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ACRONYMS AND UNITS

%	percent
AED	atomic emission detection
AFT	after flash tank
AH	amine heater
ATCC	American Type Culture Collection
BFT	before flash tank
BOD	biological oxygen demand
BTEX	benzene, toluene, ethylbenzene, and xylenes
°C	degrees Celsius
CEC	cation exchange capacity
CH ₂	methylene
CH ₄	methane
C ₂ H ₆	ethane
C ₃ H ₈	propane
C _n H _{2n} (OH) ₂	dihydric alcohols
CMOS	completely miscible organic solvents
COD	chemical oxygen demand
d	day
DEG	diethylene glycol
DOE	U.S. Department of Energy
EAE	environmentally acceptable endpoint
EERC	Energy & Environmental Research Center
EG	ethylene glycol
EPA	U.S. Environmental Protection Agency
°F	degrees Fahrenheit
FID	flame ionization detection
ft	feet
g	gram
GC	gas chromatography
GC–AED	gas chromatography–atomic emission detection
GC–FID	gas chromatography–flame ionization detection
GC–MS	gas chromatography–mass spectrometry
GD	glycol-degrading (bacteria)
GRI	Gas Research Institute
HO(C ₂ H ₄ O) _n H	ethylene glycols
hr	hour
H ₂ S	hydrogen sulfide
K _d	distribution coefficient
kg	kilogram
m	meter
mg	milligram
mg/kg	milligram per kilogram
mg/L	milligram per liter
MHC	moisture-holding capacity
min	minute
mL	milliliter
mm	millimeter

ACRONYMS AND UNITS (continued)

mmHg	millimeters of mercury
mmscfd	million standard cubic feet per day
mpn	most probable number
MS	mass spectrometry
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NCIB	National Collection of Industrial Bacteria
o.d.	outside diameter
OECD	Organization for Economic Cooperation and Development
OH ⁻	hydroxyl
OM	organic matter
PAHs	polycyclic aromatic hydrocarbons
PC	personal computer
PEG	polyethylene glycol
PG	propylene glycol
ppm	parts per million
RBCA	risk-based corrective action
RI	refractory index
rpm	revolutions per minute
RSD	relative standard deviation
SPME	solid-phase microextraction
SVI	sludge volume index
TC	total count (bacteria)
tcf	trillion cubic feet
TEG	triethylene glycol
ThOD	theoretical oxygen demand
TOC	total organic carbon
TOM	total organic matter
TREG	tetraethylene glycol
μg	microgram
μg/g	microgram per gram
μg/mL	microgram per milliliter
μL	microliter
VOC	volatile organic compound
wt%	weight percent

GLYCOL-BASED DEHYDRATION FLUIDS: WASTE CHARACTERIZATION AND SUBSURFACE TRANSPORT AND FATE

EXECUTIVE SUMMARY

In 1998, the Gas Research Institute–U.S. Department of Energy (GRI–DOE) Gas Industry Groundwater Program began to examine subsurface environmental issues associated with glycol-based gas dehydration units. A majority of these units use triethylene glycol (TEG), diethylene glycol (DEG), or ethylene glycol (EG). The research program was designed to determine 1) the character of dehydration wastes, 2) the potential that glycols may have for enhancing the mobility of condensate species due to cosolvent effects, and 3) the biodegradability of the glycols and their associated wastes. To increase the applicability of the results, many of the activities were conducted on soils collected from major gas-producing regions of North America.

The results of the characterization activities demonstrate that a variety of organic compounds (including BTEX [benzene, toluene, ethylbenzene, and xylenes], PAHs [polycyclic aromatic hydrocarbons], and alkanes) are associated with rich and lean glycol solutions. Therefore, at sites where such solutions are known to have been released to the subsurface environment, such cocontaminants may also be present. The association of these cocontaminants with the glycols may be problematic from two angles. First, data suggest the presence of cocontaminants could affect the biodegradability of glycol solutions, thereby complicating remediation efforts. Second, data also indicate the glycols in water could act as a cosolvent and greatly increase the transport of some cocontaminants (particularly the highly regulated condensate species) beyond what would normally be predicted. Failure to consider the cosolvent effects that the glycols may have on the mobility of the condensate species may result in underestimation of their transport in the subsurface, thereby leading to an underestimation of risk.

Another point to take from the research is that site-specific variables need to be considered when glycol-related subsurface contamination is assessed. In order to develop appropriate risk-based corrective action plans and/or remediation strategies, the data indicate that it is necessary to have a basic understanding of both the wastes and the native soil. For instance, the characteristics of the raw gas stream, which are typically very site-specific, can provide clues regarding the nature of the organic constituents in a rich glycol, as evidenced by the organic sulfur compounds present in the samples from the Alberta sour gas fields. Also, both the cosolvency and biodegradation data strongly suggest that soil type is a critical factor in predicting the ultimate subsurface transport and fate of glycols and their common cocontaminants. In short, the key parameters to predicting subsurface transport and fate, and assessing site-specific risk, include 1) the type and concentration of glycol; 2) the presence or absence of condensate species, especially BTEX and PAHs; and 3) the nature of the native soil, including pH, size of the microbial population, organic carbon content, and other soil-specific factors.

Key findings under this contract are as follows.

- By far, the largest constituent of dehydration fluids and wastes is the parent glycols, which typically range from 50% to 92% by weight.
- Rich glycol solutions typically include significant concentrations of BTEX (benzene, toluene, ethylbenzene, and xylene) (e.g., benzene ranged from 100 to 480 µg/g), with smaller concentrations of polycyclic aromatic hydrocarbons (PAHs) and alkanes. Lean glycol solutions typically contain lower concentrations of BTEX compared to rich solutions, but still may contain concentrations of aromatics and PAHs that are above environmental regulatory limits (e.g., benzene ranged from 0.9 to 7.9 µg/g, and naphthalene ranged from <0.1 to 15 µg/g).

- Large concentrations of glycol in water can reduce the adsorption of some condensate species on some soils. The results strongly suggest that the presence of high concentrations of glycol, such as are found in dehydration wastes, can enhance the mobility of some condensate species in soils where those same species might otherwise display low mobility.
- Organic carbon content of the soil appears to be a primary factor in controlling the mobility of the selected condensate species. In soils with low organic carbon content, the BTEX compounds were mobile to highly mobile regardless of the presence or absence of glycol. In soils with high organic carbon content, BTEX and naphthalene mobility ranged from low to immobile in the absence of glycol, but in the presence of glycol, mobility was observed to increase significantly.
- Risk-based assessments of subsurface contamination at sites where condensates and dehydration glycols may be commingled should consider the cosolvent effects that the glycols may have on the mobility of the condensate species. Failure to do so may result in underestimation of the extent of the transport of condensates in the subsurface.
- Regarding pure compounds under both aerobic and anaerobic conditions, triethylene glycol (TEG) and diethylene glycol (DEG) likely would not persist in a highly bioactive soil, but may persist in soils with poor bioactivity, especially when the TEG or DEG concentrations are high. Ethylene glycol (EG) appears to be readily biodegradable under both aerobic and anaerobic conditions in all three of the test soils and, therefore, would not likely persist under most soil conditions.
- Rich glycol solutions collected from dehydration units in Texas, Oklahoma, and Alberta were not readily biodegraded in aqueous slurries of New Mexico, Louisiana, and Alberta test soils without nutrient amendments. These results suggest that the hydrocarbon constituents present in the rich solutions can inhibit the degradation of the glycols. The addition of nutrient amendments (nitrogen and phosphorus) significantly enhanced the biodegradability of the rich glycol solutions in aqueous soil slurries.
- In order to generate an appropriate site-specific risk-based assessment and/or remediation plan for subsurface contamination associated with natural gas dehydration operations, it is necessary to have a basic understanding of both the wastes and the native soil. Key parameters to predicting subsurface transport and fate include 1) the type and concentration of glycol; 2) the presence or absence of condensate species, especially BTEX; and 3) the bioactivity and availability of nutrients (i.e., nitrogen and phosphorus) of the native soil (including soil pH, size of the microbial population, grain-size distribution, and total organic carbon content).

GLYCOL-BASED DEHYDRATION FLUIDS: WASTE CHARACTERIZATION AND SUBSURFACE TRANSPORT AND FATE

1.0 Introduction

Under normal production conditions, natural gas is saturated with water vapor. This is undesirable because when the gas is compressed or cooled, water vapor converts to a liquid or solid phase. Liquid water can accelerate corrosion and reduce gas transmission efficiency, and ice can plug valves, fittings, and gas lines. The presence of water vapor can also lead to undesirable hydrate formation. When a water-saturated natural gas stream reaches its dew point, water molecules can combine with methane, ethane, propane, and other hydrocarbons to form crystalline-structured solid hydrates. Because of the problems that may result from the formation of hydrates, it is necessary to dehydrate the natural gas stream. Dehydration commonly occurs shortly after the natural gas has come to the surface, such as at wellheads, gathering stations, and the front end of gas-processing plants.

Glycols are commonly used to dehydrate natural gas. The three major glycols used in gas dehydration processes are triethylene glycol (TEG), diethylene glycol (DEG), and ethylene glycol (EG). Glycol selection for dehydration applications can be based on many factors, including dehydration capacity, glycol losses during various process stages, and absorption of volatile organic compounds (VOCs), including benzene, toluene, ethylbenzene, and xylenes (collectively referred to as BTEX). Because of their solubility in glycols, BTEX compounds are removed along with water during dehydration. Of the three major dehydration glycols, TEG is the most commonly used, and it also has the highest affinity for BTEX absorption (Ebeling and others, 1999). DEG was used in the early days of the natural gas industry as the solvent of choice for gas dehydration. In the 1950s, TEG replaced DEG as the most commonly used glycol in gas dehydration units. TEG was preferred because of its ability to produce a dryer gas than DEG at the same conditions, its higher thermal stability, and lower evaporative losses in the absorber and reboiler (Pearce, 1982). It was only in the 1990s, in response to regulatory scrutiny under the Clean Air Act, that the problem associated with TEG's affinity for aromatic hydrocarbons was recognized. Glycol solutions containing BTEX and other VOCs may be introduced into subsurface environments as a result of spills, leaks, mechanical failures, and past disposal practices. Review of the literature indicated that wastes associated with glycols from gas dehydration operations may be of environmental concern because 1) past operation and disposal practices have resulted in the release of glycol-related wastes into subsurface environments; 2) based on their physicochemical properties, glycols may be readily transported in the subsurface and act as cosolvents; 3) very little research has been performed regarding the biodegradability of gas industry glycols and their associated wastes under environmentally relevant conditions; and 4) regulatory scrutiny of glycols has increased in recent years (Sorensen and others, 1996).

Generally, adequate data exist to confirm the presence of glycols and at least some of their biodegradation products and other associated contaminants in soils, sediments, and groundwater at former and current natural gas dehydration sites. However, sampling, analytical, and other difficulties have often prevented detailed delineation of the nature and extent of glycol-related contamination at individual sites. Because only limited work has been done to investigate the behavior of the chemical species that comprise glycol wastes, little is known about their subsurface transport and fate. Air emissions from glycol-based gas dehydration units have been regulated under the Clean Air Act for a number of years, but the U.S. Environmental Protection Agency (EPA) has recently added EG to its list of contaminants to be considered for regulation under the Safe Drinking Water Act. This places the natural gas industry in the difficult position of having to address a contamination issue that is neither well defined nor well understood.

2.0 Objectives

The objective of the research described in this report was to provide data and insights that will enable the natural gas industry to 1) significantly improve the assessment of subsurface glycol-related contamination at sites where it is known or suspected to have occurred and 2) make scientifically valid decisions concerning the management and/or remediation of that contamination. The described research was focused on subsurface transport and fate issues related to TEG, DEG, and EG. TEG and DEG were selected for examination because they are used in a vast majority of gas dehydration units, and EG was chosen because it is currently under regulatory scrutiny as a drinking water pollutant. Because BTEX compounds are often very closely associated with glycols used in dehydration processes, the research necessarily included assessing cocontaminant effects on waste mobility and biodegradation. BTEX hydrocarbons are relatively water-soluble and, because of their toxicity, are of regulatory concern. Although numerous studies have investigated the fate of BTEX, and significant evidence exists to indicate the potential biodegradability of BTEX in both aerobic and anaerobic environments (Kazumi and others, 1997; Krumholz and others, 1996; Lovely and others, 1995; Gibson and Subramanian, 1984), relatively few investigations have convincingly demonstrated in situ biodegradation of these hydrocarbons (Gieg and others, 1999), and less work has been done on investigating the fate of BTEX species in combination with miscible glycols.

To achieve the research objectives, laboratory studies were conducted to 1) characterize glycol-related dehydration wastes, with emphasis on identification and quantitation of coconstituent organics associated with TEG and EG wastes obtained from dehydration units located in the United States and Canada, 2) evaluate the biodegradability of TEG and DEG under conditions relevant to subsurface environments and representative of natural attenuation processes, and 3) examine the possibility that high concentrations of glycol may act as a cosolvent for BTEX compounds, thereby enhancing their subsurface mobility. To encompass a wide variety of potential wastes representative of different natural gas streams and dehydration processes, raw, rich, and lean glycol solutions were collected from 12 dehydration units at eight different gas-processing facilities located at sites in Texas, Louisiana, New Mexico, Oklahoma, and Alberta. To generate widely applicable environmental fate data, biodegradation and mobility experiments were performed using four distinctly different soils: three obtained from three gas-producing areas of North America (New Mexico, Louisiana, and Alberta), and one obtained from a North Dakota wetland to represent a soil with high organic matter content.

The definition of “soil” and “sediment” varies between scientific disciplines (i.e., geology versus biology). Because of the interdisciplinary nature of the research described in this report, the terms “sediment” and “soil” are used interchangeably and are meant to include shallow subsurface soils, sediments, and aquifer materials.

3.0 Glycols and the Dehydration Process

Water in natural gas can cause serious operational problems in both the transmission and processing of the gas. The natural gas industry has found that dehydration ensures smooth operation of gas transmission lines, prevents formation of gas hydrates, and reduces corrosion. The most commonly used dehydration method is absorption using desiccants such as glycols (Katz and Lee, 1990).

3.1 Past and Present Usage

The most common dehydration process used in the gas industry is the glycol absorption/stripping process. While the actual number of operating glycol units is unknown, it is estimated that as many as 40,000 units exist in the United States and about 100,000 worldwide (Grizzle, 1993). In the United States, 90%–95% of the units process less than 10 million standard cubic feet per day (mmscfd) of natural gas (Grizzle, 1993). No estimates were available on the total number of glycol dehydration units operating in Canada, but they are very commonly used in Canadian gas fields. The total volume of natural gas dehydrated in North America each year is approximately 17–18 trillion cubic feet (tcf) (Thompson and others, 1993). While no figures were available on percentage of gas dehydrated by glycol dehydrators, the literature search and discussions with gas industry personnel indicate that a large majority of the dehydration is done using glycol-based units.

EG, DEG, TEG, tetraethylene glycol (TREG), and blends of these glycol solutions have all been used in natural gas stream dehydration processes for decades. The earlier glycol dehydration units used DEG but were commonly characterized by the thermal decomposition of the DEG. Around 1950, TEG became the most commonly used glycol because its higher boiling point provided better water removal and less thermal decomposition (Pearce, 1982). Although EG and TREG are used in specialized cases, TEG is currently used at an estimated 95% of all glycol-based natural gas dehydration process units (Thompson and others, 1993). Because of its favorable physicochemical properties for dehydration applications, TEG is often added to other glycols to augment performance. For example, some DEG solutions contain 15%–30% TEG, in addition to minor amounts of antifoaming agent, inhibitor, and dye. A detailed discussion of specific applications for each of these glycols in the natural gas industry is provided by Sorensen and others (1996).

3.2 Dehydration Processes

Water and glycols are mutually soluble in the liquid phase and have very low vapor pressures. These properties enable the use of glycols to remove water vapor from a wet gas stream in an “absorber,” after which the “rich” (water-laden) glycols can be regenerated (stripped of water) in a “stripping column.” Figure 3-1 is a schematic of a typical glycol-based dehydration unit. The absorption/stripping dehydration process begins by running compressed, wet inlet gas through a 2- or 3-phase physical separation process to remove liquid and solid impurities. This is followed by sending the gas countercurrently through a liquid–glycol absorber. The “lean” (dry) glycol absorbs the water from the wet gas stream, and the dried gas leaves the absorber for further processing or transport.

The rich glycol exits the bottom of the absorber and on some units is sent through a gas-driven or electric pump to a flash tank, where much of the absorbed natural gas is separated from the glycol solution. The glycol solution is then sent through a series of heat exchangers and filters, after which water is either distilled or stripped from the glycol solution in a regenerator (Thompson and others, 1993). Rising steam in the regenerator strips the water vapor from the rich glycol solution, and the glycol, carried by the rising vapor, is condensed in the reflux section and washed back into the reboiler. A sludge is sometimes left in the vessel and eventually removed for disposal. The uncondensed steam exits the top

Figure 3-1. Typical glycol-based dehydration unit.

of the stripper as an offgas emission, together with traces of hydrocarbon condensate which may be collected. The regenerated lean glycol is then cooled and returned to the absorber (Ballard, 1986).

3.3 Glycol Chemical and Physical Properties

Glycols are aliphatic organic compounds and are members of a group of chemicals referred to as the dihydric alcohols (diols). This group has the general formula $C_nH_{2n}(OH)_2$ and is characterized by the presence of two hydroxyl (OH^-) functional groups linked to methylene (CH_2) subunits. Ethylene glycols have the general formula $HO(C_2H_4O)_nH$, where n can equal 1 (monoethylene), 2 (diethylene), 3 (triethylene), or 4 (tetraethylene). Table 3-1 lists general physical and chemical properties of glycols commonly used by the gas industry.

