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17 Dec 2001

Telomer Research Program Update and Status Report

United States Environmental Protection Agency
18 December 2001

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Agenda

- TRP General Update; TRP Meetings
- Radio-label Update
- Pharmacokinetics
- Toxicology & Descriptive Biology
- Environmental Fate & Effects
- Summary
- Discussion and Path Forward

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TRP Review with U.S. EPA -- 21 Feb. 2001

- Toxicology Program Status & Timing
- Environmental Fate & Effects Program, Protocols, & Timeline
- Radiolabel Material Status, Timeline

Telomer Research Program (TRP) : 2001 Key Events

- *January 9, 2001* : Environmental Fate & Effects Research Team Meeting
- *February 21, 2001* : U.S. EPA
- *March 9, 2001* : METI
- *March 20-22, 2001* : TRP Meeting, Osaka
- *April 19, 2001* : Environment Canada
- *May 16-17, 2001* : Environmental Fate & Effects Research Team Meeting
- *May 18, 2001* : U.K. Environment Agency
- *June-September 2001*: Sub-team meetings and Conference Calls
- *October 31 - Nov. 2, 2001* : TRP Meeting, Frankfurt

TRP Osaka Meeting Highlights 20-22 March 2001

- Updates on 8-2 Alcohol Starting Material and Quality
- Radio-label Synthesis Strategic Plan and Path Forward
- TRP Database Status
- Review and Upgrades to Toxicology and Pharmacokinetics Plans
- Review and Upgrades to EF&E Work Plans: Tier I, II, III
- Communications Team Work Plan

TRP Frankfurt Meeting Highlights 31 October - 2 November 2001

- Toxicology Data Review and Subsequent Upgrades to Overall Tox Plans; Review Data to Date and Scheduling
- Radio-label Synthesis Review, Sourcing Plan, and Timing
- Pharmacokinetics Plans due to Radio-label Schedule
- Analytical Effort Overview and Challenges
- EF&E Tier I and II Workplan Updates and Revisions; Review Data to Date and Scheduling
- Workplan for Compounds Beyond 8-2 Alcohol
- TRP Sponsorship of Outside Research
- Communication Strategy and Path Forward
- Next Meeting(s): Conference Calls and In Person

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$n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$ Analysis Profile

- Purity > 99% by G.C.
 - 99.2% $n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$
 - no other $\text{C}_n\text{F}_{2n+1}\text{CH}_2\text{CH}_2\text{OH}$ species
 - 0.8% impurity

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n-C₈F₁₇CH₂CH₂OH Selection Criteria

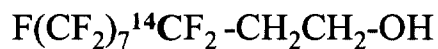
- Selection Criteria Included: Simple, Single, Pure, Representative, and Readily Characterized
- Most Common and Representative from Each of 5 Major Producers – Includes Both Type and Chain Length
- Telomer 8-2 Alcohol – F(CF₂CF₂)₄-CH₂CH₂-OH - Was Chosen To
 - Be Able to Characterize a Single Compound versus a Complex Product Mixture
 - Enable the Research Team to Study Metabolites in the Pharmacokinetics Work (ADME)
 - Study the Degradation Products and Locations in the Environmental Fate Studies
 - Enable the Team to Measure Critical Physical Property Data
 - Enable the Team to Radio-label a Single Key Compound for the Toxicology and Environmental Fate Studies

n-C₈F₁₇CH₂CH₂OH Selection Criteria

- Due to the Complexity and Non-Classic Behavior of the Fluorochemicals, Numerous Testing Protocols Had to be Developed and Validated. By Definition, a Single Compound was the Only Viable Alternative that Held Prospects to Gain Significant and Interpretable Results of Common Value
- The Ability to Analyze Products, By-Products, Metabolites, and Attain Low Detection Limits Drove the Research Team to a Single Representative Molecule, Not a Product Mixture
- Products from the 5 Major Producers Do Share Some Similarities But are for the Most Part Different
- Protocols Developed by the TRP Research Team Will Have Broad Applicability for Use by TRP Members on Other Compounds and Products of Their Choice and Relevant to their Company

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Radiolabeled 8-2 Telomer B Alcohol



