AR226-2724

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January 10, 2005

Peter D. Robertson
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Mr. Charles M. Auer
Director
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
EPA East
1201 Constitution Avenue, NW
Washington, DC 20460

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Re: Further DuPont Voluntary Response to EPA's November 4, 2004 Request
Submission to AR-226 and TSCA 8(e) Office
Miscellaneous: Analytical Methods and Environmental Monitoring/Fate

Dear Charlie:

The attached document production and information submitted with this letter represents DuPont's continued good faith effort to respond to EPA's November 4, 2004 request for voluntary submission of certain material.

Submission for January 10, 2005

The documents submitted with this letter are documents that DuPont located during this continued document review and that relate to categories for which DuPont has already made document submissions – namely, environmental monitoring, biodegradation, environmental fate, and water analytical methods. A summary of the steps taken to collect the information requested by EPA for this submission, along with the guidelines used to assess responsiveness to the EPA request, is provided on the Attachment 1 to this letter.

A summary of the contents of this submission is provided in Attachment 2 to this letter. There is confidential business information contained in this submission. In addition to a cumulative index for this submission, each individual binder also contains an index for the contents of the binder.

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TSCA 8(e) Office Submission

DuPont does not believe that the information submitted with this letter triggers reporting obligations under TSCA section 8(e). However, in the course of discussions with OPPT and the Office of Regulatory Enforcement (ORE), it has become clear to DuPont that those two offices are applying standards of reporting under TSCA section 8(e) that DuPont cannot anticipate. Accordingly, DuPont initially informed EPA that DuPont intended to submit all productions to both the AR-226 docket and the TSCA 8(e) office, as was done with the submission of November 15, 2004. EPA has, however, informed counsel for DuPont that it is not necessary to make a duplicate, formal submission to the 8(e) office in order to discharge any reporting obligations that EPA might otherwise assert. Instead, EPA has asked that DuPont make only a single submission and advised that DuPont should indicate in this cover letter that the submission is intended for both the AR-226 docket and the TSCA 8(e) office.

As such, DuPont states that the enclosed documents are intended to be a submission to both the AR-226 docket and, as a precaution, to the TSCA 8(e) office, notwithstanding DuPont's firm belief that the information does not trigger reporting obligations under that section of TSCA. This submission should not be construed as a direct or indirect admission that DuPont believes that any of the enclosed information triggers such reporting obligations. We understand that ORE has agreed that DuPont's submission shall not prevent DuPont from asserting, in any proceeding, that section 8(e) did not require submission of this information.

Continued Activity

After EPA has had the opportunity to review this letter and the documents, we are available at your convenience to discuss the documents in further detail to assist in your understanding. In the meantime, we will continue to review and produce any additional documents that are located and are relevant to the topics that are the subject of this submission, or prior submissions, and create the electronic files you requested.

Please contact me directly if you have any questions or need further clarification with respect to this submission.

Sincerely,

Peter D. Robertson

Attachments

- Attachment 1
- Attachment 2 – Summary of submission (3 volumes – 2 public, 1 CBI)

ATTACHMENT 1

The sections below provide a summary of the steps taken to collect and produce the information provided in this submission. DuPont had previously provided EPA with documents pertaining to Environmental Monitoring, Environmental Fate, and Biodegradation. For each category, please note that DuPont does not maintain a comprehensive file of all such information for the entire Company. In making the initial submissions for these categories and other categories, documents were collected either from the over one million pages of documents collected for a class action lawsuit in West Virginia or from files of individuals most likely to have documents responsive to EPA's request. Note that the documents collected for purposes of the West Virginia litigation are primarily related to PFOA. After the initial submissions were made, review continued of collected documents pertaining to the subject matter of this letter and the documents submitted with this letter are the result of that review.

For purposes of this document production, and in the interest of attempting to make this production useful and purposeful to the Agency and to avoid redundancy, DuPont followed the guidelines provided below. Please note, however, that if the Agency wants DuPont to submit the type of information that is indicated below as not being submitted herewith, please notify us and we will provide the requested information.

It is noted, in particular, that DuPont requested specific guidance on whether the Agency wanted DuPont to provide all underlying raw data and other underlying information for environmental data. The Agency stated that, at this time, it is not necessary to provide those documents. DuPont will provide such information upon request from the Agency, although the documentation is expected to be voluminous and as such, we will need sufficient time to gather it.

General Guidelines for January 10th Submission

In reviewing documents for this submission, and following discussion with EPA concerning the content of the prior submissions, DuPont focused on submitting final reports, draft reports where no final report is known to exist, and documents that contain data. This submission does not include the following types of documents:

- Draft documents where it is known that a final version exists

- Documents known to be in the possession of EPA (either at headquarters or an EPA Regional Office)
- Non-DuPont documents (includes documents from other companies, plaintiffs' counsel, etc.)
- Presentations and other documents that reference data that is in a report submitted to EPA
- Documents known to be publicly available
- Documents that are subject to attorney-client privilege
- Documents concerning work-in-progress
- Supporting reports/documents for data (e.g., lab analysis, lab notebooks, field logs, waste characterization sheets, model inputs, etc.)
- Air permits, underlying documents for the air permits, and analysis to show permit compliance
- Documents on emissions reduction, treatment, recycle, process improvements

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Summary of Collection Process for Specific Categories of Requested Information

The documents for this submission were collected from review of the documents produced in the class action litigation, along with collection of documents from individuals in the Company most likely to possess such information.

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ATTACHMENT 2

Summary of January 10, 2005 Submission

Public Version

Box no.	Volume	Part no.	Topic
1	-	-	Cumulative index for Vols. 39 and 40
1	39	-	PFOA in Water - Past Analytical Procedures
1	40	-	Environmental Fate/Monitoring

Confidential Business Information Version

Box no.	Volume	Part no.	Topic
1	40	-	Environmental Fate/Monitoring

VOL	TAB	DATE	DESCRIPTION	CATEGORY
40	1	8/30/04	Study – Activated Sludge Respiration Inhibition Test of Zonyl® DuPont Glasgow Labs	Telomer: Testing of Zonyl® for toxicity towards activated sludge
40	2	1/20/03	APFO Containment at Washington Works; Part II – Process Water	Environmental Emissions
40	3	5/22/00	Modeling Releases of Ammonium Perfluorooctanoate into the Ohio River; William R. Berti DuPont Glasgow Labs	Environmental Modeling
40	4	5/1/00	Adsorption-Desorption Screening Studies of Ammonium Perfluorooctanoate; William R. Berti DuPont Glasgow Labs	Environmental Fate: Adsorption-Desorption
40	5	9/26/97	Closing Report; Addendum #1 – Product Area: DuPont Fine Powder/Dispersion	Environmental: Waste Disposal
40	6	3/11/97	Evaluation of the Biodegradability of C-8 Using the Modified Sturm Test (OECD 301 B) DuPont Glasgow Labs	Environmental Fate: Biodegradability of C8
40	7	11/26/91	Summary – Evaluation of Surfactant C-8 Relative to Chambers Works	Environmental Fate: Fate of C8 in waste water treatment system