Glycols are similar to water in that they are clear, colorless, odorless liquids. However, when compared to water, the glycols have a greater specific gravity and viscosity at all temperatures, a higher boiling point, and a lower freezing point. Glycols are completely water-soluble and can also act as solvents for some organic compounds, including most aromatic compounds. They are generally stable in air under usual storage conditions; however, when heated, glycols tend to oxidize, forming products such as aldehydes and acids (Dow Chemical Company, 1992). All glycols are hygroscopic; that is, they have the ability to take up water from the atmosphere, a characteristic that makes them useful in removing water from natural gas streams.

Table 3-1. General Physical and Chemical Properties for Selected Glycols¹

	EG	DEG	TEG	TREG
Molecular Weight	62.1	106.1	150.2	194.2
Boiling Point @760 mmHg, °F/°C	387.3/197.4	473.8/245.5	550.0/287.8	618.1/325.6
Freezing Point, °F/°C	7.9/-13.4	16.4/-8.7	19.0/-7.2	15.1/-9.4
Flash Point, °F/°C	247/119	281/138	325/163	400/204
Specific Gravity @ 25 °C	1.110	1.111	1.120	1.123
Vapor Pressure, mmHg @ 25 °C	<0.1	<0.01	<0.01	<0.01
Aqueous Solubility	Completely miscible	Completely miscible	Completely miscible	Completely miscible
Color	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless

¹ Dow Chemical Company, 1992.

4.0 Dehydration Waste Characterization

4.1 Wastes Generated by the Dehydration Process

The operation of gas dehydration units creates a variety of potential environment-impacting wastes, including fresh and used glycol solutions, process residuals, and by-products. The gas dehydration wastes described in this section are the vehicles by which the glycols and their process-related coconstituents may be introduced into the subsurface environment. Attempts to control these vehicles of contamination include waste management techniques designed to minimize the exposure of dehydration wastes to the subsurface environment; however, wastes generated from accidental releases such as may occur from foaming events, spills, or leaks may go unmanaged. Most unplanned releases of glycols into the subsurface at dehydration locations are due to foaming events. A foaming event in a glycol-based dehydration unit is typically caused by the presence of hydrocarbons (oil) in the glycol stream, excessive use of additives, or high concentrations of degradation products. The managed and unmanaged waste streams may be composed of rich or lean glycols and/or wastes from process unit tank bottoms or system filters. Unmanaged waste streams, particularly spills during changeover operations (the process of exchanging used chemical for fresh chemical), may also include fresh glycols. Additional components of the wastes may include additives such as corrosion inhibitors and antifoaming agents, various hydrocarbons, and trace metals. Sorensen and others (1996) includes a detailed discussion of the generation, handling, and disposal of gas dehydration wastes, as well as how those wastes may be introduced into the subsurface environment.

4.2 Tri-, Di-, and Ethylene Glycol Waste Characterization

The primary objective of the characterization activities was to determine the gross chemical characteristics of rich and lean glycol solutions and glycol-associated dehydration wastes and the identities and relative amounts of any organic cocontaminants. Glycols and glycol-related wastes derived from dehydration units that use TEG and EG were characterized qualitatively and semiquantitatively, with a focus on species of high toxicological and regulatory interest like benzene and benzene derivatives toluene, ethylbenzene, and xylenes (collectively referred to as BTEX). This information will ultimately be used to estimate the potential for facilitated transport and to design experiments to determine the effect of glycols on the transport of organic cocontaminants. Since the vast majority of regulated organics are in the volatile and semivolatile range, the primary emphasis was on these compounds.

Rich and lean glycol solutions and other glycol-related dehydration wastes that are derived from EG- and TEG-utilizing dehydration units were collected for use in experimental activities. Efforts to locate a DEG-utilizing dehydration unit were not successful, thus no DEG-based samples were available. Glycol solutions that have not been used in a dehydration unit, but are stored for use as makeup fluid, or during changeover, are referred to as “raw” glycols. Twenty-nine samples of rich, lean, and raw glycol solution were collected from 12 dehydration units from eight gas-processing facilities in different gas-producing parts of North America (Louisiana, Texas, New Mexico, Oklahoma, and Alberta, Canada). Samples were collected from a variety of dehydration units in order to examine the heterogeneity between wastes that may be caused by different gas compositions, feed rates, and/or the use of various additives. Collection of samples was conducted by Energy & Environmental Research Center (EERC) personnel to ensure the consistency of sampling methods. Gas company personnel familiar with the operation of each dehydration unit were asked to fill out a questionnaire on the operating characteristics of the sampled unit and the nature of the natural gas stream itself.

The experimental plan for characterization of the samples comprised 1) glycol quantitation using gas chromatography (GC) with flame ionization detection (FID); 2) initial screening for qualitative identification of volatile and semivolatile cocontaminant species of interest using GC, mass spectrometry (MS), and atomic emission detection (AED) methods; and 3) analysis of selected samples for semiquantitation of regulated organics and other species of interest using GC–FID. Initial screening was performed on all 29 samples, and semiquantitative analysis was performed on rich and lean samples from three selected sites, for a total of six samples.

4.2.1 Initial Sample Screening

Glycol concentrations for each of the 29 samples were determined via GC using a commercially available GC column specific for quantitation of glycols. Waste samples were diluted to about 1 wt% in water and injected into the column, and glycol concentrations were calculated based on three-level GC–FID response calibrations for both TEG and EG. Initial glycol waste cocontaminant characterizations were performed based on the use of conventional methylene chloride extraction. All methylene chloride extracts were also analyzed for sulfur-, nitrogen-, chlorine-, and bromine-containing compounds using GC–AED. As shown in Table 4-1, all samples were composed primarily of either TEG or EG. Glycol concentrations ranged from 23.9% to 92.1%. Figures 4-1–4-5 show chromatograms selected to illustrate organic characteristics typical of raw, rich, and lean glycol solutions. As shown, raw solutions (see Figure 4-1) are typically relatively “clean,” rich solutions (Figures 4-2–4-5) generally contain relatively high levels of BTEX and potentially significant levels of naphthalene and other polycyclic aromatic hydrocarbons (PAHs), and lean solutions (Figure 4-5) generally contain lower levels of BTEX and similar or higher levels of PAHs compared to rich solutions.

4.2.2 Selected Sample Analysis for BTEX and Naphthalene

As expected, difficulties were encountered in attempting to quantitatively extract volatile and semivolatile organics from the glycol-based wastes using methylene chloride. Therefore, two other analytical techniques were evaluated for application to quantitation of organics in glycol wastes: the use of deuterium-labeled (deuterated) BTEX and naphthalene spikes in conjunction with hexane extraction and the use of deuterated spikes in conjunction with solid-phase microextraction (SPME) methods. Based on these limited evaluations, hexane extraction was chosen as the best (most reproducible and accurate) method for organics quantitation, since the SPME methods investigated appeared to be insufficiently selective for accurate analysis of glycol-based solutions. For analysis, 50 μ L of deuterated BTEX was blended into about 1 g of glycol waste, the mixture was extracted with 1 mL of hexane, and the extract was analyzed via GC–FID using a J&W Scientific DB-1 column. Table 4-2 lists BTEX and naphthalene concentrations determined for the six selected samples.

4.3 Summary of Glycol-Related Waste Analyses

Analyzed raw TEG and EG solutions were generally clean, although a sample from one site contained moderate levels of toluene, benzene, and naphthalene. Rich solutions typically included high concentrations of BTEX, with smaller concentrations of PAHs and alkanes, while lean solutions typically contained lower BTEX concentrations and still significant concentrations of PAHs. Overall, lean solutions appeared to vary more widely in BTEX and PAH concentrations than rich or raw solutions; in fact, at some facilities it was difficult to differentiate between lean and rich samples based on analytical results. Other notable observations included the lack of significant levels of chlorine- and bromine-containing organics in any of the 29 samples analyzed (not surprising, since halogenated solvents are not widely used in natural gas processing) and the presence of significant levels of organosulfur compounds

Table 4-1. Initial Glycol Waste Characterization

Sample Number	Site Number	Site Location	Sample Description	Color	Glycol Conc., wt%	Relative Std. Deviation, %	BTEX Concentration ¹
1	1	Texas	TEG-Raw	Clear	89.3	4.4	Low
2	1	Texas	TEG-Lean	Yellow	86.2	2.0	Low
3	1	Texas	TEG-Rich	Yellow	92.1	6.3	Low
4	2	Texas	TEG-Rich-BFT ²	Dark	73.5	6.9	High
5	2	Texas	TEG-Rich-AFT ³	Dark	75	4.9	High
6	2	Texas	EG-Rich-AH ⁴	Dark	54.2	5.6	High
7	2	Texas	TEG-Raw	Clear	86.8	4.9	Moderate
8	2	Texas	TEG-Lean	Dark	81	4.2	High
9	3	Louisiana	TEG-Lean	Dark	75	2.6	High
10	3	Louisiana	TEG-Raw	Clear	85.1	4.9	Low
11	3	Louisiana	TEG-Rich-BFT	Dark	74.9	6.4	High
12	3	Louisiana	TEG-Rich-AFT	Dark	75.6	5.5	High
13	3	Louisiana	EG-AH	Yellow	23.9	2.3	Low
14	4	Texas	TEG-Rich	Dark	74.7	4.9	High
15	4	Texas	TEG-Raw	Clear/Yellow	88	3.3	Low
16	4	Texas	TEG-Lean	Dark	84.8	3.7	Low
17	5	New Mexico	TEG-Rich	Dark	85.4	4.0	Moderate
18	5	New Mexico	TEG-Drip	Clear	0	0.0	Low
19	5	New Mexico	TEG-Raw	Clear	91.5	1.5	Low
20	5	New Mexico	TEG-Lean	Dark	91.9	3.5	Moderate
21	6	Alberta	EG-Rich	Yellow	76.5	5.2	High
22	6	Alberta	EG-Raw	Yellow	89.1	2.9	Low
23	6	Alberta	EG-Lean	Yellow	82.2	1.4	Low
24	7	Texas	TEG-Raw	Clear	93.7	3.0	Low
26	7	Texas	TEG-Rich	Dark	69.1	4.1	High
25	7	Texas	TEG-Lean	Dark	89.8	1.8	Moderate
27	8	Oklahoma	TEG-Rich	Yellow	72.8	2.9	High
28	8	Oklahoma	TEG-Raw	Clear	85.4	8.7	Low
29	8	Oklahoma	TEG-Lean	Yellow	81.7	8.5	Moderate

¹ Cumulative concentrations of benzene, toluene, ethylbenzene, and xylenes.

² Before flash tank.

³ After flash tank.

⁴ From amine heater.

in only one sample: the rich EG waste from Site 6 in Alberta. The presence of organosulfur compounds in the Alberta sample is not unexpected, because the raw natural gas stream processed at Site 6 often contains up to 25% hydrogen sulfide (H₂S).

Figure 4-1. Gas chromatogram of raw TEG solution from Site 3 (Louisiana).

Figure 4-2. Partial gas chromatogram of rich TEG solution from Site 2 (Texas) showing higher-volatility organic cocontaminants.

Figure 4-3. Partial gas chromatogram of rich TEG solution from Site 2 (Texas) showing lower-volatility organic cocontaminants.

Figure 4-4. Partial gas chromatogram of rich EG solution from Site 6 (Alberta).

Figure 4-5. Gas chromatogram of lean TEG solution from Site 2 (Texas).

Table 4-2. BTEX and Naphthalene Concentrations in Selected Glycol Wastes¹

Site	Benzene	Toluene	Ethylbenzene	<i>m</i> -, <i>p</i> -Xylene	<i>o</i> -Xylene	Naphthalene
4 – Rich	150	260	34	190	63	<0.1
4 – Lean	0.9	0.3	<0.1	<0.1	<0.1	<0.1
6 – Rich	480	250	7.5	64	27	<0.1
6 – Lean	7.9	5.0	0.6	2.3	2.3	<0.1
8 – Rich	100	160	21	80	32	28
8 – Lean	4.6	2.3	0.6	1.6	0.8	15

¹Concentrations in µg/g.

5.0 Glycol Interactions with Sediments and Mobility Predictions

5.1 Background

In addition to glycol-based wastes, natural gas “condensates” are often generated at facilities where natural gas dehydration occurs. Condensates are typically low-molecular-weight hydrocarbons coproduced with natural gas. The hydrocarbons exist in the gaseous phase under temperature and pressure conditions commonly encountered in petroleum reservoirs; however, they tend to condense as the gas cools. Natural gas production wells generate varying volumes of condensates. In some cases, the production of condensates from a well can be profitable, but in other cases, it is considered a by-product waste stream. In either case, condensates have occasionally been introduced to the shallow subsurface environment through unplanned releases. As with the dehydration process, the generation and collection of condensates often occur at production facilities and gas-processing plants.

Condensate compounds may be introduced separately or together into the subsurface environment in the vicinity of dehydration units as a result of spills, leaks, and mechanical failures. The miscibility of TEG with hydrocarbons indicates that it may act as a cosolvent for less soluble hydrocarbons, thereby increasing the subsurface mobility of condensates. To investigate the potential cosolvency effect of TEG on condensate species, a series of tests were conducted to determine the effect of TEG on the subsurface mobility of condensate species in a variety of North American soils.

5.2 Natural Gas Condensates and Condensate-Contaminated Soils

In 1998, work was conducted to thoroughly characterize the nature of natural gas condensate contamination in the shallow subsurface at gas production and processing sites. The laboratory-based research activities were particularly focused on determining the nature and concentrations of hydrocarbons present in several natural gas condensate samples and in soils with known exposure to condensates. Both condensate and contaminated soil samples were obtained from several North American gas production and processing facilities, including plants in Wyoming, Colorado, New Mexico, and Alberta, Canada.

The identification of all organics in the condensates and in the soil extracts was based on high-resolution GC–MS analysis. Percent composition of the individual organics found in the condensates was based on GC analysis with FID. The procedures and results of that work are presented and discussed in detail in Hawthorne and Miller (1998). The results of those analyses showed that compositions of specific condensates varied with location. In general, the major components of the condensates were straight-chained, branched, and unsaturated hydrocarbons in the C5 to C10 range, although the Wyoming condensate contained high concentrations of longer-chain *n*-alkanes, which had higher molecular weight. In addition to the aliphatic hydrocarbons, each of the condensates examined by the study also contained environmentally significant concentrations of BTEX. Benzene concentrations ranged from 0.15 to 3.6 wt% (of the total condensate mass), with the other BTEX compounds being present in somewhat higher concentrations. None of the condensate samples contained higher-molecular-weight aromatics (e.g., PAHs) in significant concentrations (i.e., >0.1 wt%).

5.3 Subsurface Mobility and Cosolvency

Many risk-based corrective action (RBCA) determinations and site closure plans are based to a large extent on the occurrence of BTEX. In particular, predicting the mobility of BTEX and other hydrocarbons in the subsurface is a key component of any risk-based modeling effort. Along with the

meteorological, hydrogeological, and soil characteristics of a site, these predictions are typically based on the physicochemical properties of each individual compound. Properties commonly used to predict the adsorptive tendencies of a compound include octanol–water partitioning coefficients (K_{ow} values), organic carbon partitioning coefficients (K_{oc} values), and aqueous phase–soil partitioning coefficients (K_d values). Key soil properties affecting mobility include total organic carbon (TOC), total organic matter (TOM), grain-size distribution, and pH.

Cosolvency effects caused by cocontaminants are not typically considered as part of most RBCA determinations. Cosolvency is the effect of completely miscible and/or partially miscible organic solvents on the solubility and sorption of hydrophobic organic chemicals (Rao and others, 1990). As the concentrations of organic cosolvents increase, sorption of hydrophobic organics to soil will decrease. The hydrophobic organic contaminants will, therefore, likely be present at greater concentrations in pore water. Consequently, in the presence of a cosolvent, transport of the organic contaminants would be greater than predicted by a strictly aqueous-based transport model (Rao and others, 1991). If information regarding the cosolvent effects of a potential or known cocontaminant is not included as part of the RBCA modeling efforts, then the subsurface transport of the compounds of primary concern may be significantly underestimated.

Since aromatic hydrocarbons such as BTEX can be significant components of condensate and condensates are often generated or handled at the same sites that glycols occur, experiments were conducted in 1999 to determine the cosolvent effects, if any, that TEG might have on BTEX in four different soils. The waste characterization work described in Section 4.0 had shown that BTEX can also occur in glycol-based dehydration wastes, particularly “rich” glycol streams. This gave further impetus to determining the nature of interactions between glycols and hydrocarbons in soil and water. Along with the BTEX compounds, naphthalene was also selected for evaluation because of its low vapor pressure (relative to BTEX), high K_{ow} and K_{oc} values, and occasional presence in both condensates and glycol-based dehydration wastes. Experiments were also performed to evaluate the effect of TEG on the solubility of the selected hydrocarbons in weathered condensate-contaminated soils from two natural gas-processing facilities. All experiments used an aqueous solution of 40% TEG to reflect minimum glycol concentrations commonly encountered in dehydration wastes and represent a scenario where a large volume of glycol-based fluids are released onto a soil that already contains condensate.