Needed for Pharmacokinetics & Environmental Fate Studies

Status

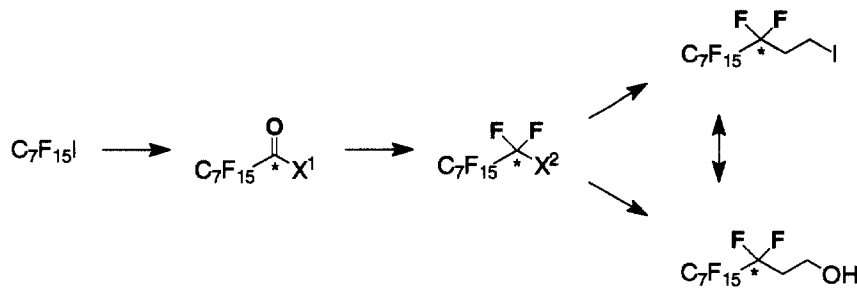
- Synthetic Route Demonstrated & Reproducible : can put ^{14}C "in"
- Necessary Documentation & Samples being finalized
- Partner identified to accomplish hot synthesis
- Timing to completion: March 2002

Synthetic Strategies for Radiolabeled Fluorotelomers



December 2001

General Synthetic Strategy



- **TFE not available as source of radiolabel**
 - practical synthesis not demonstrated; SHE issues
- **Must use readily available sources of ^{14}C**
 - e.g., CO_2 (ex. $BaCO_3$), $NaCN$, etc.
 - ample literature precedent for conversion of $C=O$ into CF_2
 - typical reagents: Et_2NSF_3 (DAST); $(MeOCH_2CH_2)_2NSF_3$ (DeoxoFluor[®])

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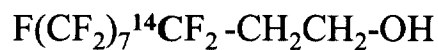
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Radiolabel Path Forward



	2001 Dec	2002 Jan	2002 Feb	2002 Mar
Chemical Synthesis Methods	done			
Package out for Bid	done			
Analytical Methods				
Cold Standards				
¹³ C Material as Standard				
¹⁴ C Study Material Delivery				

TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats 8-2 Alcohol

Objective : To determine test concentration for 90-Day toxicity study

- A) *In vivo* response
- B) Attain steady-state blood fluorine levels

Procedure : Dose Groups* of rats with 0, 5, 25 or 125 mg/kg/day of
8-2 Telomer Alcohol

- A) Monitor clinical observations, weigh rats 2x/week
- B) Total blood fluorine analysis weekly

* 10 males, 5 females / group

TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats

8-2 Alcohol

Results - Clinical Observations

- 125 mg/kg/day
 - Males: striped teeth (7/10), teeth clipped (4/10)
 - Females : striped teeth (4/5)
- No Effects : 5 - 25 mg/kg/day

TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats

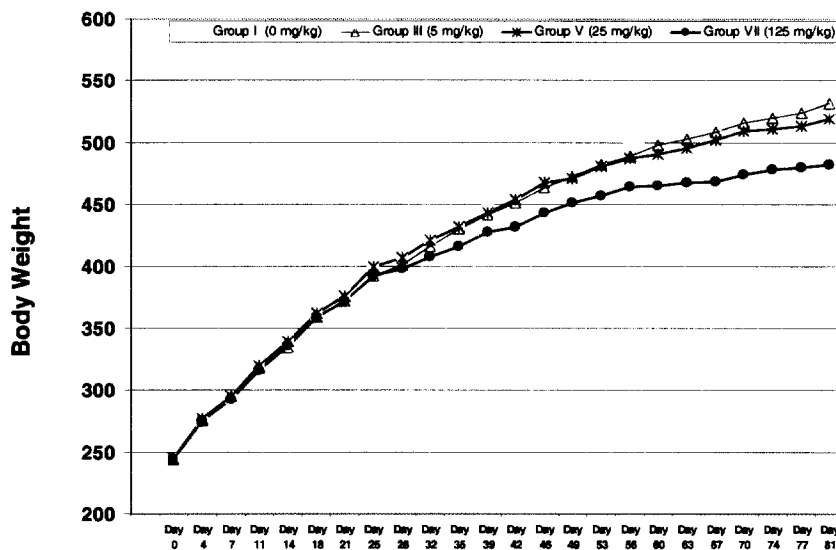
8-2 Alcohol

Results - Body Weight Gains

Dose Level (mg/kg/day)	<u>Males</u>	<u>Females</u>
0	297	80
5	288	119
25	275	115
125	238*	110