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PFOA IN WATER

PAST ANALYTICAL PROCEDURES

VOLUME 39

January 10, 2005
EPA PRODUCTION

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VOL	TAB	DATE	DESCRIPTION	CATEGORY
39	1	4/9/01	FC-143 in Water/Wastewater by SPME/GCMS – Lancaster Labs	Analytical Method for PFOA in Water This lab was used for water testing circa 1998-2001
39	2	4/3/01	Extraction of Low Level FC-143 in Water and Wastewater – Lancaster Labs	Analytical Method for PFOA in Water See Tab 1 above
39	3	10/1/91	Low Level Determination of FC-143 In Water- CH2M Hill Labs	Analytical Method for PFOA in Water CH2M Hill was the lab used to analyze water samples circa early 1990's to 1998
39	4	9/9/91	Letter to Mr. Michael McClusky from Jewell W. Smiley Re: Progress Report for FC-143 Method Development	Analytical Method for PFOA in Water Progress on method development by CH2M Hill Labs
39	5	4/3/81	Determination of Perfluorooctanoic Acid in Water Gas Chromatographic Method – DuPont Experimental Station	Analytical Method for PFOA in Water This method was developed by DuPont and was used while the CH2M Hill Lab method was developed (see Tab 3 above)

ENVIRONMENTAL FATE / MONITORING

VOLUME 40

January 10, 2005
EPA PRODUCTION

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VOL	TAB	DATE	DESCRIPTION	CATEGORY
40	1	8/30/04	Study – Activated Sludge Respiration Inhibition Test of Zonyl® DuPont Glasgow Labs	Telomer: Testing of Zonyl® for toxicity towards activated sludge
40	2	1/20/03	APFO Containment at Washington Works; Part II – Process Water	Environmental Emissions
40	3	5/22/00	Modeling Releases of Ammonium Perfluorooctanoate into the Ohio River; William R. Berti DuPont Glasgow Labs	Environmental Modeling
40	4	5/1/00	Adsorption-Desorption Screening Studies of Ammonium Perfluorooctanoate; William R. Berti DuPont Glasgow Labs	Environmental Fate: Adsorption-Desorption
40	5	9/26/97	Closing Report; Addendum #1 – Product Area: DuPont Fine Powder/Dispersion	Environmental: Waste Disposal
40	6	3/11/97	Evaluation of the Biodegradability of C-8 Using the Modified Sturm Test (OECD 301 B) DuPont Glasgow Labs	Environmental Fate: Biodegradability of C8
40	7	11/26/91	Summary – Evaluation of Surfactant C-8 Relative to Chambers Works	Environmental Fate: Fate of C8 in waste water treatment system

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Lancaster Laboratories
2425 New Holland Pike • Lancaster, PA 17601

FC-143 in Water/Wastewater by SPME/GCMS

Reference:

1. *Low-Level Determination of FC-143 in Water*, CH2M Hill, Inc., October 1991.

2. Method 8260B, Revision 2, SW-846, USEPA, December 1996. (BFB Tuning)
   Sarrion, M.N.; Santos, F.J.; Galceran, M.T.

3. *In Situ Derivatization/Solid-Phase Microextraction for the Determination of Haloacetic Acids in Water.* Analytical Chemistry, Vol. 72, No. 201, October 15, 2000, pp. 4865-4873.

4. SPME Applications Guide Bulletin 925A – Supelco.

5. Agilent Technologies Operations Manual.

Cross Reference:

The following procedure is cross referenced in this document:

MC-EX-001, "GC/MS Preventative and Corrective Maintenance."

Scope:

This method is applicable to the measurement of the ammonium perfluorooctanoate in water and wastewater. The limit of quantitation is 1.0 µg/L.

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Basic Principle:

Ethyl esters of the target and surrogate are converted from their respective acidic compounds using techniques for esterification. The sample is acidified to <3 using 1:1 HCL. Ion paring agent, diethyl sulfate, ethanol and sulfuric acid are used to convert the acid into the ethyl esters. The sample is mixed on a stir plate at a specified setting for a specific time. The sample is then absorbed onto a 100 µm PDMS fiber for 10.0 minutes and injected into a GC/MS system equipped with a capillary column. The analysis is performed using internal standard calibration by GC/MS.

Apparatus:

1. Hewlett-Packard Model 5890 (Series I and II) or 6890 Gas Chromatograph or equivalent
2. Hewlett-Packard Model 5971, 5972, and 5973 Mass Selective Detector or equivalent
3. Hewlett-Packard Chemstation Software, Thru-Put Systems Target data system or equivalent
4. Solid Phase Microextraction (SPME) – Supelco - 100 µm Polydimethylsiloxane (PDMS) fiber, fiber pen for manual injection, and injector guide, or equivalent
5. 15 mL – Clear vial with Teflon/silicon septum or equivalent
6. Stir Plate – VWR – mini stirrer, Model 200 or equivalent
7. Stir Bars – VWR Teflon stir bars ½" X 5/16" or equivalent
8. Disposable Glass pipettes
9. 10 mL Class A graduated cylinder

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10. Syringes – 1 mL, 10 mL, 10 µL, 25 µL, 50 µL

11. Timers – Capable of measuring time to 30 minutes.

12. Class A volumetric flasks, assorted sizes

13. Analytical balance – Capable of weighing to 0.0001 g

Reagents:

1. 1:1 HCL reagent grade or equivalent

2. Ethanol – AAPER Chemical – Absolute 200 Proof or equivalent

3. Chromatography column – J&W DB-5MS, 30M x 0.25 mm ID, 1.0 µm df or equivalent

4. UPC helium for carrier gas

5. Pentadecafluorooctanoic acid Ammonium salt — Fluka, 98% pure or equivalent

6. 4-Bromofluorobenzene – 2000 ppm BFB Chem Service ampulated solution in methylene chloride or equivalent, for tuning agent and internal standard

7. Heptadecafluorononanoic acid – Fluka, 97 % pure or equivalent for surrogate standard

8. Tetrabutylammonium hydrogen sulfate (TBA-HS) – Fluka, 99% pure or equivalent. Ion pairing reagent

9. Diethyl Sulfate –Aldrich, 98% or equivalent Ethylation reagent

10. Sulfuric Acid – ACS grade or equivalent

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11. Deionized water

Standards:

Note: Working stocks are prepared by diluting stock solutions to the appropriate concentration with a syringe and volumetric flask.

1. Stock Pentadecafluorooctanoic acid Ammonium salt (FC-143) – Weigh approximately 0.02 g pentadecafluorooctanoic acid ammonium salt into a 10 mL volumetric flask and dilute to volume with ethanol. Record in database or Dept 26 spikes and special mixes notebook. This is a stock solution.