Understanding the environmental transport and fate of a compound, in particular its subsurface mobility, requires an understanding of its interactions with soil and water. A key parameter in describing the interaction of any compound with soil and water is its aqueous phase–soil distribution coefficient (K_d), which quantitates the partitioning of a compound between the aqueous and adsorbed states, thus indicating its potential for mobility. The mathematical expression of K_d is as follows (Dragun, 1988):

$$K_d = C_s/C_e$$

where:

C_s = compound concentration adsorbed on soil surfaces (μg compound/g soil)

C_e = compound concentration in water (μg compound/mL water)

which means that the greater the extent of adsorption (i.e., $C_s \gg C_e$), the greater the magnitude of K_d , and the lower the mobility of the compound. K_d for a compound under specific conditions can be determined by plotting equilibrium compound solution concentration (x-axis) versus sediment uptake (y-axis) for a set of compound concentrations. When the resulting plot or isotherm is linear with a Y intercept equal to zero, the slope of the isotherm is the K_d of the compound for the soil conditions employed for the series

of measurements. Mobility classifications, according to Dragun (1988), for compounds based on K_d values are Class I (immobile: $K_d > 10$), Class II (low mobility: $K_d = 2-10$), Class III (intermediate mobility: $K_d = 0.5-2$), and Class IV (high mobility: $K_d < 0.5$).

5.4 Sediment–Aqueous-Phase Distribution Coefficient (K_d) Determinations

Uncontaminated base soils from three gas-producing regions of North America were collected for performance of laboratory tests to determine K_d values for selected hydrocarbons, both with and without TEG present. Soil samples were obtained from the southwestern United States (New Mexico), the Gulf Coast (Louisiana), and the northern Great Plains/northern Rocky Mountains (Alberta, Canada). Sediments from these areas were chosen to represent sediment types that encompass a broad range of characteristics, yet are typical of the major gas-producing regions of North America. The sediments were air-dried, sieved to remove cobbles and plant material, homogenized by mixing machines, and placed into 50-gallon polyethylene drums for storage. The barrels containing the sediment were stored in a cold room. Key sediment parameters that affect the transport and fate of hydrocarbons were quantitated for the three base sediments. The parameters include pH, TOC, cation exchange capacity (CEC), and texture. A fourth sediment from a North Dakota wetland was added to expand the range of sediment types and to include a sediment with high organic content. The characterization of these four soils is presented in Table 5-1.

Table 5-1. Base Soil Characterization Data

Parameter	Alberta Soil	Louisiana Soil	New Mexico Soil	North Dakota Soil
Sand (0.074 to 2 mm), wt%	25	15	39	NA ¹
Silt (0.005 to 0.074 mm), wt%	33	55	35	NA
Clay (<0.005 mm), wt%	42	31	26	NA
Organic Matter, wt%	3.6	1.2	0.7	7.6
Carbonate, wt%	4.7	0.2	1.6	0.9
Cation Exchange Capacity, milliequivalents/100 g	25.0	10.8	12.5	37.5
Moisture, wt%	2.0	1.7	1.3	1.3
Moisture-Holding Capacity (MHC)	46.7	28.4	25.3	NA
pH	7.6	5.2	7.7	7.2

¹ Not available.

For the experiments to determine K_d values for the hydrocarbons in uncontaminated soils, 1.5 g of soil were mixed and equilibrated with 8 mL of either pure water or water/40 wt% TEG for 72 hr. An aliquot was then removed, spiked with internal standards (deuterated BTEX and naphthalene), and analyzed using SPME with GC–MS. The results of the K_d determinations are shown in Tables 5-2 through 5-5. The greatest cosolvency effects of TEG on the selected aromatic hydrocarbons were observed in the North Dakota soil (Table 5-4). In this soil, all of the hydrocarbons displayed significant reductions in K_d when TEG was present. The mobility classification for benzene changed from intermediate to high, while each of the other BTEX species changed from low to high mobility. TEG had

Table 5-2. Effect of 40% TEG on Soil–Water Partitioning (K_d) of Aromatics in North Dakota Test Soil, 7.6% organic matter

	Water, 24 hr	Water, 72 hr	40% TEG, 72 hr
Benzene	1.5	1.4	<0.5
Toluene	3.5	3.2	<0.5
Ethylbenzene	8.4	6.4	0.6
<i>m</i> -Xylene	9.9	9.1	0.8
Naphthalene	68	46	2.1

Table 5-3. Effect of 40% TEG on Soil–Water Partitioning (K_d) of Aromatics in Alberta Test Soil, 3.6% organic matter

	Water, 24 hr	Water, 72 hr	40% TEG, 72 hr
Benzene	<0.5	<0.5	<0.5
Toluene	0.5	<0.5	<0.5
Ethylbenzene	1.6	<0.5	<0.5
<i>m</i> -Xylene	1.6	<0.5	<0.5
Naphthalene	6.6	6.3	<0.5

Table 5-4. Effect of 40% TEG on Soil–Water Partitioning (K_d) of Aromatics in Louisiana Test Soil, 1.2% organic matter

	Water, 24 hr	Water, 72 hr	40% TEG, 72 hr
Benzene	<0.5	<0.5	<0.5
Toluene	<0.5	<0.5	<0.5
Ethylbenzene	<0.5	<0.5	<0.5
<i>m</i> -Xylene	<0.5	<0.5	<0.5
Naphthalene	1.3	1.3	<0.5

Table 5-5. Effect of 40% TEG on Soil–Water Partitioning (K_d) of Aromatics in New Mexico Test Soil, 0.7% organic matter

	Water, 24 hr	Water, 72 hr	40% TEG, 72 hr
Benzene	<0.5	<0.5	<0.5
Toluene	<0.5	<0.5	<0.5
Ethylbenzene	<0.5	<0.5	<0.5
<i>m</i> -Xylene	<0.5	<0.5	<0.5
Naphthalene	<0.5	<0.5	<0.5

a tremendous effect on naphthalene mobility in this soil, changing its K_d value by an order of magnitude and causing its classification to go from immobile to low mobility. This soil was the only one with sufficiently high K_d values for all compounds for the effect of glycol to be observable. The high organic matter content of the North Dakota soil (7.6%) likely accounts for the significantly higher K_d values than those observed in the other tested soils.

In the Alberta soil (Table 5-3), BTEX K_d values were less than 0.5 with and without TEG present, indicating its high mobility under both circumstances. Naphthalene, however, experienced a significant reduction in K_d in the presence of the glycol solution, going from 6.3 (low mobility) in water to 0.5 (high mobility) in 40% TEG. Similar effects to BTEX and naphthalene were observed in the Louisiana soil (Table 5-4), although to a lesser extent. K_d values for BTEX and naphthalene in the New Mexico soil (Table 5-5) were all less than 0.5 in both the water and 40% TEG solution, indicating that those hydrocarbons are highly mobile in either case in that particular soil.

5.5 Cosolvency Effects in Contaminated Soils

The effect of TEG on the desorption of BTEX and naphthalene on the contaminated soils from gas-processing facilities in Alberta and Wyoming was determined by mixing and equilibrating (24 hr) 1.5 g of soil with 8 mL of either pure water or a mixture of water and TEG (TEG = 40% by weight) and then comparing the relative amounts of hydrocarbon in aliquots of each solution. The presence of 40% TEG in water increased the desorption of BTEX and naphthalene into water by approximately 2- to 5-fold in both soils. Reproducibilities were much better for naphthalene (RSDs [relative standard deviations] 5% to 15%) than for the lower-molecular-weight aromatics (RSDs approximately 10% to 50%) for replicate experiments. The good reproducibility of naphthalene indicates that the source of error is more likely a result of soil variations than analytical error.

The results shown in Table 5-6 are consistent with the results of the K_d evaluations for North Dakota soil, showing that 40% glycol shifted K_d values for the same compounds by about a factor of 10. Note that, as required for K_d determinations, none of the solutes exceeded their water solubility after the 72-hr exposure to the contaminated soils (Table 5-7). The results demonstrate that the presence of TEG does not just increase bulk solubility, but lowers K_d values and/or increases the rate of partitioning into water.

Table 5-6. Effect of TEG on the Desorption of BTEX and Naphthalene from Contaminated Soils, μg desorbed/g soil

	Alberta Soil ¹		Wyoming Soil ²	
	Pure Water	40% TEG	Pure Water	40% TEG
Benzene	1.7 $\pm$ 0.3	2.1 $\pm$ 1.1	2.0 $\pm$ 0.7	4.0 $\pm$ 1.5
Toluene	6.9 $\pm$ 1.2	11 $\pm$ 3	44 $\pm$ 14	137 $\pm$ 37
Ethylbenzene	0.5 $\pm$ 0.2	2.9 $\pm$ 1.3	6.1 $\pm$ 1.1	28 $\pm$ 9
<i>m</i> -Xylene	8.6 $\pm$ 5.6	29.0 $\pm$ 9	195 $\pm$ 12	570 $\pm$ 100
Naphthalene	0.90 $\pm$ 0.04	2.1 $\pm$ 0.1	14 $\pm$ 1	58 $\pm$ 9

¹ Based on triplicate determinations at each condition.

² Based on six replicate determinations at each condition.

Table 5-7. Concentrations of BTEX and Naphthalene in Pure Water after 24 hours' Exposure to Contaminated Soil

	Concentration, $\mu\text{g/mL}$		
	Alberta Soil	Wyoming Soil	Ambient Solubility, $\mu\text{g/mL}$
Benzene	0.3	0.4	1800
Toluene	1.3	8.3	530
Ethylbenzene	0.1	1.1	170
<i>m</i> -Xylene	1.9	37	160
Naphthalene	0.2	2.6	31

5.6 Summary and Implications of Cosolvency Work

The transport prediction component of risk assessment and RBCA modeling efforts is generally based on the chemodynamics of single compounds in water. In a simple aqueous-based transport model, some of these compounds, including some hydrocarbons, may have a tendency to adsorb to soil and be expected to move slowly, if at all, through the subsurface. In many cases, such transport predictions have been proven to closely reflect reality. For instance, several recent studies have shown that the length of BTEX plumes associated with gas stations throughout the United States is typically less than 250 ft, regardless of the nature of the source and other hydrogeological and meteorological variables (Rice and others, 1995; Mace and Choi, 1998; Newell and others, 1990; Groundwater Services, Inc., 1997). Since BTEX plumes associated with gas stations generally do not include any contaminants that could be classified as CMOS (completely miscible organic solvents), their short plume lengths are essentially the results of the relationship between BTEX interactions with soil and biodegradation.

However, in the realm of natural gas production and processing, a release of aromatic hydrocarbons into the environment can occasionally include any number of cocontaminants, not the least of which may be glycol. Previous characterization work and anecdotal evidence from gas industry personnel show that glycols and aromatic hydrocarbons typically associated with condensates can and do occur together not only as wastes, but as subsurface contaminants. The results of the cosolvency investigations described above clearly show that high concentrations of glycol, such as may be expected from an unplanned release of glycol-based solution from a natural gas dehydrator, can significantly enhance the mobility of BTEX and naphthalene in soils with high organic content, where it would otherwise display low mobility.

From a natural gas industry standpoint, the most serious implication of these results relates to the practice of using RBCA modeling in the development of a mitigation plan for closure of a site contaminated with natural gas condensates and glycols. If the presence of glycols and, more importantly, their cosolvency effect on at least some condensate species are not properly taken into account, then the subsurface transport of some hydrocarbons may be greatly underestimated. In the case of BTEX, which serves as an important regulatory benchmark, an erroneous prediction of transport could prove to be very costly to the responsible parties. In short, the cosolvency data suggest that rather than merely considering the well-documented mobility and biodegradability of BTEX on its own, as observed at hundreds of gas station sites, the manager of a natural gas production and/or processing site where dehydration is performed should also consider the potential role that glycol may have in the movement of a suspected plume.

6.0 Biodegradation of Dehydration Glycols and Associated Wastes

Microorganisms are ubiquitously distributed in the environment. They have broader capabilities than other forms of life to withstand environmental changes such as variations in pH, salinity, and oxygen and nutrient availability, temperature, and water deprivation. Additionally, virtually every compound that has some energy available in it will be metabolized by a microorganism. This is not to say that all microorganisms are equally capable of surviving extremes in the environment, nor do all microorganisms possess broad metabolic capacity. However, given that microbial densities in soil and water often range from 10^4 to 10^9 per g, there exists a tremendous biomass of nearly staggering diversity about which we know very little. These microbes are intimately involved in mineral transformations that result in soil fertility, aid animals in digestion of food, and degrade nearly all of the xenobiotic chemicals that humans make and sometimes dispose of without regard to their fate. Since these microbes are not homogeneous with respect to their distribution, environmental tolerances, or metabolic capabilities, concerns about the biodegradation of chemicals in the environment are warranted.

A number of attenuation mechanisms can affect the transport and fate of organic compounds in the environment, but biodegradation is often the most important of these mechanisms. Therefore, in order to understand the ultimate fate of organic compounds in the environment and the factors that control the biological processes, biodegradation studies are required. Biodegradation is typically composed of three phases: acclimation of the microbial population to a particular compound, logarithmic metabolism, and a plateau period. Physical factors that can affect biodegradation include temperature, pH, moisture content, contaminant concentrations, size of the biodegrading microbial population, the availability of oxygen or other nutrients, and other intrinsic factors specific to the soil and to the contaminant. A determination of the factors that control the biodegradation of glycols under environmentally relevant conditions is critical for assessing remedial options and predicting the ultimate environmental fate of these compounds.

The experimental activities described below were designed and performed to meet several objectives. The primary goals of the biodegradation studies were 1) to determine the biodegradation parameters for pure EG, DEG, and TEG and rich glycol-based dehydration solutions and 2) to evaluate the impact of such key variables as soil type, cocontaminant concentrations, and oxygen availability on biodegradation. The experiments were planned to assess the natural ability of three selected soils to degrade glycols. Biodegradation testing was conducted to evaluate the length of the acclimation phase; the rate constant for biodegradation; and the extent of biodegradation for TEG, DEG, and EG in soils collected from natural gas-producing regions of New Mexico, Louisiana, and Alberta, Canada. Characterization data for the three soils are presented in Tables 5-3 through 5-5.

The complete degradation of a compound to its mineral components is known as mineralization. The mineral components of the glycols and hydrocarbons identified in the characterization activities are carbon dioxide and water. Thus it is possible to indirectly follow the biodegradation of the dehydration glycols and their associated cocontaminants by measuring the evolution of carbon dioxide from a contaminated soil sample. Biodegradation of the pure glycols and selected glycol-based dehydration wastes was, therefore, evaluated by respirometric methods following those of Bartha and Pramer (1965). Rates of carbon dioxide evolution were used to determine acclimation periods and rate constants for biodegradation. The theoretical extent of biodegradation was estimated by comparing mass balances of the contaminant(s) to total carbon dioxide evolved. Carbon dioxide evolution in the contaminated (“dosed”) sample was compared to carbon dioxide evolution in an uncontaminated (“undosed”) sample under the same environmental conditions as the dosed sample. This provided data on “background” carbon dioxide evolution for each particular soil type. The data presented in the graphs in this section of the report are the result of taking the carbon dioxide evolution data generated by the dosed samples and subtracting the background carbon dioxide evolution for each soil type. The final data and associated graphs therefore represent only the carbon dioxide that evolved

from biodegradation of the contaminants. All experiments were conducted in soils at 25°C, 60% of the moisture-holding capacity, with static incubation in the dark. Glycol concentrations of 200 and 1000 mg/kg were chosen to represent environmentally significant values. Additional experiments with some glycols were conducted at doses of 500 and 1500 mg/kg. Biodegradation kinetics with respect to oxygen were evaluated to demonstrate the rates that may be obtained in unsaturated and saturated soils and in anaerobic environments. The data from this work will demonstrate the biodegradation kinetics in natural, unamended environments. A general discussion of biodegradation and a detailed review of the literature on glycol biodegradation are provided in Appendix A. Specifics on biodegradation methods used for the work reported here are provided in Appendix B.

The use of respirometric methods, which are indirect measures of biodegradation, as opposed to direct measurement by analyses for the study compound(s) in periodically taken samples, was chosen primarily because of costs. Respirometric measurements are simple, quick, and inexpensive to make. On the contrary, direct measurement of biodegradation by analyses of soil samples is time consuming, expensive, and, in the case of glycols and low-concentration hydrocarbons, requires elaborate analytical techniques that have variable detection limits. An accurate determination of biodegradation parameters such as acclimation times and rate constants also requires numerous measurements. Numerous direct analyses of material (soil or slurry) from one biometer over the course of a study period would not only be prohibitively expensive, but would also result in the loss of material from the biometer due to periodic sampling. Over extended periods of study, such a loss of material may alter the conditions within the biometer, thereby producing misleading results. In selected experiments, direct analyses for target compounds were conducted at the beginning and end of the study period to validate and verify the respirometric results.

6.1 Pure Compound Biodegradation Studies

6.1.1 TEG Biodegradation

Biodegradation of TEG was assessed aerobically at doses of 200 and 1000 mg/kg and anoxically at a dose of 1000 mg/kg using biometers. Additionally, electronic respirometry was used to assess aerobic biodegradation at doses of 500 and 1500 mg/kg. The aerobic biodegradation of TEG at a 200-mg/kg dose is shown in Figure 6-1 as cumulative carbon dioxide evolved during the 62-day incubation period. Biodegradation in the Alberta and New Mexico soil showed similar lag periods, rates, and extent of biodegradation of TEG. In contrast, the Louisiana soil showed poor biodegradation of TEG, with an extent of biodegradation that was probably complete, at 53%, but with only arithmetic kinetics. Typical observations of biological kinetics are generally described as being hyperbolic, or logarithmic, in nature. The logarithmic nature of biological kinetics is a result of the logarithmic growth that is characteristic of bacteria. Occasionally, the graphical representation of the biological kinetics will not be hyperbolic in nature, but more straight-lined, indicating little or no change in rate over time. In such a case, the kinetics are described as being arithmetic. Arithmetic kinetics may result when the substrate is not capable of supporting growth, is toxic or results in a toxic product, or when other nutrients necessary for growth are lacking. The aerobic biodegradation of TEG at the 1000-mg/kg dose is shown in Figure 6-2. At this higher dose, the Alberta soil still showed hyperbolic kinetics with a high rate and an extent of biodegradation of almost 72%. The Louisiana soil was also similar to the lower dose at 1000 mg/kg, with arithmetic kinetics, and an extent of only 23%, suggesting poor biodegradation. However, the New Mexico soil was very different at this dose, performing similarly to the Louisiana soil, rather than to the Alberta soil. It is likely that this shift to arithmetic kinetics in response to toxicity. Table 6-1 compares the biodegradation parameters for the three soils at the two doses under aerobic conditions.

