California Biobutanol Multimedia Evaluation

Tier II
Work Plan

Prepared

By

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for the

California Environmental Protection Agency
Multimedia Working Group

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1. Executive Summary

1.1. Scope

Butamax™ Advanced Biofuels, LLC[1] is seeking to commercialize biobutanol[2] for use in blends with gasoline to be offered for sale within the State of California. Under California law, a necessary prerequisite for this commercialization is completion of a Multimedia Assessment. A Tier I Multimedia Report summarizing existing knowledge on biobutanol and identifying key knowledge gaps has previously been approved by the California Multimedia Workgroup and published[3]. This document, the Tier II work plan, has been prepared as the next step in the multimedia evaluation process.

Butanol-Gasoline blends of up to 3.7wt% Oxygen (approximately 16vol%) and meeting certain additional requirements are approved by the US EPA as substantially similar to baseline gasoline under terms of the Octamix Waiver[4] issued under §211(f) of the Clean Air Act Amendments. US EPA Regulations also require completion of health effects testing (§211(b)) prior to commercialization; the Butamax team is currently working to complete this requirement.

The scope of this Multimedia Assessment is limited to gasoline/biobutanol blends containing 3.7wt% Oxygen (approximately 16vol%) in the form of iso-butanol and meeting other requirements of the Octamix Waiver and applicable California reformulated gasoline requirements. While many other fuel formulations fall within the scope of the Octamix Waiver, they are not within the scope of this Multimedia Assessment.

1.2. Background

In 2006, BP and DuPont first announced their joint efforts to develop biobutanol as a new biofuel component for use as a gasoline blendstock. The motivation for this multi-year effort is to develop a fuel that can be economically produced from renewable feedstocks and which provides superior performance and consumer value with the existing and future vehicle fleet.

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[1] Butamax™ Advanced Biofuels, LLC is a 50:50 joint venture of BP and DuPont which was formed in July 2009 for the purpose of commercializing biobutanol technology that has been jointly developed by BP and DuPont.

[2] For the purposes of this document, the term "biobutanol" is used to refer to all isomers of butanol produced from biomass. BP and DuPont are working specifically to commercialize the production of iso-butanol, one of the possible isomers. Inclusion of data on other isomers of butanol is for reference only.

[3] http://www.arb.ca.gov/fuels/multimedia/020910biobutanoltierI.pdf

[4] 53 FR 3636 (2/8/88).

Compared to ethanol, biobutanol offers several potential advantages –

- Biobutanol can be produced from the same feedstocks as ethanol through modest retro-fits of existing corn and sugarcane ethanol assets. This will allow production to be ramped up quickly by existing ethanol producers without impact to feedstock producers. As technology develops for production of ethanol from lignocellulosic feedstocks, biobutanol technology will be extended to include those feedstocks as well.

- Biobutanol's chemical properties allow it to be blended at 16vol% in gasoline while maintaining compatibility with the existing E10-capable vehicle fleet and offering at leastequivalent performance on criteria pollutant emissions.

- Biobutanol has a higher energy density than ethanol, allowing the iso-butanol in a 16vol% blend to displace about 13.6%[5] of the hydrocarbon gasoline, while the ethanol in a 10vol% blend displaces only about 6.8%[6] of the hydrocarbon gasoline.

- The water-solubility and corrosivity of biobutanol is sufficiently low that biobutanol/gasoline blends can be transported in existing pipelines without risk of phase separation.

- Biobutanol has a blending RVP[7] of 5.2psia, considerably lower than that of ethanol (blending RVP of 19 psia). As a result, biobutanol offers enhanced value to refiners who are typically RVP-constrained during summer blending season.

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[5] $\frac{16vol\% * (95500/115600)}{[84vol\% + 16vol\% * (95500/115600)]} = 13.6\%$, where iso-butanol energy content is 95,500 BTU/gal and gasoline energy content is 115,600 BTU/gal.

[6] $\frac{10vol\% * (75700/115600)}{[90vol\% + 10vol\% * (75700/115600)]} = 6.8\%$, where ethanol energy content is 75,700 BTU/gal and gasoline energy content is 115,600 BTU/gal.

[7] The blending Reid Vapor Pressure (RVP) of iso-butanol is 5.2 psia compared to CARB Phase 3 gasoline with a summertime RVP of 7.00 psia. (RVP is defined as the vapor pressure of an air-saturated sample at 100ºF and a 4:1 vapor:liquid ratio.)

The blending value (vapor pressure or octane) of a component (e.g. ethanol) determines the effect a blending component will have on a gasoline blend when it is blended into the base gasoline. A blending value of a component is not necessarily the same as that property of the pure component. Blending values are often functions of the blend composition.

Example 1:
For example, the Research and Motor Octane numbers for pure ethanol are 109 and 90, respectively, with a (R+M)/2 = 99.5. However, when blended at a 10% volume into a base gasoline, ethanol blending octane numbers are 129 and 103, respectively, with a (R+M)/2 = 116. To calculate the (R+M)/2 value of 10%

The benefits of biobutanol as an Alternative Fuel are recognized through its explicit mention in the renewable fuels components of the Federal Energy Independence and Security Act of 2007 (EISA 2007). The categorization of a specific source of biobutanol under EISA will be determined by the choice of feedstock (e.g., corn, sugarcane or lignocellulosic matter) and the lifecycle greenhouse gas benefit calculation. In their recent publication of the RFS2 Final Regulations, the US EPA has published their life cycle assessment of the corn starch to biobutanol pathway[8].

The various butanol isomers have been used in the chemicals industry for a number of years and the potential health effects have been well-studied. While commercial butanol production has largely been through petrochemical pathways, health impacts are a property of the molecule that will be substantially unchanged for butanol produced through biological mechanisms. Additional studies to be undertaken in support of this multi-media assessment will focus on release pathways characteristic of the fuels lifecycle.

2. Tier I Conclusions

The Tier I Report for biobutanol came to the following conclusions –

2.1. Conclusions of the Tier I Report

The hazardous properties of the different butanol isomers have been widely studied and reported in the technical literature. These properties are intrinsic to the molecule and independent of the production pathway.

The Butamax™ Advanced Biofuels production process for iso-butanol will be substantially similar in most respects to existing technology for bio-ethanol production,

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ethanol blended into a base gasoline with a (R+M)/2 of 88.5, the blending value of ethanol can now be used in the following simple equation:

(10%)*(116) + (90%)+(88.5) = 91.25

(Vol. % ethanol in blend)*(Blending Value of Ethanol) + (Vol. % gasoline)*(Value of Gasoline)= Final Property of Blended Gasoline

Example 2:
For example, the vapor pressure of pure ethanol at 100 F is 2.3 psia. Blending 10% ethanol into a base gasoline with a vapor pressure of 8.0 psi does not cause the vapor pressure of the gasoline to decrease to 7.43 (10%*2.3 + 90%*8.0 = 7.43). Instead the final vapor pressure of the blend is actually close to 9.1psia, meaning that the blending vapor pressure of ethanol at 100 F is actually 19 psia! (10%*19.0 + 90%*8.0 = 9.1).

[8] 58 FR 14669 (2010)

resulting in comparable carbon intensities for iso-butanol as for ethanol produced from the same feedstocks.

Limited data currently available indicate that 16vol% iso-butanol/gasoline blends will have vehicle emission characteristics similar to those of 10vol% ethanol/gasoline blends while displacing twice as much petroleum gasoline and providing consumers with comparable fuel economy.

Additional data needs focus on lifecycle aspects that are unique to the use of iso-butanol as a gasoline component —

- **Test representative elastomers for swell and hardness impacts due to exposure to mixtures of ethanol and iso-butanol blended CARB gasolines.**
- **Test for compatibility of California gasoline blended with iso-butanol with fiberglass tank resins and sealants.**
- **Determine the electrical conductivity of E10 and 16vol% iso-butanol/gasoline blends.**
- **Review of applicable terminal vapor recovery requirements.**
- **Perform exhaust and evaporative emissions testing for 16vol% iso-butanol blends in California reformulated gasoline versus 10vol% ethanol blends in California reformulated gasoline to determine whether any adjustments to the Predictive Model are required to model 16vol% iso-butanol blends. Determine impact on Ozone Reactivity and Potency-weighted Toxics emissions.**
- **Determine toxic air pollutants in automotive exhaust using EPA Section 211(b) methodology with California reformulated gasolines blended with 10vol% ethanol and with 16vol% iso-butanol.**
- **Determine the composition of the headspace of 10vol% ethanol and 16vol% iso-butanol blended California reformulated gasoline blends over a range of temperatures and calculate differences in potency-weighted toxics and reactivity. Determine permeation emissions of 16vol% iso-butanol relative to 10vol% ethanol in CARB gasoline.**
- **Complete environmental fate studies currently in progress.**
- **Complete the LCA for retrofits of typical existing grain and sugarcane based ethanol plants to iso-butanol production.**

2.2. Formulation of the Tier II Work Plan

The remainder of this document consists of a series of chapters, one for each of the knowledge gaps identified in Section 2.1. These chapters will describe the agreed work plan for closing each of the knowledge gaps.

For the purposes of this work program, testing will be done with petrochemically-derived iso-butanol. This is necessary as sufficient quantities of bio-derived iso-butanol will not be available until commercial production commences. As the chemical properties of iso-butanol, other than its life-cycle impacts, are independent of the manufacturing pathway[9], this should allow appropriate test programs to be completed prior to commercialization.

3. Impact of Biobutanol on Elastomers

3.1. Statement of the Knowledge Gap

The report from Tier I of the Biobutanol Multimedia Assessment detailed results from limited elastomer compatibility tests that have already been performed. Those tests evaluated percentage changes in the volume and hardness of elastomers upon exposure to chemical grade *iso*-butanol blended at 20% in unleaded regular gasoline. Elastomer swelling tests were conducted by soaking specimens of elastomer in fuel at ambient temperature for two weeks, with density and durometer hardness tests being performed before and after the fuel-soaking.

That testing has evaluated representative elastomers for swell and hardness impacts of exposure to mixtures of ethanol and iso-butanol blended CARB gasolines as percentage changes. Four elastomers were tested: CPE = chlorinated polyethylene, epichlorohydride, Hypalon™ and Viton™ B. For all four elastomers, a blend of 20% *iso*-butanol in unleaded regular gasoline (ULR) produced more swelling than the base ULR. However, differences between ULR and the *iso*-butanol blend were small, i.e. < 1%. Compared to ethanol blends at 10% and 20%, results varied depending on the elastomer, but differences were small <3 %. Hardness changes were generally inversely related to swelling (i.e. increased swelling gave decreased hardness) as typically observed. The differences observed for gasoline blends containing *iso*-butanol are not expected to have a negative impact on the vehicle systems. However because of the wide variety of materials used in vehicles, additional testing is planned.

- **Test representative elastomers for swell and hardness impacts due to exposure to mixtures of ethanol and iso-butanol blended CARB gasolines.**

[9] Petrochemical-derived iso-butanol and bio-iso-butanol will include different impurities due to differences in the manufacturing processes and pathway to market. Petrochemical iso-butanol is most commonly produced via the "oxo-process", this process primarily produces a mixture of n-butanol and iso-butanol which are then separated via distillation. As a result, the iso-butanol is purified to >99% purity with the primary impurity being n-butanol. Bio-iso-butanol is produced via fermentation with a highly selective yeast, the principal expected impurities are water (as fermentation is an aqueous process) and ethanol picked up either by contamination of the fermenter with wild-type yeasts or from contamination in storage and handling via logistics shared with fuel-grade ethanol (see draft specification in Appendix A.) Given the low level of anticipated impurities and the anticipated fuel properties of n-butanol and ethanol, respectively, Butamax believes that any impacts on the test results due to the presence of impurities will be well within the precision of the test methodologies employed.

3.2. Test Plan

The planned test work is designed to assess the relative risk of materials incompatibility with gasoline blends containing 16% v/v *iso*-butanol, compared to the risk of materials incompatibility with current marketplace ethanol-gasoline blends. The experiments will also include a fuel that is a mixture of ethanol and *iso*-butanol blended CARB gasolines. Properties of elastomers will be measured before and after exposure to the test fuels. Where possible, experiments are based on standard test methods (with preference given to ASTM methods).

Hardness testing of elastomers will be conducted according to ASTM D 2240 (Standard test method for rubber property – durometer hardness), before and after exposure to the test fuels. As stated in the ASTM document, this test method is based on the penetration of a specific type of indentor when forced into the material under specified conditions. The indentation hardness is inversely related to the penetration, and is dependent on the elastic modulus and viscoelastic behaviour of the material.

ASTM D 471 (Standard test method for rubber property – effect of liquids) will be employed as a basis for measuring changes in other rubber properties after immersion in test liquids. Properties such as mass, volume and breaking resistance, among others, may be determined within this test procedure. The ASTM document states that:

> *"This test method attempts to simulate service conditions through controlled accelerated testing, but may not give any direct correlation with actual part performance, since service conditions vary too widely. It yields comparative data on which to base judgment as to expected service quality."*

For comparative purposes in the testing of CARB fuels, test procedures will deviate slightly from the standard test method. Specifically, the test fluids might not include all of the ASTM reference oils, or the temperature and duration of exposure to liquid may be slightly varied. Nevertheless, Butamax will ensure that the tests are conducted with appropriate reference tests, so that a comparison can be made between elastomer compatibility with existing CARB fuels and that with CARB gasolines that contain *iso*-butanol.

Tensile properties of elastomers will be measured according to ASTM D 412 (Standard test method for vulcanized rubber and thermoplastic elastomers – tension), which covers procedures for determination of tensile stress, tensile strength, yield point and ultimate elongation.

Elastomer permeability to fuel will be measured as described in SAE J2665, which is a Surface Vehicle Recommended Practice, entitled "Test procedure to measure the fuel permeability of materials by the cup weight loss method."

Elastomers are selected to represent materials throughout the whole fuels supply chain, spanning fuel terminals, retail outlets, vehicles and small-engine appliances.

Furthermore, the selected elastomers cover a broad representation of materials in terms of ability to resist oil-induced swelling and ability to resist heat, as defined by ASTM D 2000 (Standard classification system for rubber products in automotive applications). Our experiments will be conducted on elastomer materials, which fall into two subsets of 5 materials each:

The first 5 materials cover elastomers that would typically be used in fuel system applications. These materials, listed below, will be exposed to test fuels for 1 week at 40 ºC:

- FKM: fluoro rubber of the polymethylene type that utilises vinylidene fluoride as a comonomer and has substituent fluoro, alkyl, perfluoroalkyl or perfluoroalkoxy groups on the polymer chain, with or without a cure site monomer (having a reactive pendant group), e.g. Viton®.
- ECO: Ethylene oxide (oxirane) and chloromethyl oxirane (epichlorohydrin copolymer).
- FVQM: silicone rubber having fluorine, vinyl, and methyl groups on the polymer chain.
- HNBR: hydrogenated acrylonitrile butadiene.
- NBR: acrylonitrile-butadiene.

The second 5 materials cover elastomers that might come into incidental contact with fuels. These materials, listed below, are typically expected to exhibit good resistance to alcohols but poor resistance to hydrocarbons, and they will be exposed to test fuels for 1 week at 23 ºC:

- VMQ: silicone rubber having both methyl and vinyl substituent groups on the polymer chain.
- CPE: chlorinated polyethylene.
- CR: chloroprene, e.g. Neoprene.
- SBR: styrene-butadiene.
- EPDM: terpolymer of ethylene, propylene, and a diene with the residual unsaturated portion of the diene in the side chain.

The elastomer materials must be cured and made into slabs before testing. For this test work, we will test standard compounds that are prepared to have 75 ± 5 durometer hardness rating – which is typical of rubber seals such as O-rings.

The following fuels will be used for the tests:

Fuel ID	Fuel Content	Fuel Description
CARB Fuel 1	Carson E10	Current quality RFG3
CARB Fuel 2	Cherry Point Bu16	High-Aromatic, Low-Olefin base
CARB Fuel 3	Carson Bu16	Low-Aromatic, High-Olefin base
CARB Fuel 4	50:50 mix of CARB Fuels 1 and 3	Commingled Carson E10 and Bu16

Table 3.1 Test Fuels for Elastomers Testing

These fuels are described further in Section **7.2.** This same set of fuels is to be used in the test programs described in Sections **4, 5, 7, 8** and **9**.

4. Impact of Biobutanol on Fiberglass Resins and Sealants

4.1. Statement of the Knowledge Gap

As stated in the Tier I report, it is known to the industry that ethanol blended gasoline can have a detrimental effect on automotive materials and components.[10,11] However, for several years automotive manufacturers and their suppliers have manufactured vehicles tolerant to E10 blends, including several million flexible fuel vehicles which are compatible with E85 fuel.

The effect of *iso*-butanol on automotive components is less well known. A literature search was conducted to shed light on this topic, and the search returned two articles that are somewhat relevant to this topic. These articles are described in the Tier I report. Butamax has not located any literature data on *iso*-butanol compatibility with fiberglass tank resins and sealants. Therefore testing is planned in this area.

- **Test for compatibility of California gasoline blended with iso-butanol with fiberglass tank resins and sealants.**

4.2. Test Plan

The planned test work is designed to assess the relative risk of materials incompatibility with gasoline blends containing 16% v/v *iso*-butanol, compared to the risk of materials incompatibility with current marketplace ethanol-gasoline blends. The experiments will also include a fuel that is a mixture of ethanol and *iso*-butanol blended CARB gasolines. Fiberglass resins and sealants will be exposed to test fuels for 30 days at a temperature of 60 ºC, with material properties being measured before and after exposure. The experiments are intended to determine the effects of test fuels in terms of swelling, hardness, leaching and delamination.

Testing will be performed with the same suite of fuels described in Section **7.2.** This same set of fuels is to be used in the test programs described in Sections **3, 5, 7, 8** and **9**.

Measurements of material properties will be based on standard test methods (with preference given to ASTM methods), unless suitable methods cannot be identified or

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[10] R. Pierce and P. Moses, Effects of Fuel Exposure on Physical Properties of Selected Plastics, SAE International, International Congress and Exposition (1990), 900632.

[11] Shiotani, Kinoshita, Goto, Saito, Research about Applicability of Biomass Ethanol for Motor Fuel, Society of Automotive Engineers of Japan, Academic Lecture Meeting, May 20, 2005.

easily sourced. If suitable methods are not practicably available, we may design methods in conjunction with experienced test providers (e.g. SwRI, Southwest Research Institute), or use related standard test methods, or we may include subjective assessments, such as microscopic examination by independent materials experts at SwRI. Where possible, these methods will be based on a combination of established practices from relevant industrial parties and/or modifications of existing test methods. In all cases, the tests will be designed to generate a relative comparison of the effects of existing CARB gasolines and gasoline blends that contain *iso*-butanol. We initially propose that the following measurements should be performed on fiberglass and resin sealant materials, before and after fuel exposure:

- Metallographic/microscopic examination for visual signs of leaching or delamination.
- Mass/volume/swell measurements.
- Hardness tests, by durometer hardness (ASTM D 2240), by Barcol impressor (ASTM D 2583), or by another method, whichever is deemed most suitable by SwRI. The same method of hardness testing will be used throughout the project for all materials in this section.
- Measurements of flexural strength, flexural modulus and flexural strain, by ASTM D 790.

At the time of writing, it is proving difficult to find definitive information regarding suitable composite materials for this test work. However, the choice of fiberglass tank resins and sealants will be focused on materials for underground storage tanks (USTs) in the retail section of the fuels supply chain.

Tests are planned for composites fabricated from a selection of the following materials:

- High density polyethylene (HDPE): KS-1866A
- Fluorinated HDPE: KS-1866A (surface of plastic was fluorinated in secondary process)
- Polypropylene (PP): KS-537
- Acetal homopolymer (polyoxymethylene-POM): Delrin II 150
- Acetal copolymer: Acetron GP
- Polyethylene terephthalate polyester (PETP): Ertalyte
- Polyethylene terephthalate glycol copolyester (PETG): Spectar
- Polybutylene terephthalate polyester (PBT): Hydex 4101
- Cork (blended w/ nitrile rubber)
- Nylon 6/6, 6, 11, & 12
- Polyvinylidene fluoride (PVDF): KS-5341
- Polytetrafluoroethylene (PTFE): KS-2342A
- Polyphenylene sulfide (PPS): Techtron CM
- Isophthalic polyester resin: Vipel F764 and Vipel F701
- Terephthalic polyester resin: Vipel F774
- Epoxy novolac vinyl ester resin: Vipel F085

- Epoxy resin: Epon 862/Epi-Cure 3282 (RT cured and heat cured)
- Polythiourea (free film & coated on steel): PTU
- Buna-N

These materials should be available from the following manufacturers: K-mac Plastics, Mc-Master Carr, DuPont, Arkema, Quadrant, Eastman, Ensinger-Hyde, Boedeker, Dow, Huntsman, Air Products, AOC Resins, and Specialty Products.

5. Electrical Conductivity of iso-Butanol/Gasoline Blends

5.1. Statement of the Knowledge Gap

As stated in the Tier I report, the electrical conductivity of pure ethanol and pure iso-butanol can be found in the literature[12]. Pure ethanol has a conductivity of 135 pS/m, and pure *iso*-butanol has a conductivity of 950 pS/m. However, the conductivity of E10 and 16vol% *iso*-butanol gasoline is a knowledge gap that will be addressed as part of the Multimedia Assessment. The knowledge gap was stated as:

- **Determine the electrical conductivity of E10 and 16vol% iso-butanol/gasoline blends.**

The ability of a fuel to generate and dissipate charge during fuel-handling operations depends on the fuel's electrical conductivity; the time for a static charge to dissipate is inversely related to conductivity, so a high conductivity is desirable for safety reasons. Conversely, a fuel with high conductivity could in principle facilitate galvanic corrosion (i.e. corrosion of metals having different electrochemical potentials when they are immersed in an electrolyte). It is anticipated that *iso*-butanol fuels will exhibit electrical conductivity close to the usual range of conductivities measured for existing CARB gasolines, and therefore approval of *iso*-butanol as a fuel component is not expected to introduce additional risk of static build-up, static discharge, or galvanic corrosion.

5.2. Test Plan

Experimental work will follow two relevant ASTM standard test methods, ASTM D2624 and ASTM D4308, entitled "Standard test method for the electrical conductivity of aviation and distillate fuels," and "Standard test method for the electrical conductivity of liquid hydrocarbons by precision meter," respectively. Both methods cover measurement of the 'rest conductivity,' which is the electrical conductivity when the fuel is uncharged. In other words, rest conductivity refers to the electrical conductivity in the absence of

[12] International Critical Tables of Numerical Data, Physical Chemistry and Technology (1st Electronic Edition) Edited by Washburn, E.W. Originally published from 1926-1930, and released by Knovel in 2003

ionic depletion or polarization. (Rest conductivity can therefore be measured at the initial instant of current measurement when a direct-current voltage is applied to the fuel, or by measurement of the average current when an alternating-current voltage is applied to the fuel, or continuously by use of a flow-cell.). Both ASTM methods are valid with good precision over a range of electrical conductivities up to 2000 pS/m. ASTM D4308 also offers extension of the measurement range up to 20,000 pS/m, but with lower precision, so these methods used together are expected to be suitable for measurements on the proposed test fuels.

Testing will be performed with the same suite of fuels described in Section **7.2.** This same set of fuels is to be used in the test programs described in Sections **3, 4, 7, 8** and **9**.

6. Terminal Vapor Recovery Requirements

6.1. Statement of the Knowledge Gap

- **Butamax will perform a review of applicable terminal vapor recovery requirements.**

6.2. Overview

In an effort to understand the impacts of biobutanol fuel and blends on terminal vapor control the following review is provided.

Terminal vapor control, (often generically referred to as vapor recovery) can be generally divided into four areas of system control processes. They are:

1. vapor recovery systems,
2. vapor combustion (aka vapor destruction) systems,
3. vapor balance systems,
4. membrane technology and other novel or emerging approaches (*e.g.*, dry vacuum pump regeneration, hybrid designs, etc.)

Most of these systems are pre-engineered, site specific, skid mounted package units. There may be commonality in system designs within an area, i.e. carbon bed vapor recovery systems may look similar, but carbon bed size, vacuum pumps, etc, are uniquely engineered for each individual site. For efficiencies, each unit is design and sized to meet the requirements of the facility for which it is intended and emission standards effective at time of start up. Legacy units are often modified or augmented with other systems in their service life to comply with new emission standards.

The first general requirement for terminal vapor control systems is the size of the terminal, e.e., what throughput is the system engineered to control? Part of this consideration is derived by studying the terminal operation. It is important to identify sources of vapor emissions which need to be controlled. Terminal truck loading racks are a major common element. The number of loading bays and loading arms are critical.

Peak and daily loading profiles need to be understood to properly size the units. The business disruption caused by rack downtime resulting from failure of the primary vapor control system may drive the requirement for a backup system.

At some terminals, there may be multiple product transfer locations, which require additional vapor control devices. These additional operations may include rail and marine product transfer areas.

The second requirement concerns the emission standard the system is to achieve. Most current systems are designed to meet 1 to 10 milligrams of VOC released per liter of product loaded, or 1 - 10 grams per cubic meter of vapor vented. Consideration needs to be also given to the possible requirement for continuous emission monitoring.

The third requirement that needs to be considered is safety. Vapor control at scale is not without inherent risk. Vapor control utilizing oxidation (combustion) presents unique considerations as described below. Marine and rail activities are special environments which also present unique safety considerations.

A brief summary of some vapor control technologies is provided below.

6.3. Vapor recovery systems

Vapor recovery systems are technologies that capture the product vapor and return it to a usable fuel. Within this area there are three general approaches.

6.3.1. Activated carbon adsorption, which is sometimes coupled with an absorption system. (ADAB)

These systems rely on activated carbon which has a highly porous structure and large surface area. The activated carbon adsorbs hydrocarbons from the air/hydrocarbon mixtures that are generated from terminal loading and transfer operations. The hydrocarbon molecules are adsorbed onto the carbon surface and are retained there until the carbon is regenerated. Adsorption of the hydrocarbon molecules continues until the available surface area of the carbon is saturated. The adsorbed hydrocarbons are then removed from the carbon beds on site for reuse by decreasing the pressure with a vacuum. At completion, a purge gas is introduced, normally air. These systems often have two carbon tanks, or *beds*, which allow for uninterrupted operations. The hydrocarbon vapors and any condensed hydrocarbon liquids from the regeneration process are discharged into a separator vessel. The separator vessel will separate any vacuum pump seal fluid from the recovered hydrocarbon. The seal fluid is cooled and returned to the vacuum system. Vapors are then recovered using an absorber column, a direct contact condenser or a refrigerated condenser. Hydrocarbon liquid is collected in the separator and in the recovery device and is pumped to liquid storage. The uncondensed hydrocarbon can be recycled back to the on-line carbon bed or to a vapor tank.

For low vapor concentrations or on small capacity designs the beds may be thermally regenerated by raising the carbon temperature. On small or portable systems, the carbon may be single-use and require canister or tank replacement instead of on-site regeneration.

Newer carbon adsorption designs include dry vacuum pumps or condensation units which expand the list of vapors which can be processed.

6.3.2. Refrigeration condensation systems

Refrigeration Condensation systems were one of the first vapor recovery technologies to be utilized in the terminals, but are not commonly used in terminals today. The process requires that the collected hydrocarbon vapors be chilled to a temperature where they condense into a liquid. These systems can be complicated and costly to operate. and control of hydrates can be challenging.

6.3.3. Lean oil absorption

Lean oil absorption was another early technology. Gasoline product, or *lean oil,* is forced to make contact with the hydrocarbon vapor, normally in a column where the vapor rises through the column counter flow to the liquid coming down. This is commonly now used as part of a carbon adsorption system.

6.4. Vapor combustion

These are systems that destroy product vapors by oxidation. The emissions are generally carbon dioxide and water. Most terminals today use enclosed burners so there is no visible flame. Newer units have sophisticated combustion process controls. Gas temperatures are measured and auxiliary fuel and/or air flow are adjusted automatically to maintain desired combustion conditions. A flame detection device is used to shut off all vapor streams should the flame be extinguished. Combustion air may flow into the burner by natural draft or via an air blower. With tighter control of the combustion process, higher destruction efficiencies can be obtained.

The hydrocarbon- air mixture flows through several devices designed to controlled and prevent flash back into the vapor header piping. Since it is possible and even likely that the hydrocarbon/air mixture coming from the loading operation is in the explosive range, it is critical that these safety devices be in place to prevent the flame at the burner tip from propagating back through the vapor header. Although the devices and their flow sequence can vary from manufacturer to manufacturer, a hydraulic seal in combination with a flame arrestor or detonation arrestors are common. In addition, staging valves are used to maintain the velocity of gas at the burner tip. As the vapor flow increases or decreases, more burner stages are open or closed to accommodate the flow changes. Without additional energy added to the combustion process, some hydrocarbon vapors can smoke during combustion in this type of process. The assist air blower adds more mixing energy during combustion to enhance smokeless combustion. Enclosed thermal oxidizers often called *enclosed flares*.

6.5. Vapor Balance

Vapor balance systems are closed piping networks that displace vapors between storage and transport containers/tanks during the transfer event. These may sometimes be found between storage tanks and transfer vessels.