2. Working Stock FC-143 -pentadecafluorooctanoic acid ammonium salt – Dilute the stock pentadecafluorooctanoic acid ammonium salt solution to 500 ppm using ethanol in a volumetric flask.

3. Internal standard/Tuning Solution BFB – 4-Bromofluorobenzene – Dilute 2000 ppm ampulated stock solution to 50 ppm in MeOH. This is the internal standard spike. Spike all standards and samples with 20 µL per 1 mL of final volume.

4. Stock Heptadecafluorononanoic acid surrogate – Weigh approximately 0.02 g Heptadecafluorononanoic acid into a 10-mL volumetric flask and dilute to volume with ethanol. Record in database or Dept 26 spikes and special mixes notebook. This is a stock solution.

5. Working Stock Heptadecafluorononanoic acid – Dilute the stock Heptadecafluorononanoic acid solution to 500 ppm using ethanol.

6. Ion Paring Agent – Tetrabutylammonium hydrogen sulfate (TBA-HS) weigh approximately (0.016 g to 100 mL deionized water)

7. Diethyl Sulfate solution 0.15 Molar in ethanol – 2mL of diethyl sulfate in 100 mL of ethanol.

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8. Calibration Levels

Level 1: F1432.5:	25 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
Level 2: F1435.0	50 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
Level 3: F14310.0:	100 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
Level 4: F14315.00:	150 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
Level 5: F14320.00:	200 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
Level 6: F14325.0	250 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water, 0.5 mL 1:1 HCL,
MDL/LOQ standard	10 µL of calibration and surrogate intermediate + 10 µL of internal standard	to 5.0 mLs deionized water 0.5 mL 1:1 HCL,

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Safety Precautions:

FC-143 is extremely dangerous in the neat room form. The time weighted average exposure limits is 0.1 mg/m^3. Therefore, extreme care must be used when working with this compound. Also, heating of the neat standard or any concentrated solution must be avoided. Any handling of the neat product must be done in a hood with a second analyst observing the process and acting as a safety spotter.

The compounds used as surrogate standards are also dangerous. These compounds form hydrofluoric acid upon heating or breakdown. Therefore, the exposure of the neat material to any concentrated solution must be avoided.

Sample Preservation and Holding Time:

Samples must be prepared within 7 days of date collected. Extracts should be analyzed at time of ethylation.

Extracts and standards must be kept at –20 to -10°C prior to analysis.

Personnel Training and Qualifications:

Each analyst performing the instrumental analysis will work with an experienced analyst for a period of time until they can independently calibrate the instrument, use the chromatographic data system to set up sequences, perform the calculations, interpret the chromatograms, and enter the data into the LIMS. They will also follow the department training manual for analysts. Proficiency is measured through documentation audits of the tasks listed and overchecking of data.

Extraction/Ethylation Procedure:

NOTE: Prepare Standards/ Samples only when ready for analysis as variations in ethylation time and absorption time will affect results. A timer is used which can measure two separate times, one for ethylation and one for absorption onto the fiber.

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1. Measure 5.0 mL of deionized water (standards) or sample into 15 mL clear vial and add 0.5 mL of 1:1 HCL to pH the solution below 3.

2. Spike Standards with appropriate amount of target and surrogate working stock solution and samples with 50 µL of 500 ppm Heptadecafluorononanoic acid surrogate solution. Add 10 µL of 50 ppm BFB internal standard to each vial prior to ethylation.

3. Add a rinsed stir bar and 250 µL of TBA-HS ion paring agent.

4. Add 1.0 mL of 0.15 molar Diethyl Sulfate and 1.0 mL 200 proof ethanol.

5. Cautiously add 10 drops of concentrated Sulfuric Acid.

6. Recap vial tightly and place on the VWR mini stirrer, Model 200, stir plate at speed 4 for 30.0 minutes (see NOTE below).

7. Following the 30.0 minute ethylation process, insert the 100 µm PDMS fiber in its withdrawn position at a setting of 1.0 on the fiber pen through the Teflon/silicon septum, lower the fiber into the headspace for 10.0 minutes to allow absorption.

8. After 10.0 minutes, raise the fiber into the up position and remove it from the vial. Change the pen height from 1.0 to 3.6 on the fiber pen. Insert the pen into the injection port and lower the fiber into the port. Press the START button to initiate the injection.

NOTE: Consistent speed rate and timing for the ethylation, absorption and insertion steps greatly increases the reproducibility and precision of the method. Conditions above may change to increase sensitivity, reproducibility and overall chromatography.

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GC/MS Analysis:

Instrument Setup

Detector –	Mass selective detector (MSD)
Detector temperature –	300°C
Oven Temperature Program –	45°C for 3 minutes, 5°C/minute to 85°C, then 70°C/minute to final temperature of 300°C, hold for 10 minutes
Carrier –	Helium, Constant flow mode – 1.5 mL/minute
Injection –	Fiber insertion 4 +minutes
Injector Temperature–	275°C
Injection Mode –	Splitless Purge flow to split vent 40 mL/minute at 2 minutes

The conditions listed above may be changed to improve the linearity, sensitivity, or overall chromatography on each GC/MS system.

Calibration:

A. Calibration consists of analyzing standards at 6 concentration levels and producing response factors. The relative standard deviation of the response factor determines the suitability of the average relative response factor for calculation of concentration.

1. An aliquot of 0.5 ng/µL BFB must analyzed and meet the criteria described in **Table I.** If the criteria are not met, the mass spectrometer must be retuned and BFB reanalyzed until all criteria are met. For BFB fiber analysis, Spike 50 µL of 50 ppm BFB working standard into 5.0 mL deionized water with 0.5 mL 1:1 HCL and place on the stir plate, insert the fiber pen, set to 1.0 through the septum and lower the fiber for 10.0 minutes. Raise the fiber, remove the pen and insert into the injection port. Start the injection.

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2. If the performance criteria are met for the BFB solution, perform an initial calibration by injecting the six calibration standard solutions referenced previously.

3. At the end of the calibration standard injections, inject the MDL/LOQ solution. The system must be able to detect FC-143 in the MDL/LOQ solution. If the system does not detect FC-143, then the tuning and calibration procedure must be repeated under conditions that will yield a detection for FC-143 in the MDL/LOQ solution. Instrument maintenance as outlined in MC-EX-001, may be required.

4. Due to the nature of the technique, initial calibrations may require multiple days for analysis. In the event a calibration carries into a second day, prior to resuming calibration standard injection, first inject and pass BFB as stated previously, then inject a comparison standard from the already injected standards and confirm system stability by response factor comparison. Response factors must be within 20% drift in order to continue with calibration. If the % drift exceeds 20%, then corrective action and a new initial calibration may need to be performed.