* p<0.05

Mean Body Weight for Males (Day 0 - 81)



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TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats

8-2 Alcohol

Results - Liver Weight / Ratios

Dose Level (mg/kg/day)	Liver Weight (g)		Liver/Body Weight g /100g	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
0	23.4	10.3	3.9	3.2
5	21.1	11.1	3.7	3.2
25	22.7	11.3	4.1	3.4
125	27.9	12.7	5.5*	3.6*

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* p<0.05

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TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats

8-2 Alcohol

Results - Kidney Weight / Ratios

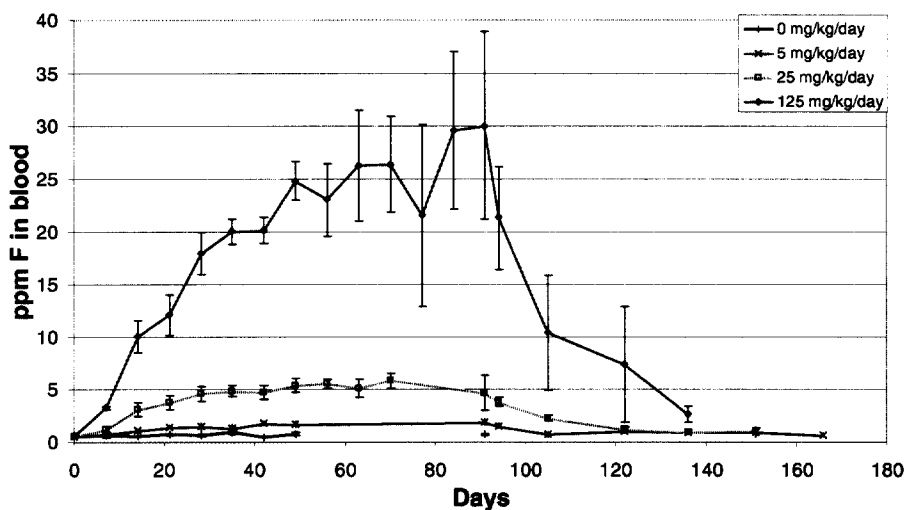
Dose Level (mg/kg/day)	Liver Weight (g)		Liver/Body Weight g /100g	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
0	4.5	2.2	0.76	0.70
5	4.3	2.4	0.76	0.71
25	4.1	2.4	0.75	0.73
125	4.4	2.7*	0.87	0.78*

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* p<0.05 Company Sanitized. Does not Contain
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Total Blood Fluorine Analysis in Male Rats Dosed with Telomer 8-2 Alcohol

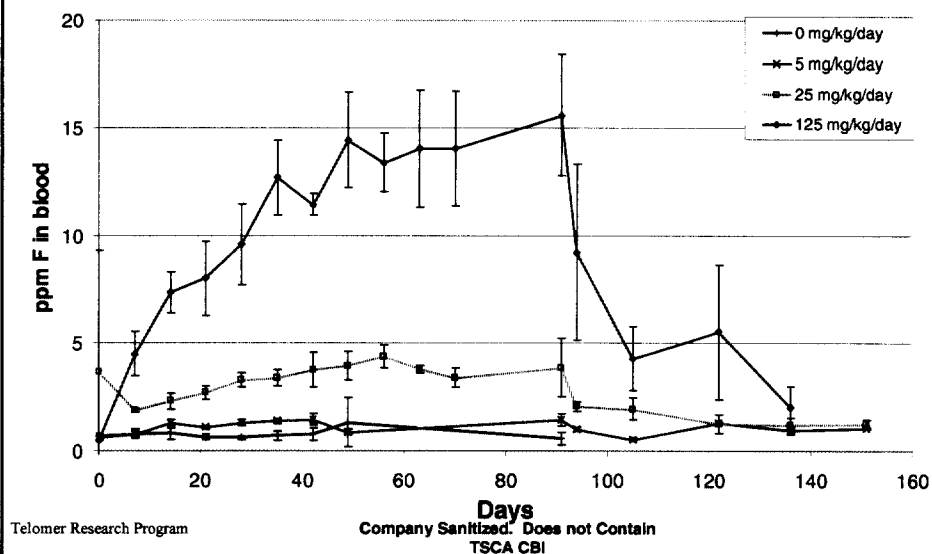


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Total Blood Fluorine Analysis in Female Rats Dosed with Telomer 8-2 Alcohol