6.6. Membrane and hybrid technologies

Emerging technologies, such asgas-vapor separation membranes, are being offered as retrofits to augment existing vapor recovery systems. Other design options like dry vacuum or adsorption - condensation units are being offered for new systems.

6.7. Other requirements.

Since these units are packages of custom and off the shelf components, the code requirements are numerous. However, general compliance with accepted terminal construction and safety codes generally assures compliance. API Standard 2610 *Design Construction, Operation, Maintenance and inspection of Terminal and Tank Facilities* is a good starting point. The DOE summary of ethanol codes also is a convenient reference of applicable requirements.

DOEethanol_codes_standards.ZIP...

Systems design for use at marine terminals will have to comply with the additional Coast Guard requirements found in

> TITLE 33--NAVIGATION AND NAVIGABLE WATERS CHAPTER I--COAST GUARD, DEPARTMENT OF TRANSPORTATION PART 154--FACILITIES TRANSFERRING OIL OR HAZARDOUS MATERIAL IN BULK Subpart E--Vapor Control Systems and
>
> TITLE 46--SHIPPING CHAPTER I--COAST GUARD, DEPARTMENT OF HOMELAND SECURITY PART 39_VAPOR CONTROL SYSTEMS

Systems designed for use at rail terminals will have to comply with the additional Bureau of Explosives requirements found in:

> BOE-6000, Hazardous Materials Regulations of the Department of Transportation
>
> BOE Circular No.17, Rules and Recommendations Relating to the Location of Loading Racks, Unloading Points, and Storage Facilities for any Flammable Liquid With Flash Point Below 20 °F (Including Gasoline, etc.)
>
> BOE Pamphlet 34, Recommended Methods for the Safe Loading and Unloading of Non-Pressure (General Service) and Pressure Tank Cars

6.8. Gasoline Bulk Terminal Emission Requirements and Limitations

The Clean Air Act Amendments of 1990 require the control of VOC emissions. These rules are modified by local California Air districts (see Table **6.1**). Each of these districts outline emission requirements in their areas for terminal operations, and the schedule for planned reductions. Control devices used for these applications needs to be CARB certified. CARB has various certification procedures which need to be followed. These are CARB CP 202, CP 203, CP 204, and CP 205. CP 203, *Certification Procedure for Vapor Recovery Systems of Terminals* is an excellent starting point. More details on these requirements can be found at: http://www.arb.ca.gov.

The systems typically have to be tested annually to ensure compliance. Inspections are common, daily using sight, sound and smell, augmented with weekly hydrocarbon (HC) analyzer tests. All liquid-filled connectors, vapor return connectors, and pressure/vacuum valves shall be vapor leak free.

Amador	Antelope Valley	Bay Area	Butte	Calaveras
Colusa	El Dorado	Feather River	Glenn	Great Basin
Imperial	Kern	Lake	Lassen	Mariposa
Mendocino	Modoc	Mojave Desert	Monterey Bay	North Coast
Northern Sierra	Northern Sonoma	Placer	Sacramento	San Diego
San Joaquin	San Luis Obispo	Santa Barbara	Shasta	Siskiyou
South Coast	Tehama	Tuolumne	Ventura	Yolo-Solano

Table 6.1. California Local Air Districts

6.9. Assessment of the Impact of Biobutanol on Terminal Emissions

There are two principle pathways through which biobutanol-blended gasoline might flow through existing gasoline distribution terminals

- **Terminal Blending** – The CARBOB currently blended with 10vol% ethanol today at the terminal will be replaced with a different CARBOB, reformulated for 16vol% iso-butanol. In this scenario, tanks currently in ethanol-CARBOB service would be re-deployed into iso-butanol-CARBOB service and ethanol tanks would be re-deployed into iso-butanol service.

- **Refinery Blending** – iso-Butanol is blended at the refinery to produce a finished gasoline which is then transported to the terminal via pipeline. Tanks currently storing CARBOB at the terminal will be placed in finished gasoline service. Tanks currently storing ethanol at the terminal will be re-deployed for other services. Truck deliveries of ethanol to the terminal will be eliminated. Bulk iso-

butanol would be shipped to refineries via pipeline. This model is essentially the same as how refineries blended MTBE when that was permissible.

The Terminal Blending pathway is likely to be employed only initially when available volumes of iso-butanol are limited and refiners explore the value of iso-butanol on a small-scale prior to the investment necessary to import and blend large volumes of iso-butanol at the refinery. Refinery blending is expected to be the dominant practice once iso-butanol is commercially proven and significant volumes become routinely available.

Trinity Consultants has developed a proposal for evaluation of the terminal emissions impacts of biobutanol blending (see attached).

Butamax - Tech Memo_Overview of A

6.9.1. Technical Assessment Approach

The potential air emissions impact assessment of iso-butanol compared to ethanol from fuel terminal operations will consist of the following basic tasks:

- Development of emission scenarios for each fuel blended product (i.e., iso-butanol vs. ethanol). This task involves following subtasks, but not limited to:
    - Collection of relevant information including physical/chemical properties of iso-butanol, ethanol, and their blended gasoline products
    - Review of applicable air emission related requirements including storage, transfer, and loading using vapor recovery and other control requirements of local, state, and federal regulatory agencies.
    - Review types of operations conducted at the fuel terminals which produce air emissions
    - Review types of air emission control measures and equipment required by respective agencies for terminal operations
    - Identification of types of emissions including criteria and toxic pollutants associated with each type of terminal operations activity
    - Review of HAP impact assessment and implications on MACT standards
    - Review of AB 2588 program requirements and compare ethanol vs. iso-butanol regulatory requirements and implications
- Quantification of air emissions from terminal operations for each selected scenario using best available information, including, but not limited to:
    - U.S. EPA AP-42 factors
    - U.S. EPA TANK 4.0 software

  - U.S. EPA's Factor Information Retrieval (FIRE)
  - California Air Toxics Emissions Factor (CATEF)
  - California AB 2588 Thresholds
  - California Air Quality Districts' requirements
  - U.S. EPA MACT and NSPS standards
  - Local district's emission factor database
  - Manufacturer's data and source test results
  - Papers, reports, rule board packages, publications, etc.
- Evaluate and compare implications of air emission estimates in various aspects, including but not limited to:
  - Comparison of air emissions quantity associated with terminal operation involving iso-butanol as opposed to ethanol blended products.
  - Comparison to demonstrate whether existing control requirements are sufficient to capture iso-butanol emissions vs. ethanol emissions.
  - Discussion of air regulatory and air quality implications of air emissions derived from terminal operation involving iso-butanol as opposed to ethanol blended products, based on the results of air emission quantifications.
  - Comparison to determine if handling of iso-butanol will add any new regulatory requirements to terminal operators.
  - Comparison to demonstrate impacts of iso-butanol relative to ethanol on carbon capture systems.

6.9.2. Scope of Assessment

Analyses will be performed around the following terminal-based sources and scenarios

Sources

- Receiving products from rail tank cars, tank trucks, and marine vessels: loading and ballasting losses (as applicable)
- Storage of product (concentrated and blended products): breathing, working, and standing storage losses (as applicable)
- Loading and blending products: loading/unloading, transit, breathing, and working losses (as applicable)
- Control systems
- Fugitives from relevant terminal operations

Scenarios

The following input parameters and factors will be evaluated and considered to develop appropriate assessment scenarios as relevant to fuel terminal operation in California and neighboring states:

- Product types
    - California reformulated gasoline blended with 10vol% ethanol
    - California reformulated gasoline blended with 16vol% iso-butanol
- Operation scenarios
    - Terminal blending and processing of fuel additives and blended gasoline[13]
    - Refinery blending and terminal processing of fuel additives and blended gasoline[14]
- Representative Locations
    - Air Districts with specific local requirements including AB 2588:
        - Bay Area Air Quality Management District (BAAQMD)
        - San Joaquin Valley Air Pollution Control District (SJVAPCD)
        - South Coast Air Quality Management District (SCAQMD)
    - Federal requirements only:
        - General California region other than the major air districts mentioned above and terminals in neighboring states

Based on the above variable parameters/factors and selected terminals' input/profile data, it is proposed that one modeling/assessment scenario be developed for each operation scenario for each product (i.e., California reformulated gasoline blended with 10vol% ethanol and with 16vol% iso-butanol) for each location (air district) for this air emission impact assessment study[15]. This will make a total of 8 scenarios for each product and 16 scenarios for all[16].

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[13] For both E10 and Bu16 blending

[14] Only applicable to Bu16 blending

[15] Butamax understands that ARB is particularly concerned about the suitability of Carbon Adsorption systems. It is expected that the range of regulatory requirements in the districts being modeled will provide a range of representative vapor control technologies for this evaluation. At least one of the terminals modeled in this study will employ carbon adsorption technology.

[16] As there is no refinery-blending scenario for E10, both Bu16 blending scenarios (refinery blending and terminal blending) will be compared to terminal-blending of E10.

Analyses

For each scenario, emission quantification and assessments will consider the following elements (but not limited to):

- Emissions basis - Potential (Permit Limit or Maximum capacity)
    - Controlled (e.g. vapor recovery system, flare, and etc.)
    - Uncontrolled
- Pollutant species
    - Criteria pollutants
    - Toxic pollutants
    - HAP pollutants
- Types of operation resulting in air emissions:
    - Loading and unloading of products
    - Storage of products in tanks
    - Blending of products (for terminal blending only)
    - Combustion emissions (flare, vapor destruction units, thermal oxidizers, and etc. as applicable)
    - Fugitives and leaks

6.9.3. Report

Since BP has established the methodologies for emissions calculations for other regulatory compliance activities, it is proposed that the same procedures and methodologies would be used. These procedures (pre-established by BP) include; but are not limited to those listed below:

- Storage tank calculations (product tanks, additive tanks, tote tanks, fire-water pump tanks, and/or sump tanks) utilizing U.S. EPA AP-42 (TANKS 4.09d software)
- Tank roof landing losses utilizing U.S. EPA AP-42 emission factors (EFs) and API guidance documents
- Tank degassing emissions using the actual degassed volume, as necessary
- Tank cleaning emissions utilizing API guidance documents
- Equipment component fugitive leak calculations utilizing U.S. EPA AP-42 default EFs. If local agency requires, refined calculations using screening or correlation values will be conducted.
- Combustion emissions from emission control equipment (flares, carbon system, vapor destruction units, thermal oxidizers, etc.) utilizing U.S. EPA AP-42 default EFs

- Product spills emissions utilizing the actual volume and speciation profiles

Based on the emission quantification results, a comparison of air emissions associated with terminal operations involving iso-butanol as opposed to ethanol blended products will be conducted. In addition, air regulatory and air quality implications of air emissions derived from terminal operation involving iso-butanol as opposed to ethanol blended products will be evaluated, based on the results of air emission quantifications. Other comparisons and/or evaluations that will be performed as part of the assessments are as follows:

- Comparison to demonstrate whether existing control requirements are sufficient to capture iso-butanol emissions vs. ethanol emissions.
- Comparison to determine if handling of iso-butanol will add new regulatory requirements to terminal operators.
- Comparison to demonstrate impacts of iso-butanol vs. ethanol on carbon capture systems.

7. Impact of Biobutanol on Exhaust and Evaporative Emissions

7.1. Statement of the Knowledge Gap

- **Perform exhaust and evaporative emissions testing for 16vol% iso-butanol blends in California reformulated gasoline versus 10vol% ethanol blends in California reformulated gasoline to determine whether any adjustments to the Predictive Model are required to model 16vol% iso-butanol blends. Determine impact on Ozone Reactivity and Potency-weighted Toxics emissions.**

7.2. Test Fuels and Vehicles

Hydrocarbon base stocks for this program will be sourced from two refineries (BP Carson and BP Cherry Point) which currently supply the California market. These two refineries have very different process configurations and, as a result, their respective products represent the range of aromatics / olefins levels typically found in CARB gasoline. Each fuel will be blended to meet current CaRFG3 specifications and pass the 31st December 2009 version of the predictive model. Fuels must be approved by ARB staff prior to testing. Descriptions of the fuels are presented in **Table 7.1.** The same group of test fuels is being employed for the test programs defined in Sections 3, 4, 5, 8 and 9.

	Fuel ID			
	CARB Fuel 1	CARB Fuel 2	CARB Fuel 3	CARB Fuel 4**
Fuel Content	Carson E10	Cherry Point Bu16	Carson Bu16	50:50 mix of CARB Fuels 1 and 3
Fuel Description	Current quality RFG3	High-Aromatic, Low-Olefin base	Low-Aromatic, High-Olefin base	Commingled Carson E10 and Bu16
RVP, psi	6.86	7.08	6.92	6.89
T50, F	214	203	213	214
T90, F	318	307	318	318
Aromtics, v%	21.4	29.8	21.4	21.4
Olefins, v5	7.3	0.7	6.1	6.7
Oxygen, wt%	3.4	3.6	3.5	3.4
Sulfur, ppm	6	4	6	6
Benzene, v%	0.69	0.81	0.74	0.72
NOx, % change*	-1.75	-1.72	-1.66	-1.76
Ozone-Forming Potential, % change*	-0.88	-0.85	-0.83	-0.75
Potency-Weighted Toxics, % change*	-2.06	-2.07	-2.13	-1.87

* as determined from California Predictive Model, spreadsheet revision of 25 Jan 2010
** estimates --- actual properties of Fuel 4 will be as result from 50:50 volume physical mix of Fuels 1 and 3
Table 7.1 Test Fuels

The vehicle fleet for this test program is being selected to include representative vehicles from the Tech III, Tech IV and Tech V vehicle technology groups as currently defined in the Predictive model. Seven vehicle models (three from Tech III, two from Tech IV and two from Tech V) are proposed as outlined in **Table 7.2**. Vehicles used in both the exhaust and evaporative programs will be procured in duplicate for a total of ten. Vehicles are subject to approval from CARB; any changes or additions must be approved by CARB prior to testing.

Vehicle Description	Year	Tech Group	Program
Buick Riviera 5.0l	1981	III	Exhaust
Nissan Sentra 1.6L	1985	III	Exhaust
Ford Crown Victoria 5.0L	1985	III	Exhaust & Evaporative
Lexus ES 300 3.0L	1992	IV	Exhaust & Evaporative
Honda Accord 2.2L	1992	IV	Exhaust
Dodge Caravan 3.3L	2005	V	Exhaust & Evaporative
Chevrolet Silverado 4.8L	2007	V	Exhaust

Table 7.2 Vehicle Fleet for Emissions Testing

7.3. Vehicle Exhaust Emissions Test Program

Objective: To determine the emissions level of a CaRFG3+ E10 fuel (CARB fuel 1), two CaRFG3 +16% iso-butanol fuels (CARB fuels 2 & 3) and a transmix (CARB fuel 4) fuel in a range of vehicles.

1. To represent the California vehicle pool as far as reasonably practicable the test vehicles will be selected from Tech Groups 3, 4 and 5. Proposed vehicle selections are detailed in **Table 7.2.**

2. When received each vehicle will be checked for general service requirements which will include but not be limited to: acceptable tires, after treatment device, exhaust leaks, transmission fluid level and proper vehicle operation on the chassis dynamometer.

   All vehicles will have their exhaust systems modified to allow the measurement of pre and post catalyst exhaust emissions.

   Each vehicle will have the following start of test services: drain the engine oil, perform a single oil flush, replace the oil filter, charge the crankcase with the manufacturers specified engine oil, replace the fuel filter and replace the air cleaner element.

   The vehicles will also undergo any manufacturer scheduled maintenance based on the current odometer reading. If unscheduled maintenance is necessary, the repairs would be made to Original Equipment Manufacturer (OEM) specifications using OEM or OEM approved parts wherever possible. Following these services each vehicle would accumulate a minimum of 100 miles of on-road stabilization.

3. The vehicle fuel system will be drained and refueled with the CaRFG3+E10 (CARB fuel 1) according to the prescribed fuel change procedure (**Figure 7.1**). (**Note:** No other fuel should be used until testing has been completed with this fuel). This fuel change procedure is based on the Auto-Oil protocol[17].

4. The vehicle's exhaust system will be prepared for connection to the Constant Volume Sampler (CVS), the chassis dynamometer coefficients will be taken from EPA's Test Car List Database. All necessary calibrations of the testing equipment will be performed and the vehicle will be run over one UDDS sequence to prepare it for testing the following day.

5. Soak vehicle overnight (12 to 36 hours).

6. The exhaust emissions and fuel economy (FE) will be determined by operating the vehicles on a chassis dynamometer over the Federal Test Procedure (FTP-75) 4 bag test.

7. Measurement of regulated emissions will include total hydrocarbons (THC), carbon monoxide (CO), oxides of nitrogen (NO_x) and carbon dioxide (CO_2). These will be determined in a manner consistent to 40 CFR parts 86 and 600. Sample for hydrocarbon speciation including aldehydes, ketones, alcohols, ethers, methane and NMHC. Sample collections can be with Tedlar bags and/or DNPH cartridges or suitable online alternative (NMOG GCMS, FTIR etc). Post test analysis will be GC

[17] Vaughn R. Burns, et al., "Description of Auto/Oil Air Quality Improvement Research Program", SAE Paper 912320, October 1991.

and HPLC. Measurement of modal raw emissions will be recorded at 1 Hz for THC, CO, NO_x and CO_2.

8. Prepare the vehicle with one UDDS sequence and repeat Steps 5 through 7.

9. After three tests are completed on a given vehicle/fuel combination its repeatability will be checked to determine if a fourth test is required. Repeatability criteria for gaseous emissions are as follows: ratio between highest and lowest; CO, 1.330; HC, 1.175; NO_x, 1.500; CO_2, 2.000.[18]

10. The vehicle will be drained and refueled with the CaRFG3+16% iso-butanol (CARB fuel 2) according to the prescribed fuel change procedure. (**Note:** No other fuel should be used until testing has been completed with this fuel). This fuel change procedure is based on the Auto-Oil protocol.

11. Prepare the vehicle with one UDDS sequence and repeat Steps 5 through 7.

12. After three tests are completed on a given vehicle/fuel combination its repeatability will be checked to determine if a fourth test is required. Repeatability criteria for gaseous emissions are as follows: ratio between highest and lowest; CO, 1.330; HC, 1.175; NO_x, 1.500; CO_2, 2.000

13. The vehicle will be drained and refueled with the CaRFG3+16% iso-butanol (CARB fuel 3) according to the prescribed fuel change procedure. (**Note:** No other fuel should be used until testing has been completed with this fuel). This fuel change procedure is based on the Auto-Oil protocol.

14. Prepare the vehicle with one UDDS sequence and repeat Steps 5 through 7.

15. After three tests are completed on a given vehicle/fuel combination its repeatability will be checked to determine if a fourth test is required. Repeatability criteria for gaseous emissions are as follows: ratio between highest and lowest; CO, 1.330; HC, 1.175; NO_x, 1.500; CO_2, 2.000

16. The vehicle will be drained and refueled with the CaRFG3 transmix (CARB fuel 4) according to the prescribed fuel change procedure. (**Note:** No other fuel should be

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[18] The figures are again based on the Auto/Oil program, this states that the difference between duplicate tests for a 95% confidence will be: -- *Difference = 2.387 x √2 x SD*

The determinations for SD were originally formulated from a GM data set; this gave the difference ratios of CO, 1.71; HC, 1.40; NO_x 1.66. These have subsequently been refined through further internal and external emissions programs and good engineering practice to the figures published here.

Louis J. Painter, James A. Rutherford. "Statistical Design and Analysis Methods for the Auto/Oil Air Quality Research Program". SAE Paper 920319, February 1992.

used until testing has been completed with this fuel). This fuel change procedure is based on the Auto-Oil protocol.

17. Prepare the vehicle with one UDDS sequence and repeat Steps 5 through 7.

18. After three tests are completed on a given vehicle/fuel combination its repeatability will be checked to determine if a fourth test is required. Repeatability criteria for gaseous emissions are as follows: ratio between highest and lowest; CO, 1.330; HC, 1.175; NO_x, 1.500; CO_2, 2.000

19. Steps 2 through 18 to be repeated for each of the test vehicles

20. Analyze all samples collected, and prepare final report detailing the exhaust emissions from butanol containing gasolines in a range of test vehicles and its influence on the California predictive model.

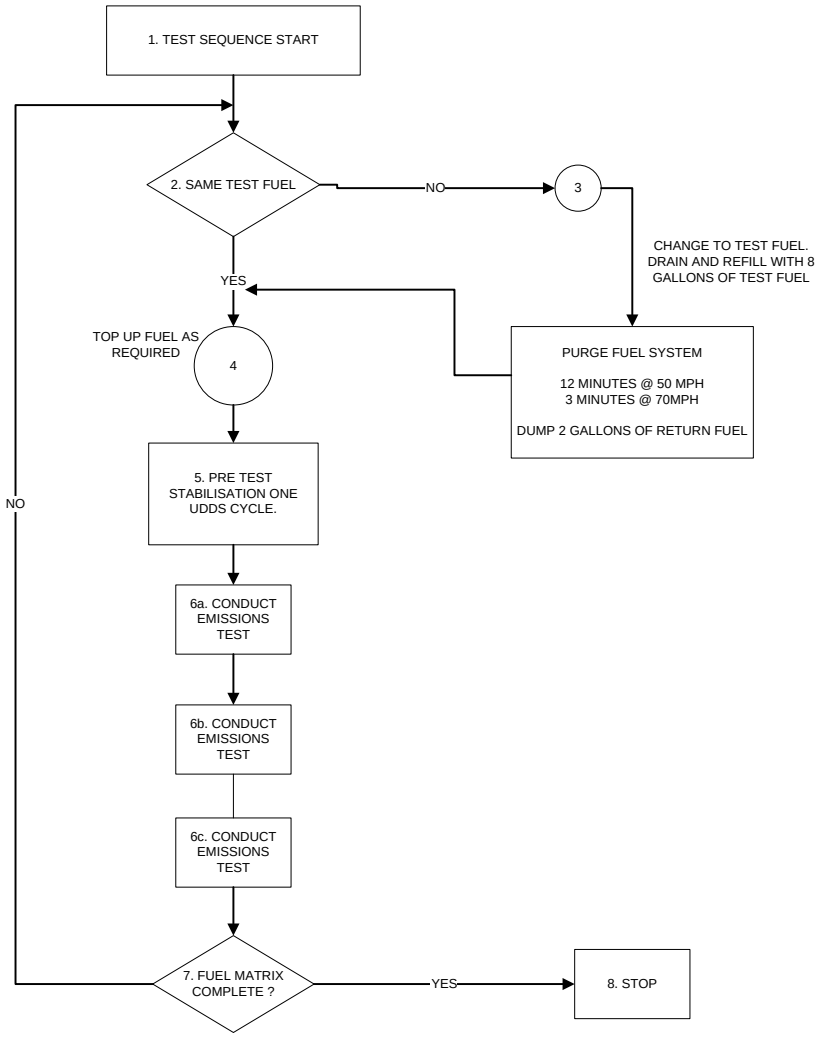

Figure 7.1 Fuel Change Procedure

7.4. Vehicle Evaporative Emissions Test Program

Evaporative emissions will be measured via CARB enhanced evaporative emission tests as described in "California Evaporative Emission Standards and Test Procedures for 2001 and Subsequent Model Motor Vehicles" with minor exceptions to the running loss test procedure (consistent with recent CRC evaporative emissions test programs) as noted below.

Each of the three vehicles (see **Table 7.2**) identified for evaporative emissions testing will receive an incoming inspection. This will include documentation of vehicle ID (VIN, Test Group, Evap Family, etc.), fuel system pressure check, thorough check of fluid levels (including oil & filter change), emission test instrumentation and road safety inspection. If the vehicle passes the acceptance tests they will be instrumented with a fuel tank surface thermocouple and means of draining the fuel from the fuel tank. The thermocouple will provide a close approximation of the liquid fuel temperature during the running loss test. Fuel temperature will also be monitored during the diurnal emission test.

It is proposed that a series of pass off tests and fuel system integrity tests be carried out prior to the test work being commenced. This will ensure that the vehicle is operating correctly and that any rectification occurs before the test programs starts. Details of these checks are set out in the protocol below.

If unscheduled maintenance is necessary, the repairs would be made to Original Equipment Manufacturer (OEM) specifications using OEM or OEM approved parts wherever possible. Unscheduled maintenance is defined as any repairs or changes required to the vehicle to return it to a state of normal operation outside of those normally deemed necessary by the manufacturer. These works will be in accordance with 40CFR 86.1834-01.

Prior to beginning the emission testing, each vehicle will need to be preconditioned / stabilized to the test fuel. Following previously established protocols, a 4 to 9 week preconditioning program will be employed. Each vehicle will be operated twice per week over the on-road LA-4 course, and two LA-4 cycles (one cold, one hot) will be driven. A baseline permeation test will be carried out to ascertain a stable permeation rate has been achieved.

Upon completing the preconditioning, each vehicle will be tested for evaporative emissions according to the ARB 3-day test sequence (Section **1.1.1.1**) and the supplemental 2-day test (Section **1.1.1.2**). Each test will be performed with "fresh" test fuel. The enhanced evaporative emissions test procedure will include the LA-4 preconditioning, fuel tank drain and 40% fill, canister load, FTP drive cycle, running loss test, hot soak and the 72-hour diurnal. Supplemental tests will include the LA-4 preconditioning, fuel tank drain and 40% fill, canister load, FTP drive cycle, hot soak and 48-hour diurnal. No off-cycle (SFTP) or refueling (ORVR) tests are required. Speciation of evaporative emissions will be performed. Consistent with recent CRC programs comparing evaporative emissions from different fuel formulations, the running loss tests

procedure will ensure appropriate increase in tank temperature over the test but will not attempt to follow vehicle-specific fuel tank temperature profiles (FTTPs)[19,20,21].

Upon completion of the emission tests and acceptance of the test data from CARB fuel #1, the fuel system of each vehicle will be drained and flushed to remove any CARB fuel #1 residual. CARB fuel #2 will be introduced and the preconditioning process will begin again from the baseline permeation. This same procedure will be followed for CARB fuels #3 and #4.

Sections **7.4.1** through **7.4.8** present a detailed task flow for the evaporative emissions test program.

7.4.1. Vehicle Procurement

1. Identify the vehicles based on CARB requirements for Tech III, Tech IV and Tech V selections.
2. Locate suitable vehicles based on history, current condition and technical details.
3. Carry out mechanical inspections to ensure there are no post manufacture modifications.
4. Arrange vehicle purchase

7.4.2. Vehicle Pass Off Tests

1. Mechanical checks of each test vehicle; including but not limited to engine operation, fuel system integrity, evaporative system and emission control system operation. Rectifications to be carried out to OEM requirements. All rectifications to be recorded
2. Determine and report fuel tank volume for use later in the test program
3. Determine and report engine number and evaporative system family.
4. Approve vehicle acceptance into program

7.4.3. Vehicle Modifications

1. Install temperature probes in fuel tank & fuel lines as required.

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[19] CRC Report No. E-77 *Vehicle Evaporative Emission Mechanisms: A Pilot Study*, p11;

[20] CRC Report No. E-77-2 *Enhanced Evaporative Emission Vehicles*, p14:

[21] CRC Report No. E-77-2c *Study to Determine Evaporative Emission Breakdown, including Permeation Effects and Diurnal Emissions, Using E20 Fuels on Aging Enhanced Evaporative Emissions Certified Vehicles*, p74.

2. Install pressure sensors in fuel tank as required.
3. Install suitable fittings to carry out fuel changes.
4. Install fittings to carry out evaporative canister charging and discharge.
5. Ensure all fittings are secure and without liquid or vapour leaks.

7.4.4. Fuel System Integrity Checks

1. Carry out fuel flush and fuel change (Commercial CA gasoline).
2. Fuel top up to 40% of defined fuel tank level.
3. MAD preconditioning (one LA4 cycle).
4. Vehicle soak 12 - 36 hours @ 68°F - 86°F.
5. Repeat 3 & 4, three times.
6. Top off fuel tank to 40%.
7. Preconditioning (one LA4 cycle).
8. 12 – 36 hour soak @ 68°F - 86°F.
9. Cold start LA4.
10. One hour hot soak @105°F.
11. Vehicle stabilized at 65°F.
12. Two day diurnal test in SHED.
13. Results evaluation.
14. Continue to 17 unless reparations are required.
15. Can vehicle be repaired or is vehicle rejected?
16. Perform 2 - 13.
17. Vehicle accepted into test program.

7.4.5. Baseline Permeation

1. Carry out fuel change if required.
2. Fuel top up to 100% of tank level (commercial CA gasoline).
3. Pre-heat SHED to 86°F.
4. Vehicle in SHED @ 86°F.
5. Ensure carbon canister and fuel tank are vented outside the SHED.
6. Door sealed, continuous sampling.
7. Conduct 1 hour permeation test.
8. Calculate permeation rate.