Electronic respirometric methods (see Appendix B) were used to evaluate aerobic TEG biodegradation at doses of 500 and 1500 mg/kg. Figure 6-3 shows the cumulative carbon dioxide evolved

Figure 6-1. Aerobic biodegradation of TEG in the three soils at a dose of 200 mg/kg.

Figure 6-2. Aerobic biodegradation of TEG in the three soils at a dose of 1000 mg/kg.

Table 6-1. Biodegradation Parameters (using biometers) for TEG in the Three Soils at 25 °C, 60% MHC, with Static Incubation in the Dark for 62 days under Aerobic Conditions

	Alberta		Louisiana		New Mexico	
Dose, mg/kg	200	1000	200	1000	200	1000
Lag Period, days	3.7	4.4	13.1	1.9	2.4	13.1
Maximum Rate, mg CO ₂ /100 g-d	2.56	5.81	0.42	0.67	2.03	0.75
Theoretical Degradation, %	99.6	71.5	53.2	23.6	93.3	31.5

Figure 6-3. Aerobic biodegradation of TEG in the three soils at doses of 500 and 1500 mg/kg as monitored by electronic respirometry.

during TEG biodegradation at 500 and 1500 mg/kg. Biodegradation of TEG in the Alberta soil shows rapid hyperbolic kinetics at these doses, as well as in the previous experiments. New Mexico soil also demonstrated rapid hyperbolic kinetics, but much less carbon dioxide was evolved than was found for the Alberta soil. The Louisiana soil showed simple arithmetic kinetics, as was found previously with TEG, suggesting growth inhibition.

Table 6-2 shows the biodegradation parameters for TEG using the electronic respirometer. Compared to the biometer method, the electronic method found longer lag times for the Alberta soil at both doses. Lag times for the New Mexico and Louisiana soils at the 500-mg/kg dose were intermediate between the 200- and 1000-mg/kg dose. However, the lag times found for New Mexico and Louisiana soils were shorter at the 1500-mg/kg dose. The shorter lag time at the 1500-mg/kg dose for these soils, along with the drop in maximum rate observed, suggests toxicity at that dose in the New Mexico and Louisiana soils. Clear evidence for toxicity was not observed in the biodegradation parameters for the Alberta soil at any dose.

Table 6-2. Biodegradation Parameters (using electronic respirometers) for TEG in the Three Soils at 25°C, 60% MHC, with Static Incubation in the Dark for 50 days under Aerobic Conditions

	Alberta		Louisiana		New Mexico	
Dose, mg/kg	500	1500	500	1500	500	1500
Lag Period, days	6.9	6.5	3.3	0.0	3.7	2.5
Maximum Rate, mg CO ₂ /100 g-d	3.66	3.73	0.26	0.19	0.85	0.74

There are several differences in the operation of biometers versus the electronic respirometry that may impact the observed kinetics. In the operation of the biometers, the evolved carbon dioxide is trapped in a potassium hydroxide solution, which is removed and titrated at intervals. Between intervals, the oxygen concentration in the atmosphere above the soil in the biometer could drop enough to change biodegradation kinetics. In contrast, in the electronic method, the headspace of the bottle is swept at regularly programmed intervals of at least four times per day. In addition, the oxygen concentrations of the headspace were measured and were noted to not drop below 20%. Recent tests with the bioreactors used for the electronic respirometer show that the data are somewhat sensitive to the ratio of soil surface area to depth and to the volume of headspace above the soil. This highlights another key difference between the respirometer and biometer methods: the respirometer chamber has a narrower diameter than the biometer. Thus a soil sample in a respirometer chamber that is of equal volume to that in a biometer will be thicker. A greater thickness of soil in the respirometer chamber may inhibit the equilibration of soil pore gas with the gas in the headspace, thereby affecting carbon dioxide production in the lower portion of the sample. Although such an effect would be minor, it may account for the differences in results between the electronic respirometer and biometer experiments. It is important to note that the trends of the data generated by the two methods are generally in agreement, and the results of each are, therefore, considered to be equally valid.

Figure 6-4 shows the anoxic biodegradation of TEG in the three test soils. TEG was biodegraded under anoxic conditions in the Alberta soil after a lag period of 13.6 days. The extent of biodegradation seen in the Alberta soil of 64.4% suggests that during the 165-day period, the TEG was completely metabolized. The extent of biodegradation is rarely 100% when measuring evolved carbon dioxide, because of incorporation of carbon into biomass. The amount incorporated into biomass is a function of the types and amounts of biomass present at the start, the organic compound, and the conditions (pH, electron acceptor, etc.). The rate of TEG biodegradation in Alberta soil was 27% of that found at the same dose under aerobic conditions.

Anoxic biodegradation of TEG in the Louisiana and New Mexico soils was much slower than for Alberta. Calculated lag periods for Louisiana and New Mexico are low, but the kinetics appear to be arithmetic, suggesting inhibition of growth in these soils. Biodegradation of TEG in the New Mexico soil showed a calculated lag period of one day, but the maximum rate was slower (about 38% of that observed with Alberta soil) and not as complete (32%) as noted for the Alberta soil. Anoxic TEG biodegradation in the Louisiana soil was poor, and only about 8% of the added TEG was mineralized to carbon dioxide. Table 6-3 compares the biodegradation parameters for TEG in the three soils under anoxic conditions.

In summary, biodegradation of TEG in the test soils is complicated. Low doses were biodegraded rapidly in the more bioactive soils, Alberta and New Mexico. The least bioactive soil, Louisiana, displayed evidence of inhibition at all doses, with long lag times, low biodegradation rates, arithmetic kinetics, and poor extents of biodegradation. Similar inhibition was also noted in the New Mexico soil at the higher doses. Biodegradation of TEG did not appear to be inhibited at any dose in the Alberta soil, with the

Figure 6-4. Anoxic biodegradation of TEG in the three soils at a dose of 1000 mg/kg during a 165-day incubation period.

Table 6-3. Biodegradation Parameters for TEG in the Three Soils at 25°C, 60% of the MHC, with Static Incubation in the Dark for 165 days under Anoxic Conditions

	Alberta	Louisiana	New Mexico
Lag Period, days	13.6	2.7	1.0
Maximum Rate, mg CO ₂ /100 g-d	1.59	0.20	0.60
Theoretical Degradation, %	64.4	8.1	32.3

exception that the extent of biodegradation decreased with increasing dose. The decrease in extent could be due to growth of the biomass. Under anoxic conditions, lag times were longer, and the rate was about 20% of the aerobic conditions in the Alberta soil. Anoxic biodegradation did occur in the New Mexico and Louisiana soils, but it was via arithmetic kinetics, suggesting inhibition. These results suggest TEG would not persist in a highly bioactive soil, but may persist in soils with poor bioactivity, especially when the TEG concentrations are high.

6.1.2 Biodegradation of TEG Plus Hydrocarbons

A number of factors can affect biodegradation of mixtures that do not impact the biodegradation of pure compounds: toxicity, substrate preference, cosolvency, bioavailability, and others. A simple experiment was performed using TEG and a model hydrocarbon to determine if these effects were present for TEG. Decane, a C₁₀ alkane, was selected as the test hydrocarbon because it is present in gas condensates and because its low volatility (vapor pressure is 2.7 mm at 20°C) allowed it to be readily tested in the experimental apparatus. A dose of 1000-mg/kg total substrate was selected. The Alberta soil was used because it was shown to be a bioactive soil that can biodegrade TEG rapidly with hyperbolic kinetics.

Incubations were performed in duplicate under aerobic conditions, with moisture set at 60% of the MHC, in the dark and static. Three conditions were tested, decane alone, TEG alone, and equal weights of TEG and decane (500 mg/kg each). The cumulative carbon dioxide evolution for these experiments is shown in Figure 6-5. It is important to note that although the TEG plus decane curve shows a larger amount of carbon dioxide evolved, the amount of carbon in decane by weight is greater than for TEG alone.

Figure 6-5. Aerobic biodegradation of TEG, decane, and TEG plus decane in Alberta soil at a dose of 1000 mg/kg.

Table 6-4 compares the biodegradation parameters for the three conditions. The TEG-alone condition shows a significant lag period, but it also had the highest rate (4.07 mg CO₂/100 g-d) and extent of biodegradation (53%). Decane biodegradation had no detectable lag period, but an extent of biodegradation of only 28%. The mixture of TEG and decane was most like decane alone. The lag period and rate for the TEG-decane mixture were similar to decane alone, while the extent of biodegradation was only slightly less than for TEG alone. The extent of biodegradation observed for TEG and decane was quite different, 53% versus 28%, respectively. These differences are probably due to the degree of oxidation of these two compounds. TEG is a fairly highly oxidized compound with a molar formula of C₆H₁₄O₄. In contrast, decane is a reduced compound with a molar formula of C₁₀H₂₂. The more highly reduced compound contains more energy that can support the development of more biomass. Carbon that is fixed into biomass will not be evolved as carbon dioxide. In summary, the data indicate that the mixture of TEG and decane did not adversely impact the biodegradation of the individual components.

Table 6-4. Biodegradation Parameters for TEG, Decane, and TEG Plus Decane in Alberta Soil

	TEG	Decane	TEG + Decane
Lag Period, days	3.2	0.1	0.0
Maximum Rate, mg CO ₂ /100 g-d	4.07	3.66	3.68
Theoretical Degradation, %	53.2	28.1	46.8

6.1.3 DEG Biodegradation

The aerobic biodegradation of DEG was assessed at doses of 200, 500, 1000, and 1500 mg/kg and under anoxic conditions at 1000 mg/kg using biometers. The aerobic biodegradation of DEG is shown in Figures 6-6 through 6-9 as cumulative carbon dioxide evolved per 100 g soil during a 90-day incubation period. At all doses, the Alberta soil was the most bioactive, followed, in decreasing order, by New Mexico and Louisiana. Biodegradation in the Alberta soil showed hyperbolic kinetics and was rapid. In the New Mexico soil, biodegradation was slower and not obviously hyperbolic. The overall rates of biodegradation in the Alberta soil were two to two and one-half times that found in the New Mexico soil. The maximum biodegradation rates in the New Mexico soil were two to two and one-half times that found in the Louisiana soil. The biodegradation in the Louisiana soil showed a prolonged lag period, with high bioactivity not initiated until about 60 days at the 500- to 1000-mg/kg doses. At the highest dose of 1500 mg/kg, the Louisiana soil did not show rapid degradation during the entire incubation period, suggesting a toxic response. The low level of bioactivity in the Louisiana soil is probably related to the low pH of that soil (5.0). The biodegradation parameters for the aerobic degradation in the three soils are shown in Table 6-5.

Anoxic biodegradation of DEG was performed at a dose of 1000 mg/kg for 90 days. Figure 6-10 shows the cumulative carbon dioxide evolved during the biodegradation of DEG. The Alberta soil showed a lag period of 38 days, followed by a period of rapid activity, ending in a plateau period, with about 17% of theoretical degradation. Biodegradation in the New Mexico soil showed a lag period of about 32 days, followed by rapid biodegradation for a short period, then a substantial change in rate from 1.02 to 0.29 mg CO₂/100 g-d for the remaining incubation period. The extent of biodegradation in the New Mexico soil was about 15%. Louisiana showed no significant activity during the incubation period. The appearance of “negative” carbon dioxide production in the Louisiana soil in Figure 6-10 is an artifact of the data reduction

Figure 6-6. Aerobic biodegradation of DEG in the three soils at a dose of 200 mg/kg.

Figure 6-7. Aerobic biodegradation of DEG in the three soils at a dose of 500 mg/kg.

Figure 6-8. Aerobic biodegradation of DEG in the three soils at a dose of 1000 mg/kg.

Figure 6-9. Aerobic biodegradation of DEG in the three soils at a dose of 1500 mg/kg.

Table 6-5. Biodegradation Parameters for DEG in the Three Soils at 25°C, 60% MHC, with Static Incubation in the Dark for 90 days under Aerobic Conditions

Soil	Dose, mg/kg	Lag Period, days	Maximum Rate, mg CO ₂ /100 g-d	Theoretical Degradation, %
Alberta	200	5.6	1.59	78.1
	500	3.9	4.43	65.5
	1000	3.4	3.06	53.5
	1500	6.2	6.17	46.4
Louisiana	200	19.6	0.55	97.4
	500	38.7	0.84	55.4
	1000	39.9	0.91	32.7
	1500	14.9	0.58	20.8
New Mexico	200	6.1	0.90	88.0
	500	2.8	1.20	53.8
	1000	4.7	1.26	28.6
	1500	3.1	2.40	33.0

Figure 6-10. Anoxic biodegradation of DEG in the three soils at a dose of 1000 mg/kg.

method. As described earlier, each set of respirometry experiments includes an undosed sample of each soil type. In the case of the Louisiana soil, for most of the study period the amount of carbon dioxide evolved in the dosed sample was less than that evolved in the undosed sample, which suggests that DEG may have even caused some inhibition of microbial activity. The biodegradation parameters are shown in Table 6-6.

In summary, under aerobic conditions, DEG is biodegraded well in all three soils, although in soils where bioactivity is low, such as the Louisiana test soil, lag periods are likely to be long. In addition, some inhibition of bioactivity is evident with the higher doses of DEG. Under anoxic conditions, DEG is not well biodegraded in the three test soils. Lag periods were long, and the extent of biodegradation was low. DEG would be expected to persist in the environment under these conditions.

Table 6-6. Biodegradation Parameters for DEG in the Three Soils at Room Temperature (ca. 22°C), 60% MHC, with Static Incubation in the Dark for 90 days under Anoxic Conditions

	Alberta	Louisiana	New Mexico
Lag Period, days	38.0	NA ^a	31.7
Maximum Rate, mg CO ₂ /100 g-d	1.29	NA	1.02
Theoretical Degradation, %	17.4	0.5	14.6

^a Not applicable.

6.1.4 EG Biodegradation

EG biodegradation testing was performed using biometers under both aerobic conditions at doses of 200 and 1000 mg/kg and under anoxic conditions at a dose of 1000 mg/kg. Aerobic biodegradation of EG at 200 mg/kg is shown in Figure 6-11, as cumulative carbon dioxide evolved in mg per 100 g soil by dry weight. In all three soils, the EG appears to be biodegraded rapidly and without a significant lag period. Table 6-7 shows the biodegradation rate, lag period, and extent of aerobic degradation of EG. The extent of biodegradation, as a percentage of the theoretical carbon dioxide possible, was 80% to 94% at the 200-mg/kg dose. Aerobic biodegradation of EG at the 1000-mg/kg dose is shown in Figure 6-12. The Alberta and Louisiana soils show a short lag period of less than 2 days, while the New Mexico soil showed a negligible lag period. The fastest biodegradation rate was found with the Alberta soil, followed by Louisiana and New Mexico, in order of decreasing rate. The biodegradation of the high dose in the New Mexico soil appears to be arithmetic, rather than the typical hyperbolic, as seen in Alberta and Louisiana. Arithmetic kinetics suggest that growth did not occur. A failure of growth is most likely due to toxicity. The extent of EG biodegradation at the 1000-mg/kg dose was about 65% for both Alberta and Louisiana, but it was only 32% for New Mexico. This suggests that EG spills in soils similar to the New Mexico soil may persist.

Figure 6-11. Aerobic biodegradation of EG in the three soils at a dose of 200 mg/kg.

Table 6-7. Biodegradation Parameters (using biometers) for EG in the Three Soils at 25°C, 60% MHC, with Static Incubation in the Dark for 31 days under Aerobic Conditions

	Alberta		Louisiana		New Mexico	
Dose, mg/kg	200	1000	200	1000	200	1000
Lag Period, days	0.0	1.6	0.2	0.4	0.5	0.3
Maximum Rate, mg CO ₂ /100 g-d	2.67	9.42	2.71	5.53	2.71	2.51
Theoretical Degradation, %	94.4	66.1	80.4	64.7	81.1	32.1

Figure 6-12. Aerobic biodegradation of EG in the three soils at a dose of 1000 mg/kg.

Two additional doses of EG were tested for biodegradation using electronic respirometry. Figure 6-13 shows the cumulative carbon dioxide evolved during biodegradation of EG at doses of 500 and 1500 mg/kg in the three test soils. Biodegradation parameters for these data are shown in Table 6-8. These data agree quite well with the data collected via biometers at the 200- and 1000-mg/kg doses. The lag times are low in all cases. The biodegradation rates increased with increasing dose with one exception, that of Louisiana at the 1500-mg/kg dose. The fact that the biodegradation of EG in the Louisiana soil showed a decrease in rate at the 1500-mg/kg dose suggests that toxicity was involved at this dose. No inhibition was noted for EG in other soils.

Anoxic biodegradation of EG was tested at a single dose of 1000 mg/kg. The course of anoxic biodegradation of EG is shown in Figure 6-14, and biodegradation parameters are provided in Table 6-9. These data show that the Louisiana soil and, to a lesser extent, the Alberta soil had hyperbolic kinetics. In contrast, the New Mexico soil showed arithmetic degradation, suggestive of toxicity. Along with the arithmetic kinetics in the New Mexico soil, no lag period was observed. The lag periods in the Louisiana soil and Alberta soil were 3.5 and 7.9 days, respectively. The degradation rates were similar in the Alberta and Louisiana soils, while the rate noted in the New Mexico soil was about one-fourth as large. Overall, the maximum rates under anoxic conditions were between one-third and one-fourth of that observed under aerobic conditions at the same dose. The extent of biodegradation noted was much lower under anoxic conditions for all three soils, ranging between 26.1% and 40.4%. It is possible that the fermentative degradation of EG yields little energy for growth under anoxic conditions. If no terminal metabolic process is active, such as methanogenesis or sulfidogenesis (sulfate reduction), metabolic intermediates may accumulate, resulting in a low extent of biodegradation.