B. Calculate the response factor for FC-143 and the PFNA surrogate for each calibration solution.

Calculation of the response factor (RF):

$$RF = \frac{[A(x) \times C(is)]}{[A(is) \times C(x)]}$$

Where:

A(x) = Area of the quantitation ion for the compound being measured

C(is) = Concentration of the specific internal standard

A(is) = Area of the quantitation ion for the specific internal standard

C(x) = Concentration of the compound being measured

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C. For FC-143 and the surrogate PFNA, calculate the mean RF from the RFs of the six calibration standards. Calculate the relative standard deviation:

$$\% RSD = \frac{SD}{Avg\ RF} \times 100$$

Where:

$$SD = \frac{\sum RF - avg\ RF}{n-1} \quad and \quad Avg\ RF = \frac{\sum RF}{n}$$

If the RSD of the response factors is less than or equal to 15%, then the average relative response factor is used for quantitation. If the %RSD exceeds 15% then a linear curve may be used for quantitation.

D. Continuing calibrations

Verify the MS tune and initial calibration at the beginning of every 12-hour work shift during which analyses are performed.

1. An aliquot of 0.5 ng/uL BFB must be analyzed and meet the criteria described in Table 1. If the criteria are not met, the mass spectrometer must be retuned until all criteria are met. See A 1. above for injection procedure.

2. Perform a continuing calibration check by injecting an aliquot of the mid-point calibration standard. Calculate the percent drift:

$$\% Drift = \frac{C(i) - C(c.)}{C(i)} \times 100$$

Where:

C(i) = Calibration check compound standard concentration

C(c) = Measured concentration using selected quantitation method

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The percent drift for FC-143 should not be greater than ± 20%. If the %drift exceeds 20%, then corrective action must be taken and a new initial calibration may need to be performed.

3. The absolute areas of the quantitation ions of the internal standard must fall within –50% to +100% of the average area from the last initial calibration. If the area for the internal standard is not within this window then corrective action must be taken and documented.

4. The MDL/LOQ standard must be injected after a compliant BFB and a compliant continuing calibration check standard. You must be able to detect FC-143 in the MDL/LOQ solution. If the system does not detect FC-143, then the tuning and calibration procedure must be repeated under conditions that will yield a detection for FC-143 in the MDL/LOQ solution. Instrument maintenance as outlined in MC-EX-001 may be required.

Analysis of Samples:

1. Analyze a 5.0 mL aliquot of each sample extract under the same conditions used for the initial and continuing calibrations.

2. At the conclusion of data acquisition, use the same software to tentatively identify peaks within the retention time window of interest. Examine the ion abundances of components of the chromatogram. If the ion abundance of the FC-143 ion used for quantitation (69 amu) exceeds the calibration range, dilute the aliquot and reanalyze. When preparing dilutions, add sufficient internal standard to maintain the same concentration as that of the ICAL.

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Qualitative Analysis:

A compound is identified by comparison of the sample mass spectrum (after background subtraction) with the mass spectrum of a standard of the target compound (standard reference spectra). In order to verify identification, the following criteria should be met:

1. The sample component retention time should be within 10 seconds of the time observed for the component when a calibration solution was analyzed.

2. All ions in the reference spectra at >10% of the most abundant ion should be present in the sample mass spectra and should agree within absolute 20%. An example spectrum for the ethylated form of FC-143 is presented in **Figure 1.**

Quantitative Analysis:

Once a compound has been identified, quantitation will be based on the internal standard technique and the integrated abundance from the extracted ion current profile (EICP) of the primary characteristic ion.

$$Concentration\ (\mu g/L) = \frac{A(x) \times I(is)}{A(is) \times RF\ V(o)}$$

Where:

A(x) = Area of the quantitation ion for the compound to be measured

I(is) = Amount of internal standard added to the water sample (in micrograms)

A(is) = Area of the quantitation ion for the appropriate internal standard

RF = Average response factor from the current initial calibration

V(o) = Original water sample volume (in liters)

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Quality Assurance:

At least one deionized water blank, laboratory control spike, and spike duplicate is analyzed with every group. A matrix spike and matrix spike duplicate are analyzed per analytical batch, unless insufficient client sample volume is supplied. In such cases, a LCSD must be substituted for the MS/MSD extraction. The spiking solution contains all analytes of interest. A surrogate standard spiking solution containing perfluorononanoic acid is also added.

The advisory QC windows are 70 – 130 for the mean recovery of the LCS/LCSD, MS/MSD, and surrogate. If the recovery falls outside these windows, corrective action may be taken based on the nature of the non-conformance. The corrective action must be documented and may include reinjection, re-extraction, instrument maintenance, and preparation of new calibration standards. Statistical QC windows will be generated and acted upon once sufficient data points have been collected.

Revision Log:

Initiated Date: 03/28/01

Ver. #	Effective Date	Change
01	APR 0 9 2001	New

0615_01.DOC
040901

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Prepared by: 383 Date: 04-09-01

Approved by: Date: 4/9/01

Approved by: Dorothy M Lave Date: 4/9/01
Quality Assurance

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Table I

BFB Key Ion Abundance Criteria

Mass	Ion Abundance Criteria
50	15% to 40% of mass 95
75	30% to 60% of mass 95
95	base peak, 100% relative abundance
96	5% to 9% of mass 95
173	less than 2% of mass 174
174	greater than 50% of mass 95
175	5% to 9% of mass 174
176	greater than 95% but less than 101% of mass 174
177	5% to 9% of mass 176

