11/01	43,029	<0.5	0.25	78.60	14.392	<10	--
SVCP-1B-022702	115.5	02/27/02	42,881	<0.5	0.19	80.73	15.439	<10	--
SVCP-1B-091002	115.5	09/10/02	41,832	0.5	0.14	81.11	16.002	<10	--
SVCP-1B-032603	115.5	03/26/03	34,599	0.6	0.33	79.93	16.012	<10	--
SVCP-1A	117.5	06/01/01	42,707	<0.5	0.31	82.67	16.189	<10	3.1
SVCP-1A-092801	117.5	09/28/01	47,678	0.7	0.67	76.96	14.089	<10	--
SVCP-1A-121101	117.5	12/11/01	46,945	0.6	0.17	77.55	14.508	<10	--
SVCP-1A-121101§	117.5	12/11/01	43,603	<0.5	1.58	77.27	13.531	<10	--
SVCP-1A-121101§	117.5	12/11/01	41,554	0.6	2.59	77.44	12.861	<10	0.70
SVCP-1A-022702	117.5	02/27/02	47,544	1.1	0.22	79.60	15.419	<10	3.0
SVCP-1A-022702-0	117.5	02/27/02	47,675	1.2	0.19	79.55	15.420	<10	--
SVCP-1A-091002	117.5	09/10/02	45,850	1.1	0.21	81.06	16.130	<10	15
SVCP-1A-032603	117.5	03/26/03	43,929	0.8	0.30	78.70	16.193	<10	0.20