Figure 6-13. Aerobic biodegradation of EG in the three soils at doses of 500 and 1500 mg/kg.

Table 6-8. Biodegradation Parameters (using electronic respirometers) for EG in the Three Soils at 25°C, 60% MHC, with Static Incubation in the Dark for 68 days under Aerobic Conditions

	Alberta		Louisiana		New Mexico	
Dose, mg/kg	500	1500	500	1500	500	1500
Lag Period, days	0.2	0.8	0.7	0.6	0.7	1.8
Maximum Rate, mg CO ₂ /100 g-d	6.06	8.29	11.59	11.11	6.50	10.37

Table 6-9. Biodegradation Parameters for EG in the Three Soils at 25°C, 60% MHC, with Static Incubation in the Dark for 68 days under Anoxic Conditions

	Alberta	Louisiana	New Mexico
Dose, mg/kg	1000	1000	1000
Lag Period, days	7.9	3.5	0.0
Maximum Rate, mg CO ₂ /100 g-d	2.50	2.90	0.72
Theoretical Degradation, %	26.1	40.4	36.0

Figure 6-14. Anoxic biodegradation of EG in the three soils at a dose of 1000 mg/kg.

In summary, EG biodegradation is rapid, with short lag periods in all soils under aerobic conditions. Biodegradation rates are much reduced under anoxic conditions, but still faster than for many compounds under aerobic conditions. EG would not be expected to persist in most soil environments.

6.2 Glycol Waste Biodegradation Studies

Three glycol-based dehydration waste solutions were selected for biological testing. These glycol solutions were selected, based on the chemical characterization studies, as representative of the two commonly encountered glycol types and waste compositions. Of the three selected waste solutions, two were from TEG systems and one was from an EG dehydrator. The two TEG-based solutions were obtained from sites operated by different producers in Texas and Oklahoma to ensure differences in gas chemistry and process-based effects. Based on the semiquantitative data, described in Section 4.0, the EG solution from Site 6 (Alberta) contained relatively high concentrations of BTEX, as did the TEG solutions from Site 2 (Texas) and Site 8 (Oklahoma). All three of the glycol-based solutions were obtained from the “rich” portion of the dehydrator stream.

6.2.1 Soil–Water–Glycol Slurries

Soil, water, and rich glycol solutions were used to establish biological slurries to determine the biodegradability of the waste solutions in the presence of each of the three test soils. The slurries contained 30% (w/w) soil, 1% waste solution, and the remainder water. No additives were added to the slurries, so that they would be representative of a release of these glycol solutions in the soil. The slurries were aerated and mixed at room temperature for 28 days. Samples were taken at the start and weekly through the incubation period for chemical oxygen demand (COD), TOC, total bacterial count (TC), and glycol-biodegrading (GD) bacterial count.

Table 6-10 shows the initial and final concentrations of the COD, TOC, and the initial and final counts of total bacteria and of GD bacteria for Alberta soil slurries. Counts of GD bacteria increased by 3 or more logs during the incubation period, indicating that this population was provided appropriate conditions for growth. However, the biodegradation of the glycols and associated contaminants was poor, based on the small or no decreases in concentrations based on COD and TOC. In fact, TOC remained essentially constant throughout the incubation. In contrast, the COD did decrease by about 3000 to 5000 mg/L. Because there was no corresponding decrease in TOC, it is probable that the organic compounds were partially oxidized only, resulting in a decrease in COD.

Table 6-10. Initial and Final Concentrations after Slurry Treatment of Three Rich Glycol Solutions with Alberta Soil

Parameter	Site 6 – Alberta	Site 2 – Texas	Site 8 – Oklahoma
Initial COD, mg/L	13,100	17,700	17,600
Final COD, mg/L	10,700	12,500	14,400
Initial TOC, mg/L	3100	3800	3800
Final TOC, mg/L	3200	3700	3200
Initial TC, mpn/mL ¹	1.3×10^5	5.4×10^5	1.6×10^6
Final TC, mpn/mL	1.1×10^8	7.9×10^7	7.9×10^7
Initial GD, mpn/mL	2.0×10^1	1.3×10^2	1.7×10^2
Final GD, mpn/mL	$>2.4 \times 10^6$	$>2.4 \times 10^6$	5.4×10^5

¹. Most probable number/mL.

The results of the slurry treatment of the three glycol solutions with the Louisiana soil (shown in Table 6-11) were similar to that found with the Alberta soil, with a few important differences. The decrease in COD was much smaller in the Louisiana soil than was noted in the Alberta soil. The increase in microbial counts in the Louisiana soil was of similar magnitude to the Alberta soil for total counts. Increases in glycol degraders in the Louisiana soil were also similar to Alberta soil, with the exception of the Site 6 (Alberta) glycol, where the increase was very small.

Biodegradation of the glycol solutions in the New Mexico soil (shown in Table 6-12) showed similarly poor results to those found in the other soils. The increase in total and glycol-degrading bacteria was about 2 to 3 logs, indicating good growth. However, decreases in COD and TOC showed poor, mixed results. The Site 6 glycol showed a 43% decrease in COD and a 31% decrease in TOC, suggesting some complete biodegradation. The Site 2 glycol showed a small decrease in COD, but no change in TOC, suggesting partial oxidation only. The Site 8 glycol showed no change in COD, but a 35% decrease in TOC. This may be an analytical error, as there is no rational explanation for this result.

In summary, the biodegradation of the rich glycol solutions in aqueous slurries of the test soils was poor. Under these conditions, the glycol solutions and their associated contaminants are likely to persist. Based on the pure compound studies described earlier, it is possible that the glycols are the cause of the inhibition. Alternatively, associated contaminants may be inhibitory or the microbes may be constrained by a lack of nutrients.

Table 6-11. Initial and Final Concentrations after Slurry Treatment of Three Rich Glycol Solutions with Louisiana Soil

Parameter	Site 6 – Alberta	Site 2 – Texas	Site 8 – Oklahoma
Initial COD, mg/L	13,600	18,300	19,000
Final COD, mg/L	12,900	16,300	18,000
Initial TOC, mg/L	3300	4500	4620
Final TOC, mg/L	3100	4090	4680
Initial TC, mpn/mL	8.0×10^4	8.0×10^4	2.0×10^4
Final TC, mpn/mL	1.1×10^9	4.9×10^8	7.9×10^7
Initial GD, mpn/mL	1.3×10^2	$<2.0 \times 10^1$	$<2.0 \times 10^1$
Final GD, mpn/mL	3.3×10^2	$>2.4 \times 10^6$	$>2.4 \times 10^6$

Table 6-12. Initial and Final Concentrations after Slurry Treatment of Three Rich Glycol Solutions with New Mexico Soil

Parameter	Site 6 – Alberta	Site 2 – Texas	Site 8 – Oklahoma
Initial COD, mg/L	14,100	18,300	18,100
Final COD, mg/L	7980	16,700	18,900
Initial TOC, mg/L	2790	3840	2830
Final TOC, mg/L	1920	3870	1850
Initial TC, mpn/mL	2.0×10^4	7.0×10^4	2.0×10^4
Final TC, mpn/mL	4.9×10^7	4.9×10^7	7.9×10^6
Initial GD, mpn/mL	1.3×10^3	3.3×10^2	4.9×10^2
Final GD, mpn/mL	2.4×10^4	$>2.4 \times 10^7$	2.8×10^5

6.2.2 Nutrient-Amended Soil–Water–Glycol Slurries

Because of the poor biodegradability noted in unamended soil slurries with the rich glycol solutions, additional tests were performed using nutrient-amended slurries. These slurries were established in a manner similar to the above soil–water–glycol slurries. Soil was added at 30% (w/w), the rich glycol solutions were added at 1% (w/w), but instead of water, a nutrient solution was used. The nutrient solution consisted of a nutrient medium with additional nitrogen and phosphorus. The nutrient medium was that used in the Organization for Economic Cooperation and Development (OECD) biodegradability testing protocols (OECD, 1981). Nitrogen and phosphorus requirements were set at a COD:N:P ratio of 100:5:1. The COD for the glycol solutions was estimated to be 20,000 mg/L, based on rounding of the mean COD of the glycol slurries used previously. Excess nitrogen was added as ammonium chloride. Excess phosphorus was added as the mono- and dipotassium phosphate salts at a pH of 7.0. The ratio of mono- to dipotassium phosphate was maintained the same as for the unamended OECD medium. The total slurry volume was 350 mL in a 1-L Erlenmeyer flask. The slurries were mixed at 75 rpm on an orbital shaker at room temperature for 30 days. Samples of the slurries were collected at the beginning, midpoint, and end of the incubation period and analyzed for glycol and COD.

The biodegradation of organic compounds in the Alberta soil slurry is shown in Figure 6-15 for COD and in Figure 6-16 for glycols. COD reductions for the Site 2 and Site 8 glycol solutions were essentially the same at about 30%. The COD reduction for the Site 6 glycol solution was much greater at 85%. The glycols were completely biodegraded to below the detection limit in all three glycol solutions in the Alberta soil slurries. Table 6-13 shows the initial and final concentrations of glycol and COD and the removals during the slurry operation.

Biodegradation in Louisiana soil slurries was poorer than noted for the Alberta soil. Figures 6-17 and 6-18 show the COD and glycol concentrations during the 30-day slurry operation with the three rich glycol solutions. COD reductions were good for the Site 6 glycol solution, but were similarly poor for the Site 2 and Site 8 samples. Table 6-14 shows the initial and final concentrations of glycol and COD and the removals during the slurry operation. The reduction in COD for the Site 6 glycol solution was 96%, compared to reductions of about 20% to 26% for the Site 8 and 2 glycol solutions, respectively. Removal of glycol from the slurries was 100% for Site 6, 96% for Site 2, but only 38.6% for Site 8.

The course of biodegradation of COD and glycols in the New Mexico soil slurries is shown in Figures 6-19 and 6-20, respectively. Table 6-15 shows the initial and final concentrations of glycol and COD and the removals during the slurry operation. Biodegradation of COD in the New Mexico soil slurries was essentially complete, with removals of 96.6%, 90.1%, and 87.6% for the Site 6, 2, and 8 glycol solutions, respectively. The removal of glycol in these solutions was 100% for all glycol solutions.

In summary, the operation of nutrient-amended soil slurries demonstrated much improved biodegradation of the rich glycol solutions compared to unamended soil slurries. There was a soil-specific effect, with the Louisiana soil, the least bioactive soil in other tests, the poorest. Also, as in previous research, the Alberta and New Mexico soils were similar with some interesting, but not understood, differences. There was also an effect due to the type of glycol solution. The Site 6 (Alberta) EG solution was biodegraded more rapidly and more completely, as expected from the pure compound

Figure 6-15. COD concentrations throughout the incubation period for the three rich glycol solutions in Alberta soil slurries.

Figure 6-16. Glycol concentrations throughout the incubation period for the three rich glycol solutions in Alberta soil slurries.

Table 6-13. Initial and Final Concentrations and Removals of COD and Glycols in the Nutrient-Amended Slurry Biological Treatment of Three Rich Glycol Solutions in Alberta Soil

Glycol	Initial COD, mg/L	Final COD, mg/L	COD Removal, %	Initial Glycol, mg/L	Final Glycol, mg/L	Glycol Removal, %
Site 6 – Alberta	12,300	1830	85.1	9070	0	100
Site 2 – Texas	16,900	11,300	33.1	13,150	0	100
Site 8 – Oklahoma	17,000	11,700	31.2	12,760	0	100

studies. Site 8 and Site 2 (Oklahoma and Texas) TEG solutions biodegraded somewhat more slowly. Pure compound studies with TEG suggested inhibition at the higher doses tested. This inhibition may have been a factor in these tests as well.

6.2.3 Nutrient-Amended Water–Glycol Slurries

Nutrient-amended aqueous glycol slurries were tested to determine the relative biodegradability of the three rich glycol solutions. These tests were designed to evaluate the biodegradation exclusive of the soil effects. The slurries were established in a manner similar to the soil–water–glycol slurries above. A nutrient-amended OECD solution was used with the glycol added at 1% (w/w). The slurries were 350 mL in a 1-L Erlenmeyer flask incubated at room temperature with shaking on an orbital shaker at 75 rpm for 30 days. Nitrogen and phosphorus additions were the same as used in the soil–water–glycol slurries. Each slurry was inoculated with a small volume of adapted slurry from previous tests.

Figure 6-17. COD concentrations throughout the incubation period for the three rich glycol solutions in Louisiana soil slurries.

Figure 6-18. Glycol concentrations throughout the incubation period for the three rich glycol solutions in Louisiana soil slurries.

Table 6-14. Initial and Final Concentrations and Removals of COD and Glycols in the Nutrient-Amended Slurry Biological Treatment of Three Rich Glycol Solutions in Louisiana Soil

Glycol	Initial COD, mg/L	Final COD, mg/L	COD Removal, %	Initial Glycol, mg/L	Final Glycol, mg/L	Glycol Removal, %
Site 6 – Alberta	12,400	502	96.0	9840	0	100
Site 2 – Texas	16,400	12,200	25.6	12,070	480	96.0
Site 8 – Oklahoma	16,600	13,300	19.9	14,650	9000	38.6

Figure 6-19. COD concentrations throughout the incubation period for the three rich glycol solutions in New Mexico soil slurries.

The decrease in COD and glycols for these slurries is shown in Figures 6-21 and 6-22, respectively. COD and glycol initial and final concentrations and removals are shown in Table 6-16. As before, the reduction in COD was better with the Site 6 (Alberta) EG solution than for the TEG solutions. COD and glycol concentrations were not reduced to the levels observed for the soil–water–glycol slurries. Glycol removal was highest in the Site 8 (Oklahoma) sample, but the corresponding COD removal in that slurry was only 18.5%. This suggests that the glycols may have been only partially oxidized. The poorer performance of COD and glycol removal in the aqueous slurries compared to those with soil may be due to several factors. It is likely that the presence of soil results in a much larger biomass than was present in the aqueous slurries. Additionally, the presence of the soil may have ameliorated toxic effects through sorption and other surface-related phenomena. In summary, biodegradation of the rich glycol solutions was poor in nutrient-amended aqueous slurries. The poor biodegradability of glycols under these conditions is probably due to inhibition of the glycols and to a low biomass concentration.

Figure 6-20. Glycol concentrations throughout the incubation period for the three rich glycol solutions in New Mexico soil slurries.

Table 6-15. Initial and Final Concentrations and Removals of COD and Glycols in the Nutrient-Amended Slurry Biological Treatment of Three Rich Glycol Solutions in New Mexico Soil

Glycol	Initial COD, mg/L	Final COD, mg/L	COD Removal, %	Initial Glycol, mg/L	Final Glycol, mg/L	Glycol Removal, %
Site 6 – Alberta	14,600	500	96.6	9068	0	100
Site 8 – Oklahoma	14,700	1830	87.6	12,500	0	100
Site 2 – Texas	14,600	1450	90.1	10,670	0	100

6.3 Biodegradation Summary and Implications

The biodegradability of dehydration glycols appears to be a function of both glycol type and soil type. Under most conditions, in all three soils, EG was the most readily biodegradable and TEG was the least biodegradable of the three dehydration glycols that were tested. In general, under aerobic conditions, the biodegradability of the glycols in soil appears to decrease as the number of ethylene moieties, or groups, increases (mono- > di- > triethylene). This observation did not necessarily hold true for the dehydration glycols under anaerobic conditions, where DEG appeared to be the least biodegradable of the glycols. Also, in all soils, the biodegradation of the three glycols appeared to be inhibited at higher concentrations (>1500 mg/kg).

With regard to the soil type, under both aerobic and anaerobic conditions, the Alberta test soil, which was characterized by a relatively even grain-size distribution, neutral pH, and reasonable organic matter (OM) content, displayed the best ability to degrade TEG and DEG and showed great ability to

Figure 6-21. COD concentrations throughout the incubation period for the three glycol solutions in aqueous slurries.

Figure 6-22. Glycol concentrations throughout the incubation period for the three rich glycol solutions in aqueous slurries.

Table 6-16. Initial and Final Concentrations and Removals of COD and Glycols in the Nutrient-Amended Slurry Biological Treatment of Three Rich Glycol Solutions

Glycol	Initial COD, mg/L	Final COD, mg/L	COD Removal, %	Initial Glycol, mg/L	Final Glycol, mg/L	Glycol Removal, %
Site 6 – Alberta	8980	4960	44.8	6440	2760	57.1
Site 2 – Texas	12,200	9940	18.5	9660	1380	85.7
Site 8 – Oklahoma	11,300	9900	12.4	9960	7250	27.2

biodegrade EG. The New Mexico test soil, which was the sandiest and had the lowest OM content of the test soils, was able to degrade EG, DEG, and TEG, although not to the extent of the Alberta soil. Aerobically, the Louisiana test soil, whose most notable features were its high silt and clay content and acidic pH, was essentially as good as the Alberta soil at degrading EG, and under anaerobic conditions, the Louisiana soil was the best. However, the Louisiana soil was strikingly unable to degrade DEG and TEG, regardless of the conditions.