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Repeated-Dose Pilot Study

Analysis of Pooled Plasma Samples Obtained at Steady State (test day 84) (PFOA, Parent Compound, and total fluorine levels)

The level of parent detected did not exceed 0.074 ppm in either males or females. The following plasma levels of PFOA were detected in males and females dosed with 0, 5, 25, and 125 mg/kg/day of 8-2 Telomer Alcohol

Dose (mg/kg/day)	Male PFOA (ppm)	Male PFOA converted to ppm F	Male total plasma F (ppm) ^a	Female PFOA (ppm)	Female PFOA converted to ppm F	Female total plasma F (ppm) ^a
0	0.07	0.05	NM	0.0	0.0	NM
5	2.19	1.51	NM	0.08	0.05	NM
25	8.47*	5.83	8.11	0.54	0.37	5.88
125	65.60*	45.16	53.24	1.85*	1.27	23.2

^aDetermined using Wickbold Torch; *mean of two separate runs; NM-not measured

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TRP Toxicology Studies

Repeated-Dose Pilot Study - Rats

8-2 Alcohol

125 mg/kg/day 8-2 Telomer Alcohol

- Produces clinical effects / body weight effects
- Blood Fluorine level reached plateau day 50<-->80
- Degree of response compatible with adequate survival

5 - 25 mg/kg/day Telomer Alcohol

- No clinical signs or weight effects
- Blood fluorine level reaches plateau day 40<-->60
- No signs of adverse response

90-Day Test Levels (suggested)

- 1 - 5 - 25 - 125 mg/kg/day

TRP Toxicology Studies

Repeated-Dose Pilot Study Conclusions

- Analysis limited to blood only
 - Significant male/female differences
 - Results confirm earlier studies results
 - Other metabolites are present & predominate in females
 - There is a significant amount of fluorinated organic material eliminated or elsewhere
- Validates need to conduct pharmacokinetic studies as planned with radiolabeled study material

TRP Toxicology and Descriptive Biology Studies: Timeline

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TRP Pharmacokinetics Studies: Timeline

✓ = Work Contracted

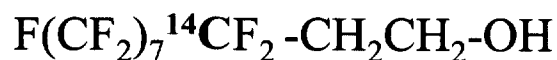
Test Material		Work	2002				2003				Contract Lab
			1Q	2Q	3Q	4Q	1Q	2Q	3Q	4Q	
8-2 Alcohol	✓	ADME Mass Balance									Haskell
	✓	In Vitro Metabolism (microsomes)									Haskell
TRP Work	✓	In Vitro Metabolism (hepatocytes)									Haskell

- Only Haskell Lab bid on entire package and will conduct the work
- Start of studies delayed 6+ months due to radio-label synthesis

TRP Pharmacokinetics Study

Radiolabeled Fluorotelomer Alcohol

^{14}C Label in perfluoro-chain (C #3)



TRP Pharmacokinetics Study

- Toxicokinetics of Telomer 8-2 Alcohol in the Rat
 - The objectives of this study are to determine the Absorption, Distribution, Metabolism and Excretion (ADME) of Telomer 8-2 Alcohol in male and female rats following oral and dermal dosing.