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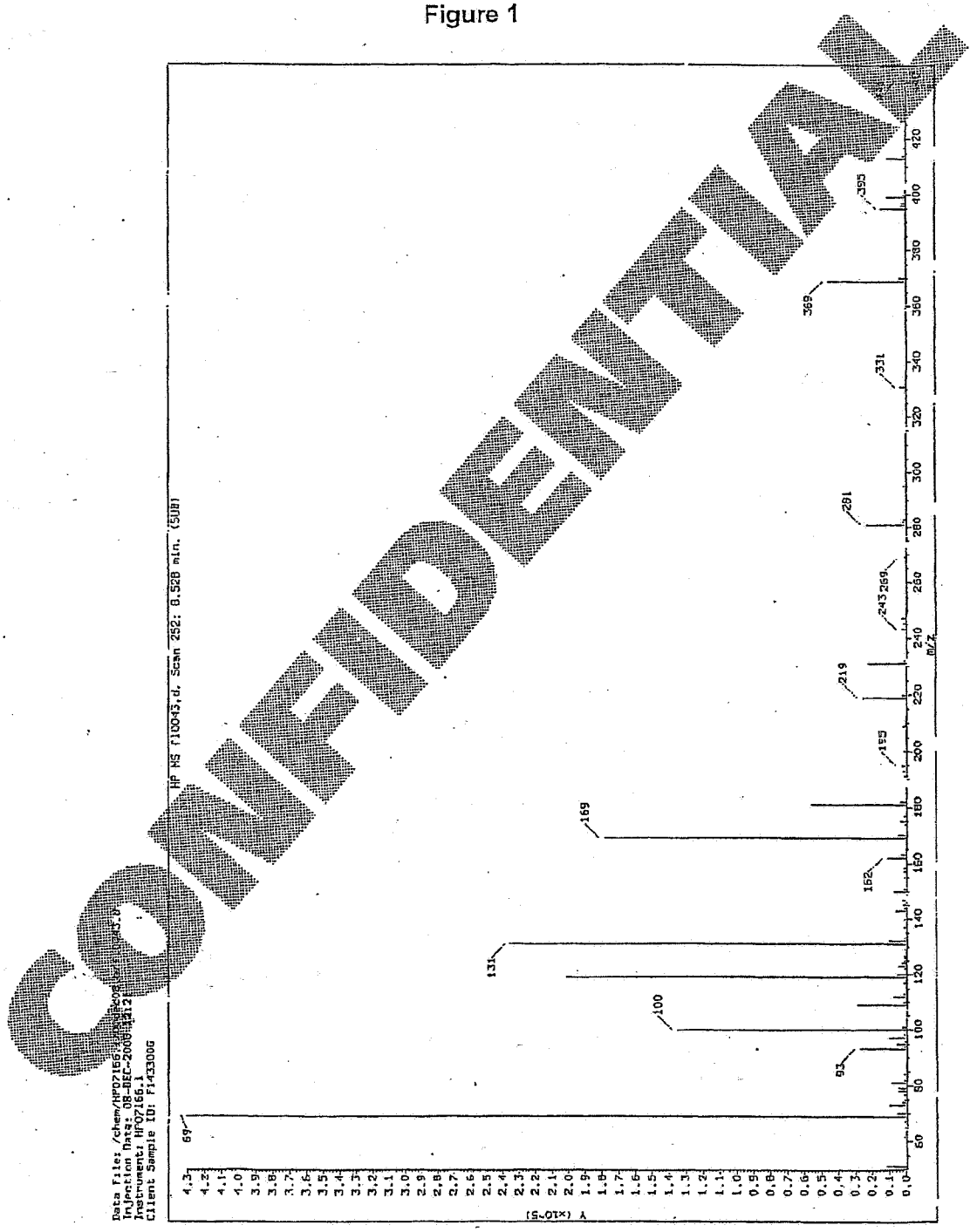
Figure 1



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Lancaster Laboratories
2425 New Holland Pike • Lancaster, PA 17601

Analysis #0813FC
Revision 01
Supersedes Date: None
Effective Date: APR 0 3 2001

Extraction of Low Level FC-143 in Water and Wastewater

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Reference:

Low Level Determination of FC-143 in Water, CH2M Hill, Inc., October 1991.

Cross Reference:

The following procedure is cross-referenced in this document:

SOP-OE-001, "Glassware Cleaning for Organic Extractions."

Scope:

This method is designed to extract FC-143 (ammonium perfluorooctanoate) from water.

Basic Principles:

FC-143 is extracted from the sample at a pH <1.5 into MTBE. The MTBE is concentrated on a steam bath. The sample is then concentrated and exchanged to ethanol. Sulfuric acid is added to the vial and then heated at 65°C for 1 hour to derivatize the compounds. Following this step, the sample is washed multiple times with deionized water and placed into an autosampler vial for analysis.

Holding Times/Storage Conditions:

Sample should be extracted within 14 days of collection. All samples should be stored at 2° to 4°C prior to extraction.

Apparatus:

1. 2-L separatory funnel for extracting organic components from an aqueous matrix
2. Kuderna-Danish (K-D) assembly with appropriate ampule for extracting the solvent used during the extraction
3. Glass pipettes
4. N-evap with nitrogen supply
5. Water bath or S-evap
6. Glass vials, various sizes
7. Centrifuge
8. Graduated cylinders – Class A, assorted sizes
9. Volumetric flasks – Class A, assorted sizes
10. Glass bottles
11. Wash bottles
12. Scriber
13. Pipettes – Class A, assorted sizes
14. Pipettes – Disposable
15. pH paper – Assorted ranges

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16. Solvent pumps

17. Automatic shaker – Capable of holding 2 L separator funnels

18. Beakers – Assorted sizes

19. Reacti therm vials

20. Screw cap GC vials

21. Glass wool

22. Amber crimp top GC vials

23. Balance – Capable of weighing to 0.01 g

Reagents:

1. Methylene chloride – Pesticide grade or equivalent

2. MTBE – Pesticide grade or equivalent

3. Acetone – Pesticide grade or equivalent

4. Hexane – Pesticide grade or equivalent

5. Sodium sulfate – Reagent grade or equivalent. Bake at 400°C for 4 hours in a shallow pan to remove contaminants. Store in a glass jar for up to 1 year after baking.

6. Sulfuric acid – ACS grade or equivalent (To prepare 1:1 solution, using graduated cylinders, add equal amount concentrated acid to deionized water in a volumetric flask. Never add water to acid. Store for up to 1 year.)

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7. Sodium hydroxide – ACS grade or equivalent – 10 N – Add 40 g NaOH to approximately 80 mL of deionized water in a volumetric flask. Shake until the NaOH goes into solution. Using a wash bottle, dilute to 100 mL with deionized water. Store at room temperature in a glass bottle. Reagent is stable 1 year. If 1 N NaOH is needed a dilution of 10× can be made of the 10 N solution.

8. Alumina N (neutral) super I activity – Use as received.

9. Teflon™-boiling chips – Rinse thoroughly with methylene chloride prior to use to remove contaminants.

Preparation of Glassware:

See SOP-OE-001. Prior to use, the glassware is rinsed with either ethyl ether or hexane to remove contaminants. See Table I with a list of glassware and the appropriate rinse solvent.

Safety Precautions:

Always add acid to water to reduce fuming. The sulfuric acid and sodium hydroxide solutions get hot when prepared. Always wear gloves when handling these solutions.

FC-143 is extremely dangerous in the neat form. The time weighted average exposure limit is 0.1 mg/m^3. Therefore, extreme care must be used when working with this compound. Also, heating of the neat standard or any concentrated solution must be avoided. Any handling of the neat product must be done in a hood with a second analyst observing the process and acting as a safety spotter.

The compounds used as surrogates standards are also dangerous. These compounds form hydrofluoric acid upon heating or breakdown. Therefore, the exposure of the neat material to any concentrated solutions must be avoided.

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The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available, such as fume hoods, lab coats, safety glasses, and gloves.

Always wear gloves when handling any samples or standards. Also, routine laboratory safety equipment must be utilized (lab coat, safety glasses, etc.).

Since the extracts are concentrated on a steam bath, caution must be exercised while working around this apparatus. All solvent waste generated from this preparation must be collected for recycling (if applicable) or must be disposed of in the designated containers. These will then be transferred to the lab-wide disposal facility. Any solid waste material (disposable pipettes, broken glassware, pH paper) may be disposed of in the normal solid waste collection containers.