The difference in abilities of the three soils to degrade the glycols is likely due to the physical and chemical characteristics of each specific test soil and the types of microorganisms in the soils. The Alberta test soil had the highest organic matter content of the three soils, a moderate pH, and a relatively even distribution of sand, silt, and clay. These conditions are good for the development and maintenance of a viable microbial population. They provide a well-balanced substrate in which bioactivity can be optimized. The Louisiana test soil, on the other hand, was distinguishable from the other soils by a low pH (5.2), which was likely inhibitory to microbial activity under even the best of conditions.

In contrast to the pure compounds, rich glycol solutions collected from dehydration units in Texas, Oklahoma, and Alberta were not readily biodegraded in aqueous slurries of New Mexico, Louisiana, and Alberta test soils without nutrient amendments. There was little significant difference in biodegradation between the three test soils. These results suggest that the presence of cocontaminants (such as condensate species) and/or high concentrations of glycols present in the wastes can inhibit the degradation of the glycols, a hypothesis which is supported by the pure compound data. As with the pure compound studies, it is apparent that soil type is likely a primary controlling factor in determining the biodegradability of a rich glycol dehydration waste in the subsurface. The availability of oxygen and soil characteristics such as organic matter content, pH, and other soil-specific factors are likely the key parameters that determine the ultimate fate of dehydration wastes in soil and groundwater.

The addition of nutrient amendments (nitrogen and phosphorus) significantly enhanced the biodegradability of the rich glycol solutions in aqueous soil slurries. In fact, the results of the nutrient-amended slurry experiments essentially reflect the results of the pure compound experiments in that biodegradation was most effective in the Alberta soil and least effective in the Louisiana soil, with the New Mexico soil falling somewhere in between the two. The results of the slurry experiments indicate that the addition of nutrient amendments may be a necessary component of any biologically based remedial strategy, regardless of the soil type.

7.0 Glycol-Based Dehydration Waste Environmental Transport and Fate Implications

It is important to note that the implications discussed in this report are based on the assumption that the soils used in these research activities are reasonably representative of the regions from which they came. The authors recognize that soil characteristics can vary dramatically within a specific region, depending on local differences in geology, topography, and climate. However, the authors themselves have spent considerable time working in the selected regions (northern Louisiana, northwestern New Mexico, central Alberta, and eastern North Dakota) and made a point to consult a variety of other academic and industry personnel in each region who are intimately familiar with the natural settings of their respective areas. In the cases of the U.S. locations, soil survey maps were also used as aids to selecting sampling sites. With this in mind, the authors consider the soil types at the sampling locations to reasonably reflect the characteristics of each region as a whole, so that the resulting data are assumed to generally represent the “typical” subsurface environment in each region. However, it is equally important to caution against applying the generalizations described in this report towards the prediction of subsurface transport and fate of dehydration-related materials at any specific site. The purpose of the data is to illustrate that differences (or lack of such, as the case may be) in subsurface environmental behavior can be expected of the dehydration glycols in different natural settings.

The biodegradability studies on pure glycol compounds indicated that, in most natural settings, an unplanned release of raw or lean glycol-based dehydration fluid may be expected to biodegrade rapidly. However, a release of rich glycol-based dehydration fluid would likely have elevated concentrations of hydrocarbon-based coconstituents, which studies showed may severely limit its biodegradability in the subsurface. In this case, such a release may be doubly problematic in that the waste may not only persist in the subsurface environment, but may also act as a cosolvent for some of the coconstituent hydrocarbon species, thereby enhancing their mobility beyond what would otherwise be predicted in a strictly aqueous solution.

For instance, data suggest the potential ramifications of an unplanned release of raw or lean glycol-based dehydration fluid into the subsurface at a dehydration unit in Louisiana may be much different than a similar release in New Mexico or Alberta. Most notably, in an acidic soil with a high clay content, such as is typically found in Louisiana, there is a strong possibility that the glycols themselves will not readily biodegrade, while in soil types typically found in New Mexico and Alberta, the glycols may be expected to degrade rapidly. From a remediation perspective, persistent contaminants are generally more difficult and, therefore, more costly to remove. Thus it is anticipated that remediation of glycol-contaminated soil at typical sites in Louisiana may be more difficult to achieve than similar sites in New Mexico and Alberta.

On the other hand, if the released glycol-based fluid contains significant concentrations of hydrocarbons (i.e., BTEX, PAHs, alkanes), as observed in samples of rich dehydration solution, then the potential impacts to the environment appear to be much more severe. In contrast to the pure compounds, none of the rich dehydration fluids was readily biodegraded in any of the aqueous soil slurries without nutrient amendments, suggesting that they may be recalcitrant in the natural subsurface environment which is less conducive to biodegradation than a slurry environment. These results suggest that the high glycol concentrations and/or hydrocarbon coconstituents present in the rich solutions can inhibit the degradation of the fluids regardless of the soil type. The lack of degradability then opens the door for the rich glycol solutions to act as a cosolvent for certain condensate species (i.e., BTEX and naphthalene). The extent to which the cosolvency effect of glycol can enhance condensate mobility is largely dependent on soil type. The data suggest that cosolvent effects will be most pronounced on soils with

high organic carbon content (i.e., Alberta and North Dakota) where mobility of BTEX and naphthalene is generally low under normal conditions.

To illustrate the potential impact that high concentrations of TEG could have on benzene and toluene mobility, the K_d data developed for benzene and toluene (with and without TEG present) in the North Dakota soil were plugged into two widely available groundwater transport models, SSGPlume and 3DADE, that are part of GRI's Showflow modeling software package. Benzene and toluene were selected because they typically are of great concern to regulators. The K_d data derived from the North Dakota soil were selected because they showed the greatest differences between the K_d values of each hydrocarbon and the K_d values of each hydrocarbon with 40% TEG.

SSGPlume simulates steady-state two-dimensional groundwater transport of solutes assuming a Gaussian plane source. The results of the SSGPlume runs suggest that the presence of 40% TEG may cause an approximate 50% increase in toluene concentrations 50 m from the source over what would be predicted without the TEG. For benzene, the model predicted that the cosolvency effect of TEG could result in a 33% increase in benzene concentrations 50 m from the source. The SSGPlume model does not include a time component in its results and, thus, does not directly predict the rate of contaminant transport. 3DADE models the transient transport of solutes with a plane or cube source in three-dimensional flow. The 3DADE modeling runs predicted the movement of benzene and toluene plumes, with and without 40% TEG present, 1 year after their release to the subsurface. The results showed that the concentrations of toluene in the center of the plume, affected by the presence of TEG, were predicted to be an order of magnitude lower than those in the center of the toluene-only plume. Also, the center of the TEG-affected plume was predicted to travel approximately 75% further downgradient than the toluene-only plume. The center of a plume of benzene and TEG was predicted to have an order of magnitude lower concentrations and to have moved 60% further downgradient than a benzene-only plume.

The SSGPlume and 3DADE modeling efforts described above were conducted to roughly predict the potential impact that glycol cosolvent effects could have on benzene and toluene mobility in a generic aquifer setting. Both models are rather simplistic when compared to other contaminant groundwater modeling software such as Bioplume. For instance, neither SSGPlume nor 3DADE takes into account the effects that TEG biodegradation might have on the hydrogeochemistry of an aquifer over time. Changes in aquifer conditions, such as BOD and COD, due to glycol biodegradation may have positive or negative influences on the transport and fate of BTEX. Because of the lack of field data for verification and the limitations of the SSGPlume and 3DADE transport models, the reliability and accuracy of their predictions should be considered to be preliminary at best and perhaps even questionable. However, the results are included in this discussion to illustrate the point that the data generated by the laboratory-based cosolvency investigations suggest it is quite possible that high concentrations of glycol cocontaminants may significantly enhance the subsurface mobility of BTEX. Future research activities should include field-based investigations coupled with rigorous modeling efforts to verify and validate the laboratory-based results and predictions described in this report.

In general, the results generated by the research program are perhaps best applied to the development of RBCA plans and/or remediation strategies at natural gas dehydration and processing sites. Putting the results from all three areas of research into this context, the association of materials such as condensate species and organosulfur compounds with the glycols appears to be problematic from two angles. First, data suggest the presence of such constituents could affect the biodegradability of glycol solutions, thereby complicating remediation efforts. Second, data also indicate the glycols in water could act as a cosolvent and greatly increase the transport of some cocontaminants (particularly the highly regulated condensate species) beyond what would normally be predicted. Failure to consider the

cosolvent effects that the glycols may have on the mobility of the condensate species may result in underestimation of their transport in the subsurface, thereby leading to an underestimation of risk. In order to appropriately develop RBCA and/or remediation plans, the data indicate that it is necessary to have a basic understanding of both the wastes and the native soil. Key parameters to predicting subsurface transport and fate include 1) the type and concentration of glycol; 2) the presence or absence of condensate species, especially BTEX, in either the dehydration solution or the receiving subsurface; and 3) the chemical, physical, and biological characteristics of the native soil, including pH, size of the microbial population, grain-size distribution, total organic carbon content, cation exchange capacity, and a variety of other soil-specific factors.

8.0 Recommendations for Future Research

In order to address issues raised by the research activities described in this report, future activities should be conducted in the following four areas: 1) field-based investigations of subsurface contamination associated with glycol-based natural gas dehydration systems, 2) continued laboratory-based investigations of the cosolvent effects of glycols on selected condensate species in a variety of North American soils, 3) additional laboratory-based evaluations of the biodegradability of glycol-based dehydration wastes, and 4) computer modeling of subsurface transport and fate of dehydration wastes using data generated in the laboratory and the field.

The primary objectives of such work would 1) provide the natural gas industry with detailed environmentally relevant data regarding the nature, occurrence, and natural attenuation of subsurface releases of wastes from natural gas dehydration units; 2) lead to the development of remediation strategies that are technically and economically viable; and 3) provide industry personnel and regulatory agencies with data that can be used as an integral part of personal computer (PC)-based predictive transport and fate and/or RBCA and environmentally acceptable endpoint (EAE) models.

Specifically, laboratory-based column studies and determinations of distribution coefficients (K_d values) for condensates in soils with and without glycol present could be used to more precisely describe the extent to which glycols can affect the mobility of condensate species in soil, thereby providing higher-quality data for RBCA modeling efforts. Regarding additional biodegradation evaluations, examinations of the impacts of condensate–glycol mixtures on microbial populations and metabolism should be performed. Such examinations would provide data regarding the subsurface fate of rich dehydration wastes as well as the remediation of such materials in soils. The data generated by additional biodegradation investigations would provide the gas industry with previously unavailable insight regarding the biological attenuation of dehydration wastes. The data may also be a key component of PC-based site-specific transport prediction and risk assessment models. The data are also necessary for the development of viable remediation strategies for soil and groundwater contaminated by dehydration wastes.

Perhaps the most critical information needed to properly judge the environmental relevance of the data generated by laboratory investigations is that which is generated by field-based investigations at sites where dehydration wastes have been introduced into the subsurface environment. To generate data that will be applicable to a broad variety of geological and climatological settings, field sites should be sought in at least three different natural gas-producing regions of North America. Since soils used in GRI–DOE-sponsored activities were collected from the San Juan Basin of New Mexico, northern Louisiana, and the foothills region of central Alberta, sites in those regions would be ideal for data comparison purposes. The data collected at the field sites would provide the gas industry with real-world information regarding the effects of dehydration wastes on subsurface environments in a variety of geological and climatological settings. Such data are necessary to verify and validate the transport and fate data generated in laboratory investigations. The field data could also be used to calibrate PC-based subsurface transport and fate computer models that could be used for site-specific risk assessments.

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APPENDIX A

Review of Glycol Biodegradation

A1.0 Introduction

Biodegradation has been determined by a number of different methods. The method used may be indirect, such as a measure of change in the biochemical oxygen demand (BOD), or direct, as in the loss of added compound. The implications of the method used can be profound. For example, if an indirect measure such as evolution of carbon dioxide is used, the investigator may note a rapid evolution of CO₂, with a final mass balance of, say, 40% of the total found. Analysis by parent compound might determine that very little of the parent compound has been degraded, and the CO₂ found might be the result of toxic effects on the biomass.

Table A-1 summarizes the most frequently used methods for determining biodegradation of a compound. Note that all indirect methods are susceptible to error due to impurities in the test compound. Frequently, a compound cannot be purchased in purity greater than about 90%. Additionally, many compounds use carrier solvents to solubilize them or contain other additives. For example, pesticides are often mixed with emulsifiers, oils, and detergents. These other compounds may falsely indicate biodegradation. It is important to distinguish between types of biodegradation. The test compound added in a biodegradation test may simply be transformed to a slightly different form, without utilization of oxygen, loss of carbon, etc. This transformation may be important since it may increase or decrease toxicity, but it may also suggest biodegradation if monitoring is only by loss of test compound. The complete degradation of a compound to its mineral components (water, carbon dioxide, ammonia, sulfate, etc.) is known as mineralization. A fraction of the test compound is usually converted into cell mass. Hence, even a readily degraded compound will not result in 100% degradation on the basis of a measure such as BOD, but analysis of test compound should indicate complete loss. Mineralization is the biodegradation usually assumed and desired in biodegradation testing. However, it cannot be assumed to have occurred without a careful mass balance and chemical analysis.

The inocula used to supply the microbes for biodegradation is very important, both in terms of numbers of microbes and the metabolic capabilities of the population. If the metabolic capacity is not known, it is best to acclimate the microbes to the test compound prior to inoculation. Acclimation is of two types: induction of enzymes required for biodegradation usually occurs within a matter of hours, while growth of the biodegrading population to numbers large enough to impact biodegradation is usually measured in terms of days for aerobic microorganisms, but may take months for anaerobic microbes. In most cases, both types of acclimation are necessary. Another important factor to be considered in biodegradation testing is the use of killed controls. These controls allow the separation of biotic and abiotic effects and can be a check on the methods used for estimating biodegradation. In some cases where abiotic mechanisms are known to be insignificant, killed controls are not necessary.

A2.0 Glycol Biodegradation

The most common glycols are ethylene glycol (EG), diethylene glycol (DEG), and triethylene glycol (TEG). In addition to these glycols, the polymeric glycol, polyethylene glycol (PEG), is used in some applications. Since the route of biodegradation of PEG may involve depolymerization followed by monomer degradation, it is useful to evaluate the literature on their biodegradation as well. Lamb and Jenkins (1952) reported on the biodegradation of EG as measured by BOD at a dose of 2.5 mg/L. The percent of the theoretical oxygen demand after 5, 10, 15, and 20 days was 12.5%, 51.8%, 71.0%, and 78.0%, respectively.

Table A-1. Methods for Determining Biodegradation¹

Method	Type	Comments
Biochemical oxygen demand (BOD), usually reported as 5-, 10-, or 20-day	Indirect – measures consumption of oxygen over an incubation period	Requires high concentrations of test compounds
Chemical oxygen demand (COD)	Indirect – measures amount of oxygen required for chemical oxidation	Requires high concentrations of test compounds
Total organic carbon (TOC)	Indirect – measures total soluble carbon	Requires high concentrations of test compounds
Respirometry	Indirect – measures CO ₂ evolution	Requires high concentrations of test compounds
Radiorespirometry	Indirect – usually a mass balance of radioactivity from the test compound	Can be performed with low concentrations; allows a mass balance
Electron acceptors	Indirect – measures concentrations of electron acceptors (e.g., sulfate, methane)	High concentrations of test compounds
Metabolite concentrations	Indirect – release of halogen, or other part of the test compound	Medium-to-high concentrations of test compounds
Growth	Indirect – changes in turbidity or cell numbers	High concentrations of test compounds
Parent compound	Direct – analysis of loss of test compound	Medium-to-low concentrations – depends on analytical method

¹ Zitco, 1984; Painter and King, 1985; Baker and Herson, 1994.

Hatfield (1957) described the biodegradation of a variety of organic compounds, including EG, DEG, and TEG. He used batchwise fill-and-draw activated sludge reactors to evaluate the biodegradability of the test compounds. The bioreactors were acclimated to each test compound by slowly increasing the concentration in the feed to a final concentration of 333 mg/L. Oxidation of the glycols was followed by the indirect measures of BOD and COD. Of two test runs made using EG, one test showed virtually no reduction, on the basis of BOD, in 8 hours of aeration. The second run with EG using a biomass that had been acclimated at a slower rate showed about a 75% reduction in 8 hours. When a biomass adapted with DEG was used, no reduction in BOD or COD was noted in the 8-hour aeration period. The TEG-adapted sludge showed a slight BOD and COD reduction of TEG in the 8-hour aeration period. Hatfield concluded that a properly acclimated culture can oxidize the three glycols at a slow rate.

Abeles and Lee (1961) studied the metabolism of EG under anaerobic conditions with an *Aerobacter aerogenes* (American Type Culture Collection [ATCC] 8724). The major product of anaerobic metabolism of EG by these cell suspensions was acetaldehyde. Data were presented to show that this transformation required a cobamide coenzyme. Fincher and Payne (1962) isolated a bacterium from soil using TEG as the carbon source. The isolate was a gram-negative aerobic non-spore-forming rod. It could grow on EG, DEG, TEG, and some of the PEGs (PEG₂₀₀ to PEG₄₀₀ and PEG₁₅₀₀) as the sole carbon source. However, the glycols were not utilized if a preferential carbon source was available (yeast extract), indicating diauxie. The implication for this phenomenon is that with mixed wastes containing glycols, the glycols may not be metabolized until the other organic compounds have been used.

Fincher and Payne (1962) also measured oxygen consumption of the glycols by manometry of resting, adapted cells. The percent of theoretical oxygen consumption found for each of the glycols was EG 67%, DEG 114%, and TEG 92%. PEG was metabolized, but only at very slow rates. The rate of dehydrogenation of TEG was measured with respect to pH and found to be fastest at a pH of 8.0 and slower at more acidic pHs. The authors showed that oxidation rates were faster with increasing size of the glycols (TEG > DEG > EG); however, the cell yield per gram of carbon decreased slightly with increasing glycol size.