TRP Pharmacokinetics Study

Toxicokinetics of Telomer 8-2 Alcohol (TBA) in the Rat

Objective: Determine the absorption, distribution, metabolism and excretion of Telomer 8-2 Alcohol in male and female rats following oral and dermal dosing

Method:

- * Conduct pilot experiments
 - ** Radiolabeled and cold TBA used as test material
 - ** 2 male and 2 female rats
 - ** Determine plasma kinetics following a single high oral dose of TBA
 - ** Determine excretion and metabolism following a single high oral dose of radiolabeled TBA
- * Conduct absorption experiments
 - ** TBA test material
 - ** 4 male and 4 female rats
 - ** Single low and single high oral dose
 - ** Single low and single high dermal exposure (8 hrs)
 - ** Single low intravenous dose
 - ** Follow plasma concentrations of test material

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TRP Pharmacokinetics Study

Toxicokinetics of Telomer 8-2 Alcohol (TBA) in the Rat (Continued)

- * Distribution experiments
 - ** Radiolabeled TBA test material
 - ** 4 male and 4 female rats
 - ** Conduct oral and dermal exposures
 - ** Evaluate tissue distribution of test material at T_{max} and T_{max}/2
- * Excretion and metabolism experiments
 - ** Radiolabeled TBA test material
 - ** 4 male and 4 female rats
 - ** Single low and high oral doses
 - ** Single low and high dermal doses
 - ** Daily dose of TBA with last dose being radiolabeled TBA
 - ** Identify excretion rates and metabolic profiles

Results:

- * Metabolic profiles of TBA
- * Qualitative and quantitative description of biodistribution of TBA
- * Description of absorption characteristics of TBA
- * Sex linked differences in TBA handling in rats
- * Description of TBA metabolism at varying concentrations and between sexes

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TRP Pharmacokinetics Study

- *In Vitro* Microsomal Metabolism Study
 - The objective of this study is to determine the rate of metabolism of a test compound in microsomes prepared from livers of a number of species (normally rat, rabbit, dog, monkey, and human) and to identify the metabolites produced in each species.
- Metabolism of Telomer 8-2 Alcohol by Mammalian Hepatocytes
 - The objective of this study is to determine the rate of metabolism of a test compound in cultured liver cells prepared from a number of species (normally rat, rabbit, dog, monkey, and human) and to identify the metabolites produced in each species.

TRP Pharmacokinetics Study

Metabolism of Telomer 8-2 Alcohol by Mammalian Microsomes

Objective: Determine the rate of test material metabolism and metabolic product identification in liver microsomes prepared from several mammalian species.

Method:

- * Microsomes prepared from rat, rabbit, dog, monkey and human
- * Samples analyzed by HPLC-MS
- * Determine linearity of reaction rate and protein concentration
 - ** Reaction media contains an NADP regeneration system, cofactors, microsomes, test compound and phosphate buffer at pH 7.4, 37C
 - ** Determines kinetics of test compound
 - ** Reactions carried out at constant initial test material concentration and at a constant protein concentration
 - ** Time course metabolism by microsomes evaluated

Results:

- * Rate of microsomal metabolism of test compound by each species tested
- * Identity of metabolic products
- * Estimates of test material half-life *in-vitro* and intrinsic clearance

TRP Pharmacokinetics Study

Metabolism of Telomer 8-2 Alcohol by Mammalian Hepatocytes

Objective: To evaluate the rate of metabolism and identification of metabolic products in cultured liver cells from several mammalian species.

Method:

- Prepare hepatocytes from rat, mouse, rabbit, monkey, dog and human
- Negative (vehicle) and positive (ethoxycoumarin) controls included
- Hepatocytes and compound incubated at 37C for 2 hrs
- Samples analyzed by HPLC-MS for test compound and metabolites

Results:

- Qualitative and quantitative description of metabolic activity toward test material in several species
- Description of metabolism in a metabolically "complete" system
- Identity of test material metabolites in a whole cell system

TRP Pharmacokinetics Study

Development of a Biologically-Based Kinetic Model:

- Objective:
 - Quantitatively Relate Exposure to Internal Dose Using a Biologically-based Kinetic Model
- Critical Information Requirements:
 - Partitioning in Biological Tissue
 - Qualitative and Quantitative Aspects of Biotransformation
 - Mass Balance and Biodistribution
 - Uptake and Elimination Kinetics

TRP Toxicology Studies

Developmental Toxicity - Rats 8-2 Alcohol

Status: Initiate 3Q 2002

- Pregnant rats
- Dose days 6-19 of gestation
- Doses from 90-day study (project 1<-->10, 10<-->50, 50<-->125 mg/kg)
- Maternal parameters
 - clinical signs, body weights, liver weight
- Fetal parameters
 - external examination, internal organ examination, liver weight, skeletal examination
- Compare maternal/fetal response