Personnel Training and Qualifications:

All personnel performing these techniques should have performed a quad study that yielded acceptable recoveries for FC-143. Personnel should spend several days working with an experienced preparation technician who has demonstrated their proficiency of the extraction.

Also, several batches of herbicide water samples should be performed under the direct observation of another experienced preparation technician to assure the trainee is capable of independent preparation.

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Procedure:

1. Set up a 2-L separatory funnel for each sample and QC sample to be extracted. For field samples which are using 1 full bottle, etch the outside of the bottle with a scriber at the meniscus of the sample volume. Shake the bottle vigorously and then pour the contents into a 2-L separatory funnel. Fill the bottle to the marked level with water and transfer the water to a graduated cylinder to determine the initial volume. Alternatively, weigh the empty bottle and tare the balance. Fill the bottle to the marked level with water and place the bottle on the tared balance. This weight rounded to a whole number is the initial sample volume. For all samples using a specified volume, shake each bottle vigorously and then use a clean graduated cylinder to measure the desired volume. Transfer the sample to a 1-L graduated cylinder. Record the sample volume and any comments about the samples in the extraction log and transfer to the 2-L separatory funnel.

2. Using pipettes, add surrogate standards and spiking solutions to the aqueous sample in the separatory funnel. Be certain the standard drips directly into the aqueous sample without touching the glass side of the separatory funnel or poor recoveries may result.

   a. Surrogates – Add 1 mL FC-143 surrogate (perfluorononanoic acid) to all samples, blanks, and spikes.

   b. Spiking solutions – Add 1 mL FC-143 spiking solution to the laboratory control sample (LCS), LCSD if applicable, matrix spike, and matrix spike duplicate samples.

3. Using a disposable pipette, adjust the pH of the sample to >12 with 10 N NaOH. Check the pH using pH paper to ensure it is >12.

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4. Using a solvent pump, add 100 mL of methylene chloride to the separatory funnel, cap the funnel, invert it, and vent immediately. Place the sample on the automatic shaker and shake at the designated speed for 2 minutes with the stopcocks closed.

   NOTE: Shaker speeds vary greatly between instruments so the proper setting is marked on each.

5. Allow the phases to separate for at least 10 minutes. Open the stopcock and drain the methylene chloride (bottom layer) into a waste container and discard.

6. Using a disposable pipette, adjust the pH of the samples to <1.5 with 1:1 sulfuric acid. Check the pH using pH paper to ensure it is <1.5.

7. Using a solvent pump, rinse each sample bottle or graduated cylinder with 100 mL of MTBE and pour into the separatory funnel.

8. Shake the separatory funnel for 2 minutes on the automatic shaker.

9. Allow the phases to separate for 5-10 minutes before draining the aqueous (lower) layer back into a 2-L beaker.

10. Drain the MTBE extract through approximately 10 cm of sodium sulfate into a K-D apparatus containing a Teflon™-boiling chip.

11. Return the aqueous sample to its respective separatory funnel. Using a solvent pump, rinse the sample bottle or graduate with 60 mL of fresh, MTBE before adding the MTBE to the separatory funnel.

12. Repeat steps 8 through 11.

13. Repeat steps 8 through 10.

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14. Attach a 3-ball Snyder column to the K-D, wet with MTBE, and concentrate at 80° to 85°C to less than 4 mL.

15. Using a pipette, add 5 mL of ethanol to the ampule and N-Evap the extract at 35° ± 2°C to 1 mL.

16. Place 10 drops from a disposable pipette of concentrated sulfuric acid into a reacti therm vial, then transfer the extract to the vial. Cap tightly and swirl.

17. Place the sample on a rack into an oven at 63° to 67°C for 1 hour to derivatize the compounds of interest to their respective ethyl esters.

18. Remove the samples from the oven and immediately place the reactor vials in the freezer until cold, approximately 15 minutes. Remove the cap and using pipettes, add 1.0 mL of methylene chloride and 1.0 mL of deionized water. Recap and shake vigorously for several seconds. Allow the phases to separate and using a disposable pipette remove the methylene chloride (lower) layer.

    Place the methylene chloride layer into a screw cap GC vial containing 1.0 mL of deionized water. Cap the vial and shake vigorously for several seconds. Allow the phases to separate. Remove the methylene chloride (lower) layer. Run the extract through a disposable pipette containing a plug of glass wool and sodium sulfate. Bring the extract to exactly 1.0 mL with methylene chloride.

19. Place the extract in an amber crimp top GC vial. Cap the GC vial securely and store in the freezer. Record the final volume in the extraction log.

Calculations:

See analysis method.

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Statistical Information:

See analysis method.

Quality Assurance:

Each day samples are extracted, a blank, laboratory control sample (LCS) (deionized water blank spiked with all compounds of interest carried through the entire procedure), and LCSD if applicable must be prepared. In addition, a matrix spike and matrix spike duplicate must be extracted. If there is limited sample, which prevents the preparation of the MS/MSD, the LCSD must be prepared instead. Also, if the batch contains only field or equipment blank samples, the LCC/LCSD QC pairing should be used. A batch is defined as the samples to be extracted on any given day, but not to exceed 20 field samples. If more than 20 samples are prepared in a day an additional batch must be prepared. If any client, agency, or state has more stringent QC or batch requirements, these must be followed instead.

Revision Log:

Initiated Date: 03/22/01

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Prepared by: _____ (OSR) Date: 4-2-01

Approved by: _____ Date: 4-2-01

Approved by: _____ Date: 4/2/01

Approved by: _____ Date: 4/3/01
Quality Assurance

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Table I

FC-143 Prep Rinsing List

Rinse 2× with acetone and then 2× with MTBE

- 2-L separatory funnels
- K-Ds
- Ampules
- Sodium sulfate columns
- Snyder columns
- 2-L beakers
- Teflon™ stoppers
- 250 mL beakers
- Reacti-vials/caps/liners

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Lancaster Laboratories
2425 New Holland Pike • Lancaster, PA 17601

Analysis #0813FC
Revision 01
Supersedes Date: None
Effective Date: APR 0 3 2001

Extraction of Low Level FC-143 in Water and Wastewater

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Reference:

Low Level Determination of FC-143 in Water, CH2M Hill, Inc., October 1991.

Cross Reference:

The following procedure is cross-referenced in this document:

SOP-OE-001, "Glassware Cleaning for Organic Extractions."

Scope:

This method is designed to extract FC-143 (ammonium perfluorooctanoate) from water.

Basic Principles:

FC-143 is extracted from the sample at a pH <1.5 into MTBE. The MTBE is concentrated on a steam bath. The sample is then concentrated and exchanged to ethanol. Sulfuric acid is added to the vial and then heated at 65°C for 1 hour to derivatize the compounds. Following this step, the sample is washed multiple times with deionized water and placed into an autosampler vial for analysis.

Holding Times/Storage Conditions:

Sample should be extracted within 14 days of collection. All samples should be stored at 2° to 4°C prior to extraction.