7.4.6. Fuel change to test fuel

1. Fuel drain and fill.
2. Vehicle preconditioning.
3. Fuel drain and fill.

7.4.7. Vehicle Stabilization

1. Fill fuel tank with test fuel to between 90% and 100% of capacity.
2. Soak vehicle between 68°F and 86°F.
3. Drive 2 LA4's one hot start one cold start.
4. Soak vehicle between 68°F and 86°F.
5. Drive 2 LA4's one hot start one cold start.
6. Parts 2 to 5 should be completed within 1 week and soak periods should be a minimum of 36 hours.
7. Complete parts 2 to 6 three more times (4 weeks elapsed time).
8. Determine baseline permeation rate.
9. If permeation rate has stabilized continue to evaporative emission tests. Baseline permeation has deemed to have stabilized if the 3 week moving average no longer declines.
10. If permeation has not stabilized repeat parts 2 to 6 then retest permeation weekly until stabilized or a maximum of 9 weeks. (An interim fuel change may be required at 6 weeks).
11. Continue to evaporative emissions tests.

7.4.8. Evaporative Emission Tests

The following tests will be performed as per the "California Evaporative Emission Standards and Test Procedures for 2001 and Subsequent Model Motor Vehicles".

1.1.1.1. 3 Day Diurnal Test

1. Fuel drain and fill.
2. Cold Soak.
3. Vehicle preconditioning.
4. Fuel drain and fill.
5. Cold soak, canister purge and load.
6. Cold start emissions test.
7. Hot start emissions test.

8. Fuel tank stabilization 105°F.
9. Running loss test - UDDC NYCC UDDC NYCC at 105°F.
10. Hot soak enclosure test at 105°F.
11. Vehicle soak last 6 hours at 65°F.
12. Diurnal test 72 hours variable SHED temp 65°F to 105°F.
13. Test complete.

1.1.1.2. 2 Day Diurnal Test

1. Fuel drain and fill.
2. Cold Soak.
3. Vehicle preconditioning.
4. Fuel drain and fill.
5. Cold soak, canister purge and load.
6. Cold start emissions test.
7. Hot start emissions test.
8. Hot soak enclosure test at 68°F 86°F.
9. Vehicle soak last 6 hours at 65°F.
10. Diurnal test 48 hours variable SHED temp 65°F to 105°F
11. Test complete.

7.5. Data Analysis and Reporting

Following analysis of the data generated, a final report will be prepared. The report will include the following elements –

- Technical details for all test vehicles
- Measurements of relevant properties for all test fuels
- A review of all tests conducted and their results
- A detailed statistical evaluation of all emissions measured including treatment of any outlier data
- Calculation of any impacts on ozone reactivity for Bu16 relative to E10 using Carter Maximum Incremental Reactivity (MIR) methodology.
- Calculation of Potency-Weighted Toxics (POT) emissions
- Additionally, an assessment will be made of the measured total hydrocarbon (THC), nitrogen oxides (NO_x) and potency-weighted toxics (POT) emissions compared to those predicted by the California Predictive Model to evaluate its applicability to gasoline/iso-butanol blends.

8. Impact of Biobutanol on Toxic Air Pollutants

8.1. Statement of the Knowledge Gap

- **Determine toxic air pollutants in automotive exhaust using EPA Section 211(b) methodology with California reformulated gasolines blended with 10vol% ethanol and with 16vol% iso-butanol.**

8.2. Test Fuels and Vehicle

This test program will be run with two of the fuels described in Section **7.2** and used in Sections 3**,** 4**,** 5**, 7.2**, 8 and 9. Specifically, the program will be run with the 10vol% ethanol fuel and one of the 16vol% iso-butanol fuels. See Table **8.1**.

Per EPA methodology[22], the testing is done with a single, high-production recent vehicle model. The vehicle chosen for this program is identified in Table **8.2**.

Fuel ID	Fuel Content	Fuel Description
CARB Fuel 1	Carson E10	Current quality RFG3
CARB Fuel 3	Carson Bu16	Low Aromatic High Olefin base

Table 8.1 Test Fuels for 211(b) Testing

Vehicle Description	Year
Toyota Camry 2.4 L	2009

Table 8.2 Test Vehicle for EPA 211(b) Testing

8.3. Test Plan

'Unregulated' Emissions Test Program [EPA 211(b)]

Objective: To determine the performance of a vehicle emissions system when using CaRFG3+E10 fuel (CARB fuel 1) and a CaRFG3+16% iso-butanol (CARB fuel 3)

1. The test vehicle will be selected from a group as per the EPA requirements for fuel / fuel additive registration; the test vehicle will be obtained with less than 500 miles on the odometer. (see Table **8.2**)

2. When the vehicle is received it will be checked for an intact after treatment device, exhaust leaks, acceptable tires, proper oil level, proper transmission fluid level and proper vehicle operation on the mileage accumulation dynamometer (MAD). The vehicle fuel system will be drained and refueled with the CaRFG3+E10 (CARB fuel 1) fuel. (**Note:** No other fuel should be used until testing and mileage accumulations have been completed with this fuel). Mileage accumulation for 4,000 miles will be

[22] 40 CFR 79.50 subpart F.

performed using a MAD or suitable alternative[23]. All scheduled maintenance will be performed according to the manufacturer's recommendations. If unscheduled maintenance is necessary, the repairs would be made by Original Equipment Manufacturer (OEM) specifications using OEM or OEM approved parts. To ensure that the post-maintenance emission levels are within 20 percent of the pre-maintenance emission levels, baseline emissions will be measured before resuming mileage accumulation after any unscheduled maintenance.

The mileage accumulation cycle for each test fuel will also serve for collection of samples for Health Effects evaluation by Ames and Comet assays. (Section **8.4.9**). Sample collection over an extended duration (approximately the first 650 miles of the total 4000-mile accumulation on each fuel) is required because it is not possible to collect enough sample from the 3 replicate gaseous emission test cycles which follow the mileage accumulation (described below).

3. For emission testing after the first 4,000 miles, the vehicle's exhaust system will be prepared for connection to the Constant Volume Sampler (CVS), the chassis dynamometer coefficients would be taken from EPA's Test Car List Database. All necessary calibrations of the testing equipment will be performed and the vehicle would be run over one UDDS sequence to prepare it for testing the following day.

4. Soak vehicle overnight (12 to 36 hours).

5. Perform a 4-bag Federal Test Procedure (FTP75). Measurement of regulated emissions will include total hydrocarbons (THC), carbon monoxide (CO), oxides of nitrogen (NO_x), carbon dioxide (CO_2) and methane (NMHC) would be determined in a manner consistent to 40 CFR parts 86 and 600. Sample for hydrocarbon speciation including aldehydes, ketones, alcohols, ethers, PAH and NPAH. Sample collections can be with Tedlar bags and/or DNPH cartridges or suitable online alternative (NMOG GCMS, FTIR etc). Post test analysis would be GC and HPLC. Analytical techniques for the speciation are described in Sections **8.4.1** through **8.4.7**.

   Additionally, measurement of particulate size and distribution will be performed per Section **8.4.8**.

6. Measurement of modal raw emissions would be recorded at 1 Hz for THC, CO, NO_x and CO_2.

7. Repeat Steps 5 and 6 two additional times on different days.

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[23] Mileage accumulation will be achieved using the US EPA Standard Road Cycle (SRC) as defined in Appendix V to 40 CFR 86. The Standard Road Cycle is a standardized whole vehicle aging cycle. The SRC consists of seven laps of 3.7 miles each. The average speed on the SRC is 46.3 mph, the maximum cruise speed is 75 mph, and the acceleration rates range from light to hard accelerations. Most accelerations are moderate and there are no wide-open-throttle accelerations. The SRC contains 24 fuel-cut decelerations. The deceleration rates range from coast-down (no brake force applied) to moderate.

8. Remove the catalytic converter; replace with an uncoated, non-functioning catalyst monolith of similar size or a blank spool piece and repeat Steps 4 through 7.

9. Perform fuel change procedure (This fuel change procedure is based on the Auto-Oil protocol[24].) using the CaRFG3+16% iso-butanol (CARB fuel 3). Change fuel filters, purge fuel supply, etc. (**Note:** No other fuel should be used until testing with this fuel has been completed). Change oil. Replace spool piece with original catalyst monolith & perform mileage accumulation for 4,000 miles using the same criteria listed in Step 3 above.

10. Repeat Steps 4 through 8.

11. Analyze all samples collected, and prepare final report.

8.4. Analysis of emissions

The analysis of the regulated and speciated emission from Sections 7 and 8 will be conducted in the following manner.

Exhaust constituents would be analyzed as follows:

Constituent	Analysis Method
Total Hydrocarbon[25]	Flame Ionization Detector
Carbon Monoxide[25]	Non-Dispersive Infrared Detector
Carbon Dioxide[25]	Non-Dispersive Infrared Detector
Oxides of Nitrogen[25]	Chemiluminescent Detector
Methane	Gas Chromatograph

Methods for sampling and analyzing unregulated emissions (from the test in Section **8.3**) will be as described below.

Equipment for sampling volatile-phase hydrocarbon compounds, aldehydes, ketones, alcohols, and ethers and semi-volatile emissions for both volatile- and particulate-phase PAH are described in **Table 8.3**.

Regulated THC, NMHC, CO, NO_X, and Particulate	Speciation $C_1 - C_{12}$	PAH		Alcohols and Ethers	Aldehydes and Ketones
		Particulate	Volatile		
Cont., Bag, 90mm Filter	Bag	20X20 Filter	PUF	Bubbler	Cartridge

Table 8.3 Sampling Methodologies

[24] Vaughn R. burns, et al., "Description of Auto/Oil Air Quality Improvement Research Program", SAE Paper 912320, October 1991.

[25] Modal emissions measurements for these constituents in Section 7.3 (Vehicle Exhaust Emissions Test Program)

Hydrocarbon speciation (C_1 to C_{12} hydrocarbons, aldehydes and ketones) will be conducted on exhaust emissions samples to detect the presence of more than 200 different exhaust species. Four gas chromatography (GC) procedures and one High Performance Liquid Chromatography (HPLC) procedure will be used to identify and quantify specific compounds. One GC is used for the measurement of methane, a second for C_2-C_4 species, and a third for C_5-C_{12} species including three ethers (methyl tertiary butyl ether – MTBE, ethyl tertiary butyl ether – ETBE, and di-isopropyl ether – DIPE). A fourth GC is used to measure 1-methylcyclopentene, benzene, toluene, 2,3-dimethylhexane, cyclohexane, and 2,3,3-trimethylpentane, which co-elute and cannot be accurately quantified by other methods. Analysis of all emission "sample" bags will begin within 30 minutes of sampling and before the "background" bags, so that reactive exhaust compounds could be analyzed as quickly as possible. Data is reported as background corrected. A brief description of these procedures is given in the following sections. A full list of species to be quantified is provided with each of the analytical procedures described in sections **8.4.1** through **8.4.7** below. A list of designated TAC's[26] determined in this testing with reference to the test procedure employed is provided as Table **8.4**.

[26] List derived from "Appendix A: Hot Spots Unit Risk and Cancer Potency Values" published at http://oehha.ca.gov/air/hot_spots/2009/AppendixA.pdf. Species tested include all of the designated TACs which are hydrocarbons, oxygenated hydrocarbons or nitro-PAHs.

Table 8.4 TACs Quantified

Chemical Species	CAS Number	Analytical Method
Acetaldehyde	75-07-0	Section 8.4.5
Benz[*a*]anthracene	56-55-3	Section 8.4.7
Benzene	71-43-2	Section 8.4.4
Benzo[a]pyrene	50-32-8	Section 8.4.7
Benzo[b]fluoranthrene	205-99-2	Section 8.4.7
Benzo[j]fluoranthrene	205-82-3	Section 8.4.7
Benzo[k]fluoranthrene	207-08-9	Section 8.4.7
1,3-Butadiene	106-99-0	Section 8.4.2
Chrysene	5120-73-19	Section 8.4.7
Dibenz[a,h]acridine	226-36-8	Section 8.4.7
Dibenz[a,j]acridine	224-42-0	Section 8.4.7
Dibenzo[a,e]pyrene	192-65-4	Section 8.4.7
Dibenzo[a,h]pyrene	189-64-0	Section 8.4.7
Dibenzo[a,i]pyrene	189-55-9	Section 8.4.7
Dibenzo[a,l]pyrene	191-30-0	Section 8.4.7
7H-Dibenzo[c,g]carbazole	194-59-2	Section 8.4.7
1,6-Dinitropyrene	42397-64-8	Section 8.4.7
1,8-Dinitropyrene	42397-65-9	Section 8.4.7
Ethyl benzene	100-41-4	Section 8.4.3
Formaldehyde	50-00-0	Section 8.4.5
Indeno[1,2,3-cd]pyrene	193-39-5	Section 8.4.7
Methyl tert-butyl ether (MTBE)	1634-04-4	Section 8.4.3
5-Methylchrysene	3697-24-3	Section 8.4.7
Naphthalene	91-20-3	Section 8.4.3
6-Nitrochrysene	7496-02-8	Section 8.4.7
2-Nitrofluorene	607-57-8	Section 8.4.7
1-Nitropyrene	5522-43-0	Section 8.4.7
4-Nitropyrene	57835-92-4	Section 8.4.7

8.4.1. Methane Speciation

Methane levels are determined for proportional exhaust gas samples collected in Tedlar® bags. A GC equipped with a flame ionization detector (FID) is utilized for the analyses, and used in accordance with SAE J1151 procedures. The GC system is equipped with a packed column to resolve methane from other hydrocarbons in the sample. Samples are introduced into a 5-mL sample loop via a diaphragm pump. For analysis, the valve is switched to the inject position, and the helium carrier gas sweeps the sample from the loop toward the detector through a 61 cm × 0.3 cm Porapak N column in series with a 122 cm × 0.3 cm molecular sieve 13X column. As soon as the methane peak passes into the molecular sieve column, the helium flow is reversed through the Porapak N column to vent. For quantification, sample peak areas are compared to those of external

calibration standards. Detection limits for the procedure are on the order of 0.05 mg/bhp-hr in dilute exhaust.

8.4.2. C_2-C_4 Species

With the aid of a DB-WAX pre-column and a 10-port switching valve, this procedure allows the separation and determination of exhaust concentrations of C_2-C_4 individual hydrocarbon species, including: ethane; ethylene; acetylene; propane; propylene; trans-2-butene; butane; 1-butene; 2-methylpropene (isobutylene); 2,2-dimethypropane (neopentane); propyne; 1,3-butadiene; 2-methylpropane; 1-butyne; and cis-2-butene. Bag samples are analyzed with a GC system which utilizes a Hewlett-Packard Model 5890 Series II GC with an FID, two pneumatically operated and electrically controlled valves, and two analytical columns. The first column separates the C_2-C_4 hydrocarbons from the higher molecular weight hydrocarbons and the polar compounds. These higher molecular weight hydrocarbons (and water and alcohols) are retained on the pre-column while the C_2-C_4 hydrocarbons are passed through to the analytical column. At the same time, the C_2-C_4 hydrocarbons are separated on the analytical column, the pre-column is back-flushed with helium to prepare for the next analysis. The carrier gas for this analysis is helium. Analysis for the C_2-C_4 hydrocarbons is typically begun within 30 minutes after sample collection is complete. Detection limits for the procedure are on the order of 0.05 mg/bhp-hr in dilute exhaust for all compounds, with a quantification limit of 0.1 mg/bhp-hr.

8.4.3. C_5-C_{12} Species

Bag samples are analyzed using a gas chromatograph equipped with an FID. The GC system utilizes a Hewlett-Packard Model 5890 Series II GC with an FID, a pneumatically operated and electrically controlled valve, and a DB-1 fused silica open tubular (FSOT) column. The carrier gas is helium. Gaseous samples are pumped from the bag through the sample loop and then introduced into a liquid nitrogen cooled column. The column oven is programmed to provide a maximum temperature of 200° C. Detection limits for the procedure are in the order of 0.05 mg/bhp-hr in dilute exhaust for all compounds, and a quantification limit of 0.1 mg/bhp-hr. This procedure provides separation and exhaust concentrations for more than 100 C_5-C_{12} individual hydrocarbon compounds. The species quantified by this method are listed in Table **8.4**.

COMPOUND	COMPOUND	COMPOUND
1,1,2-TRIMETHYLCYCLOPENTANE	2,4,4-TRIMETHYLHEXANE	CIS-3-HEXENE
1,1,3-TRIMETHYLCYCLOHEXANE	2,4-DIMETHYLHEPTANE	CIS-3-NONENE
1,1,3-TRIMETHYLCYCLOPENTANE	2,4-DIMETHYLHEXANE	CYCLOHEXANE
1,1-DIMETHYLCYCLOHEXANE	2,4-DIMETHYLOCTANE	CYCLOHEXENE
1,1-DIMETHYLCYCLOPENTANE	2,4-DIMETHYLPENTANE	CYCLOPENTADIENE
1,2 DIETHYLBENZENE	2,5-DIMETHYLHEPTANE	CYCLOPENTANE
1,2,3,4-TETRAMETHYLBENZENE	2,5-DIMETHYLHEXANE	CYCLOPENTENE
1,2,3,5-TETRAMETHYLBENZENE	2,6-DIMETHYLHEPTANE	DECANE
1,2,3-TRIMETHYLBENZENE	2-BUTYNE	DI-ISOPROPYL ETHER
1,2,4,5-TETRAMETHYLBENZENE	2-METHYL-1,3-BUTADIENE	DODECANE
1,2,4-TRIETHYLBENZENE	2-METHYL-1-BUTENE	ETBE
1,2,4-TRIMETHYLBENZENE	2-METHYL-1-HEXENE	ETHYLBENZENE
1,2-DIMETHYL-3-ETHYLBENZENE	2-METHYL-1-PENTENE	ETHYLCYCLOHEXANE
1,2-DIMETHYL-4-ETHYLBENZENE	2-METHYL-2-BUTENE	ETHYLCYCLOPENTANE
1,3,5-TRIETHYLBENZENE	2-METHYL-2-HEXENE	HEPTANE
1,3,5-TRIMETHYLBENZENE	2-METHYL-2-PENTENE	HEXANE
1,3,-DIMETHYL-5-ETHYLBENZENE	2-METHYLBUTANE (ISOPENTANE)	HEXYLBENZENE
1,3-DIETHYLBENZENE	2-METHYLBUTYLBENZENE (sec AMYLBENZENE)	INDAN
1,3-DIMETHYL-2-ETHYLBENZENE	2-METHYLHEPTANE	ISOBUTYLBENZENE
1,3-DIMETHYL-4-ETHYLBENZENE	2-METHYLHEXANE	ISOPROPYLBENZENE (CUMENE)
1,4-DIETHYLBENZENE	2-METHYLOCTANE	ISOPROPYLCYCLOPENTANE
1,4-DIMETHYL-2-ETHYLBENZENE	2-METHYLPENTANE	m-& p-XYLENE
1-CIS,2-TRANS,3-TRIMETHYLCYCLOPENTANE	3,3-DIMETHYL-1-BUTENE	METHYLCYCLOHEXANE
1-DECENE	3,3-DIMETHYLHEPTANE	METHYLCYCLOPENTANE
1-HEPTENE	3,3-DIMETHYLHEXANE	METHYLPROPYLBENZENE (sec butylbenzene)
1-HEXENE	3,3-DIMETHYLPENTANE	MTBE
1-METHYL-1-ETHYL-CYCLOPENTANE	3,4 DIMETHYLCUMENE	NAPHTHALENE
1-METHYL-2-ETHYLBENZENE	3,4-DIMETHYL-1-PENTENE	NONANE
1-METHYL-2-ISOPROPYLBENZENE	3,4-DIMETHYLHEPTANE	N-PENT-BENZENE
1-METHYL-2-N-PROPYLBENZENE	3,4-DIMETHYLHEXANE	n-PROPYLBENZENE
1-METHYL-3-ETHYLBENZENE	3,5-DIMETHYLHEPTANE	OCTANE
1-METHYL-3-ISOPROPYLBENZENE	3-ETHYL-CIS-2-PENTENE	o-XYLENE
1-METHYL-3-N-PROPYLBENZENE	3-ETHYLHEXANE	PENTANE
1-METHYL-4-ETHYLBENZENE	3-ETHYLPENTANE	STYRENE
1-METHYL-4-ISOPROPYLBENZENE	3-METHYL-1-BUTENE	TERT-1-BUT-2-METHYLBENZENE
1-METHYL-4-N-PROPYLBENZENE	3-METHYL-1-HEXENE	TERT-1-BUT-3,5-DIMETHYLBENZENE
1-METHYLCYCLOPENTENE	3-METHYL-1-PENTENE	TERT-1-BUTYL-4-ETHYLBENZENE
1-NONENE	3-METHYL-CIS-2-PENTENE	TERT-AMYL METHYL ETHER
1-OCTENE	3-METHYLCYCLOPENTENE	TERT-BUTANOL
1-PENTENE	3-METHYLHEPTANE	TERT-BUTYLBENZENE
1-TRANS-2-CIS-3-TRIMETHYLCYCLOPENTANE	3-METHYLHEXANE	TRANS-1,2-DIMETHYLCYCLOHEXANE
1-TRANS-2-CIS-4-TRIMETHYLCYCLOPENTANE	3-METHYLOCTANE	TRANS-1,2-DIMETHYLCYCLOPENTANE
2,2,3-TRIMETHYLBUTANE	3-METHYLPENTANE	TRANS-1,3-DIMETHYLCYCLOHEXANE
2,2,3-TRIMETHYLPENTANE	3-METHYL-TRANS-2-PENTENE	TRANS-1,3-DIMETHYLCYCLOPENTANE
2,2,4-TRIMETHYLHEXANE	3-METHYL-TRANS-3-HEXENE	TRANS-1,4-DIMETHYLCYCLOHEXANE
2,2,4-TRIMETHYLPENTANE	4,4-DIMETHYLHEPTANE	TRANS-1-METHYL-2-ETHYLCYCLOPENTANE
2,2,5-TRIMETHYLHEXANE	4-ETHYLHEPTANE	TRANS-1-METHYL-3-ETHYLCYCLOPENTANE
2,2-DIMETHYLBUTANE	4-METHYL-1-PENTENE	TRANS-2-HEPTENE
2,2-DIMETHYLHEPTANE	4-METHYL-CIS-2-PENTENE	TRANS-2-HEXENE
2,2-DIMETHYLHEXANE	4-METHYLHEPTANE	TRANS-2-NONENE
2,2-DIMETHYLOCTANE	4-METHYLOCTANE	TRANS-2-OCTENE
2,2-DIMETHYLPENTANE	4-METHYL-TRANS-2-PENTENE	TRANS-2-PENTENE
2,3,3-TRIMETHYLPENTANE	CIS-1,2-DIMETHYLCYCLOHEXANE	TRANS-3-HEPTENE
2,3,4-TRIMETHYLHEXANE	CIS-1,2-DIMETHYLCYCLOPENTANE	TRANS-3-HEXENE
2,3,4-TRIMETHYLPENTANE	CIS-1,3-DIMETHYLCYCLOHEXANE	TRANS-3-NONENE
2,3,5-TRIMETHYLHEXANE	CIS-1,3-DIMETHYLCYCLOPENTANE	TRANS-4-OCTENE
2,3-DIMETHYL-2-PENTENE	CIS-1-METHYL-2-ETHYLCYCLOPENTANE	UNDECANE
2,3-DIMETHYLBUTANE	CIS-1-METHYL-3-ETHYLCYCLOPENTANE	UNIDENTIFIED C5 OLEFINS
2,3-DIMETHYLHEPTANE	CIS-2-HEPTENE	UNIDENTIFIED C6
2,3-DIMETHYLHEXANE	CIS-2-HEXENE	UNIDENTIFIED C7
2,3-DIMETHYLPENTANE	CIS-2-OCTENE	UNIDENTIFIED C8
2,4,4-TRIMETHYL-1-PENTENE	CIS-2-PENTENE	UNIDENTIFIED C9-C12+
2,4,4-TRIMETHYL-2-PENTENE	CIS-3-HEPTENE	

Table 8.5. C5 - C12 Species Quantified

8.4.4. Benzene and Toluene

This analytical procedure uses a separate system configured similarly to the third GC method (with a DB-5 analytical column in place of a DB-1 FSOT column) to resolve individual concentrations of benzene and toluene according to the CRC Auto/Oil Phase II Protocols. Separation of benzene and toluene from co-eluting peaks is carried out by fine-tuning the column head pressure to give benzene a retention time of 22 to 23 minutes. The GC is calibrated daily using a CRC 7-component calibration mixture. Detection limits for the procedure are 0.05 mg/bhp-hr in dilute exhaust for all compounds, with a quantification limit of 0.1 mg/bhp-hr.

8.4.5. Aldehydes and Ketones

An HPLC procedure is used for the analysis of aldehydes and ketones. The method is similar to CARB SOP MLD 104. Samples are collected in DNPH cartridges at a nominal flowrate of 2 L/min and eluted with acetonitrile. Samples are extracted from the cartridges using pure acetonitrile, transferred into volumetric flasks with ground glass joints, and either analyzed immediately or stored in ground glass stopped vials at 0°C for no more than one week prior to analysis. For analysis, a portion of the acetonitrile solution is injected into a liquid chromatograph equipped with an ultra-violet (UV) detector. External standards of the aldehyde and ketone DNPH derivatives are used to quantify the results. The aldehydes and ketones shall include formaldehyde, acetaldehyde, acrolein, acetone, propionaldehyde, crotonaldehyde, isobutyraldehyde/methylethylketone (not resolved from each other during normal operating conditions, and so split equally between the two compounds), benzaldehyde, valeraldehyde, o-tolualdehyde, m-tolualdehyde/p-tolualdehyde (not resolved from each other during normal operating conditions, and so reported together), hexanaldehyde, and 2,5-dimethylbenzaldehyde. Detection limits for this procedure are in the order of 0.05 mg/bhp-hr aldehyde or ketone in dilute exhaust, with a quantification limit of 0.1 mg/bhp-hr.

8.4.6. Alcohols and Ethers

The measurement of alcohols in exhaust is accomplished by bubbling the exhaust through glass impingers containing deionized water. Water soluble alcohols are detected using two glass impingers in series to collect exhaust samples for the analysis. The two glass impingers contain 25 ml of deionized water each and are able to collect 99+ percent of the lower molecular weight alcohols which are soluble in water. Table **8.6** lists a number of alcohols and ethers that range in solubility from miscible to slightly soluble in water. The temperature of the collection impingers is maintained at 0 to 5° C with an ice water bath, and the flow rate through the impingers was maintained at 4 L/min by the sample pump. A dry gas meter is used to determine the total flow through the impingers. The temperature of the gas stream is monitored by a thermocouple immediately prior to the dry gas meter. A drier is included in the system to prevent condensation in the pump, flow meter, dry gas meter, etc. The flow meter in the system allows for continuous monitoring of the sample to ensure proper flow rates during the sampling. The Teflon

line connecting the CVS and the solenoid valve is heated to approximately 235° F to prevent water from condensing in the sample line.

The exhaust sample is collected continuously during each cold- and hot-start test cycle. Upon completion of each transient cycle, the impingers are removed, and the contents transferred to a 30 ml polypropylene bottle and capped. Analysis of samples begins within four hours of sampling. The analytical method is similar to CARB SOP MLD 101. For analysis, a 1.0 µl portion of the aqueous solution is injected into a GC equipped with a FID and an autosampler. The analytical column is a 30 m × 0.53 mm i.d. capillary column of 1 µm DB-Wax film thickness. The carrier gas is helium and set to give optimum separation (18 ml/min.). To quantify the results, the sample peak areas are compared to peak areas of standard solutions. External standards containing methanol, ethanol, isopropanol, n-propanol, isobutanol, and n-butanol in deionized water are used to quantify the results. Sample chromatograms will also search for the presence of a number of other alcohols using predetermined retention times. The search list will include: tert-butanol (CAS# 75-65-0), 2-methyl-2-butanol(CAS# 75-85-4), 2-butanol (CAS# 78-92-2), 3-pentanol (CAS# 584-02-1), 3-methyl-3-pentanol (CAS# 77-74-7), 3,3-dimethyl-2-butanol (CAS# 464-07-3), 2-pentanol (CAS# 6032-29-7), 4-methyl-2-pentanol (CAS# 108-11-2), 2-methyl-1-butanol (CAS# 137-32-6), 3-methyl-1-butanol (CAS# 123-51-3), 1-pentanol (CAS# 71-41-0), 2-methyl-1-pentanol (CAS# 105-30-6), and 2-ethyl-1-butanol (CAS# 97-95-0). Detection limits with this procedure are in the order of 0.05 mg/bhp-hr in dilute exhaust, with a quantification limit of 0.1 mg/bhp-hr.