TRP Toxicology Studies

Acute Toxicity - Rats 8-2 Alcohol

Status: Completed

Rat - Male and Female

- 5/sex/group
- Single oral dose of 2,000 mg/kg (0.5% aqueous methylcellulose)
- Results
 - No mortality
 - No clinical signs including body weight
 - Lethal dose >2,000 mg/kg

TRP Toxicology Studies

90-Day Toxicity Study - Rats

8-2 Alcohol

Additions to Protocol

1. Test levels (4 instead of 3) 1, 5, 25, and 125 mg/kg
2. Extend recovery period - 90 days
3. Add measure of peroxisome proliferation - β -oxidation
4. Add thyroid weights, plasma and urinary [F]

TRP Toxicology Studies

Genetic Toxicity

8-2 Alcohol

Status: Draft Reports

- Salmonella/Escherichia coli Assay
 - TA 98, TA 100, TA 1535, TA 1537 WP2uvrA
 - With/without S9 activation
 - To 5,000 μ g/plate
 - Negative (draft report to issue 12/01)

TRP Toxicology Studies

Genetic Toxicity

8-2 Alcohol

- *In Vivo* Rat Micronucleus Study
- 0, 500, 1,000, 2,000 mg/kg single dose oral gavage
- 6 male rats/group (examined 5) - harvest at 24 and 48 hours
- 60 mg/kg cyclophosphamide positive control
- Micronucleated cells/2000/animal

<u>Dose (mg/kg)</u>	<u>24 hrs</u>	<u>48 hrs</u>
0	0.04	0.07
CP-60	3.10	0.78
8-2 Alcohol - 500	0.04	0.09
-1000	0.15	0.09
-2000	0.10	0.09

- **Conclude:** No increase in micronucleated cells
(draft report available 12/5/01)

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TRP Toxicology Studies

Reproductive Toxicity - Rats

Multigeneration Reproduction - 8-2 Alcohol

Status: Pending Outcome of 90-Day Study/Developmental Toxicity

- Rat - dosing levels/control
 - treat 70 days pre-mating
 - 2nd generation from F1 offspring
- Parameters - estrous cycling/sperm assessment
 - fertility/reproductive function
 - gestational/lactational performance
 - pup growth/survival/structural integrity
 - P1/F1 parental pathology (focus on reproductive system)

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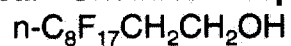
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TRP Environmental Fate & Effects Studies

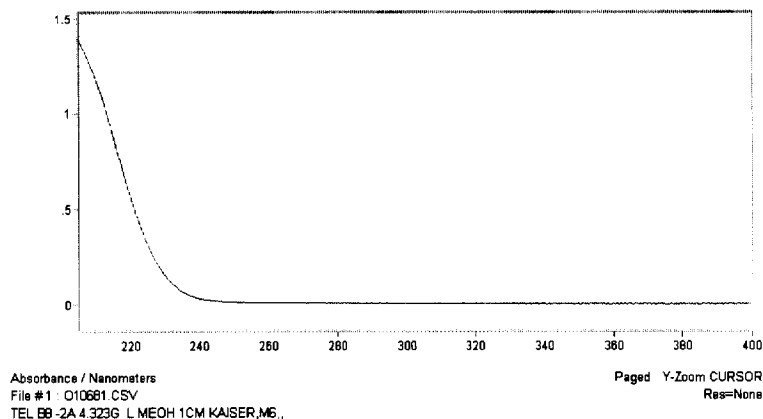
- Physical - Chemical Properties
- Environmental Fate
- Environmental Effects
- Bioconcentration