The capabilities of the acetic acid bacteria for oxidation of glycols was studied by Kersters and DeLey (1963). They found that *Glucobacter oxydans* (*suboxydans*) could oxidize a wide variety of glycols, including EG, DEG, and TEG. Resting cells of *G. oxydans* (*suboxydans*) and *Acetobacter aceti* (*liquifaciens*) produced glycolic acid from EG. They showed that at least three enzymes were involved in the oxidation of these glycols: a soluble NAD (nicotinamide adenine dinucleotide)-linked primary alcohol dehydrogenase, a soluble NAD-linked secondary alcohol dehydrogenase, and one or more particulate oxidative systems. The primary alcohol dehydrogenase acts on EG and related alcohols to produce the corresponding aldehyde. The secondary alcohol dehydrogenase acts on glycols with a secondary OH group and produces the corresponding ketone. The particulate oxidative system is probably linked to the cytochrome system, with the products being the corresponding alcohol, then the acid.

The utilization of EG by an anaerobic bacterium was reported by Gaston and Stadtman (1963). A new species of bacterium, *Clostridium glycolicum* (deposited with ATCC as 14880), a gram-positive, anaerobic, non-spore-forming rod that can use EG as the sole carbon and energy source was isolated from mud using enrichment culture. This bacterium was believed to metabolize EG to equal amounts of acid and alcohol (acetic acid and ethanol). Gonzalez and others (1972) isolated a salt-requiring bacterium from a brine pond near the Great Salt Lake that could use EG as the sole carbon source. The bacterium, designated T-52, is a gram-negative, nonmotile rod that requires at least 0.85% NaCl for growth. Although the bacterium has not been identified, it was deposited and is available from ATCC (27042).

Gonzalez and others (1972) used 1,2-¹⁴C-ethylene glycol as a tracer to follow respiration and assimilation by bacterium T-52. The best growth by this bacterium was observed in 2.5% NaCl or 12.15% Na₂SO₄ (ca. 1.08 M Na⁺) at a neutral pH. Oxidation experiments with whole cells grown with EG or glucose showed that EG-grown cells oxidize EG much faster than glucose-grown cells. The presence of glucose reduced metabolism of EG to near zero until the glucose was consumed. Gonzalez and others (1972) proposed a pathway for glycol metabolism in bacterium T-52 based on its use of glycolate and glyoxylate, as well as EG, as sole carbon sources, plus the suggested pathways for glycolate metabolism in *Escherichia coli* (Kornberg and Sadler, 1961). EG is converted to glycolic acid, then glyoxylic acid. Glyoxylic acid with acetyl-CoA can be converted into malate for entry into the tricarboxylic acid cycle, or the glyoxylic acid may be decarboxylated into tartronic semialdehyde. Tartronic semialdehyde is reduced to glyceric acid, which is then phosphorylated to 3-phosphoglyceric acid for entry into the glycolytic cycle.

Grabińska-Łoniewska (1974a,b) evaluated the biodegradation of EG and other compounds by activated sludge bacteria. The activated sludge reactor used as a source of bacteria was treating industrial wastewater from a plant producing polyester fibers. Most of the bacteria isolated from the activated sludge reactor were capable of using EG as the sole carbon and energy source, including members of the following genera: *Acetobacter*, *Achromobacter*, *Alcaligenes*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Pseudomonas*, *Sarcina*, and *Xanthomonas*. However, not all members of these genera could grow with EG, nor could they all grow equally well on EG.

Evans and David (1974) evaluated the biodegradation of EG, DEG, and TEG in river waters. They collected water from four different river sources and tested the biodegradation of the glycols at doses of 0, 2, and 10 mg/L and at temperatures of 4°, 8°, and 20°C. The tests were performed in batch and aerated with humidified air. Analysis was for disappearance of the glycols. The data showed that EG degraded in 3 days at 20°C in all river waters, while the samples at 4° and 8°C demonstrated variability between waters, but required less than 14 days for complete degradation. DEG and TEG degradation was more complex. Biodegradation in some river waters was complete at about 4 days, regardless of the temperature, while in other samples, no significant degradation was observed during the 14-day incubation period. The theoretical oxygen demand (ThOD) for EG is 1.30 mg O₂/mg. The chemical oxygen demand (COD) was found to be 1.29 mg O₂/mg (Price and others, 1974). Additionally, Price and others determined the biodegradability of EG in freshwater and in artificial seawater. Domestic sewage was the seed, and batch tests were conducted using doses of 3, 7, and 10 mg/L. Dissolved oxygen depletion was followed for 20 days. The results are shown in Table A-2.

Table A-2. Biodegradation of EG in Freshwater and Synthetic Seawater¹

	Freshwater, % biodegraded	Seawater, % biodegraded
Day 5	34	20
Day 10	86	60
Day 15	92	65
Day 20	100	77

¹Price and others, 1974.

Haines and Alexander (1975) examined the biodegradability of a series of glycols, including EG, DEG, TEG, tetraethylene glycol, and PEGs. Biodegradation was followed via BOD during tests with soil microorganisms. EG was degraded within 2 days. DEG, TEG, tetraethylene glycol, and PEG₄₀₀ showed extensive degradation by 5 days. A number of bacteria were isolated from these soils able to use PEG_{20,000} as the sole carbon source. One selected microorganism, identified as *Pseudomonas aeruginosa*, was studied further. The *Ps. aeruginosa* studied was found to elaborate an extracellular enzyme that could degrade high-molecular-weight PEGs to lower-molecular-weight compounds, which could serve as growth sources for the bacterium. It was not known if the lower-molecular-weight compounds were EG, DEG, or oligomers.

Harada and Nagashima (1975) isolated bacteria using several alkylether compounds as the sole carbon sources. Bacterium MC11 isolated with ethylene glycol monomethylether and a bacterium isolated with TEG could also grow on EG, DEG, TEG, and tetraethylene glycol. Bacterium PE18 was isolated on PEG₄₀₀ and could grow on TEG and tetraethylene glycol, but not on EG or DEG. These isolates could also grow on glycolate and acetate. The bacteria were all tentatively identified as members of the genus *Alcaligenes*. The authors note that although alkylether compounds are not common in nature, the ability to degrade them appears to be widely distributed.

Pitter (1976) conducted biodegradation testing of a variety of organic compounds using batch-activated sludge reactors. The inocula were adapted to the test compound prior to inoculation. Biodegradation was followed using COD analysis, with the test compound added at a COD of 200 mg/L. He found that EG, DEG, and TEG were rapidly biodegraded with removals of 96.8%, 95.0%, and 97.7%,

respectively. The removal rates observed were 41.7, 13.7, and 27.5 mg COD/g-h, respectively. Bedard (1976) evaluated the biodegradation of EG, antifreeze, and other organic compounds. He estimated a refractory index (RI), defined as the ratio of the ultimate BOD to the ThOD. Domestic sewage was used as inoculum and oxygen depletion measured over 30 to 40 days. EG and antifreeze were both judged to be readily degradable, with RI values of 0.76 and 1.12, respectively.

Jones and Watson (1976) reported on the activities of an *Acinetobacter* sp. isolated from sewage using enrichment culture techniques. This bacterium could grow on EG, DEG, TEG, PEG₂₀₀, and PEG₄₀₀ as the sole carbon sources. Cell-free extracts of EG-grown cells have high activities of EG dehydrogenase that can use NAD⁺ or NADP⁺ with a pH optimum of 9.8. EG dehydrogenase activity in cells grown on succinate was low. Growth on EG also induced production of an NAD-dependent glycolaldehyde dehydrogenase that also had activity with acetaldehyde and glyceraldehyde. They did not detect metabolism of glycolate, but glyoxylate was detected from EG. Tartronic semialdehyde activity was not found from glyoxylate, but hydroxypyruvate reductase activity was identified. This suggests that hydroxypyruvate, rather than tartronic semialdehyde, may be the first C₃ compound in this bacterium.

Additional studies by Watson and Jones (1977) with *Acinetobacter* SC25, *Pseudomonas* sp. KW 8, and *Flavobacterium* sp. BT 1 showed conflicting degradation patterns. The *Acinetobacter* isolated with PEG₄₀₀ could grow on EG, DEG, TEG, PEG₂₀₀, and PEG₄₀₀ and very slowly degrade PEG₆₀₀, PEG₈₀₀, and PEG₁₀₀₀. The *Pseudomonas* sp. could degrade EG and PEGs up to 400 and slowly degrade PEG₆₀₀, but not longer PEGs. The *Flavobacterium* sp. isolated with PEG₁₅₀₀ could degrade PEG₂₀₀ to PEG₁₅₀₀, but not EG, DEG, or TEG. Kawai and others (1978) isolated a mixed culture consisting of *Flavobacterium* and a *Pseudomonas* species that grew synergistically on PEGs. They identified a PEG dehydrogenase in the particulate fraction of extracts that produced an aldehyde. This enzyme had wide substrate specificity and could act on glycols from DEG to PEG_{20,000}. They propose a pathway of PEG degradation proceeding by reduction of the terminal alcohol to the aldehyde, then to the acid, followed by cleavage of a two-carbon moiety, analogous to β -oxidation of fats.

Another *Flavobacterium*, isolated from pond water by enrichment with EG and designated National Collection of Industrial Bacteria (NCIB) 11171, was found to grow on EG and produce glycolate, glyoxylate, tartronic semialdehyde, and glycerate (Child and Willets, 1978). The glycerate feeds into the glycolytic pathway. The proposed pathway was elucidated by testing proposed intermediates as growth substrates, by comparing the rate of oxidation of the proposed intermediates by cells grown with EG and other substrates, and by determining activities of a variety of enzymes. The proposed pathway is shown in Figure A-1.

Cox (1978) reviewed the biodegradation of PEG. The early literature on PEG indicated that this material was biorefractory. However, recent studies using long-term adapted biomass and pure cultures have shown that the higher-molecular-weight PEGs can be biodegraded. He raised the concern that abiotic reactions of the glycols have not been thoroughly accounted for in most studies. Additionally, most glycols contain by-products and precursor materials that can give erroneous results. Bieszkiewicz and others (1979) evaluated the effect of EG on activated sludge treating a synthetic wastewater. The wastewater contained acetone, acetate, and glycerol. The EG was added in increasing concentration over a period of about 6 weeks. Treatment efficacy was monitored via COD and oxygen uptake. He concluded that wastewater containing EG could be treated by activated sludge, providing the concentration did not exceed 1000 mg/L. Decreasing COD removals were noted as EG concentrations increased above ca. 500 mg/L. In addition, at the higher concentrations sludge-settling was impaired, as measured by the sludge volume index (SVI), apparently as a result of decreased floc formation.

Figure A-1. Pathway suggested for metabolism of EG by *Flavobacterium* NCIB 11171 (after Child and Willets, 1978).

The fate of PEG in three bacterial isolates was followed by Jenkins and others (1979). The three isolates were obtained by enrichment culture on EG, DEG, and PEG₄₀₀ and were designated Z, R, and O, respectively. Isolate Z grew only on EG, while isolates R and O grew on low- and high-molecular-weight PEGs. Data with isolate R using PEG₂₀₀ suggested that the lower-molecular-weight components were selectively degraded, and the remaining components were converted into carboxylated derivatives that accumulated in the media.

Toraya and others (1979) examined several genera of the bacterial family *Enterobacteriaceae* to determine the mode of metabolism of EG used under anaerobic conditions. They found that in *Klebsiella pneumoniae* (ATCC 8724, formerly *Aerobacter aerogenes*), EG was readily metabolized under anaerobic conditions. The enzyme appeared to be a diol dehydratase using a pathway essentially as suggested by Abeles and Lee (1961) and described by Child and Willets (1978; see Figure A-1). However, under aerobic conditions, very little activity was noted. They suggest that the diol dehydratase is not active under aerobic conditions, principally because of low levels of coenzyme B₁₂ (cobamide). Other *Enterobacteriaceae* tested were able to ferment 1,2-diols as well.

Thélu and others (1980) isolated a *Pseudomonas* using PEG₄₀₀ as the sole carbon source. This bacterium was found to grow on DEG and TEG, as well as on glycollate and glyoxylate, but could not grow on EG or PEG₆₀₀ or larger. The investigators detected a dehydrogenase activity on PEG₄₀₀ that did not act at the terminal alcohol group. They suggested that the enzyme formed a double bond between the two ethylene carbons, followed by hydrolysis to form a hydroxyl. Additionally, they suggested that the use of PEG with molecular weights greater than 400 was not detected because of limitations of pore sizes on the bacterial membranes. Larger PEGs presumably require extracellular enzymes to produce fragments small enough to enter the cell.

Pearce and Heydeman (1980) isolated twenty strains of bacteria from soil, sewage, and river water. They noted that the glycol utilization pattern of these isolates closely matched their source. Only isolates from sewage could grow on PEG₆₀₀ or above. River isolates could grow on EG and were the only ones that could grow with glycine and glycolate. No isolates used acetaldehyde, glycolaldehyde, or glyoxal for growth. EG was produced in equimolar amounts to the disappearance of DEG. Small amounts of ethanol and acetate and traces of acetaldehyde were noted as well. Under anaerobic conditions or in the presence of cyanide, DEG disappeared as rapidly as aerobically, but the product was ethanol. Crude enzyme isolated from active cells was able to remove ethylene oxide units from DEG and higher glycols and did not exhibit oxygen dependence as others have noted (Jones and Watson, 1976). Moreover, this enzyme cannot utilize EG, as did the enzyme described by Haines and Alexander (1975). A pathway was suggested to fit these data (Figure A-2). The enzyme catalyzing this reaction was named di(ethylene)glycolase.

Figure A-2. Metabolism of DEG by a gram-negative bacterium (after Pearce and Heydeman, 1980).

A *Mycobacterium*, designated strain E44, was isolated from ditch water using ethylene as the sole carbon source (Wiegant and de Bont, 1980). Cell-free extracts of organisms grown on EG contained a diol dehydratase that required K^+ , NH_4^+ , and B_{12} for activity. A proposed pathway for the metabolism of EG, based on substrate oxidation studies, growth, and enzyme activities, is shown in Figure A-3.

Means and Anderson (1981) measured the biodegradability of a number of organic compounds using five test methods. The test methods included BOD, CO_2 evolution, activated sludge, shake flask, and the Gledhill test. All five test regimes used adapted seed, and the test compound was the sole carbon source. EG biodegraded readily in all tests after a lag period of up to 3 days.

Tabor reported additional data on the salt-requiring bacterium, T-52 (Caskey and Tabor, 1981). Cells grown on EG had elevated enzyme activities for EG dehydrogenase and glycolate oxidase. Additionally, cells grown on EG, glycolate, or glyoxylate could oxidize glycolate and glyoxylate at rates about six times greater than cells grown with glycerol. They suggest a pathway of EG degradation to

Figure A-3. Metabolism of EG by *Mycobacterium* sp. E44
(after Wiegant and de Bont, 1980).

glycolate to glyoxylate to tartronic semialdehyde to glycerate. Willets (1981) examined the effects of oxygen availability on the metabolism of EG by *Flavobacterium* NCIB 11171. Under strongly aerobic conditions, the primary mechanism of EG metabolism was oxidative via EG oxidase to glycolate, glyoxylate, tartronic semialdehyde, glycerate, etc. (see Figure A-4). Under conditions of low oxygen availability, EG dehydratase was the key metabolic enzyme. The products of the dehydratase pathway were acetaldehyde, acetate, and ethanol (Figure A-5).

Kaplan and others (1982) examined the biodegradability of DEG, TEG, and some other glycols using aerobic and anaerobic batch cultures. The inocula were activated sludge and anaerobic digester sludge from sewage treatment plants. Analysis was by gas chromatography of the glycols. DEG was degraded under both aerobic and anaerobic conditions, but it appeared to be abiotic degradation. DEG sterile controls were observed to have DEG loss at rates similar to the inoculated samples. Moreover, the loss of DEG was the same in distilled water, nutrient broth, or mineral salts supplemented with glucose. Similar results were found for TEG. The fact that the loss of these glycols occurred under aerobic and anaerobic conditions suggests abiotic hydrolysis. The hydrolysis products are likely to be biodegraded as well as or better than the parent glycols.

Dwyer and Tiedje (1983) examined the biodegradation of EG, DEG, and PEGs by methanogenic consortia. Ethanol, acetate, methane, and EG (with PEGs) were the products. They suggest that ethylene oxide units of the PEGs ($\text{HO}-[\text{CH}_2-\text{CH}_2\text{O}]_x\text{H}$) were dismutated to acetate and ethanol. The ethanol was subsequently oxidized to acetate. The rates of degradation were inversely related to the number of ethylene oxide monomers per molecule. The enrichments were shown to best oxidize glycols close to the molecular weight on which they had been enriched. The pathway they suggested is essentially the same as the one described by Wiegant and de Bont (1980 – see Figure A-3) shown in Figure A-6.

Figure A-4. Aerobic metabolism of EG by *Flavobacterium* NCIB 11171 primarily uses ethylene glycol oxidase (after Willets, 1981).

Figure A-5. Microaerophilic metabolism of EG by *Flavobacterium* NCIB 11171 uses ethylene glycol dehydratase as the primary enzyme (after Willets, 1981).

Figure A-6. Metabolism of EG under anaerobic conditions using a methanogenic consortia (after Dwyer and Tiedje, 1983).