Compound	Empirical Formula	Compound	Empirical Formula
Water Soluble Alcohols			
Methanol	CH_4O	3-Methyl-2-butanol	$C_5H_{12}O$
Ethanol	C_2H_6O	Neopentanol (2,2-dimethyl-1-propanol)	$C_5H_{12}O$
2-Propyn-1-ol	C_3H_4O	1-Pentanol	$C_5H_{12}O$
Allyl alcohol (2-propen-1-ol)	C_3H_6O	2-Pentanol	$C_5H_{12}O$
Isopropanol	C_3H_8O	3-Pentanol	$C_5H_{12}O$
n-Propanol	C_3H_8O	tert-Pentanol (2-methyl-2-butanol)	$C_5H_{12}O$
Crotyl alcohol (2-buten-1-ol)	C_4H_8O	Phenol	C_6H_6O
n-Butanol	$C_4H_{10}O$	3-Methyl-1-pentyn-3-ol	$C_6H_{10}O$
Isobutanol (2-methyl-1-propanol)	$C_4H_{10}O$	Cyclohexanol	$C_6H_{12}O$
sec-Butanol (2-butanol)	$C_4H_{10}O$	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$
tert-Butanol (2-methyl-2-propanol)	$C_4H_{10}O$	2,2-Dimethyl-1,3-dioxolane-4-methanol	$C_6H_{12}O_3$
Furfuryl alcohol	$C_5H_6O_2$	2,5-Tetrahydrofurandimethanol	$C_6H_{12}O_3$
2-Methyl-3-butyn-2-ol	C_5H_8O	1-Hexanol	$C_6H_{14}O$
Cyclopentanol	$C_5H_{10}O$	2-Methyl-1-pentanol	$C_6H_{14}O$
Tetrahydrofurfuryl alcohol	$C_5H_{10}O_2$	3-Methyl-3-pentanol	$C_6H_{14}O$
Isopentanol (3-methyl-1-butanol)	$C_5H_{12}O$	4-Methyl-2-pentanol	$C_6H_{14}O$
2-Methyl-1-butanol	$C_5H_{12}O$	3.3-Dimethyl-2-butanol	$C_6H_{14}O$
3-Methyl-1-butanol	$C_5H_{12}O$	2-Ethyl-1-butanol	$C_6H_{14}O$
Water Soluble Ethers			
Methyl ether	C_2H_6O	Ethyl ether	$C_4H_{10}O$
Methyl ethyl ether	C_3H_8O	Methyl propyl ether	$C_4H_{10}O$
Vinyl ether	C_4H_6O	Isopropyl ether	$C_6H_{14}O$
Cyclopropyl methyl ether	C_4H_8O	Propyl ether	$C_6H_{14}O$

Table 8.6 Selected C_1 to C_6 Alcohols and Ethers That Have Some Solubility in Water

8.4.7. PAH

In addition to the regulated and C_1 to C_{12} hydrocarbon exhaust emissions, semi-volatile (volatile- and particulate-phase) PAH compounds will also be determined for each fuel. The analytical method is similar to CARB SOP MLD 429 using an isotope dilution technique. The 19 PAH target compound list includes: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, benzo[e]pyrene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, 2-methylnaphthalene, naphthalene, phenanthrene, perylene, and pyrene. A 400 in^2 fluorocarbon-coated glass fiber filter (20×20-inch Pallflex filter) is used to collect the particulate-phase PAH, and a PUF/XAD/PUF sandwich adsorbent trap for the volatile-phase PAH. The PUF/XAD/PUF traps contain a layered sampling media consisting of a 1.25 inch deep layer of polyurethane foam (PUF), a 0.5 inch deep layer of XAD-2 resin, and a second 1.25 inch deep layer of PUF. The XAD-2 resin is incorporated to improve the trapping efficiency for the lighter PAH compounds.

Volatile-phase PAH samples present a particular problem for light-duty sampling because conventional sampling techniques do not allow for sufficient sample to be gathered to meet EPA detection requirements. Commercially available sampling media and hardware are of insufficient size to allow for the collection of sample volumes needed to meet these detection limits. Sampling media size is also limited by the ability to extract and concentrate the samples obtained. Therefore, the following approach has been devised involving both custom built sampling hardware and a modified sampling plan. The PUF/XAD/PUF traps are sized to allow a media diameter of 4 inches, rather than the conventional 2.5 inches. This larger diameter allows a much higher flowrate to be used, while maintaining the face velocity within recommended levels for the smaller, conventional sampling media. This volume of dilute exhaust sample is sufficient for the analysis to meet a detection threshold of 1 ng/hp-hr.

Prior to sampling, both the XAD-2 and PUF sample media are cleaned. First, the XAD-2 is cleaned by siphoning four times with water using a Soxhlet. The residual water is then removed under vacuum. The XAD-2 Soxhlet is extracted three times: once with methanol for 24 hours, once with toluene for 48 hours, and finally with methylene chloride again for 48 hours. The residual methylene chloride is removed by purging with heated nitrogen. For PUF cleaning, each foam disk is Soxhlet extracted three times: once for 24 hours with acetone, once for 48 hours with hexane/ether, and finally for 24 hours with acetone.

Volatile- and particulate-phase PAH samples are obtained using a separate secondary dilution tunnel, this is operated in parallel with the smaller secondary dilution tunnel to obtain the 90-mm filter samples for particulate mass determinations. The PAH tunnel is considerably larger than the 90-mm system in order to allow for the use of 20×20-inch Pallflex sampling media to collect particulate-phase PAH compounds and to allow the use of a specially designed PUF/XAD/PUF trap to collect the volatile-phase PAH compounds. Filter and PUF/XAD/PUF trap samples will be generated during each cold-start and a hot-start test. Background PAH sample sets are obtained by operating the

sampling systems for about two hours with sampling media loaded, but without operating the vehicle.

Following testing, sample sets are delivered to the analytical laboratory for extraction and analysis. In cases where immediate extraction is not possible, samples are stored at 4° C. Just prior to extraction of the PUF/XAD/PUF samples, the material is placed in a Soxhlet, and 25 µL of a PAH internal standard (IS) spiking solution containing 18 deuterated PAHs is spiked onto each PUF/XAD/PUF sample. This spiked solution is used as an internal standard to verify sample recovery during the extraction process. The samples are then extracted for 16 hours with methylene chloride. After extraction, the methylene chloride extract will be reduced to approximately 20 mL with a rotary evaporator and water bath held at 35° C. The concentrated extract will be split into two equal portions: one for storage as a reserve and the other for analysis. Samples for analysis are solvent exchanged to hexane and cleaned with acid or base wash and silica gel fractionation; and the solvent volume reduced to 1000 µL. Each extract is then spiked with 25 µL of a recovery standard (RS) which is a mixture of 1-methylmaphthalene-d10 and p-terphenyl-d14 just prior to analysis by GC/MS. After analysis for the lower molecular weight PAH, the 1000 µL extract is carefully blown down to 100 µL. This reduced sample is then analyzed for benzo(a)anthracene and higher molecular weight PAHs.

The filter extract is treated similarly to the trap extract. One half of each filter is extracted separately, and the unextracted filter half saved as a reserve. Just prior to extraction, 20 µL of the PAH IS is spiked onto each filter after placement in the Soxhlet. Filter samples will be extracted for 16 hours with toluene. After extraction, the methylene chloride extract is reduced to approximately 20 mL with a rotary evaporator and water bath held at 95^{o}C. Samples are then solvent exchanged to hexane and cleaned with acid or base wash and silica gel fractionation; and the solvent volume reduced to 150 µL. Each extract is then spiked with 10 µL of RS just prior to analysis by GC/MS.

Samples for both the volatile- and the particulate-phase PAH are analyzed by GC/MS using an Agilent 5973N MSD 30 m by 0.25 mm i.d. DB-5 column with a 0.25 µm film thickness. A calibration curve consisting of at least five points will be obtained prior to sample analysis to ensure linearity in the range from 3 pg/µL to 1000 pg/µ, and a mid-point continuing calibration performed each day after the initial five point calibration. All PAH IS and RS are at a concentration of 250 pg/µL. For each analysis, a 1 µL aliquot of the sample extract is injected into the instrument. Analysis for PAH compounds is performed using the positive ion/electron ionization (PI/EI) mode. Two or three characteristic ions for each PAH and one or two characteristic ions for each IS are monitored. Each target compound should meet the criterion of a 30 percent relative response factor (RRF) and 30 percent deviation in relation to the mean RRF obtained in the initial and continuing calibrations. A solvent or lab blank will also be analyzed immediately after the last calibration to ensure no carryover. Quantitation limits (QL) should range from 2 to 10 ng per PAH for the PUF/XAD/PUF samples and 2 ng per PAH for the filter samples.

8.4.8. Particulates

Ultrafine, PM 2.5 and particle size distribution will be measured using a rotating disk diluter or other suitable piece of measuring equipment such as an EEPS machine. This equipment typically has a measuring range of 2.5 nm–1000 nm. These techniques use the following principles of operation.

Rotating disc diluter- A portion of the raw exhaust is captured by each cavity of the rotating disk and transported to the measurement channel where it is mixed with HEPA-filtered, particle-free dilution air. The dilution ratio is a linear function of the disk calibration factor (corresponding cavity volume and number of cavities per disk), the rotation frequency and the flow rate of the dilution air:

EEPS- A sample of the exhaust flow is drawn into the measuring device where the particles are positively charged using a corona charger. These particles are then introduced to the measurement region near the centre of a high-voltage electrode column and transported down the column surrounded by HEPA-filtered sheath air. A positive voltage is applied to the electrode and creates an electric field that repels the positively charged particles outward according to their electrical mobility. Charged particles strike the respective electrometers and transfer their charge. A particle with higher electrical mobility strikes an electrometer near the top; whereas, a particle with lower electrical mobility strikes an electrometer lower in the stack. This multiple detector arrangement using highly sensitive electrometers allows for simultaneous concentration measurements of multiple particle sizes.

Particulate data for the E10 and Bu16 fuels will be compared for significant differences with consultation as required from experts in particulate measurements. If differences between the fuels tested are deemed to be significant, the health implications of those differences will be assessed by suitable consultants.

8.4.9. Health Effects

Test work for health effects determination will utilise the Ames[27] Bacterial Reverse Mutation Assay and the *in vitro* Comet Assay in Human Peripheral Blood Lymphocytes. While these tests do not comprise a measure of absolute hazard, they are useful tools for assessing relative hazard and thereby consistent with the overall relative hazard methodology of the Multimedia Assessment. Accordingly, it is proposed to compare results of Ames and Comet genotoxicity assays on combustion emission residues from the proposed fuel (Bu16) and the current market fuel (E10). Comparison of the assay results will be used to deterimine if further testing is warranted. Taken together with the detailed chemical composition analysis of the E10 and Bu16 exhaust emissions being generated in this program, the assay results will provide sufficient data for toxicologists familiar with gasoline engine exhaust to evaluate the utility of animal testing in further informing the evaluation of the multimedia impacts of Bu16.

[27] AMES test procedure is EPA 712–C–98–247 1998

Preparation of the sample quantity required for the assays is difficult due to the low level of tailpipe emissions from automobiles that are compliant with current regulatory requirements. This difficulty in sample generation limits the feasibility of running a broader portfolio of toxicological screening tests.

Each pair of assays (Ames and Comet, one for each fuel) requires about 1 gram of the heaviest constituents of the vehicle exhaust. The target sample size was specified by the independent laboratory chosen to perform the assays based on (1) the high volatility and low water solubility of engine exhausts and (2) an expected number of replicates and doses to calculate the mutagenicity index. Because the amount of condensable species in the exhaust is expected to be only a few milligrams per mile, it is necessary to operate the vehicle for an extended driving distance to generate this quantity of sample.

As described in Section 8.3, the test vehicle will be driven 4000 miles on a chassis dynamometer using the EPA Standard Road Cycle on each fuel. Gaseous emissions tests for each fuel occur at the end of each 4000-mile cycle. The assay samples will be collected from the vehicle exhaust onto filter media at the beginning of each 4000-mile SRC segment. The vehicle exhaust will be connected to a standard light-duty emissions measurement system comprising a full-flow dilution tunnel and Constant Volume Sampler controlled by critical-flow venturi. A 500mm x 500mm Pallflex filter holder will be assembled between the dilution tunnel and the CVS blower / exhaust. This system will allow the Pallflex filter will capture particulates and condensable species from the full flow of diluted vehicle exhaust. The dilution flow rate will be chosen to maintain the filter face temperature below 52 °C per EPA requirements for exhaust particulate sampling.

Butamax completed trial experiments using this sampling arrangement to estimate accumulation rate on the sample filters. The trial experiments indicated approximately 25 repetitions of the Standard Road Cycle (about 650 total miles) will be required to produce a 1 g sample. To monitor accumulation rate and avoid vehicle exhaust restriction from pressure drop, a new 500mm x 500mm Pallflex filter will be used for each 26-mile SRC segment (i.e., the mileage accumulation will be interrupted to change filters at the end of each SRC cycle). Accumulation rate and total sample accumulation will be determined by carefully weighing each filter before and after exposure to the diluted vehicle exhaust. Once the filter weights indicate and aggregate sample of 1g, mileage accumulation will continue to the 4000-mile target for the gaseous emissions tests without the sample filter apparatus.

For extraction, each 20×20-inch filter will be placed in a separate Soxhlet extractor and refluxed for at least 8 hours with toluene/ethanol. The solvent will be removed from the extract material by the use of a roto-evaporator and by a stream of dry nitrogen at ambient temperature. Extracts for each fuel will be combined into a single sample and alternatively dried and weighed until a constant weight is obtained and recorded.

The collected extracts for each fuel will be transferred to BioReliance, Inc for completion of the Ames and Comet assays. BioReliance will follow Good Laboratory Practice Regulations as specified in US EPA GLP Standards 40 CFR 792 and OECD Principles of

Good Laboratory Practice (C(97)186 Final). Complete protocol templates for the assays are embedded below.


Ames Assay Protocol Template.doc


Comet Assay Protocol Template.do

8.5. Data Analysis and Reporting

Following analysis of the data generated, a final report will be prepared. The report will include the following elements –

- Technical details of the test vehicle
- Measurements of relevant properties for the test fuels
- A review of all test conducted and their results
- A detailed statistical evaluation of all emissions measured, including treatment of any outlier data
- Review of the findings of the Ames testing
- Any further analyses warranted by the test program findings
- Discussion of the significance of the results and their implications

9. Impact of Biobutanol on Gasoline Headspace

9.1. Statement of the Knowledge Gap

- **Determine the composition of the headspace of 10vol% ethanol and 16vol% iso-butanol blended California reformulated gasoline blends over a range of temperatures and calculate differences in potency-weighted toxics and reactivity.**

The impact of iso-butanol on evaporative emissions is not currently understood. It is anticipated that the presence of 16% *iso*-butanol will have no substantial impact on the composition of the headspace gases when compared to an E10 gasoline (10% ethanol) at the same vapor pressure and seasonal volatility class (ASTM 4814). The only significant difference that is expected is the presence of *i*-butanol in the headspace rather than ethanol.

9.2. Test Procedure

9.2.1. Overview

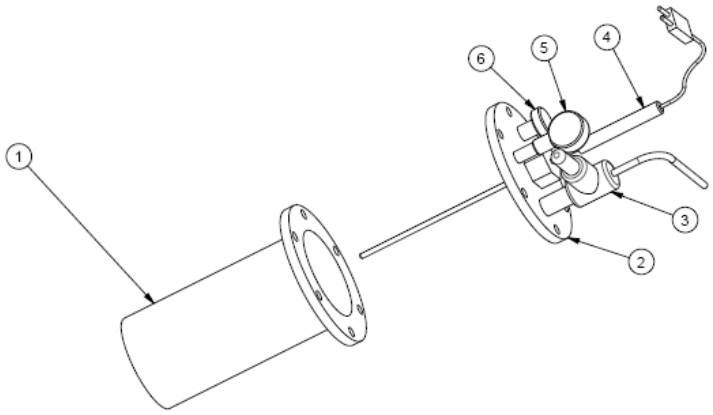
Evaporative emissions will be generated using an evaporative emissions generator (EEG) and subsequently speciated a using a gas chromatograph equipped with a flame ionization detector (FID).

9.2.2. Evaporative Emissions Generator

An Evaporative Emissions Generator (EEG) is a fuel tank or vessel which is heated to cause the volatile portion of the fuel or fuel additive to evaporate at a desire rate. A vessel has been designed and constructed by Southwest Research Institute® (SwRI®) in accordance with the requirements of the Code of Federal Regulation (CFR) Title 40 – Protection of Environment, Part 79 – Registration of Fuels and Fuel Additives, Subpart F – Testing Requirements for Registration, Section 79.57 – Emission Generation.

The EEG is a stainless steel cylinder with a flange on the top for the introduction of the fuel sample. A bleed valve, closed-tip thermocouple which extends to the liquid volume, pressure gauge, and septum-type sampling port are mounted on the top flange. The top flange is bolted onto the main portion of the vessel at the start of each test. A Teflon® ring is used to provide a seal between the bottom and top of the vessel. The assembled

vessel is wrapped with a custom-made thermal blanket. The thermal blanket is connected to a temperature controller to maintain the desired test temperature. **Error! Not a valid bookmark self-reference.** shows a schematic of the EEG vessel.



#	Item
1	Stainless steel chamber bottom
2	Stainless steel chamber top
3	Bleed valve
4	Thermocouple
5	0-15 PSI Pressure gauge
6	Sampling port assembly

Figure 9.1 Evaporative Emissions Generator

9.2.3. Sample Generation

The EEG is filled to 40 ± 5 % of its internal volume (375ml); 150ml of the fuel or additive/fuel mixture being tested is added using a Class A graduated cylinder, and the vessel is sealed. The remainder of the volume, 225ml, is ambient air. The temperature of the fuel in the vessel is then raised to the desired temperature (see table 1 for fuel and temperature test permutations). This temperature is maintained for two hours. During this equilibration period the pressure inside the vessel is monitored and maintained within 10 percent of the ambient atmospheric pressure. Headspace samples are collected after two hours and then at one hour intervals for a total of three samples.

9.2.4. Sample Collection and Analysis

For the purpose of characterizing the evaporative emissions, duplicate samples are collected from the vapor space of the EEG.

First, a 100 µl sample is withdrawn with a syringe, injected into a Tedlar® bag filled with 0.5 ft³ of nitrogen and gently mixed to obtain a homogeneous mixture. A sample of this homogenous mixture is immediately analyzed for hydrocarbon species. Hydrocarbons (C_1-C_{12}) speciation will follow a 3 column GC method based the procedures outlined in the CRC Auto/Oil Phase II methods discussed in SAE Paper No. 930142[28].

Second, a 500 µl sample is withdrawn from the vapor space and introduced into a 50 ml volumetric flask filled with the distilled water. Contents of the flask are vigorously shaken and a sample is analyzed for alcohols. Alcohols (C_1-C_8) speciation will be

[28] Siegl, Walter O., et. al. Improved Emissions Speciation Methodology for Phase II of the Auto/Oil Air Quality Improvement Research Program - Hydrocarbons and Oxygenates, SAE Paper No. 930142, 1993.

performed following the guidelines outlined in the California Air Resources Board, SOP No. MLD 101, revision 2, January 2005[29].

The concentration of all speciated hydrocarbon's and alcohol's will be reported as a percentage of the sampled headspace.

9.3. Test Matrix / Permutations

Two base fuels, representative of the extremes of Californian gasoline, have been selected for the purpose of the evaporative emissions and combustions emissions testing. A more aromatic gasoline from the BP Cherry Point Refinery has been selected; a Bu16 will be prepared from this BOB. A more olefinic (low aromatic) BOB from the BP Carson Refinery has also been identified. From this BOB a Bu16 and an E10 will be blended. All fuels will be blended in compliance with the ARB predictive model (2009). In addition to the evaporative headspace testing, these same fuels are also being used for the test programs described in Sections 3**,** 4**,** 5**,** 7 and 8.

Evaporative emissions will be generated from each fuel at three different test temperatures: 70 F, 105F and 130F (see **Table 9.1**).

Base Fuel	Oxygenate	Test Temperatures (ºF)
CARB Fuel 1	Carson E10	70, 105 and 130
CARB Fuel 2	Cherry Point Bu16	70, 105 and 130
CARB Fuel 3	Carson Bu16	70, 105 and 130
CARB Fuel 4	50:50 Carson E10 : Carson Bu16	70, 105 and 130

Table 9.1 Test Fuels and Temperatures

9.4. Data Analysis and Report

Following analysis of the data generated a final report will be prepared. The report will include the following elements—

- Description of the test apparatus
- Measurements of relevant properties for all test fuels
- A review of all tests conducted and their results
- A detailed statistical evaluation of headspace composition measurements including treatment of any outlier data
- Calculation of any impacts on ozone reactivity for Bu16 relative to E10 using Carter Maximum Incremental Reactivity (MIR) methodology
- Calculation of Potency-Weighted Toxics detected in the headspace samples
- Discussion of the significance of the test findings and any follow-up analyses suggested from review of the data.

[29] "Determination of Alcohols in Automotive Source Samples by Gas Chromatography," California Air Resources Board, SOP No. MLD 101, revision 2, January 2005.

10. Impact of Biobutanol on Permeation Emissions

10.1. Statement of the Knowledge Gap

- **Determine permeation emissions of 16vol% iso-butanol relative to 10vol% ethanol in CARB .**

10.2. Work Plan

10.2.1. Test Fuels

Four test fuels will be involved in this program:

1. Non-Oxygenated comparison fuel - E0
2. 10 V% Ethanol fuel - E10
3. 16 V% Butanol Blend - Bu16
4. 50:50 blend (E10/Bu16)

The blend RVP and Aromatics levels of the test fuels have been matched as closely as possible with oxygen content levels targeted for 3.7 wt% in the oxygenated blends

During new vehicle certification, special procedures are required to accurately measure alcohol concentration and mass in an evaporative or exhaust emission sample. These procedures were used during the E-65 testing for ethanol, and will be required to measure butanol in this program. This will necessitate purchase of a known n-butanol standard for calibration of the SHED FID and the gas chromatograph (GC). A 5-ppmV standard is being employed for this purpose.

During each SHED test, periodic samples will be withdrawn from the enclosure and analyzed with a GC to determine butanol ppmV concentrations. New vehicle certification procedures will be used to separate the butanol concentration from the total HC concentration. This requires determination of a response factor of the SHED FID for butanol, subtraction of the corrected butanol ppm from the FID total, independent computation of the mass of butanol, and mass of remaining HC's using average density for each SHED measurement. The two masses (butanol and HC) are then summed to provide the total SHED mass.

10.2.2. Test Vehicles

The seven vehicles being used in this study are listed in **Table 10.1**. This includes the three vehicles from phase 1 testing (the 2006 Chevrolet Impala, the 2005 Dodge Caravan and the 2003 Toyota Camry) which evaluated the permeation impact associated with a 2.0 wt% oxygen content fuel . Four additional vehicles were added for this study to cover older vehicle groups. Each vehicle was inspected at time of purchase for any indication of non-representative operation, major repair, or for any modifications to the body or fuel systems. The vehicles are all high-volume, representative in-use samples.

Make/Model	Cylinders	Displacement	Certification
2006 Chevrolet Impala	6	3.5L	Tier 2 Bin 8
2005 Dodge Caravan	6	3.3L	LEV II
2003 Toyota Camry	4	2.4L	ULEV
1994 Ford Taurus	6	3.0L	Tier 0
1991 Honda Accord	4	2.2L	Tier 0
1985 Nissan Sentra	4	1.6L	Tier 0
1981 Buick Riviera	8	5.0L	Tier 0

Table 10.1 Vehicle Fleet for Permeation Study

Test vehicles will be drained and filled with non-oxygenated fuel when they arrive at the laboratory. The vehicles will be operated on the dynamometer for a minimum of 45 minutes (2 X LA-4 preconditioning) at least once per week until dismantled.

An incoming vehicle inspection will document vehicle condition and all identification data including odometer, VIN, engine size, emission identification data, and a description of emission control systems. Digital photographs will be included in the vehicle log.

An evaporative system evaluation test will be performed and reported before the vehicle is dismantled. The test will consist of an LA-4 preconditioning, drain and 40% fill with non-oxygenated 7.0 psi fuel, 12 - 36 hour soak, a bag-only FTP exhaust emission cycle, a one-hour hot soak evaporative test, and one 24 hour 65-105°F California diurnal test.

All inspection results will be reviewed with the Program Manager and Test Sponsor before continuing.

Following approval, each vehicle's fuel system will be carefully removed from the vehicle and mounted on a custom constructed aluminum frame for testing (the "rig"). A photo of a typical "test rig" is included below as Figure **10.1**..

Figure 10.1 Typical Fuel Test Rig

For E-65, the fuel tank, fuel inlet and cap, vapor control canister, vapor and purge lines, and fuel lines to the inlet at the manifold were tested. The intake manifold, fuel injectors,

and other engine parts were not included in the permeation test. This same protocol is planned for this study.

Fuel caps will be fitted with a stainless steel Swagelok® adapter to permit venting of the fuel tank vapor space. The outlet of the fuel vapor evaporative control canister will be sealed and fitted with a similar adaptor. During testing, fuel vapors will be directed from the two rig outlets to matching bulkhead fittings in the SHED wall, using non-permeable Teflon® hoses. Provisions will be made to activate the fuel pump, permitting weekly circulation of fuel in the rig through all OEM lines and hoses. Similar adaptors to operate purge control valves will be installed for weekly system purge simulation. All openings, other than the two vent outlets, will be fitted with caps and/or valves to positively seal the rigs during testing.

The express purpose and intent of this operation is to insure that only the low-level fuel system permeation emissions existing on the whole vehicle are measured during the test program. No repairs or additional parts are to be added, and no joints or connections on the vehicle's fuel system will be broken during transfer from vehicle to fuel test rig. The resulting rig will represent the fuel system as it previously existed on the production vehicle.

10.2.3. Test Procedures

Each rig will be tested with the base fuel (E0), the 10 V% Ethanol fuel (E10), the 16 V% Butanol fuel (Bu16) and a 50:50 Blend (E10/Bu16). For each fuel, the test rig will receive a fuel flush with the upcoming fuel, stabilization, and weekly evaluations at 105°F. A final evaluation with a California two-day diurnal test will follow.

The stabilization period begins with a fuel flush and 100% fill with the appropriate test fuel. Once each week, the rig will be transferred to a SHED for a 5-hour steady-temperature permeation stability check. The weekly result, and a running three-week average result, will be reported. A rig is considered stable when the last of three consecutive running average results "reverses" the trend seen in the previous two. For example, a rig's first two running averages may show a steady decrease in readings, whereas the third running average will reverse this trend and show an increase. At this time, the rig is considered stabilized with the new fuel.

A conservative estimate of nine weeks maximum has been incorporated into project cost estimates for stabilization purposes.

When all agree that the rig and fuel have stabilized, a final diurnal test will be performed. Fresh fuel will be used for the diurnal test. A two-day California diurnal temperature cycle (65 – 105°F) will be performed. The SHED atmosphere will receive detailed speciation analysis for each diurnal day. When all test results are complete, the rig will then be readied for the next fuel evaluation. The process will be repeated until all three fuels have been tested and results deemed complete.

The following provides a synopsis of the detailed test procedures proposed:

10.2.4. Fuel Change Procedure

1. Drain existing fuel
2. 10% fill with fuel for upcoming test
3. Rock rig to wash walls
4. Activate fuel pump to circulate
5. Drain & fill 10%
6. Repeat rig rocking and fuel circulation
7. Drain and fill 100%
8. Place rig in soak

10.2.5. Weekly Fuel Circulation – 2 days before test

1. Extend a fuel line from fuel outlet to tank inlet
2. Activate fuel pump for 5 minutes – ensure fuel is flowing
3. Restore rig to standard condition

10.2.6. Weekly Canister and vapor space purge – 2 days before test

1. Verify canister outlet is open (OBDII valve open, if required)
2. Connect vacuum pump inlet to purge line, pump outlet to lab exhaust
3. Activate vacuum pump for 15 minutes (0.8-CFM minimum)
4. Restore rig to standard condition

10.2.7. Steady State Stability test

1. Set temperature to 105°F
2. Evacuate and fill SHED volume compensation bag
3. Transfer rig to SHED.
4. Connect fuel cap and canister outlets to SHED bulkhead fittings.
5. Verify Teflon lines do not have condensation to block free flow.
6. Locate ambient temperature probe under fuel tank on rig
7. Allow SHED temperatures to stabilize

8. Seal door and open volume compensation bag
9. Start data logger
10. Zero and span FID
11. Allow rig to stabilize a minimum of one hour before 1st reading
12. Collect GC sample; record initial FID, temperature, and barometer
13. At hour 3 and hour 5 repeat GC sample, SHED readings
14. Check linearity of first 3 observations, continue for additional 2 hours if indicated
15. Return rig to 105°F soak

10.2.8. Diurnal Test

1. Set SHED temperature to 65°F
2. Drain the tank, fill to 40% with fresh fuel, transfer to SHED when complete
3. Connect fuel cap and canister outlets to SHED bulkhead fittings.
4. Verify Teflon lines do not have condensation to block free flow.
5. Locate ambient temperature probe under fuel tank
6. Allow rig to soak a minimum of 8 hours at 65°F
7. Evacuate and fill Volume compensation bags
8. Start data logger
9. Zero and span FID
10. Start temperature profile
11. At t = 0 hours, t = 24 hours, and t = 48 hours collect GC bag sample and record FID, barometer and temperature
12. Store rig in main soak room after diurnal test until approved

10.3. Data Analysis and Reporting

Following analysis of the data generated, a final report will be prepared. The report will include the following elements –

- Technical details for all test rigs
- Measurements of relevant properties for all test fuels
- A review of all tests conducted and their results
- A detailed statistical evaluation of all emissions measured and treatment of any outlier data

- Test data will be compared across the various vehicle fuel systems to determine the impact of permeation from iso-Butanol blends and compare them to equivalent Ethanol blends.
- Speciated data from the permeation will be used to quantify the oxygenate contribution to the permeation emissions and the ozone reactivity of the permeate for comparison across fuels and vehicle systems.
- Hydrocarbon profiles will be developed from the fuel to the permeation emissions for each fuel type.
- The Ozone reactivity for each fuel will be calculated using the Carter Maximum Incremental Reactivity (MIR) methodology to estimate the ozone forming potential of the permeation emissions.
- Potency-weighted toxics will be calculated for each of the permeates and any differences found between fuels will be identified
- The permeation emissions will also be compared across technology groups to determine impacts of fuel system technology on each fuel type. Significance of test findings will be reviewed along with any additional analyses warranted upon review of the data. Questions to be answered include:

    1) Does iso-Butanol increase permeation when compared to Ethanol at 3.5 to 3.7wt% oxygen content?
    2) Is there a synergistic impact of commingling Ethanol and iso-Butanol blended fuels? What is that synergistic impact?
    3) What impact does iso-Butanol have on ozone reactivity of the permeation emissions?