Apparatus:

1. 2-L separatory funnel for extracting organic components from an aqueous matrix
2. Kuderna-Danish (K-D) assembly with appropriate ampule for extracting the solvent used during the extraction
3. Glass pipettes
4. N-evap with nitrogen supply
5. Water bath or S-evap
6. Glass vials, various sizes
7. Centrifuge
8. Graduated cylinders – Class A, assorted sizes
9. Volumetric flasks – Class A, assorted sizes
10. Glass bottles
11. Wash bottles
12. Scriber
13. Pipettes – Class A, assorted sizes
14. Pipettes – Disposable
15. pH paper – Assorted ranges

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16. Solvent pumps

17. Automatic shaker – Capable of holding 2 L separator funnels

18. Beakers – Assorted sizes

19. Reacti therm vials

20. Screw cap GC vials

21. Glass wool

22. Amber crimp top GC vials

23. Balance – Capable of weighing to 0.01 g

Reagents:

1. Methylene chloride – Pesticide grade or equivalent

2. MTBE – Pesticide grade or equivalent

3. Acetone – Pesticide grade or equivalent

4. Hexane – Pesticide grade or equivalent

5. Sodium sulfate – Reagent grade or equivalent. Bake at 400°C for 4 hours in a shallow pan to remove contaminants. Store in a glass jar for up to 1 year after baking.

6. Sulfuric acid – ACS grade or equivalent (To prepare 1:1 solution, using graduated cylinders, add equal amount concentrated acid to deionized water in a volumetric flask. Never add water to acid. Store for up to 1 year.)

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7. Sodium hydroxide – ACS grade or equivalent – 10 N – Add 40 g NaOH to approximately 80 mL of deionized water in a volumetric flask. Shake until the NaOH goes into solution. Using a wash bottle, dilute to 100 mL with deionized water. Store at room temperature in a glass bottle. Reagent is stable 1 year. If 1 N NaOH is needed a dilution of 10× can be made of the 10 N solution.

8. Alumina N (neutral) super I activity – Use as received.

9. Teflon™-boiling chips – Rinse thoroughly with methylene chloride prior to use to remove contaminants.

Preparation of Glassware:

See SOP-OE-001. Prior to use, the glassware is rinsed with either ethyl ether or hexane to remove contaminants. See **Table I** with a list of glassware and the appropriate rinse solvent.

Safety Precautions:

Always add acid to water to reduce fuming. The sulfuric acid and sodium hydroxide solutions get hot when prepared. Always wear gloves when handling these solutions.

FC-143 is extremely dangerous in the neat form. The time weighted average exposure limit is 0.1 mg/m^3. Therefore, extreme care must be used when working with this compound. Also, heating of the neat standard or any concentrated solution must be avoided. Any handling of the neat product must be done in a hood with a second analyst observing the process and acting as a safety spotter.

The compounds used as surrogates standards are also dangerous. These compounds form hydrofluoric acid upon heating or breakdown. Therefore, the exposure of the neat material to any concentrated solutions must be avoided.

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The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available, such as fume hoods, lab coats, safety glasses, and gloves.

Always wear gloves when handling any samples or standards. Also, routine laboratory safety equipment must be utilized (lab coat, safety glasses, etc.).

Since the extracts are concentrated on a steam bath, caution must be exercised while working around this apparatus. All solvent waste generated from this preparation must be collected for recycling (if applicable) or must be disposed of in the designated containers. These will then be transferred to the lab-wide disposal facility. Any solid waste material (disposable pipettes, broken glassware, pH paper) may be disposed of in the normal solid waste collection containers.

Personnel Training and Qualifications:

All personnel performing these techniques should have performed a quad study that yielded acceptable recoveries for FC-143. Personnel should spend several days working with an experienced preparation technician who has demonstrated their proficiency of the extraction.

Also, several batches of herbicide water samples should be performed under the direct observation of another experienced preparation technician to assure the trainee is capable of independent preparation.

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Procedure:

1. Set up a 2-L separatory funnel for each sample and QC sample to be extracted. For field samples which are using 1 full bottle, etch the outside of the bottle with a scriber at the meniscus of the sample volume. Shake the bottle vigorously and then pour the contents into a 2-L separatory funnel. Fill the bottle to the marked level with water and transfer the water to a graduated cylinder to determine the initial volume. Alternatively, weigh the empty bottle and tare the balance. Fill the bottle to the marked level with water and place the bottle on the tared balance. This weight rounded to a whole number is the initial sample volume. For all samples using a specified volume, shake each bottle vigorously, and then use a clean graduated cylinder to measure the desired volume. Transfer the sample to a 1-L graduated cylinder. Record the sample volume and any comments about the samples in the extraction log and transfer to the 2-L separatory funnel.

2. Using pipettes, add surrogate standards and spiking solutions to the aqueous sample in the separatory funnel. Be certain the standard drips directly into the aqueous sample without touching the glass side of the separatory funnel or poor recoveries may result.

   a. Surrogates – Add 1 mL FC-143 surrogate (perfluorononanoic acid) to all samples, blanks, and spikes.

   b. Spiking solutions – Add 1 mL FC-143 spiking solution to the laboratory control sample (LCS), LCSD if applicable, matrix spike, and matrix spike duplicate samples.

3. Using a disposable pipette, adjust the pH of the sample to >12 with 10 N NaOH. Check the pH using pH paper to ensure it is >12.

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4. Using a solvent pump, add 100 mL of methylene chloride to the separatory funnel, cap the funnel, invert it, and vent immediately. Place the sample on the automatic shaker and shake at the designated speed for 2 minutes with the stopcocks closed.

   NOTE: Shaker speeds vary greatly between instruments so the proper setting is marked on each.

5. Allow the phases to separate for at least 10 minutes. Open the stopcock and drain the methylene chloride (bottom layer) into a waste container and discard.

6. Using a disposable pipette, adjust the pH of the samples to <1.5 with 1:1 sulfuric acid. Check the pH using pH paper to ensure it is <1.5.

7. Using a solvent pump, rinse each sample bottle or graduated cylinder with 100 mL of MTBE and pour into the separatory funnel.

8. Shake the separatory funnel for 2 minutes on the automatic shaker.

9. Allow the phases to separate for 5-10 minutes before draining the aqueous (lower) layer back into a 2-L beaker.

10. Drain the MTBE extract through approximately 10 cm of sodium sulfate into a K-D apparatus containing a Teflon™-boiling chip.

11. Return the aqueous sample to its respective separatory funnel. Using a solvent pump, rinse the sample bottle or graduate with 60 mL of fresh, MTBE before adding the MTBE to the separatory funnel.

12. Repeat steps 8 through 11.

13. Repeat steps 8 through 10.

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14. Attach a 3-ball Snyder column to the K-D, wet with MTBE, and concentrate at 80° to 85°C to less than 4 mL.