Eichler and Schink (1985) tested the anaerobic fermentation of several compounds, including EG, with a variety of anaerobic bacteria. The bacterial strains used were isolated from sewage sludge, freshwater mud, and marine sediments. Bacteria from all three groups, I. homoacetogenic bacteria, II. propionic acid forming bacteria, and III. bacteria-disproportionating acetoin and 1,2-diols to acids and primary alcohols, were capable of growth on EG. On the basis of assay of enzymes from EG-grown cells, the following enzyme activities were thought to be involved: alcohol dehydrogenase, aldehyde dehydrogenase, acetate kinase, and phosphate acetyltransferase. The products of EG degradation were largely acetate and methane. Most-probable-number estimates of the EG-degrading populations in freshwater creek mud and sewage sludge found 4.6×10^6 and 4.6×10^3 cells per mL, respectively. The pathway of EG metabolism is essentially that shown in Figure A-3 (diol dehydratase to acetaldehyde to ethanol to acetylphosphate, etc).

Additional anaerobic testing of glycol degradation was done by Dwyer and Tiedje (1986). They identified a *Bacteroides* sp. and *Desulfovibrio desulfuricans* from an anaerobic PEG-degrading culture. The *D. desulfuricans* culture could metabolize glycols ranging from EG to tetraethylene glycol. The *Bacteroides* culture metabolized glycols from DEG to PEG_{20,000}. Both strains produced acetaldehyde, acetate, ethanol, and hydrogen as products. Koganovskii and others (1987) examined the biodegradation of DEG using microbes isolated from soils at glycol manufacturing plants and from a gas industry pilot plant. Tests were conducted using batch and continuous culture, as well as immobilized and unimmobilized bacteria. The bacteria were identified as various species of *Bacillus* and *Pseudomonas*. Mixed cultures gave much better removals compared to that observed with pure cultures. The best removals were found with cells immobilized on a polypropylene filter with mixing. The results of this study were used to design a treatment system for DEG-contaminated wastewater.

Flathman and others (1989) remediated EG-contaminated groundwater. An estimated 4000 gallons (15,140 liters) of cooling water containing approximately 25% (v/v) EG leaked from a lagoon. A contaminant plume approximately 180×45 feet (54.8×13.7 m) was identified, with mean EG concentrations of 1440 ppm. The mean pH of the groundwater in the plume was 4.5, probably a result of

the accumulation of acetate under anaerobic conditions. However, acetate analysis was not reported. A biofeasibility analysis indicated that the groundwater contained a viable microbial population of from 10^2 to 10^6 colony-forming units per mL. Electrolytic respirometry was used to evaluate the oxygen demand, toxicity, degradation rate, and abiotic reactions. The results showed that EG was degraded completely without accumulation of incomplete oxidation products. No lag period in EG oxidation was noted, indicating that the indigenous microbes were adapted to EG. No toxicity was noted. The first-order degradation rate, based on BOD, was 0.3 days^{-1} .

Field bioremediation of the EG spill was conducted using a combination of an on-site activated sludge system and enhanced in situ treatment. Water from recovery wells was pumped to an aeration tank where nutrients were added and the pH was adjusted to neutral. Treated water was clarified and pumped into the subsurface in a fashion to flush through the contaminated zone. This injected water provided nutrients, adjusted the pH, added large numbers of bacteria, and provided hydraulic control of the plume. EG concentrations were reduced by 85% and 92% in two downgradient wells in the first 26 days of treatment. Treatment continued until EG levels were below detection. Batch bioreactor tests for degradation of EG and DEG using acclimated biomass were reported by ARCO Chemical Company (1990). Using initial concentrations of 2000 and 2100 mg/L, after 24 hours the concentrations had been reduced by 92% and 24%, respectively (as COD). The rate of DEG degradation was only about 30% of that observed for EG.

McGahey and Bouwer (1992) examined the biodegradation of EG in simulated subsurface environments. They conducted batch studies using water amended with EG and supplemented with nutrients and with inocula from primary settled wastewater effluent, soil, and groundwater. EG concentrations of 100, 1000, and 10,000 ppm were used. Data collected included biomass and EG concentrations and CO_2 evolution. EG concentrations were measured using a radioisotope dilution technique. A small amount of radiolabeled EG ($\text{U-}^{14}\text{C}$) was added, with the remainder unlabeled EG. First-order kinetic constants were estimated using plots of $\log_e[\text{EG}]$ versus time. These studies all found a lag period of adaptation ranging from 0 to 3 days, followed by exponential decreases in EG. The typical first-order kinetic rate constant was $1.13 \pm 0.34 \text{ days}^{-1}$. The data showed that increasing EG concentrations resulted in decreased transformation rates. Leaving out the nutrients resulted in a slightly longer lag period and reduced removal rate.

The soil used for inocula had a clear effect on biodegradation. The first-order kinetic rate constants were 1.01 for the Mississippi sandy loam (0.94% organic carbon) and 2.90 for the Texas sandy silt loam (3.25% organic carbon). The half-lives ($\log_2/k$) under each of the test conditions were estimated. Half-lives range from 0.35 to 0.92 (13.3 days for the 10,000-ppm dose; McGahey and Bouwer, 1992). Batch experiments using the Mississippi soil as inocula and EG concentrations of 100, 1000, and 10,000 ppm showed that the first-order rate constants decreased with increasing EG concentration. The removal of EG at 10,000 ppm was minimal. Additional batch tests using the Texas soil as inocula evaluated temperature and nutrient effects. The degradation rate at 25°C was 2.90, but decreased to 1.19 days^{-1} at 10°C . EG removal at both temperatures was nearly complete. The addition of nutrients resulted in a small decrease in the removal rate from 2.90 to 2.00 days^{-1} (McGahey and Bouwer, 1992). Decreasing the temperature from 25° to 10°C for a given soil retarded the biodegradation rate by a factor of 2.44. Even at the reduced temperature, EG removal was greater than 99% in less than 7 days of incubation. The effect of added nutrients was minimal. Groundwater amended with EG showed transformation rates similar to those observed with soils. Overall lag periods ranged from 0 to about 3 days, they followed first-order kinetics, and the half-lives of EG were from 0.24 to 0.92 days (at 10,000 ppm, the half-life was 13.3 days).

Klečka and others (1993) examined biodegradation of glycols in soils at low temperatures. They used soils from near aircraft runways and aircraft deicing mixtures containing EG, PEG, or polypropylene glycol. Glycols were added at doses from 0.05% to 0.5% (v/w) to soils in serum bottles. Loss of glycols was determined via gas chromatography and by measuring carbon dioxide production. They found the glycols to be mineralized from all deicing mixtures tested, at all temperatures, ranging from -2° to 25°C , although rates were very low at temperatures below freezing. No lag period was observed; however, the soil used probably had previous exposure to glycols. Biodegradation rates for all three glycol compounds were similar, with average rates at 8°C from 19.7 to 27.0 mg/kg soil per day and at 25°C from 66.3 to 93.3 mg/kg soil per day. Rates observed at -2°C were about one-sixth to one-eighth of the rate at 8°C . Nitschke and others (1996) investigated the biological treatment of wastewater containing glycols from deicing. They found that adapted laboratory activated sludge units were able to adequately treat wastewater containing up to 88% glycol at 18°C . Shock loadings of DEG into an unadapted activated sludge unit resulted in immediate decrease in COD removal. Inhibition of the activated sludges was related to temperature and concentration of glycol.

A3.0 Summary of the Literature on Glycol Biodegradation

Acclimation of the biomass is important. The best results are found when acclimation is with the glycol that is to be degraded. The pathway of aerobic metabolism is via EG and produces glycolate, glyoxylate, tartronic semialdehyde, and glycerate. The glycerate enters central metabolism and is completely oxidized aerobically. Anaerobic metabolism has been demonstrated as well. The principal products of anaerobic metabolism are acetic acid and ethanol. Under methanogenic conditions, methane is produced from the ethanol and hydrogen. The intermediates in the metabolism of glycols identified in this review are not likely to accumulate in the environment. These compounds are all metabolized by an even broader range of microbes than can utilize the glycols.

If glycols are present with other organic compounds, preferential usage of the other organic compounds may be observed. This is similar to the catabolite repression seen with mixtures of glucose and another organic compound. If the other organic compound is inducible, the glucose must be completely oxidized before the enzymes for degrading the inducible compound are even synthesized (Neidhardt and others, 1990). If this phenomena occurs with glycols, even though they appear to degrade very well and rapidly by a large variety of microbes, they may be transported far from the source before they can be biodegraded. However, the data collected by McGahey and Bouwer (1992) on soils suggest that organic matter in soil does not suppress EG degradation.

Both aerobic and anaerobic metabolism may use an enzyme that requires vitamin B_{12} (cobamide). Vitamin B_{12} is an enzyme cofactor that contains cobalt. Certain environments may be low in cobalt, which limits the initial degradation of glycols. Abiotic reactions in the degradation of glycols may be significant in some situations, especially with DEG and TEG. Since many investigators have not analyzed for parent compound or used adequate controls, the extent of abiotic loss of these glycols, or the conditions that may affect rates, are not known. The hydrolysis of these glycols probably improves their biodegradability and reduces the potential for their transport.

A wide variety of microbial types have been shown to biodegrade glycols, including acetic acid bacteria, anaerobes, salt-requiring bacteria, and others, including members of the following genera: *Acetobacter*, *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Bacteroides*, *Clostridium*, *Desulfovibrio*, *Flavobacterium*, *Glucobacter*, *Klebsiella*, *Micrococcus*, *Mycobacterium*, *Pseudomonas*, *Sarcina*, and *Xanthomonas*. Clearly the metabolic capacity to degrade glycols is widely distributed. However, the degradation capacity is inducible, and the segment of the microbial population that can degrade glycols in an environment without previous exposure is probably very small. Exposure results in rapid growth of the appropriate population of microbes.

Reduced temperatures lead to variability in biodegradation results and overall much reduced rates. Biodegradation rates in seawater are less than in freshwater. EG was shown to be inhibitory in activated sludge treatment at concentrations greater than 500 mg/L. The principal effects were reduced COD removals and poor sludge settling. It is clear that using adapted biomass at concentrations of glycols of less than 1000 to 2000 mg/L, biodegradation proceeds rapidly and completely in a matter of hours to days.

A4.0 References

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APPENDIX B

Biodegradation Test Methods Employed

B1.0 Introduction

Biodegradation tests were performed with pure compounds and with spent (waste) glycol mixtures collected at dehydration sites using soils from Alberta, Louisiana, and New Mexico. Compounds of the highest obtainable purity were obtained from commercial vendors. Spent glycols were obtained from operating dehydrators, as previously described, and were stored at 3°–4°C until used. Soil–slurry treatment tests were performed using shake flasks and slurry bioreactors. Shake flask slurry treatments were performed using 30% (w/w) soil in water with or without nutrient supplementation. One-liter borosilicate Erlenmeyer flasks filled to no more than 20% of the volume and capped with aluminum foil were used for shake incubations. The slurries were incubated at room temperature on a gyratory shaker at 75 rpm.

Twelve-liter, baffled, borosilicate, round-bottom flasks were used for the slurry reactors. The top of the reactor was fitted with a three-hole standard taper top that contained an air inlet, a stirring adaptor, and an Allihn condenser. Humidified air was metered at approximately 80 mL/min into the bioreactor through a 5-mm-o.d. (outside diameter) borosilicate tube drawn to a fine capillary and placed about 5 cm below the surface of the slurry. Stirring was performed with an IKA Labortechnik Model RW20 stirrer via a stainless steel shaft and agitator through the Teflon-lined adaptor at approximately 150 rpm. The exhaust air from the bioreactor passed through the condenser, which was cooled to 10°C to reduce loss of water and volatile contaminants.

B2.0 Biometer/Wet Chemical Respirometry

Respirometric methods employed followed those of Bartha and Pramer (1965). Respirometric vessels used were 250-mL flasks with a sidearm containing KOH for absorption of carbon dioxide. The flask contained 100 g of test soil, and distilled water was added to achieve the desired level of moisture. Typically, water was added to provide 60% of the moisture-holding capacity (MHC). At 60% MHC, the soil has adequate water for microbial activities and sufficient space in the soil to promote good air exchange. After the soils were added to the flask and wetted, they were incubated at the desired temperature for approximately 10 days without the addition of chemicals or additives. This period of time was meant to allow the soil, typically stored in an air-dried condition, to become microbially active again. During this period of reactivation, there is often a burst of microbial activity that could obscure the testing program. The burst of activity generally subsides in about 7 to 10 days.

After the 10-day preincubation, the test chemical was added, the soil was mixed to incorporate the chemical, KOH was added to the sidearm, and the biometer was sealed. The biometers were incubated at 25°C without mixing and with precautions to prevent exposure to light. An adequate supply of oxygen in the biometers was assured by weekly flushing of the flasks with carbon dioxide-free air. At approximately weekly intervals, the KOH was removed from the sidearm and mixed with excess BaCl₂ to “fix” the absorbed carbonate, and the remaining KOH was titrated with standardized HCl. Equation B-1 shows the absorption of carbon dioxide as bicarbonate to form potassium carbonate. When barium chloride is added, the potassium carbonate is converted into barium carbonate and potassium chloride (Equation B-2). The quantity of absorbed carbon dioxide was calculated from the amount of spent KOH.

Anaerobic biodegradation experiments were similar to the aerobic experiments, except for provisions taken to exclude oxygen. Anaerobic respirometry was conducted in widemouth jars sealed with Teflon-lined screwcaps. The KOH was introduced and replaced at intervals by unscrewing the jar and placing a vial containing the KOH into a center well in the jar. The incubation and manipulation of anaerobic respirometers took place in a controlled-atmosphere glove box. The glove box was maintained at approximately room temperature (23°C) with an atmosphere of approximately 90%–95% nitrogen and the remainder hydrogen. A fan circulated the air through a bed of catalyst and alumina that removed oxygen and water to very low concentrations.

In general, four types of biodegradation curves are found, as shown in Figure B-1. Curve I represents a compound that is degraded rapidly without a lag period. Curve II represents a compound that is degraded rapidly with a lag period of about 7 days. Curve III represents a compound that is biodegraded slowly, without a lag period. However, the compound in Curve III shows only an arithmetic degradation, not hyperbolic. This suggests that Compound III cannot support growth or it is toxic. Curve IV represents a compound that is not biodegraded. All four types of curves might be found with the same compound, depending on factors such as concentration, soil type, and availability of water, nutrients, and electron acceptors.

Biodegradation parameters from curves such as those shown in Figure B-1 are evaluated as follows: 1) lag times are estimated graphically as the x intercept of a linear regression of the biodegradation rate during the period of rapid carbon dioxide evolution on the basis of a plot of net cumulative carbon dioxide evolution, 2) the biodegradation rate is estimated by linear regression of the data during the rapid carbon dioxide evolution phase, and 3) the extent of biodegradation is estimated from the plateau of carbon dioxide evolution. Figure B-2 shows a typical example of a compound that is biodegraded after a lag period of about 6.5 days with hyperbolic kinetics. The completeness of biodegradation of DEG in a soil can be calculated as follows. The amount of CO₂ produced in total for a 1000-mg DEG/kg soil dose is the quantity of CO₂ found experimentally minus the CO₂ produced in the blank = mg CO₂/kg soil. The molecular weight of CO₂ is 44. The molecular weight of carbon is 12.011.

Figure B-1. Biodegradation parameters from curves.

Figure B-2. Example of a compound biodegraded after a lag period of about 6.5 days with hyperbolic kinetics.

The molecular weight of DEG is 106.12. The carbon fraction in DEG is 0.45 by weight. The amount of carbon in a 1000-mg/kg dose of DEG is the dose times the carbon fraction of the test compound times the ratio of the molecular weight of carbon dioxide to carbon ($1000 \text{ mg} \times 0.45 \times [44/12.011] = 1650 \text{ mg CO}_2/\text{kg}$). Complete mineralization of DEG at 1000 mg/kg would give 1650 mg of carbon dioxide. If the amount of CO_2 found was 1000 mg/kg, then the biodegradation was 60.3% complete ($1000 \text{ mg/kg} \div 1650 \text{ mg/kg} \times 100$). Biodegradation completeness is rarely 100%, largely because of incorporation of carbon into biomass. Typically 50% to 60% or better of carbon dioxide expected is considered complete (Alexander, 1994). Extents of biodegradation less than about 50% suggest partial or otherwise incomplete biodegradation.

B3.0 Electronic Respirometry

Continuous constant-volume electronic respirometry was performed with some test chemicals using a system of analyzers and multiplexers and a data acquisition system from SableSystems (Henderson, NV). Two-hundred-fifty-gram dry weight samples of soil were placed in 500-mL glass bottles fitted with an airtight two-hole cap. The soils were wetted to 60% of the MHC, preincubated for 10 days, then dosed with the test chemical. Incubations were static, in the dark at $25 \pm 0.1^\circ\text{C}$. Outside air was mixed in a 25-liter carboy, then filtered through a Drierite® column and a Drierite®–Ascarite® column, to remove water and water plus carbon dioxide, respectively. Enough air to completely flush each bottle was metered through each bottle via a mass flow controller in turn using a multiplexed set of solenoid valves at timed intervals. Time intervals were selected to provide sufficient data and to prevent fluctuations in oxygen concentrations. Generally, four or more flushes were performed per day. Air exiting the bottles was dried using a Peltier-effect condenser, then analyzed for carbon dioxide using an infrared analyzer (Sable CA-1), dried further using a molecular sieve (Alltech, 13X and 4A) column, and analyzed for oxygen using a fuel cell analyzer (Sable FC-1). The respirometry system, as configured, can sequentially monitor 24 bottles. The analyzers were calibrated twice per week using outside air as

20.95% for oxygen and using a certified ($\pm 5\%$) calibration gas of 7.49% carbon dioxide, with the balance nitrogen (Scott Specialty Gases). The data on carbon dioxide and oxygen concentrations of the flushing of each bottle were logged and reduced to concentrations over time using Datacan V and ConVol 1.1, respectively (Sable Systems).

B4.0 References

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