Lastly, all vehicle fuel system data across technology groups and fuels will be combined to determine a fleet impact of Isobutanol blends compared to ethanol blends.

11. Environmental Fate of Biobutanol

11.1. Statement of the Knowledge Gap

- **Complete environmental fate studies currently in progress.**

Iso-Butanol Environmental FateButamax has undertaken a series of experiments to facilitate the modeling of a BTEX (Benzene, Toluene, Ethyl-Benzene, and Xylene) plume if an iso-butanol blended gasoline were to be accidentally released underground. These experiments will generate the data necessary to better understand both biodegradation of iso-butanol blended gasoline in anaerobic conditions, and the transport properties of this same fuel. Although the project is still in progress, the biodegradation experiments that feed into this environmental-fate work are underway.

Program objectives are as follows --

- Determine biodegradation characteristics of iso-butanol blended gasoline under different environmental conditions.
- Analyze biodegradation pathways of pure iso-butanol under different environmental conditions and determine potential formation and kinetics of metabolites (e.g. iso-butyric acid, iso-butylaldehyde)
- Determine the distribution coefficients for BTEX and iso-butanol in water and gasoline.
- Determine the adsorption coefficients of pure iso-butyric acid[30], a key iso-butanol metabolite, in various soil types.
- Develop a model to evaluate the impact of iso-butanol on elongation of BTEX plumes if iso-butanol blended gasoline were to be released underground.

The five objectives of this study are to be completed through a series of four separate, but related, experiments. These four experiments and the subsequent modelling work are outlined below in Sections **11.2** through **11.6.**

The output from the below experiments and modelling work will feed into an evaluation of soil cleanup impacts comparing a potential spill of an iso-butanol blended gasoline to an ethanol blended gasoline, and this evaluation will be included in the Tier III report.

[30] Biodegradation studies have shown that iso-butanol rapidly degrades to iso-butylaldehyde which, in turn, very rapidly degrades to iso-butyric acid. Accordingly, the adsorption work focuses on iso-butyric acid as the species which will be most meaningful to study.

11.2. Biodegradation of Iso-Butanol Blended Gasoline under Different Environmental Conditions

This study will determine the biodegradation properties of an iso-butanol blended gasoline in both aerobic and anaerobic conditions. Butamax regards this study as crucial to evaluate the environmental risks of iso-butanol blended fuel associated with potential groundwater contamination scenarios (e.g. assessment of dissolved plume length, migration and transport of fuel molecules). Shaw Environmental was contracted by BP to perform this biodegradation study and this work has already been published[31].

11.2.1. Soil and Groundwater Collection

Vandenberg Air Force Base in Santa Barbara County, California was selected as an ideal site to collect the soil and groundwater needed for this study. One reason was that this site has been studied extensively by the academic community and remediation industry. This site was the location of an accidental MTBE leak, and then a controlled release of an ethanol blended gasoline by Professor Douglas MacKay from the University of California at Davis.

11.2.2. Microcosm Preparation and Monitoring

The biodegradation microcosms were established in two main groups, aerobic and anaerobic. The aerobic and anaerobic microcosms were prepared using different portions of soil, water, and headspace.

The microcosms were designed to study the influence of iso-butanol and ethanol on BTEX degradation at two concentration levels of BTEX and alcohols. These two levels were established to simulate the "high" level of BTEX and alcohol that would likely be present in the close proximity to the source area. The other level was designated as "low" and intended to simulate the concentration of BTEX and alcohol further from the source. Below in Table **11.1** is a matrix showing the levels of iso-butanol, ethanol, and BTEX in the "high" and "low" microcosms.

	Benzene	Toluene	Ethylbenzene	Xylenes	Isobutanol	Ethanol
Low	1.4	0.3	0.4	0.8	5	500
High	14	3.0	4.0	8.0	250	

Table 11.1 Concentration of BTEX, iso-Butanol and Ethanol in Microcosms (mg/L)

[31] Schaefer, C.E., Yang, X., Pelz, O., Tsao, D.T., Streger S.H., Steffan, R.J., 2010. Anaerobic biodegradation of iso-butanol and ethanol and their relative effects on BTEX biodegradation in aquifer materials. Chemosphere, In Press, Corrected Proof, Available online 27 September 2010. http://dx.doi.org/10.1016/j.chemosphere.2010.09.002

Schaefer, C.E., Yang, X., Pelz, O., Tsao, D.T., Streger S.H., Steffan, R.J., 2010. Aerobic biodegradation of iso-butanol and ethanol and their relative effects on BTEX biodegradation in aquifer materials. Chemosphere, In Press, Corrected Proof, Available online 27 September 2010. http://dx.doi.org/10.1016/j.chemosphere.2010.09.003

For clarity, every microcosm that contained high iso-butanol also contained high BTEX, i.e. there was no mixing between high and low concentrations of components. The only exception to this rule was the ethanol microcosms, where only one concentration of ethanol was used due to its relatively high solubility in water. This was done on the assumption that ethanol would be at a "high" concentration throughout the plume.

As a control, several of the microcosm treatments included a killed control. For these microcosms, the soil and groundwater is sterilized[32] to ensure that all of the microorganisms present in the soil were killed. These control experiments are designed to test whether or not there are any abiotic losses of BTEX and alcohol, e.g. the iso-butanol does not react with an unknown soil component. If there are no abiotic processes present, the concentration of BTEX and the alcohols in the KC microcosm should remain constant throughout the study.

To simulate multiple soil conditions that occur naturally, several different electron acceptors were added to the groundwater, including nitrate, sulfate, and iron(III). The term "unamended" is used for microcosms in which no electron acceptors were added. In these microcosms, the only electron acceptors present were those in the groundwater and soil when they were collected.

A list of all the microcosm treatments is below in Table **11.2**. For the purposes of this table, the following definitions of soil types are used –

- **Uncontaminated Soil** - This soil had not previously been exposed to hydrocarbons or oxygenates, and should have been virgin soil. For the Vandenberg samples, the contaminated soil was taken upstream of the underground water flow that traveled through the spill sight.

- **Remediated Soil** - This soil sample from the Vandenberg site was previously exposed to HC and oxygenates. However, the soil has been remediated by Shaw to reduce BTEX levels to acceptable levels. The microbes living in this area have potentially adapted to more readily consume HC and oxygenates. Further in some cases, you may expect the soil to be depleted of nutrients and reducers (e.g. nitrates) that microbes can use to consume HC and oxygenates.

[32] Sterilization is effected through treatment with mercuric chloride (700 mg/L) followed by 1% v/v formaldehyde.

Aerobic Microcosm - Remediated Soil		
	Low Concentration BTEX	
		KC with BTEX
		KC with BTEX and IBA
		Live with BTEX
		Live with BTEX and IBA
		Live with BTEX and ethanol
	High Concentration BTEX	
		KC with BTEX
		KC with BTEX and IBA
		Live with BTEX
		Live with BTEX and IBA
	Additional High Conc. BTEX	
		KC with BTEX and ethanol
		Live with BTEX and ethanol
		Live with BTEX and IBA
Anaerobic Microcosm - Remediated Soil		
	Low Concentration BTEX	
		KC with BTEX and IBA
		KC with BTEX and ethanol
		Live-Unamended with BTEX and IBA
		Live-Unamended with BTEX and ethanol
		Live-Nitrate reducing with BTEX and IBA
		Live-Iron reducing with BTEX and IBA
		Live-Sulfate reducing with BTEX and IBA
		Live-Sulfate reducing with BTEX and ethanol
	High Concentration BTEX	
		KC with BTEX
		KC with BTEX and IBA
		Live-Unamended with BTEX
		Live-Unamended with BTEX and IBA
		Live-Nitrate reducing with BTEX
		Live-Nitrate reducing with BTEX and IBA
		Live-Iron reducing with BTEX
		Live-Iron reducing with BTEX and IBA
		Live-Sulfate reducing with BTEX
		Live-Sulfate reducing with BTEX and IBA
Anaerobic Microcosm - Uncontaminated Soil		
	Low Concentration BTEX	
		KC with BTEX and IBA
		Live-Unamended with BTEX and IBA
	High Concentration BTEX	
		KC with BTEX
		KC with BTEX and IBA
		Live-Unamended with BTEX
		Live-Unamended with BTEX and IBA

Table 11.2 Complete List of All Microcosm Treatments (IBA = iso-butanol)

The first order biodegradation rates for iso-butanol and ethanol were determined from these microcosm experiments. However, the detailed kinetics of iso-butanol degradation and its metabolites will be determined in the evaluation described in Section **11.3.**.

11.2.3. Enrichment Study

Enrichment testing was also conducted on aerobic iso-butanol and ethanol degrading bacteria. The purpose of enrichment testing was to grow specific strains of microorganisms for further evaluation. For example, a microorganism that degrades iso-butanol can be grown, isolated, and identified. One benefit to this characterization is that if it is discovered that the microorganism is common in various soil environments, then the conclusions of this biodegradation evaluation are applicable to more than just Vandenberg AFB.

11.2.4. Analytical Methods

In Table **11.3** below, are some of the analytical methods that Shaw Environmental used for the biodegradation evaluations.

Analysis	Method	Matrix	Approx. Sample Volume
VOCs	GC-FID	Headspace	10 - 200 µL
VOCs	GC-MS (EPA Method 8260)	Aqueous / Soil	8 ml / 20 - 40 g
Alcohols	GC-FID (EPA Method 8015B	Aqueous	1 µL - 2 ml
SVOCs	GC-MS (EPA Method 8260)	Aqueous / Soil	500 ml / 20 - 40g
GRO	GC-FID	Aqueous / Soil	500 ml / 20 - 40g
TOC	EPA Method 415.1	Aqueous / Soil	500 ml / 20 - 40g
Oxygen	TCD	Headspace	10 - 200 µL
Anions	IC (EPA Method 300)	Aqueous	5 ml
Methane	GC-FID	Headspace / Aqueous	10 - 200 µL / 4 mL
Total and dissolved Fe and Mn	ICP	Aqueous	20 - 100 ml
Dissovled Fe (in microcosms)	Hach Kit	Aqueous	2 ml
Hardness	EPA Method 130.2	Aqueous	50 ml
Alkalinity	EPA Method 310.1	Aqueous	100 ml
Total Nitrogen	EPA Method 351.3	Aqueous	500 ml

Table 11.3 Analytical Methods Used by Shaw Environmental

GC – Gas Chromatograph / MS – Mass Spectrometer / FID – Flame Ionization Detector

TCD – Thermal Conductivity Detector / IC – Ion Chromatography / ICP – Inductively Coupled Plasma

11.3. Degradation Pathways and Kinetics of Iso-Butanol

Existing literature indicates that iso-butanol biodegrades rapidly under aerobic conditions. However, biodegradation data under anaerobic conditions could not be found, and first order reduction rates were not available when Butamax submitted the Cal EPA MMA Tier I report. Since then, BP, DuPont, and Shaw Environmental did determine first order reduction rates as part of work described in Section **11.2**, and these values will be published. However, the planned study described in this section involves the use of radioisotope labelling to determine detailed degradation pathways, kinetics, and potential problematic metabolites of iso-butanol degradation under both aerobic and anaerobic conditions in pristine and contaminated environments (e.g. soils from Vandenberg AFB as well as other potential sources).

In addition, the odor and taste concerns for potential iso-butanol metabolites are at the trace level (pbb, ug/L) and thus it is analytically challenging to detect the metabolites of concern[33]. The radioisotope labeling facilitates the detection and measurement of these metabolites at very low concentrations. Since one of the potential biodegradation metabolites, iso-butylaldehyde, is an irritating chemical of unpleasant odor, which at excessive doses may be absorbed into the body via all routes of exposure, it is important to determine if such metabolites may accumulate during iso-butanol biodegradation under certain environmental conditions.

BP and DuPont's joint Biofuels Product Stewardship Team (PST) has recommended this evaluation be performed by DuPont's Haskell Global Centers for Health and Environmental Sciences (HGC), and below is the protocol recommended by HGC.

Soil biodegradation of ^{14}C-Labeled Iso-butanol under aerobic and anaerobic conditions

11.3.1. Introduction

Using ^{14}C-labeled iso-butanol to study its biodegradation in the environment offers several advantages over using non-radioisotopic labeled iso-butanol:

1) Achieve better mass balance. It allows quantifying the portions of ^{14}C-labeled iso-butanol and potential transformation products that are bound to the soil (which can not be extracted with an organic solvent). It also allows capturing $^{14}CO_2$ lost to the headspace due to iso-butanol biodegradation. These two portions can account for ~50% of initially-dosed iso-butanol during biodegradation.

[33] *Butamax will conduct a literature survey to determine if the taste and odor threshold for the iso-butanol metabolites are already known. This information, combined with the known concentrations and persistence of these metabolites (measured as part this Tier II WP), will determine the need for future odor and taste studies. If needed, the protocol would be similar to the iso-butanol odor and taste study submitted as part of Butamax Tier I report.*

2) Allow unequivocally identify ^{14}C-labeled transformation products that are extremely difficult to be identified with non-labeled iso-butanol as the starting material.

3) Enhance detection limit to allow identifying and quantifying low levels of potential transformation products.

11.3.2. Study Objectives

The [methyl-^{14}C] iso-butanol biodegradation pathway under various environmental conditions was investigated to determine iso-butanol biodegradation potential in soil. Specific objectives included: 1) Quantify iso-butanol primary biodegradation rate; 2) Identify potential transformation products; 3) Determine molar yields of each individual transformation product; and 4) Establish iso-butanol biodegradation pathways under aerobic and anaerobic conditions.

Study material - Custom synthesized [methyl-^{14}C] iso-butanol with 99.9% radiochemical purity (specific activity = 52.9 mCi/mmol) was used to study iso-butanol biodegradation.

Biodegradation equipment - Glass serum bottles (129-mL volume) containing 10g soil, 1-10 mL deionized water, and 0.4 g iso-butanol kg^{-1} soil were crimp-sealed with butyl rubber stoppers and incubated at 14 – 15 °C under dark. For anaerobic studies, headspace gas pressure was controlled within 1 bar to prevent stopper bulging and gas leakage.

Shaw lab studies described in Section 11.3 used non-radiolabeled isobutanol and ~ 40 g soil per sample bottle.

Soils

- **Aerobic**: Two surface soils, one from Delaware and one from California, were used within 3 months after collection and storage at ~ 4 °C. The water content of aerobic soil was ~ 20%. One to two mL of distilled water was added to each bottle containing 10 g soil.

- **Anaerobic-sulfate reducing**: Subsurface (at least 8 ft below ground) soil was collected from California. The water content of the soil was approx. 22%. Ten mL of ground water (from California) and 0.020 mL of sterile $MgSO_4 \cdot 7H2O$ stock solution (37.2 g/L) were added to each bottle containing 10 g soil.

- **Anaerobic-methanogenic condition**: The same subsurface (at least 8 ft below ground) soil was collected from California. Ten mL of ground water (from California) was added to each bottle containing 10 g soil.

Duration of the Studies

- **Aerobic soil** – at least 90 days. Isobutanol is biodegradable and it will be degraded within 90 days.

- **Anaerobic soil** – 180 days or longer. Degradation under anaerobic condition is slower than that under aerobic, 180 days or longer will ensure its complete degradation.

Sample Processing – The headspace O_2 content was monitored periodically in sample bottles. When oxygen content within the bottles dropped below 10%, the headspace was purged with air to ensure aerobic conditions. At each sampling time point, some sample bottles were sacrificed for sampling and processing. The headspace gas of each sample bottle was purged through two ^{14}C-volatile traps (consisted of 20 - 40 mL cold methanol or ethanol in each glass serum botttle to trap volatile isobutanol and other potential volatile transformation products.) and two $^{14}CO_2$ traps (each contained 20 – 40 mL of 50 mN NaOH) mounted in tandem to capture and analyze volatile ^{14}C lost to the headspace. After headspace sampling, the soil in the sample bottles was extracted by a suitable solvent (e.g. acetonitrile) for further analysis. The headspace O_2 content (aerobic conditions) and CO_2, CH_4, and H_2 gases (anaerobic conditions) were monitored in control bottles (contained non-labeled iso-butanol only).

Sample Analysis - ^{14}C-volatile traps and soil extracts from all samples were used for liquid scintillation counting for total ^{14}C counts from different compartments, LC/ARC (Liquid Chromatography/Accurate Radioisotope Counting) and for LC/MS/MS or GC/MS analysis to quantify and identify ^{14}C-labled iso-butanol and potential ^{14}C-labeled transformation products. The non-extractable portions of ^{14}C remaining in the soil were either combusted or further extracted with acetonitrile plus concentrated HCl to accounted for soil-bound ^{14}C.

It should be noted that the ^{14}C-labeled material is not physically separated from the cold ^{12}C in this analysis. The starting material contained both cold (^{12}C) and ^{14}C-labeled isobutanol and both forms are subject to biodegradation by the microorganisms in the same manner.

The amount of ^{14}C is differentiated from ^{12}C through different measurements on the CO_2 traps. Scintillation counting gives the amount of ^{14}C and analysis of total carbon trapped provides the sum of ^{12}C + ^{14}C. The amount of ^{12}C can then be determined by difference.

11.4. Environmental Transport of Iso-Butanol and BTEX from Spills and Leaking USTs

If a gasoline mixture is accidently released underground, the gasoline components may mix into the local groundwater. The concentration of the hydrocarbon components in the ground water is termed enhancement. A series of simple batch experiments will be conducted to assess the enhancement of BTX in groundwater. The primary goal of these

experiments will compare the enhancement of BTX when iso-butanol is used as an alternative oxygenate to ethanol.

11.4.1. Cosolvency Effects of Iso-butanol on Gasoline Solubility in Water

To test the effects of iso-butanol on the equilibrium partitioning coefficients of benzene, toluene, m-xylene (BTX), 1,2,4-trimethylbenzene (TMB), and iso-octane (see Table **11.4**), a synthetic gasoline mixture will be created to contain 16% (v/v) iso-butanol. The composition of the mixture is summarized in Table **11.5** and approximates the expected commercial grade iso-butanol-blended gasoline fuel. Identical experiments will also be run using the synthetic gasoline made with ethanol instead of iso-butanol, and also without any alcohol added. The compositions of these mixtures are also summarized in Table **11.5** using iso-octane to make up the volumetric balance. Bulk mixtures of each synthetic gasoline will be created and analyzed at BP's Global Fuels Technology Laboratory in Naperville, IL prior to initiating the partitioning studies.

These fuel mixtures will be contacted with varying amounts of a synthetic groundwater created from distilled water containing 0.005 M $CaCl_2$ (He and Rixey, 2004). Sixty (60) ml solutions of the synthetic fuel and water will be prepared in clear, pre-weighed 60-ml EPA VOA vials ("Test Vials") with open top caps and PTFE silicone septa (Fisher-Scientific #340-60C and #300-125, respectively; see Figure **11.1** EPA VOA Vials with Open Top Caps (left) and PTFE Silicone Septa (right)). The water:fuel (ml:ml) volumetric ratios to be prepared will include 10:50, 20:40, 30:30, 40:20, and 50:10 (zero headspace). The appropriate volume of fuel will be injected first into the Test Vial and then weighed, followed by injecting the synthetic groundwater, and then weighed again. These recorded masses will be used to calculate the total mass of synthetic fuel and groundwater in each Test Vial. The mixtures will be injected into the Test Vials using dedicated (one per fuel or groundwater) 50-ml Hamilton® gas tight syringes with PTFE luer lock and stainless steel 22-gauge needles (Sigma-Aldrich #20707 and #21746, respectively; see Figure **11.2**). Each solution will be prepared in triplicate and agitated on a platform shaker overnight. After settling, each Test Vial will be inverted (cap on the bottom) and each batch will be allowed to equilibrate for one week in a non-agitated state at room temperature (24±1 °C).

Figure 11.1 EPA VOA Vials with Open Top Caps (left) and PTFE Silicone Septa (right)

Chemical	CAS Number	Source	Purity
Iso-butanol	78-83-1	Acros	99+%
Ethanol	64-17-5	Acros	99.50%
Benzene	71-43-2	Fisher Scientific	99.50%
Toluene	108-88-3	Acros	99.50%
m-Xylene	108-38-3	Acros	99+%
1,2,4-trimethylbenzene	95-63-6	Acros	98%
Iso-octane[34]	540-84-1	Acros	99+%
Anhydrous CaCl2	10043-52-4	Acros	96%

Table 11.4 Purity and Sources for Stock Chemicals

[34] Typical isomer used in fuel compositions

Constituent	Molecular Weight, MW (g/mol)	Aqueous Solubility, $S_{aq,\,i}$ (mg/L)	Relative Density (g/ml)	Weight Fraction, x_i (g/g)	Iso-butanol Blend Volume Fraction (100 ml basis)	Ethanol Blend Volume Fraction (100 ml basis)	Petroleum Blend Volume Fraction (100 ml basis)
Iso-butanol	74.12	87,000	0.80	0.16[35]	16	--	--
Ethanol	46.07	miscible	0.79	0.14[36]	--	14	--
Benzene	78.11	1,780	0.88	0.004	0.4	0.4	0.4
Toluene	92.14	535	0.87	0.05	4.6	4.6	4.6
m-Xylene	106.20	135	0.86	0.13	12	12	12
TMB	120.19	57	0.88	0.32	29	29	29
Iso-Octane	114.22	2.1	0.70	balance	38	40	55
Iso-butanol Blend	157.74		0.80	1.00	100		
Ethanol Blend	153.10		0.80			100	
Petroleum Blend	152.03		0.79				100

Table 11.5 Composition of the Synthetic Gasoline Mixtures Containing 16% (m/m) iso-Butanol, 14% (m/m) Ethanol or Without Alcohol

Figure 11.2 Hamilton(r) Gas Tight Syringe with PTFE Luer Lock (above) and 22-Gauge Stainless Steel Beveled-Tip Needle (below)

Upon completion of the equilibration, one (1) ml of the aqueous phase in a Test Vial will be withdrawn using a 1-ml Hamilton® gas tight syringe with PTFE luer lock and stainless steel needle (Sigma-Aldrich #20997 and #21746, respectively). After withdrawing into the syringe, the aqueous solutions will be injected directly into pre-weighed 40-ml VOA vials ("Sample Vials") containing an appropriate preservative supplied from a commercial laboratory. Samples will be taken from lowest (i.e. 50:10 water:fuel) fuel concentration to highest (10:50 water:fuel). One syringe will be used for each synthetic fuel and one removable needle will be used for each set of replicates. After injecting, the Sample Vial will be weighed again. The syringe and needle will be rinsed between uses with three to five, full draw-volumes of deionized water. The remaining headspace will be eliminated by injecting additional deionized water using a dedicated 50-ml Hamilton®

[35] 16% (w/w) iso-butanol is approximately equivalent to 16% (v/v) iso-butanol

[36] Composition based after He and Rixey, 2004

gas tight syringe with PTFE luer lock and stainless steel needle (Sigma-Aldrich #20707 and #21746, respectively). Three (3) Sample Vials will be prepared from each Test Vial, as required for analysis at the commercial laboratory. The triplicate Sample Vials will be immediately stored on ice (<4 °C) and shipped overnight for analysis of each constituent using EPA Method 8260B (GC/MS) with Method 5030 (purge and trap).

Each shipment will also contain QA/QC samples (pre- and post-experimental method/equipment blanks, and a trip blank) and one duplicate analytical sample from each set of water:fuel ratios. The solutions for the pre-experimental method/equipment blanks will be prepared using sixty (60) ml of 100% synthetic groundwater (0.005 M $CaCl_2$ solution) at the beginning and at the conclusion when the fuel/water mixtures are prepared and will otherwise be treated as a fuel mixture and sampled for analysis at the conclusion. Likewise, post-experimental method/equipment blanks will also be prepared using the 100% synthetic groundwater at the beginning and at the conclusion when the aqueous samples are collected after the one week equilibration. Duplicate samples will be collected at the same time the aqueous samples are collected at the conclusion of the equilibration. Trip blanks will be prepared using 100% synthetic groundwater after the post-experimental method/equipment blanks, aqueous, and duplicate samples are collected. Each of these QA/QC samples will be produced in triplicate for analysis, as required for the commercial laboratory.

For each synthetic gasoline mixture (iso-butanol, ethanol, or without alcohol), the number of analyses is 25 (5x3 aqueous replicates + 5 duplicates + 2 QA/QC pre-experimental method/equipment blanks + 2 QA/QC post-experimental method/equipment blanks + 1 QA/QC trip blank). The samples from each of the three different synthetic gasoline mixtures will be shipped separately to avoid cross-contamination. The total number of sample vials shipped will be at least 225: (5x3 Test Vials + 5 duplicates + 5 QA/QC) x 3 triplicate Sample Vials x 3 shipments. A complete list of equipment, materials, and external services is provided in Table **11.6**.

Chemical, Equipment, or Service	Minimum Quantity
Ethanol (0.14 x 150 ml per mixture set [37] x 3 replicates)	63 ml
Biobutanol (0.16 x 450 ml)	72 ml
Benzene (0.004 x 450 ml per fuel x 3 fuels)	5.4 ml
Toluene (0.046 x 1,350 ml)	62.1 ml
m-Xylene (0.12 x 1,350 ml)	162 ml
TMB (0.29 x 1,350 ml)	391.5 ml
Iso-Octane (450 ml per fuel x [0.38 + 0.40 + 0.55] fractions in each fuel)	598.5 ml
Anhydrous $CaCl_2$ (0.555 g/L x [450 + 300 ml QA/QC] per fuel x 3 fuels)	1.25 g in 2,250 ml DI water
Deionized Water	As needed
60-ml EPA VOA Vials with Open Top Caps ([5x3 + 5 QA/QC[38]] per fuel x 3 fuels)	60 = 1 Case (of 144)
PTFE silicone septa	60 = 4 Cases (of 24)
50-ml Hamilton® Gas Tight Syringes with PTFE Luer Lock (1 per bulk fuel x 3 fuels + 1 groundwater + 1 DI water)	5
1-ml Hamilton® Gas Tight Syringes with PTFE Luer Lock (1 per fuel x 3 fuels)	3
22-Gauge Stainless Steel Beveled-Tip Needles (1 per bulk fuel x 3 fuels + 1 groundwater + 1 DI water + [5 + 1 QA/QC [38,39]] per fuel x 3 fuels)	23 = 4 Cases (of 6)
SW846 5030/8260 Sample Preparation and Analytical	75 (including QA/QC)

Table 11.6 Summary with Equipment and Material Quantities and Laboratory Serices

[37] Five (5) water:fuel mixture sets equal 150mL (50+40+30+20+10 mL) fuel and DI water per replicate

[38] QA/QC blanks are created/treated as fuel mixtures and then sampled for analysis

[39] QA/QC samples for analysis are extracted using a dedicated needle per fuel

11.4.2. Links to laboratory supply vendor sites

- http://www.fishersci.com/wps/portal/ITEMDETAIL?ru=http://prodwcsserver:9060/webapp/wcs/stores/servlet/FisherItemDisplay&catalogId=29104&productId=3327711&parentProductId=745995&langId=-1&distype=0&fromCat=[Ljava.lang.String;@13a1e7b&catCode=RE_SC&brCategoryId=null&highlightProductsItemsFlag=Y&fromSearch=Y&fromProductCatalogPage=Y&crossRefPartNo=null&crossRefData=null

- http://www.fishersci.com/wps/portal/ITEMDETAIL?ru=http://prodwcsserver:9060/webapp/wcs/stores/servlet/FisherItemDisplay&catalogId=29104&productId=3521829&parentProductId=802647&langId=-1&distype=0&fromCat=[Ljava.lang.String;@1bf845&catCode=RE_SC&brCategoryId=null&highlightProductsItemsFlag=Y&fromSearch=Y&fromProductCatalogPage=Y&crossRefPartNo=null&crossRefData=null

- http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=20997|SIAL&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC&lang=en_US

- http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=20707|SIAL&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC&lang=en_US

- http://www.sigmaaldrich.com/catalog/ProductDetail.do?lang=en&N4=21746|SIAL&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC

11.4.3. Reference to publications used in developing this protocol:

The Impact of Gasohol and Fuel-Grade Ethanol on BTX and Other Hydrocarbons in Ground Water: Effect on Concentrations Near a Source, Results From Bench-Scale Partitioning and Column Studies; API Bulletin No. 23, December 2005; William G. Rixey, Xiaohong He, and Brent P. Stafford, Dept. of Civil and Environmental Engineering, University of Houston.