Physical - Chemical Properties



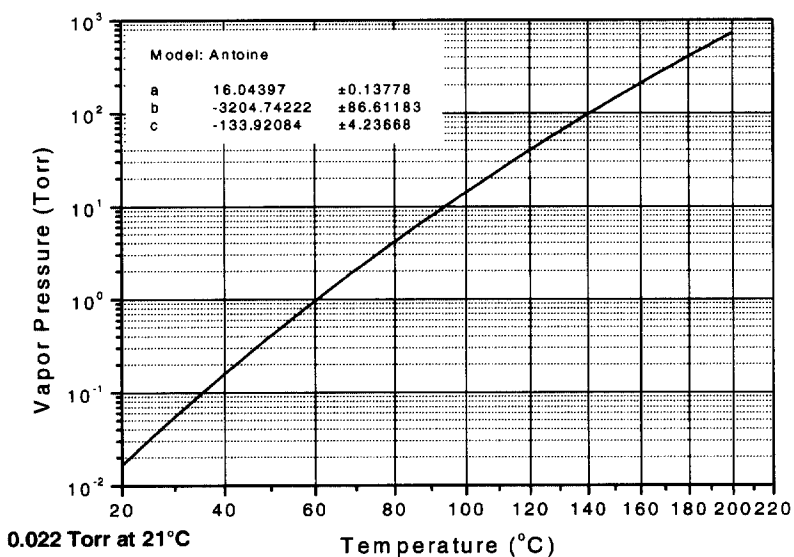
- UV / Visible Absorption Spectrum
- Vapor Pressure
- Sorption
- Water Solubility
- Henry's Law Constant
- Log K_{ow} (Octanol - Water Partition)

UV-Visible Spectra : $n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$



- No absorbance in visible light range, 350-800nm
- Direct photolytic degradation not expected
- Indirect Photolysis [OH Radical] work underway

Vapor Pressure : $n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$



Physical - Chemical Properties : Sorption
 $\text{n-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

- rapid sorption from saturated aqueous solution
 - glass, silanized glass, plastic, PTFE, stainless steel
- *evidence of time-dependent adsorption with decreasing ability to recover material from surface(s)*
- specific recovery methods had to be developed and validated
 - weeks for each sample to reach equilibrium with continuous analysis
 - 8 to 20 samples replicated to establish method and analysis confidence
- *makes water solubility measurement much more complicated and mass balances difficult to achieve*

Physical - Chemical Properties : Water Solubility
 $\text{n-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

- pH 3, 7, 9 ; T = 12, 25, 37, 60°C
- see no differences in solubility versus pH
- *material is adsorbed to the container, not in the water, when excess material is no longer present*
 - sorbed to the container surfaces

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Physical - Chemical Properties : Water Solubility
 $n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

	Solubility $\mu\text{g} / \text{mL}$ (ppm)			
	<u>12°C</u>	<u>25°C</u>	<u>37°C</u>	<u>60°C</u>
$\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$	0.134 ± 0.029	0.150 ± 0.005	0.318 ± 0.176	
APFO	> 10 wt%			
PFOS	680			

- De-ionized water measurements

Physical - Chemical Properties : Henry's Law Constant
 $n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

- Computed from Water Solubility and Vapor Pressure
- Non-linearity and Non-traditional behavior
- Surface Tension Driving Behavior?
- Measure utilizing Static Headspace Method

Physical - Chemical Properties : Conclusions

$n\text{-C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

- Water Solubility varies with T but not pH and is in the 100's of ppb
- Highly sorptive onto many surfaces and may remain highly sorbed and difficult to remove
- Preliminary VP data do not exhibit anomalies
- No visible light absorbance
- These properties present *significant* method development and analysis challenges going forward and will require use of radiolabeled material for more studies than expected

Environmental Fate & Effects Work Plan



Physical Properties

- UV/Visible Spectrum
- Water Solubility
- Adsorption to Glass
- Air-Water Partition Coefficient

Environmental Fate : Abiotic

- Hydrolysis
- Adsorption / Desorption *
- Indirect Photolysis in Air

Environmental Fate : Biotic

- Anaerobic Biodegradation*
 - Aerobic Biodegradation*
- (* Radiolabeled Material)

Environmental Effects

- Bioconcentration Factor
- 90 Day Early Life Stage Study – Rainbow Trout
- 21 Day Invertebrate Daphnia Study

TRP Environmental Fate & Effects Studies : Timeline

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TRP Path Forward Items

- Toxicology & Descriptive Biology
- Pharmacokinetics
- Radiolabel
- Environmental Fate
- Environmental Effects
- Mass Balance Effort

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Discussion and Path Forward

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