Technical Protocol for Evaluating the Natural Attenuation of MtBE, Regulatory and Scientific Affairs Department; API Publication 4761, May 2007.

Personal Communications from William G. Rixey, University of Houston, to Michael Foster and David Tsao at BP March 2010.

11.5. Sorption Coefficients of Pure Iso-butanol and Iso-butyric Acid in Soil

This evaluation will determine the sorption of iso-butanol and its key metabolite, iso-butyric acid, in a variety of soil and aquifer materials (sandy, silt, clay, etc.) One of the candidate soils being considered is the uncontaminated soil from Vandenberg AFB. This soil has been highly characterized from previous work (Section **11.2**) and would provide continuity with other experiments.

Following are test protocols that DuPont's Haskell Global Center (HGC) will perform to characterize sorption of both iso-butanol and iso-butyric acid.

11.5.1. Iso-Butyric Acid: Estimation of the Adsorption Coefficient (K_{oc}) using High Performance Liquid Chromatography (HPLC)

INTRODUCTION

This protocol describes a test method to assess the adsorption behavior of (1- ^{14}C)-Isobutyric Acid, sodium salt, on soils. The information developed is used to determine a sorption value to predict partitioning of the test substance in the environment.

The study will be performed in two phases:

- Phase 1 is a preliminary phase to determine solubility of the test substance in 0.01M CaCl2, adsorption to the test vessels, the soil/solution ratio, the equilibrium time for adsorption and desorption, and the amount of test substance adsorbed at equilibrium. The distribution coefficients kd, kom, and koc will be determined during this phase.
- Phase 2 is performed to study adsorption in three different soils at three concentration of the test substance using the soil : aqueous solution ratio and equilibration time determined in phase 1. In this phase the Freundlich adsorption isotherms are determined to establish the influence of concentration on the extent of adsorption on soils. The study of desorption by means of Freundlich desorption isotherms is also part of this phase.

OBJECTIVE

The objective of the study is to determine the adsorption/desorption characteristics of the test substance in three soils with varying characteristics types. The study will be conducted to meet the requirements of the OECD Guideline 106 (January 2000).

It is designed to provide data for determining the following parameters:

- adsorption or distribution coefficient, k_d,
- adsorption coefficient as a function of organic matter, k_{om},
- adsorption coefficient as a function of organic carbon, k_{oc},
- Percent desorbed

- Freundlich adsorption isotherm in test soils with >10% adsorption

REGULATORY COMPLIANCE AND TEST GUIDELINES

This study will be conducted in compliance with U.S.EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current Good Laboratory Practices.

This study will be conducted in compliance with the following test guideline:

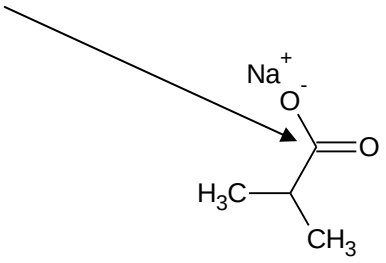
- Organization for Economic and Cooperative Development (OECD) Guideline for the Testing of Chemicals 106, Adsorption/Desorption (January 21, 2000).

TEST SUBSTANCES

Radiolabeled

(1- ^{14}C)-Isobutyric Acid, sodium salt (hereafter referred to as 'test substance') has the following structure:

Position of radiolabel



Chemical Name:	Isobutyric acid, sodium salt (1-^{14}C)
CAS Name, non labeled	Propanoic acid, 2-methyl-, sodium salt (1:1)
CAS Number, non-labeled:	996-30-5
Radiochemical Purity:	99.4% (HPLC)
Specific Activity:	59.5 mCi/mmol
	185,000 ng /mL
Concentration:	0.1mCi/mL in sterile water solution
Molecular Weight:	110.09 g/mole
Chemical Formula:	$C_4 H_8 O_2$. Na

The radiochemical purity and the stability of the test substance in the dosing solvent will be validated at DuPont HGC.

Non-radiolabeled

Non-radiolabeled test substance will be obtained as needed and will be documented in the study records and final report.

TEST DESIGN

Test System

Soil Types, Preparation, Characterization, and Storage

Three soil types will be used to meet the requirements of the OECD Guideline 106 (January 2000). The soils will differ in organic carbon content, clay content, soil texture and pH.

Soil Type*	Soil ID	pH (0.01M $CaCl_2$)	Organic carbon, %	Clay, %	Soil texture#
5	Newark CRD 17,235	4.3	2.1	13	Sandy Loam
3	California Aerobic (Surface) Soil CRD 17,792	6.9	4.3	17	Sandy Loam
5 and 6	California Anaerobic Soil CRD 17,793	7.4	0.3	10	Loamy Sand

* Soil type is most similar to the selection criteria in Table 1 of the OECD TG106.

Texture according to US Department of Agriculture system.

Prior to characterization and use, test soils will be thoroughly mixed and passed through a 2 mm mesh sieve. Soils will be stored air-dried at ambient temperatures. Each soil will be uniquely identified and labeled.

At a minimum, test soils will be characterized for the following characteristics within three years of use in the study:

- pH (0.01M $CaCl_2$)
- organic matter content, %
- organic carbon content, %
- particle size distribution and textural class (U.S. Department of Agriculture)
- cation exchange capacity (mmol/kg)

The source of the test soils, approximate collection date and location, processing and storage conditions, and characterization data and methods will be documented in the study records and final report. All soils will be sterilized by gamma irradiation.

Before use the moisture content of each soil will be determined by drying at *ca* 105°C. In the procedures that follow, the weight of soil always refers to the dry weight equivalent.

Experimental Procedures

Test Conditions

All phases of the test will be conducted at room temperature between 20° and 25° C. Centrifugation conditions will be calculated to remove particles larger than 0.2 µm from the portion of the solution from which aliquots are taken.

Solubility

Non-labeled test substance, (^{14}C)-test substance, and 0.01 M $CaCl_2$ will be mixed to prepare a homogenous 2000 µg/mL stock solution of the test substance. Complete solubilization of the test substance will be determined by liquid scintillation counting (LSC) after centrifugation. If the test substance is shown not to form a homogenous solution, a lower concentration will be tested. No co-solvents will be used.

Adsorption to Containers

The potential for adsorption to test vessels (e.g., PTFE, polypropylene, polypropylene co-polymer) will be assessed. A 0.01 M $CaCl_2$ solution containing the lowest proposed use concentration (i.e., 2 µg/mL) will be added to duplicate test vessels containing no soil. The vessels will be shaken on an end-over-end shaker for 24 h and the solution re-assayed. The amount of radioactivity lost from the solution will indicate the amount of test substance adsorbed onto the surface of the test vessel. The lowest concentration used will preferably be two orders of magnitude lower than the highest concentration.

If significant adsorption to the test vessels occurs, attempts will be made to eliminate it by changing to another type of test vessel, coating the test vessels with non-labeled test substance, lining the inside of test vessel caps with aluminum foil, etc.

Ratio of Soil to Aqueous Phase Test

The purpose of this phase of the test is to find a ratio of soil to aqueous phase such that >20% and preferably >50% of the test substance is adsorbed onto the soil. All soil types, three soil/solution ratios and the highest test substance concentration (i.e., 200 µg/mL) will be used.

The following ratios of soil and volumes of 0.01 M $CaCl_2$ solution will be used for the tests:

- 1:1 Ratio: 10 g of soil and 10 mL of solution
- 1:5 Ratio: 5 g of soil and 25 mL of solution

- 1:20 Ratio: 1 g of soil and 20 mL of solution.

These ratios may be adjusted based on the estimation method outlined in paragraphs 38 to 41 of the OECD 106 test guidelines. If less than three ratios are studied, the protocol will be amended and the reason for doing so will be justified in the report. Each sample will be labeled with a unique code.

The air-dried soil samples will be re-equilibrated by shaking with a calculated volume of 0.01 M $CaCl_2$ solution overnight before the day of the experiment. The volume will be such that when mixed with the stock solution, the soil : solution ratio will be correct and the concentration of test substance in the 0.01 M $CaCl_2$ solution will be 200 ng/mL. The calculated volume of stock solution will be added after re-equilibration. The pH of the stock solution used for dosing (test substance dissolved in 0.01 M $CaCl_2$ solution) will be measured before adding it to the soils.

Duplicate test units will be prepared for each soil ratio studied. The weights of soil and 0.01 M $CaCl_2$ solution will be recorded. The mixtures will be capped and shaken continuously on an end-over-end shaker for 24 h at a speed sufficient to ensure that the soil remains in suspension.

After 24 h the soil/solution mixtures will be centrifuged and the percentage adsorption will be determined. The aqueous layer will be removed to minimize disturbance of the soil, for example, by using a pipette. Radioactivity determined by liquid scintillation counting (LSC). The pH of the aqueous solution will also be determined. Soil remaining in the test vessels will be extracted with acetonitrile (CASN 79-05-8) or other suitable solvent (details to be provided in the study records and report) and radioactivity determined by LSC. Following extraction, the soils will be air-dried, ground using a mortar and pestle, and then triplicate aliquots of each soil type used will be determined using a Harvey oxidizer or equivalent method and the radioactivity determined by LCS.

If the radioactive material balance is <90%, the study will be stopped until the cause of the lost of test substance is determined and appropriate changes made to the protocol to prevent the lost during the study.

Adsorption Equilibrium Time Determinations

The adsorption equilibrium time will be selected as the time at which the percentage of adsorption reaches a plateau. It will be determined for all soils.

Duplicate test units will be prepared for each soil at the chosen ratio at the highest test concentration (i.e., 200 µg/mL). The ratio of soil to 0.01 M $CaCl_2$ solution that will be used has previously been determined. The mixtures will be capped and shaken on an end-over-end shaker for up to 48 h at a speed sufficient to ensure that the soil remains in suspension.

Each test vessel will be centrifuged after 3, 6, 24, and 48 h mixing, for example, and the radioactivity content of the supernatant will be determined by LSC. The volume of each aliquot taken for LSC will be <1% of the total volume and the volume of the aliquots taken will be replaced with an equal volume of 0.01 M $CaCl_2$ solution. Before resuming end-over-end shaking to the next sampling interval, the test vessels will be shaken vigorously by hand to break up the soil packed at the bottom of the vessel and to remix it with the solution.

The concentration of test substance in the adsorption supernatant will be calculated at each time point on the basis of radioactivity measurements. These concentrations will be plotted against time to estimate the achievement of the equilibrium plateau.

If a plateau is not reached within 48 h, the above procedure will be repeated, extending the mixing period beyond 48 h. On the other hand, if a steady decrease in supernatant concentration is found instead of reaching a plateau, this may be due to complicating factors such as biodegradation or slow diffusion into the soil. The study will be stopped until the cause of the loss is determined and appropriate changes made to the protocols.

Desorption Equilibrium Time Determinations

The desorption equilibrium time will be determined on all soils using a similar method as in the adsorption equilibrium time determination study except that:

- No aliquots will be removed during the adsorption phase. At the selected adsorption equilibrium time, the soil/solutions will be separated by centrifugation and as much aqueous phase as possible will be removed. It will be replaced with an equal volume of fresh 0.01 M $CaCl_2$ solution. The test vessels will be shaken vigorously to break up the soil packed at the bottom of the vessel and remix it with the solution prior to resuming end-over-end shaking.

- Each test vessel will be centrifuged and aliquots will be taken at appropriate times based on the adsorption equilibrium time and including a point at twice the selected adsorption equilibrium time.

The concentration of test substance in desorption supernatant will be calculated at each time on the basis of radioactivity measurements. These concentrations will be plotted against time to estimate the achievement of the equilibrium plateau. The time to reach desorption equilibrium will be the time taken to reach this plateau.

If the desorption equilibrium is attained within twice the time of the adsorption equilibrium and the total desorption is >75% of the amount adsorbed, the adsorption is considered to be reversible.

Adsorption Isotherms

Isotherms will be determined on all three soils. Three concentrations of the test substance will be selected preferably covering two orders of magnitude (e.g., 0, 2.0, 20, 200 µg/mL) . In selecting the concentrations, previous study results will be taken into account. The same soil : solution ratio per soil, determined previously, will be kept throughout the study.

The test will be performed in duplicate as described above except that the aqueous phase will only be analyzed once at the adsorption equilibrium time determined previously. The equilibrium concentrations in the solution will be determined and the amount of test substance adsorbed will be calculated from the depletion of radioactivity from the supernatant. The adsorbed mass per unit mass of soil will be plotted as a function of the equilibrium concentration of the test substance.

The pH of the stock solution used for dosing (test substance dissolved in 0.01 M $CaCl_2$ solution) will be measured before adding it to the soils. The pH of each adsorption supernatant will also be measured after centrifugation for the highest and lowest concentrations of test substance used (e.g., 2.0 and 200 µg/mL).

Desorption Isotherms

The supernatant will be removed from each duplicate sample (i.e., three soils) at the highest concentration of test substance from the adsorption study and replaced with an equal volume of 0.01 M $CaCl_2$ solution. The test vessels will be shaken vigorously to break up the soil packed at the bottom before resuming end-over-end shaking for the desorption equilibrium time determined previously. The tubes will then be removed, centrifuged and the radioactivity content of the supernatant determined by LSC.

Determination of Radioactivity

Weights of all samples will be measured where appropriate. Volumes of $CaCl_2$ solution and volumes of supernatant will be determined from their corresponding weights using a density of 1.00 g/mL.

Duplicate portions of supernatants, test substance solutions and extracts will be added directly to the scintillation cocktail and the radioactivity content determined by LSC.

To allow determination of a radioactive material balance, triplicate aliquots of the air-dried and ground soil samples from the highest test substance concentration of each soil type used will be determined using a Harvey oxidizer or equivalent method and the radioactivity determined by LSC.

DATA ANALYSIS

Individual and mean data will be tabulated and presented in graphical form as appropriate. The following parameters will be reported:

- the concentrations and quantities of test substance, as measured by radioactivity, in solution and in soil and the soil/solution concentration ratio for each soil at equilibrium
- the Freundlich constants K_F and (1/n) for each adsorption and desorption isotherm, calculated from the following relationship:
  $Concentration\ in\ soil = K_F \times Concentration\ in\ water^{(1/n)}$
- the Freundlich adsorption coefficient calculated as a function of the organic carbon content of the soil (K_{oc})
- radioactive material balance for each soil type reported in duplicate.

REPORTING

The final report will include the information and data required by Good Laboratory Practice standards and relevant guideline reporting requirements. Corrections or additions to a final report will be in the form of an amendment by the study director. The amendment will clearly identify that part of the final report that is being added to or corrected and the reasons for the correction or addition, and will be signed and dated by the person responsible.

AMENDMENTS TO THE PROTOCOL

All amendments to this protocol must be approved by the study director prior to implementation. The study sponsor representative will be notified of the amendment. Amendments shall be prepared by the testing facility and shall contain the description of the study changes, the reasons for the amendment, the dated signatures of the study director and sponsor, and the impact of the changes on the study.

PROTOCOL DEVIATIONS

Any deviations from the study protocol will be identified in writing. Deviations will be documented in the study records and in the final report. Any statement regarding a deviation shall include the reason for the deviation, its date of occurrence, its anticipated effect on the outcome of the study, and the dated signature of the study director.

RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained by the performing laboratory.

STUDY PERSONNEL

Study Director:	Robert F. Vavala
	Staff Chemist
Management:	William R Berti
	Group Technical Leader

STUDY DATES

Proposed Experimental Start: September 2010

Proposed Experimental Termination: December 2010

REFERENCES

Organization for Economic and Cooperative Development (OECD) Guideline for the Testing of Chemicals 106, Adsorption/Desorption (January 21, 2000).

U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances (OPPTS), Fate, Transport and Transformation Test Guidelines, OPPTS 835.1110 Activated Sludge Sorption Isotherm, EPA 712-C-98-298, January 1998.

11.5.2. (^{14}C)-Iso-butanol: Adsorption/Desorption in Soil Test Protocol

INTRODUCTION

This protocol describes a test method to assess the adsorption behavior of Isobutanol, [Methyl-^{14}C]-, on soils. The information developed is used to determine a sorption value to predict partitioning of the test substance in the environment.

The study will be performed in two phases:

- Phase 1 is a preliminary phase to determine solubility of the test substance in 0.01M $CaCl_2$, adsorption to the test vessels, the soil/solution ratio, the equilibrium time for adsorption and desorption, and the amount of test substance adsorbed at equilibrium. The distribution coefficients k_d, k_{om}, and k_{oc} will be determined during this phase.

- Phase 2 is performed to study adsorption in three different soils at three concentration of the test substance using the soil : aqueous solution ratio and equilibration time determined in phase 1. In this phase the Freundlich adsorption isotherms are determined to establish the influence of concentration on the extent of adsorption on soils. The study of desorption by means of Freundlich desorption isotherms is also part of this phase.

OBJECTIVE

The objective of the study is to determine the adsorption/desorption characteristics of the test substance in three soils with varying characteristics types. The study will be conducted to meet the requirements of the OECD Guideline 106 (January 2000).

It is designed to provide data for determining the following parameters:

- adsorption or distribution coefficient, k_d,
- adsorption coefficient as a function of organic matter, k_{om},
- adsorption coefficient as a function of organic carbon, k_{oc},

- Percent desorbed

Freundlich adsorption isotherm in test soils with >10% adsorption

REGULATORY COMPLIANCE AND TEST GUIDELINES

This study will be conducted in compliance with U.S.EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current Good Laboratory Practices.

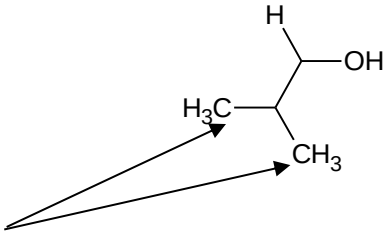
This study will be conducted in compliance with the following test guideline:

- Organization for Economic and Cooperative Development (OECD) Guideline for the Testing of Chemicals 106, Adsorption/Desorption (January 21, 2000).

TEST SUBSTANCE

Test Substance, Radiolabeled

Isobutanol, [Methyl-^{14}C]- (hereafter referred to as 'test substance') has the following structure:



Positions of radiolabel

Chemical Name:	Isobutanol, [Methyl-^{14}C]-
CAS Number, non-labeled:	78-83-1
Radiochemical Purity:	99.00% radiochemical pure
Specific Activity:	52.90 mCi/mmol
Concentration:	0.50mCi/mL in water solution
Molecular Weight:	74.12 g/mole
Chemical Formula:	$C_4 H_{10} O$

The radiochemical purity and the stability of the test substance in the dosing solvent will be validated at DuPont HGC.

Test Substance, Non-radiolabeled

Non-radiolabeled test substance will be obtained as needed and will be documented in the study records and final report.

TEST SYSTEM

Soil Types, Preparation, Characterization, and Storage

Three soil types will be used to meet the requirements of the OECD Guideline 106 (January 2000). The soils will differ in organic carbon content, clay content, soil texture and pH.

Soil Type*	Soil ID	pH (0.01M $CaCl_2$)	Organic carbon, %	Clay, %	Soil texture#
5	Newark CRD 17,235	4.3	2.1	13	Sandy Loam
3	California Aerobic (Surface) Soil CRD 17,792	6.9	4.3	17	Sandy Loam
5 and 6	California Anaerobic Soil CRD 17,793	7.4	0.3	10	Loamy Sand

* Soil type is most similar to the selection criteria in Table 1 of the OECD TG106.

Texture according to US Department of Agriculture system.

Prior to characterization and use, test soils will be thoroughly mixed and passed through a 2 mm mesh sieve. Soils will be stored air-dried at ambient temperatures. Each soil will be uniquely identified and labeled.

At a minimum, test soils will be characterized for the following characteristics within three years of use in the study:

- pH (0.01M $CaCl_2$)
- organic matter content, %
- organic carbon content, %
- particle size distribution and textural class (U.S. Department of Agriculture)
- cation exchange capacity (mmol/kg)

The source of the test soils, approximate collection date and location, processing and storage conditions, and characterization data and methods will be documented in the study records and final report. All soils will be sterilized by gamma irradiation.

Before use the moisture content of each soil will be determined by drying at *ca* 105°C. In the procedures that follow, the weight of soil always refers to the dry weight equivalent.

EXPERIMENTAL PROCEDURES

Test Conditions

All phases of the test will be conducted at room temperature between 20° and 25° C. Centrifugation conditions will be calculated to remove particles larger than 0.2 µm from the portion of the solution from which aliquots are taken.

Solubility

Non-labeled test substance, (^{14}C)-test substance, and 0.01 M $CaCl_2$ will be mixed to prepare a homogenous 2000 µg/mL stock solution of the test substance. Complete solubilization of the test substance will be determined by liquid scintillation counting (LSC) after centrifugation. If the test substance is shown not to form a homogenous solution, a lower concentration will be tested. No co-solvents will be used.

Adsorption to Containers

The potential for adsorption to test vessels (e.g., PTFE, polypropylene, polypropylene co-polymer) will be assessed. A 0.01 M $CaCl_2$ solution containing the lowest proposed use concentration (i.e., 2 µg/mL) will be added to duplicate test vessels containing no soil. The vessels will be shaken on an end-over-end shaker for 24 h and the solution re-assayed. The amount of radioactivity lost from the solution will indicate the amount of test substance adsorbed onto the surface of the test vessel. The lowest concentration used will preferably be two orders of magnitude lower than the highest concentration.

If significant adsorption to the test vessels occurs, attempts will be made to eliminate it by changing to another type of test vessel, coating the test vessels with non-labeled test substance, lining the inside of test vessel caps with aluminum foil, etc.

Ratio of Soil to Aqueous Phase Test

The purpose of this phase of the test is to find a ratio of soil to aqueous phase such that >20% and preferably >50% of the test substance is adsorbed onto the soil. All soil types, three soil/solution ratios and the highest test substance concentration (i.e., 200 µg/mL) will be used.

The following ratios of soil and volumes of 0.01 M $CaCl_2$ solution will be used for the tests:

- 1:1 Ratio: 10 g of soil and 10 mL of solution
- 1:5 Ratio: 5 g of soil and 25 mL of solution
- 1:20 Ratio: 1 g of soil and 20 mL of solution.

These ratios may be adjusted based on the estimation method outlined in paragraphs 38 to 41 of the OECD 106 test guidelines. If less than three ratios are studied, the protocol will be amended and the reason for doing so will be justified in the report. Each sample will be labeled with a unique code.

The air-dried soil samples will be re-equilibrated by shaking with a calculated volume of 0.01 M $CaCl_2$ solution overnight before the day of the experiment. The volume will be such that when mixed with the stock solution, the soil : solution ratio will be correct and the concentration of test substance in the 0.01 M $CaCl_2$ solution will be 200 ng/mL. The calculated volume of stock solution will be added after re-equilibration. The pH of the stock solution used for dosing (test substance dissolved in 0.01 M $CaCl_2$ solution) will be measured before adding it to the soils.

Duplicate test units will be prepared for each soil ratio studied. The weights of soil and 0.01 M $CaCl_2$ solution will be recorded. The mixtures will be capped and shaken continuously on an end-over-end shaker for 24 h at a speed sufficient to ensure that the soil remains in suspension.

After 24 h the soil/solution mixtures will be centrifuged and the percentage adsorption will be determined. The aqueous layer will be removed to minimize disturbance of the soil, for example, by using a pipette. Radioactivity determined by liquid scintillation counting (LSC). The pH of the aqueous solution will also be determined. Soil remaining in the test vessels will be extracted with acetonitrile (CASN 79-05-8) or other suitable solvent (details to be provided in the study records and report) and radioactivity determined by LSC. Following extraction, the soils will be air-dried, ground using a mortar and pestle, and then triplicate aliquots of each soil type used will be determined using a Harvey oxidizer or equivalent method and the radioactivity determined by LCS.

If the radioactive material balance is <90%, the study will be stopped until the cause of the lost of test substance is determined and appropriate changes made to the protocol to prevent the lost during the study.

Adsorption Equilibrium Time Determinations

The adsorption equilibrium time will be selected as the time at which the percentage of adsorption reaches a plateau. It will be determined for all soils.

Duplicate test units will be prepared for each soil at the chosen ratio at the highest test concentration (i.e., 200 µg/mL). The ratio of soil to 0.01 M $CaCl_2$ solution that will be used has previously been determined. The mixtures will be capped and shaken on an end-over-end shaker for up to 48 h at a speed sufficient to ensure that the soil remains in suspension.

Each test vessel will be centrifuged after 3, 6, 24, and 48 h mixing, for example, and the radioactivity content of the supernatant will be determined by LSC. The volume of each aliquot taken for LSC will be <1% of the total volume and the volume of the aliquots taken will be replaced with an equal volume of 0.01 M $CaCl_2$ solution. Before resuming end-over-end shaking to the next sampling interval, the test vessels will be shaken vigorously by hand to break up the soil packed at the bottom of the vessel and to remix it with the solution.

The concentration of test substance in the adsorption supernatant will be calculated at each time point on the basis of radioactivity measurements. These concentrations will be plotted against time to estimate the achievement of the equilibrium plateau.

If a plateau is not reached within 48 h, the above procedure will be repeated, extending the mixing period beyond 48 h. On the other hand, if a steady decrease in supernatant concentration is found instead of reaching a plateau, this may be due to complicating factors such as biodegradation or slow diffusion into the soil. The study will be stopped until the cause of the loss is determined and appropriate changes made to the protocols.

Desorption Equilibrium Time Determinations

The desorption equilibrium time will be determined on all soils using a similar method as in the adsorption equilibrium time determination study except that:

- No aliquots will be removed during the adsorption phase. At the selected adsorption equilibrium time, the soil/solutions will be separated by centrifugation and as much aqueous phase as possible will be removed. It will be replaced with an equal volume of fresh 0.01 M $CaCl_2$ solution. The test vessels will be shaken vigorously to break up the soil packed at the bottom of the vessel and remix it with the solution prior to resuming end-over-end shaking.

- Each test vessel will be centrifuged and aliquots will be taken at appropriate times based on the adsorption equilibrium time and including a point at twice the selected adsorption equilibrium time.

The concentration of test substance in desorption supernatant will be calculated at each time on the basis of radioactivity measurements. These concentrations will be plotted against time to estimate the achievement of the equilibrium plateau. The time to reach desorption equilibrium will be the time taken to reach this plateau.

If the desorption equilibrium is attained within twice the time of the adsorption equilibrium and the total desorption is >75% of the amount adsorbed, the adsorption is considered to be reversible.

Adsorption Isotherms

Isotherms will be determined on all three soils. Three concentrations of the test substance will be selected preferably covering two orders of magnitude (e.g., 0, 2.0, 20, 200 µg/mL) . In selecting the concentrations, previous study results will be taken into account. The same soil : solution ratio per soil, determined previously, will be kept throughout the study.

The test will be performed in duplicate as described above except that the aqueous phase will only be analyzed once at the adsorption equilibrium time determined previously. The equilibrium concentrations in the solution will be determined and the amount of test substance adsorbed will be calculated from the depletion of radioactivity from the supernatant. The adsorbed mass per unit mass of soil will be plotted as a function of the equilibrium concentration of the test substance.

The pH of the stock solution used for dosing (test substance dissolved in 0.01 M $CaCl_2$ solution) will be measured before adding it to the soils. The pH of each adsorption supernatant will also be measured after centrifugation for the highest and lowest concentrations of test substance used (e.g., 2.0 and 200 µg/mL).

Desorption Isotherms

The supernatant will be removed from each duplicate sample (i.e., three soils) at the highest concentration of test substance from the adsorption study and replaced with an equal volume of 0.01 M $CaCl_2$ solution. The test vessels will be shaken vigorously to break up the soil packed at the bottom before resuming end-over-end shaking for the desorption equilibrium time determined previously. The tubes will then be removed, centrifuged and the radioactivity content of the supernatant determined by LSC.

Determination of Radioactivity

Weights of all samples will be measured where appropriate. Volumes of $CaCl_2$ solution and volumes of supernatant will be determined from their corresponding weights using a density of 1.00 g/mL.

Duplicate portions of supernatants, test substance solutions and extracts will be added directly to the scintillation cocktail and the radioactivity content determined by LSC.

To allow determination of a radioactive material balance, triplicate aliquots of the air-dried and ground soil samples from the highest test substance concentration of each soil type used will be determined using a Harvey oxidizer or equivalent method and the radioactivity determined by LSC.

DATA ANALYSIS

Individual and mean data will be tabulated and presented in graphical form as appropriate. The following parameters will be reported:

- the concentrations and quantities of test substance, as measured by radioactivity, in solution and in soil and the soil/solution concentration ratio for each soil at equilibrium
- the Freundlich constants K_F and (1/n) for each adsorption and desorption isotherm, calculated from the following relationship:
  $Concentration\ in\ soil = K_F \times Concentration\ in\ water^{(1/n)}$
- the Freundlich adsorption coefficient calculated as a function of the organic carbon content of the soil (K_{oc})
- radioactive material balance for each soil type reported in duplicate.

REPORTING

The final report will include the information and data required by Good Laboratory Practice standards and relevant guideline reporting requirements. Corrections or additions to a final report will be in the form of an amendment by the study director. The amendment will clearly identify that part of the final report that is being added to or corrected and the reasons for the correction or addition, and will be signed and dated by the person responsible.

AMENDMENTS TO THE PROTOCOL

All amendments to this protocol must be approved by the study director prior to implementation. The study sponsor representative will be notified of the amendment. Amendments shall be prepared by the testing facility and shall contain the description of the study changes, the reasons for the amendment, the dated signatures of the study director and sponsor, and the impact of the changes on the study.

PROTOCOL DEVIATIONS

Any deviations from the study protocol will be identified in writing. Deviations will be documented in the study records and in the final report. Any statement regarding a deviation shall include the reason for the deviation, its date of occurrence, its anticipated effect on the outcome of the study, and the dated signature of the study director.

RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained by the performing laboratory.

STUDY PERSONNEL

Study Director: Robert F. Vavala
Staff Chemist
Management: William R Berti
Group Technical Leader

STUDY DATES

Proposed Experimental Start: September 2010

Proposed Experimental Termination: December 2010

REFERENCES

Organization for Economic and Cooperative Development (OECD) Guideline for the Testing of Chemicals 106, Adsorption/Desorption (January 21, 2000).

U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances (OPPTS), Fate, Transport and Transformation Test Guidelines, OPPTS 835.1110 Activated Sludge Sorption Isotherm, EPA 712-C-98-298, January 1998.

11.6. Modelling

The objective of groundwater modelling is to use a mathematical/computer model to evaluate the differential impact of iso-butanol compared to ethanol on the potential elongation of BTEX plumes if iso-butanol blended gasoline were to be released underground

Most commercially available models (e.g. U.S. EPA's Footprint model, U.S. Geological Survey MODFLOW in combination with the Reactive Transport in 3 Dimensions, RT3D, model) have been used to evaluate the potential impacts of ethanol-blended fuels on benzene plume elongation. In order to evaluate iso-butanol blended fuels, BP's Remediation Management recommended contracting Professor Pedro Alvarez of Rice University to develop a user interface whereby RT3D could be used to numerically solve the transport conditions, based on water flow and chemical properties, while incorporating biodegradation using the General Substrate Interactions Module (GSIM). The model interface was designed to allow incorporation of a broader electron acceptor pool besides just aerobic and methanogenic conditions (e.g. nitrate-, iron-, and sulphate reducing conditions) and consider the impacts of iso-butanol on the elongation of other alkylbenzene (TEX) plumes under these conditions. The full proposal from Prof. Alvarez is included as Appendix B (Section 16.2.)

This modelling approach was recently published and used to simulate the effects of various fuel alcohols on the natural attenuation of benzene[40]. Other researchers have likewise adopted these techniques[41]. The experimental data generated from the work outlined in Sections **11.2** through **11.5** will be used to model the plume elongation of BTEX when an iso-butanol blended gasoline is accidentally spilled underground. Specifically, model parameters (e.g. sorption, diffusivity, solubility, biodegradability) derived from experimental data generated from the work outlined in Sections **11.2** through **11.5** will be incorporated into the model with the subsurface characteristics summarized in Tables **11.7** and **11.8**.

Gasoline solutes that will be simulated and compared are listed in Table **11.7**.

Solute	Iso-butanol gasoline amount	Ethanol gasoline amount	Comparison
Benzene	0.6%	0.6%	Like vs like plume extent by conc.
Toluene	20%	20%	Like vs like plume extent by conc.
Xylenes	25%	25%	Like vs like plume extent by conc.
Ethyl benzene	5%	5%	Like vs like plume extent by conc.
Alcohol	16%	10%	plume extent by concentration

Table 11.7. Gasoline Solutes in Modelling Study

Furthermore, the effects and persistence of iso-butanol degradation byproducts, namely iso-butyric acid and iso-butylaldehyde, will be modeled as well. The sensitivity of the model outputs to different initial values of the input parameters will be studied by simulations as summarized in Table **11.8**. For ethanol, the best measured/literature parameters will be used whereas for iso-butanol, parameters will be a combination of

[40] Gomez D. and P.J.J. Alvarez (2010). Comparison of the Effects of Various Fuel Alcohols on the Natural Attenuation of Benzene Plumes: A Simulation Analysis Using RT3D with the General Substrate Interaction Module. _J. Contam. Hydrol. 113, pp. 66-76.

[41] Modelling Fate of Groundwater Contaminants Resulting from Butanol-Blended Fuel Leaks; Khai H. Vuong, Mark N. Goltz (mark.goltz@afit.edu), and Charles A. Bleckmann (Air Force Institute of Technology, Wright Patterson AFB, OH), Junqi Huang (U.S. EPA R.S. Kerr Laboratory, Ada, OK), Douglas M. Mackay (University of California, Davis, CA). Poster at Battelle's Seventh International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, CA, May 24-27, 2010.

literature values and those generated in the experiments summarized in Sections **11.2** through **11.5**. In addition, some of the parameters will be altered one at a time in a stepwise manner from the base case until an increase in plume size of about 10% is calculated or the parameter becomes unrealistic. This approach is taken because the magnitude of change of a particular parameter to produce a given effect is unknown. The combined studies will result in a wide range of effects of iso-butanol compared to ethanol on potential BTEX plume elongation.

Model Parameter	Value 1	Value 2
Soil Type	Ref. Soil 1 - Vandenberg, CA (see Section **11.2**)	Ref. Soil 2 - Newark, DE (see Section **11.2**)
Iso-butanol sorption/desorption coefficients	By Soil Type (see Section **11.2**)	By Soil Type (see Section **11.2**)
Spill Amount	30 gallons	
Enhanced BTX solubility (cosolvency)	No enhancement[b]	Measured; optimal mixing (see Section **11.2**)
Aerobic iso-butanol degradation rate	Measured; microcosms (see Sections **11.2**, **11.2**) [a]	
Anaerobic iso-butanol degradation rate (e.g. nitrate-reducing, sulfate-reducing, methanogenic conditions)	Measured; microcosms (see Sections **11.2**, **11.2**) [a]	
Iso-butanol degradation byproduct kinetics (e.g. iso-butyric acid and iso-butylaldehyde)	Measured; microcosms (see Sections **11.2**, **11.2**)	

[a] Iso-butanol degradation rate constants were published in Schaffer, et al.[42],[43].

[b] Depends on retention of isobutanol in the vadose zone.

Table 11.8. Modeling Parameters and Test Values

[42] Schaffer C.E., Yang X., Pelz O., Tsao D.T., Streger S.H., and R.J. Steffan (2010a). Aerobic biodegradation of iso-butanol and ethanol and their relative effects on BTEX biodegradation in aquifer materials. Chemosphere 81(9), pp. 1104-1110.

[43] Schaffer C.E., Yang X., Pelz O., Tsao D.T., Streger S.H., and R.J. Steffan (2010b). Anaerobic biodegradation of iso-butanol and ethanol and their relative effects on BTEX biodegradation in aquifer materials. Chemosphere 81(9), pp. 1111-1117.

While model results considering biofuels different than ethanol, and contaminants different than benzene, should only be considered as theoretical, a comparison of the developed model output to prior ethanol/benzene model results will be made in order to provide some validation and calibration (see Appendix B, section 16.2.5 for more details). While no field data are available, some calibration of the model may be conducted using literature data.

The above modeling work is directed to the groundwater (saturated zone). While not identified as a gap in the Tier I report, there is recent interest about ethanol from a high-concentration ethanol fuel spill being retained and degraded in the vadose zone to form methane that could have environmental consequences. As an additional piece of work, a study will be conducted to determine whether the "buoyancy effects" that result in ethanol being retained in the vadose zone from high-concentration ethanol blended fuels occur with iso-butanol from a 16% iso-butanol blended fuel. This evaluation will be conducted using dyed alcohol and petroleum constituents in aboveground two-dimensional tanks similar in construct to those used by Professor Bill Rixey at the University of Houston[44]. Should the less hydrophilic iso-butanol be likewise retained in the vadose zone similar to ethanol, the relative rate of methane formation from iso-butanol as compared to ethanol will be evaluated as a second step. This subsequent evaluation, if necessary, will be based on experimental designs accepted and published in the scientific literature.

[44] Stafford, B.P. (2007). Impacts to Groundwater from Releases of Fuel Grade Ethanol: Source Behavior. Dissertation. Department of Civil and Environmental Engineering, University of Houston.

12. Lifecycle Analysis of Biobutanol

12.1. Statement of the Knowledge Gap

- **Complete the LCA for retrofits of typical existing grain and sugarcane based ethanol plants to iso-butanol production.**

12.2. Work Plan

The LCA will be completed using CA-GREET, as defined in the LCFS ruling, to determine the well-to-wheel energy use, greenhouse gas (GHG) emissions and other available emissions for producing iso-butanol. Because of the similarities with the corn dry grind and sugarcane ethanol processes, many of the existing inputs, such as corn grain and sugarcane production, will be used from the CA-GREET model. The current coproduct credit methodology used in the CA-GREET models for bioethanol will be utilized for the iso-butanol LCA. Information from the iso-butanol process model for yield, fuel use, electricity use, and DDGS produced will be entered into the CA-GREET model initially to determine LCA results. The iso-butanol CA-GREET model will be updated with data from the pilot plant as it becomes available. The higher energy density of butanol will need to be accounted for in the fuel transport models. Also, results from the testing of fugitive emissions and combustion emissions being determined as part of this multimedia evaluation will need to be included in the modifications to the CA-GREET model. It is expected that indirect land use change effects will be very similar to the bioethanol results, but could vary slightly with differences in yield of fuel energy per mass of corn or sugarcane. The running of the GTAP model to determine the indirect land use change impact will be left to CARB, per the LCFS instructions.

Iso-butanol process options can then be compared to external benchmarks like conventional gasoline and bioethanol in order to compare LCA results to other potential fuels and fuel additives on the market. This comparison is done on the basis of a unit of energy delivered by the fuel. Greenhouse gas emissions and non-renewable energy use are the environmental indicators of primary focus.

LCA results will need to be approved by ARB using method 2B described in the LCFS Final Regulation. New pathways will be developed for all likely retrofit process designs. The CA-GREET modifications will be completed with input from CARB. The methodology will be developed initially using any testing data available and inputs from the latest process model. As pilot plant results become available, that data will replace modeled input data. Using this approach, quantitative LCA results will be available in the final version of the Biobutanol Multimedia Evaluation Report. A detailed discussion of greenhouse gas emission results at each point in the value chain for iso-butanol compared to bioethanol will also be included.

If the results of the test programs described in this section indicate significant persistence of iso-butanol or its degradation products in soil or water, implications for remediation techniques will be reviewed with findings presented in the Tier III report.

13. Management of Genetically Modified Microorganisms

13.1. Statement of Knowledge Gap

At the time of the Tier I report was submitted, the identity of the host microorganism could not be disclosed. With the announcement of *Saccharomyces cerevisiae* as the host microorganism to make iso-butanol, the applicable regulatory framework and requirements can be discussed in greater detail (Section **13.2**).

13.2. Description of Country Specific Regulatory Framework and Requirements

All country specific regulatory requirements are very similar in that they are based on human health and environmental risk assessments conducted on the host microorganism and facility requirements for containing and inactivating the genetically engineered microorganism. Approvals have already been obtained for the iso-butanol pilot facility located UK. The UK regulations are based on the EU Contained Use Directive for Genetically Modified Microorganisms (GMMs).

Since initial commercial production of iso-butanol is planned for the United States and Brazil, the regulatory framework and requirements for these countries will be described in Section **13.2.1** for the US and Section **13.2.2** for Brazil.

As background, *S. cerevisiae* has a history of safe use in food processing and is widely used for the production of lipids, amino acids, vitamins and proteins including enzymes. *S. cerevisiae*, also known as Bakers's Yeast, has been used for centuries as a leavening agent for bread, as a fermenter of alcoholic beverages and more recently to convert sugars and starches from renewable feedstocks into fuel ethanol. It is non-pathogenic and has been recognized globally as a Biosafety Level 1 (BSL-1) microorganism.

13.2.1. United States Regulations

U.S. EPA has conducted and published a human health and environmental risk assessment on *S. cerevisiae*[45] and has determined that it is eligible for an exemption under the Toxic Substances Control Act[46] based on its history of safe use and low potential for adverse effects on human health or the environment. Qualification for this exemption is based on meeting EPA criteria for the introduced genetic material and for the physical containment of the manufacturing facility. EPA's stated approach for the Tier 1 Exemption is to 1) list host microorganisms which have little to no potential for

[45] EPA Biotechnology Program under TSCA, 1996: Final Risk Assessment of *Saccharomyces cerevisiae*, http://www.epa.gov/biotech_rule/pubs/fra/fra002.htm .

[46] Microbial Products of Biotechnology (40 CFR Parts 700, 720, 721,723 and 725)

adverse effects; 2) specify criteria for the introduced genetic material which will not likely increase potential for adverse effects and then, as further assurance; 3) specify physical containment and inactivation requirements to minimize release of microorganism to the environment[47].

EPA Criteria for the Introduced Genetic Material

The genetic material introduced into *S. cerevisiae* to produce iso-butanol will meet all of the following criteria (as described in Part 725.421): 1) limited in size, 2) well-characterized, 3) poorly mobilizable and 4) free of toxin encoding sequences. In other words, those criteria ensure that genetic modifications are well understood, with minimum risk of potential adverse effects of the microorganism to humans and the environment. The genetically modified *S. cerevisiae* is being developed based on these design criteria. A Certification Notification will be submitted to EPA per the requirements for the Tier 1 Exemption[48] prior to commencing initial manufacturing of iso-butanol using the genetically engineered *S. cerevisiae*.

Description of Containment and Measures to Minimize Environmental Release

To qualify for the EPA Tier 1 exemption, the manufacturer must meet all of the following physical containment and control criteria as listed in 40 CFR 725.422:

1) Use a structure designed and operated to contain the new microorganism;

2) Control access to the structure;

3) Provide written, published and implemented procedures to protect workers

4) Use inactivation procedures demonstrated and documented to reduce viable microorganism in liquid or solid wastes by a minimum 6 log reduction (99.9999%);

5) Use controls known to be effective in minimizing viable microorganism in aerosols and exhaust gases;

6) Use systems for controlling dissemination of new microorganism through other routes (e.g. pest and other vectors); and

7) Have in place emergency clean-up procedures.

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[47] EPA Biotechnology Program under TSCA, 1996: Final Decision Document: TSCA Section 5 (H) (4) Exemption for *Saccharomyces cerevisiae*, http://www.epa.gov/biotech_rule/pubs/fra/fd002.htm.

[48] Part 40 CFR 725.424 (4) including recordkeeping (Part 725.424(5)

Figure **13.1** below is a block diagram of the iso-butanol process based on corn as the feedstock. The unit operations that will meet EPA containment requirements are outlined in red. Physical and operational containment will include all areas with structures that effectively surround and enclose viable genetically modified microorganisms. For the butanol process, this includes the fermentation train (e.g. laboratory, seed and production fermentors) and the downstream product recovery and refining lines and vessels that contain viable microorganisms. Inactivation of the genetically modified microorganism occurs in the Integrated Recovery block.

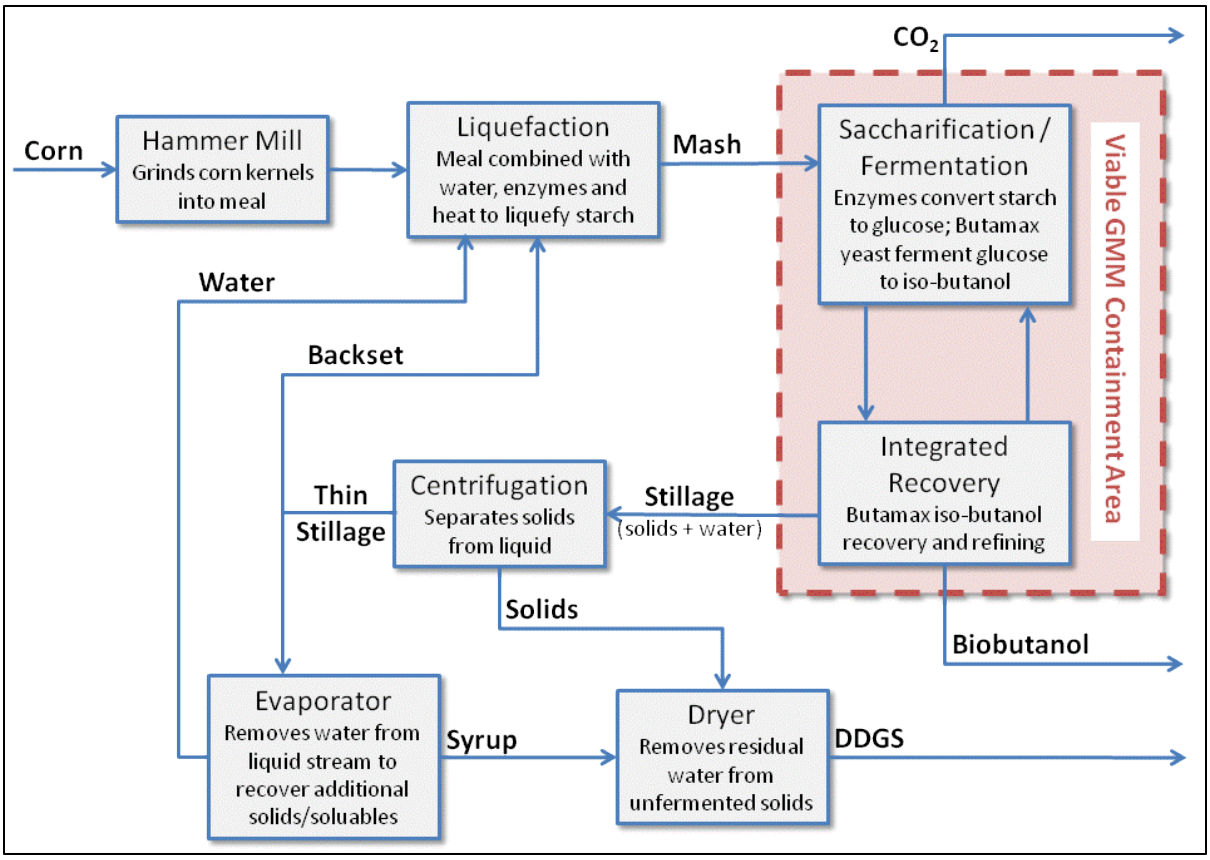

Figure 13.1 Corn Process Block Flow Diagram

Prior to leaving containment, process liquids and solid wastes will be inactivated with heat and/or pH to achieve the 99.9999% or greater reduction criterion. Operating conditions will be controlled and monitored to achieve the inactivation criteria and samples will be collected, analyzed and records maintained to validate that the containment requirements are met.

Controls for exhaust gases from the fermentors and other containment structures will be provided to minimize release of viable organisms as aerosols. The specific controls provided will depend on the size and final design of the facility and include options such as filters, scrubbers and thermal oxidizers.

The refining process will prevent the presence of any inactivated GMMs in the butanol product consistent with the specifications required for its use as a transportation fuel.

Stillage containing inactivated GMM will undergo centrifugation to remove the solids (corn residues and inactivated yeast cells) and the resulting thin stillage (which is mainly water containing soluble salts, and material from the corn and yeast) is sent back to liquefaction and/or evaporated to recover water and solubles. Evaporator solubles (commonly known as syrup) is then backmixed and then dried with the solids from the centrifugation step to produce distiller dry grain with solubles (DDGS) which is used in the animal feed industry. The final use of the inactivated genetically modified microorganism is as a small component (less than 5% by weight) of the DDGS. Acceptance of the butanol DDGS as an animal feed falls under the auspices of the FDA Center for Veterinary Medicine.

A Tier I exemption also requires the operating procedures to protect workers and emergency control and cleanup procedures in case of spills that release viable GMM to the environment. Spill response plans will be prepared based on facility and site specific considerations but generally will consider assessment of potential release points, spill scenarios and the measures to mitigate each.

Existing corn-to-ethanol facilities are based on either a dry or wet mill design. The corn-to-butanol process technology is capable of either retrofitting existing facilities or building new ones. Wet corn mills often involve permitted wastewater treatment whereas dry mills are usually "zero wastewater discharge". Regardless of the mill design, the corn-to-butanol process will be designed to meet the containment requirements of the EPA Tier 1 Exemption and all applicable air, water and solid waste environmental regulations.

13.2.2. Brazil Regulations

Brazil regulations are based on a series of resolutions which are administered by the National Technical Biosafety Commission (CTNBio). Laboratory and pilot facilities are regulated through a licensing procedure (Biosafety Quality Certificate) under Normative Resolution No. 1. Normative Resolution No. 2 (NR-02) contains a microorganism classification scheme based on risk. *S. cerevisiae* meets Risk Class 1 ("low individual risk and low risk for the community"). NR-02 also includes facility specific containment requirements for pilot and large scale activities. Commercial uses of genetically modified microorganisms that involve more than one facility are regulated by Normative Resolution No. 5. NR-05 requires applicants to submit a formal human health and environmental risk assessment to CTNBio for review and approval.

Figure **13.2** below is a block diagram of the iso-butanol process based on sugar cane as the feedstock. Differences do exist in the handling and preparation of the feedstocks and in the composition and uses of the coproducts, specifically vinasse. However, the iso-butanol cane process is very similar to the corn based process as it pertains to the containment and inactivation of the GMM.

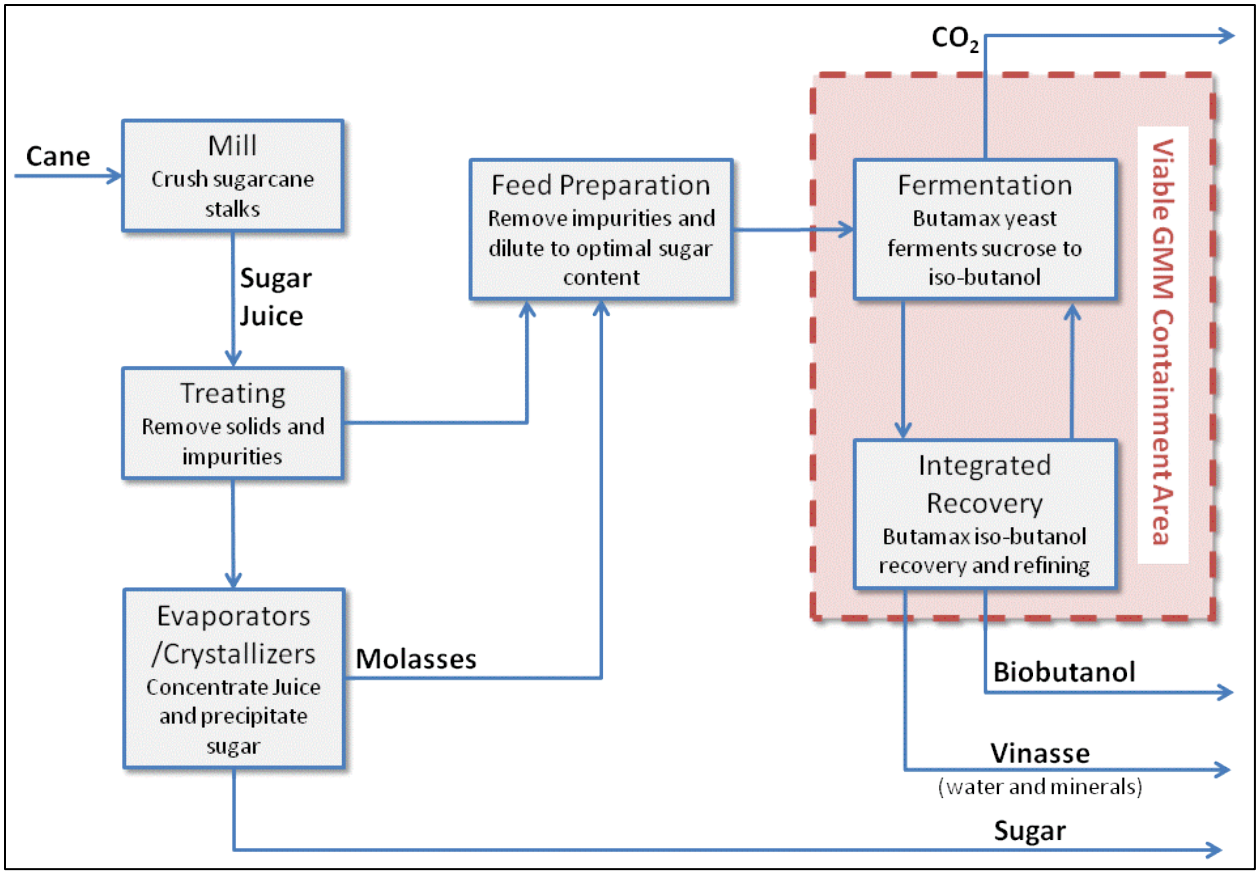

Figure 13.2 Cane Process Block Flow Diagram

In the current Brazil ethanol industry, *S. cerevisiae is* used to convert cane sugar to ethanol and the resulting vinasse is land applied for its fertilizer value In the Butamax process, a genetically modified *S. cerevisiae* will be used to convert sugar to iso-butanol, the GMM will be inactivated within the containment area and the vinasse will be land applied for its fertilizer value. In both cases, there will be residual amounts of yeast in the vinasse; however, in the butanol process the yeast in the vinasse will be inactivated before leaving containment and being land applied. While the GMM is under the authority of CTNBio, the vinasse will be regulated by applicable environmental permitting regulations.

13.3. Work Plan

Butamax continues to monitor regulatory requirements in the US and Brazil. The Tier III Report will include any updates on applicable GMM regulations.

14. Certification of Fuel-Dispensing Equipment and Compatibility of UST Leak Detection Systems

14.1. Statement of Knowledge Gap

Certification of gasoline dispensing equipment and compatibility of underground storage tank leak detection (UST-LD) systems were not cited as knowledge gaps in the Tier I report. However, the Multimedia Working Group has expressed interest in these areas in the months since the Tier I report was approved. This chapter describes work Butamax is pursuing outside the Multimedia process, in conjunction with fuel-dispensing standards / listing organizations and national working groups, to verify the compatibility of isobutanol blended gasoline with existing fuel-dispensing and leak detection systems.

14.2. Overview

Regulations in most US states require key equipment in fuel-dispensing systems to be certified or 'listed.' Although exact definitions for 'listed' vary in detail, the common intent is to require fuel-dispensing facilities to select equipment from a list published by a nationally recognized testing laboratory whose listing indicates the equipment complies with applicable nationally recognized standards, or has been tested and found suitable for use in the specified application, or both.

The listing organization / testing laboratory must also be acceptable to the relevant Authority Having Jurisdiction (AHJ). For retail fuel-dispensing equipment including, the most commonly accepted listing organization is Underwriters Laboratories (UL). The relevant AHJs vary state by state, but often include the state fire marshal, agriculture department, and state EPA or natural resource departments. Listing requirements can include but are not limited to equipment such as integrated meter/dispenser units, hose, nozzles, piping, pumps, and storage tanks.

California regulations require use of listed fuel-dispensing equipment. The California Fire Code (Title 24, Part 9, Chapter 22) as administered by the Office of the State Fire Marshall requires electrical equipment, dispensers, hose, nozzles, and submersible or subsurface pumps used in fuel-dispensing systems to be listed. Because of UL's dominance in developing standards and certifications for fuel-dispensing, existing fuel-dispensing equipment in California is most likely listed by UL.

Compatibility of UST leak detection systems with 16% isobutanol blends (as well mid-level and flex-fuel ethanol blends E15 to E85) is currently under study by industry groups. The Environmental Technology Verification UST LD stakeholder committee is working with Battelle in developing a revised Technology Assessment to help inform the National Working Group on Leak Detection Evaluations (NWGLDE) and EPA's Office of Underground Storage Tanks (OUST) on applicability of UST-LD technologies in these gasoline-alchohol blends. This work will provide a technology assessment of the impact of 16% isobutanol gasoline blends (as well as ethanol blends with alcohol content ranging from E0 to E85) on the functionality of leak detection equipment.

14.3. UL Guidance for Isobutanol Fuels

UL certifications for fuel-dispensing equipment are specific by fuel type. For example, many dispensers in use today were certified to UL Standard 87 which can include applications in gasoline containing up to 10% ethanol. Since about 2010, newer dispensing equipment may be listed under UL Subject 87A which provides two levels of certification in ethanol fuels: (1) for gasoline blends containing up to 25% ethanol and (2) for flex-fuels up to E85.

Although UL does not modify existing certifications *per se* for additional fuel types, UL has developed methods for advising AHJs so as to permit use of new biofuels in previously-certified fuel-dispensing equipment. As an example, UL recently provided guidance on the use of diesel fuels containing up to 5 vol% biodiesel.[49] In providing this guidance, UL investigated properties of the new fuel and found no adverse impact to safety or performance against UL standards. In the investigation, UL found material compatibility analyses sufficient for evaluating safety performance of the systems using the new biofuel and equipment tests were not required.

UL also investigated the use of mid-level ethanol blends (e.g., E15, E25) in equipment certified to UL Standard 87 for E10. In studies including the US Department of Energy and its national laboratories, UL evaluated the effects of mid-level ethanol blends on storage and dispensing system materials including elastomers, plastics, and metals[50] as well as the performance of dispensing system components.[51] These investigations established essential links between the observation of standard compatibility evaluations of dispensing system materials and performance of dispensing equipment in certification tests, thereby forming a basis for predicting equipment performance from materials compatibility observations.

Butamax has engaged UL to develop the data required for advising AHJs on the use of 16% isobutanol gasoline in equipment certified to UL standards. These studies will follow the principles and learnings from the foregoing biodiesel and mid-level ethanol investigations discussed above. The hypotheses investigated, program design, and test execution are at UL's discretion with Butamax acting as research project sponsor. As the investigations proceed, UL will determine if material compatibility tests need to be augmented with device testing in order to prepare their advice statement. UL also encouraged Butamax to engage Oak Ridge National Laboratory (ORNL) for additional investigation of material compatibility with 16% isobutanol blends. As described below, ORNL's scope of work will compliment UL's own investigations by replicating previous

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[49] Underwriters Laboratories Announces Position on Use of B5 Biodiesel Blends
http://www.ul.com/global/eng/pages/offerings/industries/appliancesandhvac/gasoilsolidfuel/release/

[50] Compatibility Study for Plastic, Elastomeric and Metallic Fueling Infrastructure Materials Exposed to Aggressive Formulations of Ethanol Blended Gasoline. ORNL/TM-2012/88, M. D. Kass, et al. May 2012

[51] Dispensing Equipment Testing with Mid-Level Ethanol/Gasoline Test Fluid. NREL/SR-7A20-49187, Kenneth Boyce and J. Thomas Chapin, November 2010.

work on mid-level ethanol materials compatibility for isobutanol blends for a broad range of materials found is fuel storage and dispensing systems.

14.4. Fuel-Dispensing Equipment Compatibility Testing

UL standards for certification of fuel dispensing equipment include performance tests using 'aggressive' test fluid surrogates instead of actual fuels. In addition to forming a consistent and reproducible basis for testing, these test fluids are intended to provide an additional margin of safety by requiring equipment to perform in fluids with increased potential for materials incompatibility. In the case of equipment to be used in gasoline / alcohol fuel service, the test fluids include (1) ASTM Fuel C (50/50 iso-octane / toluene) as hydrocarbon base, (2) elevated alcohol level compared to the intended service level, and (3) contaminants in the alcohol components as specified by SAE J1681 Recommended Practice for fuel surrogates to be used in materials compatibility testing.[52]

As shown in the Tier 1 report, isobutanol / gasoline blends are often less aggressive to materials in fuel-dispensing systems than comparable blends made with lighter alcohols.[53] These data have been reviewed with UL and have been used to form a hypothesis for further investigations on compatibility of isobutanol blends with fuel-dispensing equipment: 'aggressive' isobutanol test fluids should show similar or improved compatibility with fuel-dispensing system materials compared to the ethanol-based test fluids already in use for certification testing of equipment. UL postulate that results from testing this hypothesis will be suitable for UL to advise AHJs on the use 16% isobutanol fuels in fuel-dispensing systems. Butamax is sponsoring two experimental programs investigating this hypothesis.

14.4.1. UL Compatibility Testing

UL have conducted an investigation entitled "Swelling Study of Elastomers Exposed with Isobutanol / Gasoline Fuel Blends" which measured compatibility of isobutanol / gasoline blends and compared the results with corresponding data on ethanol blends. While the focus of the study was swelling, hardness changes, compositional changes (thermal gravimetric analysis, pyrolysis GC/MC, etc.) of elastomer sealing materials, and electrical conductivity of the test fuels were also collected and compared for physical property impact and metal corrosion potential.

The study covered blends of ASTM Fuel C with isobutanol in concentrations from 10 to 30 vol%, including an 'aggressive' isobutanol formulation following the precedent for aggressive alcohols from SAE J1681, 10 vol% and 15 vol% ethanol. Fifteen elastomer materials were evaluated including:

- Fluoro rubbers FKM (Viton™ A and B)

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[52] Surface Vehicle Recommended Practice J1681 Gasoline, Alcohol, and Diesel Fuel Surrogates for Materials Testing, Society of Automotive Engineers.

[53] Biobutanol Multimedia Evaluation Tier 1 Report, Butamax™ Advanced Biofuels, pp 24 – 27.

- acrylonitrile butadiene rubbers NBR – 5 grades
- chloroprene rubber (neoprene) CR
- styrene butadiene rubber SBR
- polyurethane PU
- silicone rubber FMQ
- fluorosilicone rubber FVMQ
- natural rubber NR
- NBR-impregnated cork
- epichlorohydrin rubber (ECO)-impregnated cork

Elastomer samples were exposed at laboratory ambient conditions for three weeks with pre- and post-measurement of volume (swelling), mass, and hardness. Thermogravimetric analysis was used to identify degradation and stability of the elastomer samples before and after test fluid exposure for comparison of material degradation with samples exposed to ethanol blends under similar conditions. Pyrolysis GC/MS was used to identify degradation compounds of elastomers in the test fuels, e.g., dissolution of elastomer additives into the test fluids.

Butamax™ will include results from this study in the Biobutanol Multimedia Tier 3 report.

14.4.2. Oak Ridge National Laboratory Compatibility Testing

The Fuels, Engines, and Emissions Research Center at Oak Ridge National Laboratory recently completed studies on the compatibility of intermediate ethanol blends on fuel-dispensing infrastructure materials.[54] These studies included a broad range of elastomer, metal, and plastic materials exposed to aggressive ethanol surrogate fuels at elevated temperatures. In addition to the elevated intermediate ethanol levels of future interest, the study also baselined compatibility of these materials in E0 and E10 test fluids. Under the advice of UL, Butamax has commissioned a study of essentially identical content using aggressive isobutanol surrogate fuels. This study will provide additional data for UL's consideration in evaluating the compatibility of fuel-dispensing equipment with isobutanol fuels, especially as compared to the ethanol fuels.

The study made use of two environmental stir chambers originally constructed for the intermediate ethanol compatibility studies. One chamber exposed specimens to an aggressive formulation of ASTM Fuel C with 16% isobutanol, while the other extended the study range by using an aggressive 24% isobutanol test fluid. Elastomer and metal specimens were exposed for 4 weeks and evaluated for changes in mass, volume, elastomer hardness, glass transition temperature, and metal corrosion. Plastic specimens were exposed for 16 weeks and evaluated for changes in mass, volume, hardness and glass transition temperature. Test specimens were exposed in either the liquid or vapor space of the chambers as appropriate, and the chambers were maintained at 60°C during

[54] Compatibility Study for Plastic, Elastomeric and Metallic Fueling Infrastructure Materials Exposed to Aggressive Formulations of Ethanol Blended Gasoline. ORNL/TM-2012/88, M. D. Kass, et al. May 2012.

the exposure period. Materials evaluated were similar to those used in the intermediate ethanol studies and are listed in Table 14.1.

Butamax will include results from this study in the Biobutanol Multimedia Tier 3 report.

Metals/Alloys	Elastomers	Plastics
304 stainless steel	Viton™ A (FKM)	High-density polyethylene (HDPE)
1020 carbon steel	Viton™ B (FKM)	Polypropylene (PP)
1100 aluminum	Acrylonitrile butadiene rubber (NBR)	Polyoxymethylene (POM)
Cartridge brass	Silicone rubber (FMQ)	Nylon (PA)
Phosphor bronze	Fluorosilicone rubber (FVMQ)	Polyvinylidene fluoride (PVDF)
Nickel 201	Neoprene rubber (CR)	Polytetrafluoroethylene (PTFE)
Terne-plated steel	Styrene butadiene rubber (SBR)	Polyphenylene sulfide (PPS)
Galvanized steel	Polyurethane (PU)	Polyethylene terephthalate (PET)
Cr-plated brass	Rubberized cork (NBR type)	Polybutylene terephthalate
Cr-plated steel	Rubberized cork (ECO type)	Polythiourea
Ni-plated aluminum		Epoxy vinyl ester resin
Ni-plated steel		Terephthalic ester resin
Zn-plated steel		

Table 14.1 Materials for ORNL isobutanol compatibility study

14.5. Leak Detection Equipment Testing

The Battelle project on Environmental Technology Evaluation (ETV) UST LD will evaluate three technology categories of equipment in order to revise and finalize the Technology Assessment. The approach will be similar to that used by Ken Wilcox Associates for the biodiesel study for the NBB (National Biodiesel Board). A Quality Assurance Program Plan (QAPP) will be prepared and relevant data will be collected; published literature and data will be reviewed.

The QAPP experimental testing will be conducted in three phases:

- Bench-scale Test Set
  - Small-scale studies (< 1 L samples)
  - Purpose: Determine intrinsic (belonging to samples by their nature) and intensive properties (not depending on sample size)
  - Six ethanol blends (0%, 10%, 15%, 30%, 50%, 85%) and one isobutanol blend(16%) tested for non-additive volume changes and interface determination (position vs water concentration by absorbance).
  - Six ethanol blends (0%, 10%, 15%, 30%, 50%, 85%) and one isobutanol blend(16%) each mixed with 5 levels of water (0.00%,

0.25%, 0.50%, 2.50%, 5.00%) to generate 35 blends tested for pH, density, conductivity, viscosity and thermal expansion (5 to 30 ºC).

- Laboratory-scale Test Set
  - Meso-scale studies (~10 L)
  - Purpose: Simulate water ingress in a small-scale tank and evaluate potential effects on detection ability of various LD technologies (ATG and two others) in biofuels to inform operation and predict performance on an operational scale
  - Three ethanol blends (0%, 15%, 85%) and one isobutanol blend (16%) tested using laboratory equipment for continuous water ingress with and without a splash for minimum detection height and smallest detection increment.
  - Three ethanol blends (0%, 15%, 85%) and one isobutanol blend (16%) tested using laboratory equipment for quick water dump followed by fuel dump to induce and observe phase separation.

- Full-scale Test Set
  - Full-scale studies (10,000s L)
  - Purpose: Evaluate leak detection performance data collected from LD technologies' (ATG and two others) operation in a real-world biofuel environment
  - Data collected under tight-tank conditions (59 runs) and simulated-leak conditions (0.1, 0.2 and 0.3 gal/hr leak; 10 runs)

Laboratory testing is scheduled for June 2013.

The overall study will allow direct comparison of isobutanol 16% gasoline blend with E0, E10, E15 and E85. Battelle will revise and finalize the Technology Assessment based on this study. The Technology Assessment will help inform NWGLDE (National Working Group on Leak Detection Evaluations) and EPA-OUST with making decisions on applicability of leak-detection technology listings.

15. References

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16.2. Appendix B – E-Fate Modeling Proposal from Professor Pedro Alvarez

Modeling dissolved BTX plume centerline lengths for groundwater impacted by releases of various biofuel mixtures

Research Proposal to BP America

Pedro J. Alvarez, Rice University

16.2.1. Summary

An advanced (numerical) computer module - designated the "General Substrate Interaction Module" (GSIM) - was recently developed to evaluate the impact of ethanol on benzene plume elongation [*Gomez et al.*, 2008], for use with the RT3D (Reactive Transport in 3-Dimensions) model [*Clement et al.*, 1998]. We will adapt this model to consider other types of biofuels (e.g., butanol, and novel proprietary compounds) at various concentrations in gasoline, consider their impact on the elongation of alkylbenzene (TEX) plumes, and consider a broader electron acceptor pool by including other possible anaerobic degradation pathways besides aerobic and methanogenic degradation (e.g., nitrate-, iron- and sulfate-reducing pathways).

16.2.2. Introduction

Previous models addressing the effect of ethanol on benzene plume length have typically considered important fate and transport processes, such as advection, dispersion, sorption, aerobic and anaerobic biodegradation, and ethanol-driven O2 depletion. *Heermann and Powers* [1996] considered 2D transport, with focus on cosolvency and mass transfer effects, and obtained a 10% increase in the length of a simulated m-xylene plume. *McNab et al.* [1999] considered 3D aqueous transport from a finite source release zone and assumed that no anaerobic benzene degradation would occur following oxygen depletion exerted by ethanol, which resulted in a benzene plume elongation on the order of 100%. *Molson et al*. [2002], considered 3D transport and microbial growth following Monod kinetics, including competition for oxygen between ethanol and hydrocarbon degraders. These simulations showed benzene plume elongation of up to 150%.

Although valuable insight into how ethanol influences hydrocarbon plume dynamics, including competitive inhibition processes (*Lu et al.*, 1999), most of these models have not simulated potentially important substrate interactions that influence catabolic enzyme induction (i.e., the synthesis of an enzyme by the cell, when in the presence of a specific substrate) and the metabolic flux of the target pollutants (i.e., the rate at which a pollutant such as benzene is metabolized per unit of biomass, which is analogous to the specific utilization rate). These interactions can cause slower BTEX degradation rates at sites with high ethanol concentrations [*Lovanh and Alvarez*, 2004], although this negative effect can be offset by higher microbial concentrations resulting from the presence of ethanol as an additional substrate [*Lovanh et al.*, 2002].

GSIM expands on these models by incorporating catabolite repression, metabolic flux dilution, cosolvency and microbial population dynamics. Although GSIM is calibrated to work only with ethanol and benzene under aerobic and strongly anaerobic (methanogenic) conditions, the model is flexible enough that it can simulate any BTEX plumes under the effect of different biofuels and with multiple electron accepting pathways.

16.2.3. Objectives

The objective of this research is to develop and utilize a user-friendly mathematical model, based on the GSIM model, to accomplish the following deliverables.

1) Simulate the dissolved plume centerline lengths of biofuel-gasoline mixtures (with the main constituents being ethanol, butanol or other biofuel and monoraomatic hydrocarbons, BTEX) following releases to groundwater. The baseline GISM model can achieve this goal, with recalibration of co-solvency, sorption and degradation kinetic parameters needed for different biofuel and TEX molecules.

2) Consider organic/water phase partitioning, adsorption, retardation, and other common groundwater fate and transport mechanisms (currently implemented through the native RT3D features).

3) Consider important substrate interactions that influence degradation rates and the growth of specific degraders, through the use of an external module (currently GSIM)m which include: (1) metabolic flux dilution (MFD), which is defined as a decrease in the specific benzene utilization rate due to non-competitive inhibition when ethanol is present [*Lovanh and Alvarez,* 2004]; (2) catabolite repression, which is defined as the repression of inducible enzymes that degrade the target pollutant (e.g., benzene) by the presence of a preferred carbon source (e.g., ethanol) [*Madigan et al.,* 2000]; (3) Cosolvency effects of ethanol on source zone dissolution and water-soil partitioning of dissolved BTEX components; and (4) proliferation of different microbial populations in response to changes in electron acceptor and substrate availability.

4) Include biodegradation kinetics of the species involved (specific growth rate, biomass yield and half-saturation coefficients). These values can be obtained from laboratory experiments or the literature. If this data is not readily available, first-order degradation coefficients can be easily used in lieu of Monod kinetics. Currently the model has parameters calibrated for ethanol and benzene only, but it is flexible enough to include TEX and any number of other compounds, if biodegradation parameters are available.

5) Expand electron acceptor pool to be considered, and include additional electron acceptor pathways (denitrifying, iron reducing and sulfate reducing) to simulate the impact of limited mass of electron acceptors along the groundwater flow pathway. The model already simulates depletion of oxygen with rapid transition to strongly anaerobic (methanogenic) conditions.

6) Consider sorption-related retardation of BTEX and the biofuel molecule as sole attenuation mechanism, without biodegradation. This is easily achieved in the current model just by turning off biodegradation processes in the input file.

A final report and user manual will also be prepared.

16.2.4. Methodology

Contaminant Partitioning and Transport

Contaminant advection, dispersion and adsorption to aquifer material were simulated using existing models, RT3D (Reactive Transport in 3-Dimensions; *Clement et al.,* 1998) and the USGS flow model MODFLOW (MODular three-dimensional finite-difference ground-water FLOW) model [*Harbaugh et al.,* 2000]. RT3D describes reactive-flow and transport of multiple mobile and/or immobile species by solving the 3D reactive advection dispersion equation that governs these processes:

$$\frac{\partial C}{\partial t} = \left[D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2} \right] - \left[\bar{v}_x \frac{\partial C}{\partial x} + \bar{v}_y \frac{\partial C}{\partial y} + \bar{v}_z \frac{\partial C}{\partial z} \right] + r \qquad (1)$$

where D_i is the coefficient of hydrodynamic dispersion along the *i* axis (m^2/d), *C* is the contaminant aqueous-phase concentration (mg/l), $\bar{v}_i$ is the seepage velocity along the *i* axis (m/d), and *r* are all the reactions that occur in the aqueous and solid phases (mg/l-d).

RT3D uses the solvers for advection and dispersion from the 1997 Department of Defense version of MT3D, and requires MODFLOW to compute variations in groundwater head distribution (groundwater flow). It is a generalized multi-species version of the U.S. Environmental Protection Agency (EPA) transport code, MT3D [*Zheng,* 1990].The transport equation considers changes in concentration due to advection (water flow), dispersion, molecular diffusion, and external sources/sinks and reactions on the water/solid phase. GSIM calculates the value of the reaction term *r* explicitly, for each time step of the model, using a time-splitting approach. RT3D has been previously validated by comparing the code results against various numerical and analytical solutions [*Clement et al.,* 2000; *Sun and Clement,* 1998; *Sun et al.* 1998].

16.2.5. Substrate Interactions and Biodegradation

One of the main advantages of RT3D is that it has a user-defined reaction option that can be used to simulate any type of user-specified reaction kinetics [*Clement,* 1998]. This capability allows the development of custom biodegradation reaction modules without changing the coded flow and transport processes.

A unique feature of the GSIM biodegradation module for RT3D is that it incorporates metabolic flux dilution (MFD) and catabolite repression (CR). The metabolic flux of a compound is defined as the rate at which it is metabolized per unit biomass. Therefore, the specific substrate utilization rate (i.e., the degradation rate per unit biomass, U (g-substrate g-cells^{-1} hr^{-1}), is a direct measure of metabolic flux. Metabolic flux dilution is a form of non-competitive inhibition that decreases the specific rate of utilization of one substrate due to the utilization of another substrate [*Lovanh and Alvarez,* 2004]. Previous laboratory studies have shown that the metabolic flux of a compound in a mixture is proportional to its relative availability, expressed as a fraction of the available organic carbon [*Egli et al.,* 1993; *Lovanh et al.,* 2002].

Limitations to benzene biodegradation rates caused by MFD are incorporated into GSIM through the variable f_S, which is calculated as the aqueous concentration of a substrate S (benzene in this case) divided by the total concentration of other dissolved organic species, expressed on a total organic carbon (TOC) basis after excluding biomass:

$$f_S = \frac{S_{TOC}}{T_{TOC}} \qquad (2)$$

where f_S is the metabolic flux dilution factor (dimensionless), S_{TOC} is the substrate concentration as total organic carbon (mg/l) and T_{TOC} is the total organic carbon concentration (mg/l). The specific substrate utilization rate of the substrate in the absence of ethanol (U_S, [g/g-d]) is multiplied by f_S [*Lovanh and Alvarez,* 2004] to obtain the corrected rate (U_S^*, [g/g-d]). That is,

$$U_S^* = f_S \cdot U_S \qquad (3)$$

Thus, as the concentration of ethanol increases, f_S decreases, and the specific substrate utilization rate of benzene is increasingly diminished, potentially leading to longer plumes.

Catabolite repression (CR) was modeled as a modulated mechanism in which the induction of a hydrocarbon catabolic gene decreases with increasing concentrations of the repressor (i.e., ethanol) and increases with the relative availability of the inducer (i.e., benzene) in the mixture, as shown by *Lovanh and Alvarez* [2004]. Thus, CR was considered by assuming that U_S^* is proportional to f_S. Recalling that MFD separately implies that U_S^* is also proportional to f_S (eq. 3), a simple (multiplicative) empirical equation was used to combine the effects of MFD and CR [*Lovanh and Alvarez,* 2004]:

$$U_S^{*} = f_s^{2} \cdot U_S \quad (4)$$

Cosolvency effects of ethanol on benzene are implemented by the relationship (Rao et al, 1985):

$$Log(K_m) = Log(K_w) - \alpha \cdot \sigma \cdot f_c \quad (5)$$

Where Km is the distribution ratio in presence of the cosolvent, Kw is the distribution ratio with pure water. This relationship was later refined, (Rao et al, 1991):

$$Log(\frac{K_d^{'}}{K_d}) = -\alpha \cdot \beta \cdot \sigma \cdot f_c \quad (6)$$

Where Kd is the distribution ratio for pure water, and Kd' accounts for the presence of a cosolvent. fc is the cosolvent content as volume fraction, in this case, ethanol. σ is the cosolvent power. This relationship is valid for ethanol volume fractions of 1 to 40%. This value can be calculated if needed by (Rao et al, 1991):

$$\sigma = Log(\frac{Sc}{Sw}) \quad (7)$$

Where Sc is the solute solubility in pure cosolvent and Sw in pure water. In the case of ethanol, we will obtain the values from the literature.

The product $\alpha\beta$ in equation 6, is measured empirically and depends on various molecular interactions between cosolvent and sorbent (α), and cosolvent and solute (β). There is no documented relationship for these values and soil parameters, so they have to be measured experimentally in a case by case basis. In the case of α, the more it deviates from 1, the more the cosolvent interacts with the sorbent (soil). If the soil is relatively inert and low in organic content, then this value should approach 1. α and β have been assumed to be 1 for simplicity (conservative approach), and the values for σ for the BTEX compounds are (Reckhorn et al, 2001): 2.96, 3.58, 5.26 and 4.65 for benzene, toluene, ethylbenzene and xylene respectively. Then, for an inert soil with low organic content: (assuming $\alpha\beta = 1$)

$$K_d^{'} = K_d * 10^{-\sigma \cdot f_c} \quad (8)$$

Expressed in terms of retardation factor used in equations 10 and 11, (*li et al.*, 2000):

$$R_s^{'} = \frac{R_S - 1}{10^{-\sigma \cdot f_c}} + 1 \quad (9)$$

Substrate biodegradation is modeled using a system of equations based on multiplicative Monod kinetics that incorporate MFD plus CR (eq. 3 and 4), recognizing that the overall degradation rate (*r*) is the product of the specific degradation rate (*U*) and

the microbial concentration (*X*). Thus, degradation rate equations are derived for both aerobic (eq. 10) and anaerobic conditions (eq. 11). Oxygen consumption (eq. 12) and anaerobic electron acceptor consumption (eq. 13) [*Borden and Bedient*, 1986], aerobic biomass growth (eq. 14) and anaerobic biomass growth (eq. 15) are also considered. All biomass is assumed to be attached in the form of immobile micro-colonies that behave as fully-penetrated biofilms (*Chen et al.*, 1992), as is the case for at least 99% of subsurface microorganisms (*Harvey et al.*, 1984; *Lehman et al.*, 2001).

The reaction term (*R*) in equation 1, translates directly into equations 10 to 13, while microbial growth is represented in equations 14 and 15.

$$r_{S,Aer} = \left[\frac{dS}{dt}\right]_{Aer} = -\frac{f_S{}^2}{R_S}\left[\frac{\mu_{mS,Aer} X_{Aer}}{Y_{S,Aer}}\left(\frac{S}{K_{S,Aer}+S}\right)\left(\frac{O}{K_O+O}\right)\right] \quad (10)$$

$$r_{S,An} = \left[\frac{dS}{dt}\right]_{An} = -\frac{f_S{}^2}{R_S}\left[\frac{\mu_{mS,An} X_{An}}{Y_{S,An}}\left(\frac{S}{K_{S,An}+S}\right)\left(\frac{N}{K_N+N}\right)\right] \quad (11)$$

$$r_O = \frac{dO}{dt} = \left[r_{S,Aer} F_{S,O}\right] \quad (12)$$

$$r_N = \frac{dN}{dt} = \left[r_{S,An} F_{S,N}\right] \quad (13)$$

$$r_{X,Aer} = \frac{dX_{Aer}}{dt} = -\left[r_{S,Aer} Y_{S,Aer}\right]\left(1-\frac{\eta_{bio}}{\gamma \cdot n}\right) - b_{Aer} X_{Aer} \quad (14)$$

$$r_{X,An} = \frac{dX_{An}}{dt} = -\left[r_{S,An} Y_{S,An}\right]\left(1-\frac{\eta_{bio}}{\gamma \cdot n}\right) - b_{An} X_{An} \quad (15)$$

where $r_{S,Aer}$ and $r_{S,An}$ are the aerobic and anaerobic reaction rates (mg/l-d), *S* is the substrate concentration (mg/l), *O* is oxygen concentration (mg/l), *N* is anaerobic electron acceptor concentration (mg/l), X_{Aer} and X_{An} are the aerobic and anaerobic microbial populations (mg/l), $F_{S,O}$ and $F_{S,N}$ are the electron acceptor utilization rate. $\mu_{mS,Aer}$ and $\mu_{mS,An}$ are the maximum specific growth rate of aerobic biomass and anaerobic biomass respectively (day^{-1}), $Y_{S,Aer}$ and $Y_{S,An}$ are the aerobic and anaerobic biomass yield coefficients (g-biomass/g-substrate), $K_{S,Aer}$ and $K_{S,An}$ are the half-saturation coefficients of the substrate under aerobic and anaerobic metabolism (mg/l) (N being the concentration of any anaerobic electron acceptor). Equations 10 and 11 describe the loss of substrates due to aerobic and anaerobic biodegradation, which is conservatively assumed to occur only in the liquid phase. Catabolite repression and metabolic flux dilution, as well as soil adsorption, are accounted for through the f_S terms and retardation factor R_S. Equations 12 and 13, describe the loss of oxygen by aerobic biodegradation processes and of the appropriate electron acceptor (i.e. sulphate etc) by anaerobic biodegradation processes. Equations 14 and 15, describe aerobic and anaerobic biomass growth. The new values of

substrate, electron acceptor, and biomass concentrations at the end of each time step in each grid block are then returned to RT3D as initial values for the subsequent time step. This process is repeated for each time step of simulation.

Model verification and calibration

GISM was tested by comparing the output of MODFLOW/RT3D/GSIM with BIOSCREEN [*Newell et al.,* 1996] applied to a field study, Keesler Air Force Base (SWMU 66), with extensive data characterization. GSIM simulations considered flow and transport of BTEX, under the same set of parameters as BIOSCREEN, and biodegradation parameters from the literature, maintaining the approximate first order degradation coefficients used in that model. Hydrogeological data and biokinetic parameters used to model this site are readily available from the user's manual [*Newell et al.,* 1996]. This simulation was used to calibrate the domain simulation parameters for stability (cell size and time step), and to verify the behavior of the system of equations of the model under known conditions and results.

The simulation was run for 6 years, and the BTEX source concentration was simulated as a constant concentration of 13.7 mg/l [*Newell et al.,* 1996]. Data was compared with a first order model, thus, values of biokinetic parameters for GSIM were manipulated to simulate first-order reactions. The concentration profiles of the two presented similar results, and with an R^2 of 0.98, the GSIM module fits the data slightly better than BIOSCREEN (R^2 of 0.96).

Validation of the microbial kinetics module was done by comparing simulated benzene and ethanol concentrations with results from laboratory microcosm studies by Hunt et al. (1997). The simulations matched ethanol data with an R^2 of 0.96, and benzene data with a R^2 of 0.94. Thus, model outputs for benzene degradation in the presence of ethanol closely matched laboratory data.

Any model results considering biofuels different than ethanol, and contaminants different than benzene, should only be considered as theoretical and would require laboratory or field validation. Recalibration of the model in the absence of such data would be based on available literature data.

16.2.6. Model Limitations and software requirements

Model requires the following software to run correctly:

- Modflow : "Modflow96.exe" and associated files must be used. Version 96 can be obtained at http://water.usgs.gov/software/MODFLOW-96/
- RT3D : "rt3dv25dll.exe" must be used. Version 2.5 can be obtained at http://bioprocess.pnl.gov/rt3d.htm
- Input files for modflow and rt3d can be created manually, but it is a tedious and non-trivial process. It is recommended that they be created using GMS (Groundwater modeling system). We currently work with GMS 3.1, license obtained from http://www.ems-i.com/.

- GSIM model is a biodegradation module, and as such it does not calculate NAPL source zone dynamics. To calculate the appropriate source zone concentrations of different components (Ethanol, MTBE, butanol, BTEX, etc) different software should be used. For ethanol, we use a spreadsheet developed at Rice that is included in the model. For compounds that don't have a cosolvent effect at the source zone, the API-LNAST (http://www.api.org/ehs/groundwater/lnapl/lnapl-guide.cfm) model is recommended.

The module has several limitations that are important to highlight:

- Microbial activity is assumed to occur attached to the soil matrix. The model does not consider transport of microbial biomass or attachment/detachment kinetic.
- Substrate degradation is conservatively assumed to occur only in the liquid phase, ignoring potential decay of sorbed contaminants.
- Total organic carbon (TOC) is assumed to be completely available for degradation processes, and is only used to calculate the metabolic flux dilution factor f_s, not to calculate the specific substrate utilization rate U.
- The operator splitting solution scheme of the model requires that small time steps be used in the simulations (< 0.005 days) due to convergence and stability issues.

A personal computer and licenses of software programs required by the user friendly package will be purchased by BP America for their use and application of the software. We will load up the software and provide training as needed.

16.2.7. Budget

This one-year project requests support for salaries of scientific personnel and associated fringe benefits, amounting to $25,000 plus 51% F&A costs. The total requested budget is $ $37,750, as detailed in the attached budget forms.

16.2.8. References

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