15. Using a pipette, add 5 mL of ethanol to the ampule and N-Evap the extract at 35° ± 2°C to 1 mL.

16. Place 10 drops from a disposable pipette of concentrated sulfuric acid into a reacti therm vial, then transfer the extract to the vial. Cap tightly and swirl.

17. Place the sample on a rack into an oven at 63° to 67°C for 1 hour to derivatize the compounds of interest to their respective ethyl esters.

18. Remove the samples from the oven and immediately place the reactor vials in the freezer until cold, approximately 10 minutes. Remove the cap and using pipettes, add 1.0 mL of methylene chloride and 1.0 mL of deionized water. Recap and shake vigorously for several seconds. Allow the phases to separate and using a disposable pipette remove the methylene chloride (lower) layer.

    Place the methylene chloride layer into a screw cap GC vial containing 1.0 mL of deionized water. Cap the vial and shake vigorously for several seconds. Allow the phases to separate. Remove the methylene chloride (lower) layer. Run the extract through a disposable pipette containing a plug of glass wool and sodium sulfate. Bring the extract to exactly 1.0 mL with methylene chloride.

19. Place the extract in an amber crimp top GC vial. Cap the GC vial securely and store in the freezer. Record the final volume in the extraction log.

Calculations:

See analysis method.

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Statistical Information:

See analysis method.

Quality Assurance:

Each day samples are extracted, a blank, laboratory control sample (LCS)(deionized water blank spiked with all compounds of interest carried through the entire procedure), and LCSD if applicable must be prepared. In addition, a matrix spike and matrix spike duplicate must be extracted. If there is limited sample, which prevents the preparation of the MS/MSD, the LCSD must be prepared instead. Also, if the batch contains only field or equipment blank samples, the LCC/LCSD QC pairing should be used. A batch is defined as the samples to be extracted on any given day, but not to exceed 20 field samples. If more than 20 samples are prepared in a day an additional batch must be prepared. If any client, agency, or state has more stringent QC or batch requirements, these must be followed instead.

Revision Log:

Initiated Date: 03/22/01

Ver. #	Effective Date	Change
01	APR 0 3 2001	New

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Prepared by: _____ (OSR) Date: 4-2-01

Approved by: _____ Date: 4-2-01

Approved by: _____ Date: 4/2/01

Approved by: _____ Date: 4/3/01
Quality Assurance

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Table I

FC-143 Prep Rinsing List

Rinse 2× with acetone and then 2× with MTBE

- 2-L separatory funnels
- K-Ds
- Ampules
- Sodium sulfate columns
- Snyder columns
- 2-L beakers
- Teflon™ stoppers
- 250 mL beakers
- Reacti-vials/caps/liners

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AR 226 - 2727

LOW LEVEL DETERMINATION OF FC-143 IN WATER

October 1991

CH2M HILL, INC.
MONTGOMERY, ALABAMA 36116

LOW LEVEL DETERMINATION FC-143 IN WATER

Scope and Application

1.1 This method uses capillary gas chromatography with electron capture detection (GC/ECD) to analyze water samples for the presence of FC-143. FC-143 is a technical grade of ammonium perfluorooctanoate whose most abundant component has CAS Number 3825-26-1. The use of this method should be restricted to only those analysts who can demonstrate acceptable precision and accuracy with four spike replicates into laboratory water at the 1 ppb level. Sample results are reported without blank subtraction and without correction for surrogate recoveries.

1.2 The estimated limit of detection for FC-143 is lower than 0.1 ug/L.

Summary of the Method

2.1 A one-liter aliquot of sample is fortified with surrogates, acidified, and extracted by partitioning with an organic solvent. The extract is concentrated to small volume before adding ammonium hydroxide and further concentrating to dryness. Alcoholic HCl is added to the dry residue and heated for one hour to form the ethyl ester. Several cleanups are described as part of the overall procedure to remove interferences from the extract before analysis using GC/ECD.

Safety

3.1 The hazard of each reagent used in this method is not accurately known. Exposure to the chemicals should be held to the smallest practical level. Safety data sheets for all materials must be made available to the personnel involved in the chemical analysis.

3.2 Any unfamiliar water sample may offer dangerous native contents beyond the list of chemicals listed in this method.

Apparatus and Materials

4.1 Samples should be collected in the field in accordance with the quality assurance project plan. Sample containers should be amber glass or similar inert material which will not offer interferences for the analysis.

4.2 Glassware

4.2.1 Optional all glass continuous liquid/liquid extractor

designed to extract one liter of water sample using dichloromethane solvent.

4.2.2 Separatory funnel, 2-L size, PTFE stopcock.

4.2.3 Erlenmeyer flask, 1-L size with calibration mark etched at the 1.0 liter volume.

4.2.4 Concentrator tube, Kuderna-Danish, 10-mL size, graduated, ground glass joint.

4.2.5 Evaporative flask, Kuderna-Danish, 500-mL size, purchased with a 24/40 ground glass stopper for the upper joint.

4.2.6 Snyder column, Kuderna-Danish, three-ball macro, ground glass joint, floodless type.

4.2.7 Snyder column, Kuderna-Danish, two-ball micro, ground glass joint, floodless type.

4.2.8 Pipets, various sizes and types.

4.2.9 Autosampler vials, 2-mL size, PTFE-faced septum.

4.2.10 Screw-cap vials, 4-dram size, PTFE-lined cap.

4.2.11 Micro Reaction Vessels, graduated 5-mL size (Catalog #3-3299M, Supelco Inc., Bellefonte, PA).

4.2.12 Replacement PTFE-faced Rubber Septa for Micro Reaction Vessels, 20 mm size. (Catalog #2-3264M, Supelco Inc., Bellefonte, PA)

4.3 Analytical Balance.

4.4 Centrifuge, IEC HN-SII or equivalent.

4.5 Heated water bath, steam delivery and temperature should be controllable, concentric ring covers for openings.

4.6 Nitrogen blowdown apparatus.

4.7 Gas Chromatographic System, ECD, split/splitless capillary injector and pneumatics, complete with a data system for collecting and processing chromatographic data.

4.8 Rtx-200 Primary column or equivalent, 100% trifluoropropyl methyl polysiloxane stationary phase, 30 meters in length, 0.32 mm inside diameter, 0.5 um film thickness. (Catalog #15039, Restek Corporation, Bellefonte, PA).

4.9 HP-5 Confirmation column or equivalent, 5% phenyl methyl polysiloxane stationary phase, 25 meters in length, 0.32 mm inside diameter, 0.5 um film thickness. (Hewlet Packard part number 10091J-112, Telephone 1-800-227-9770).

Reagents

5.1 Blank water which does not produce an interference for parameters of interest.

5.2 Ether, pesticide quality or equivalent.

5.3 Hexane, pesticide quality or equivalent. Cleaned prior to use by briefly shaking 100 mL of hexane against 2 grams of Activity I Neutral Alumina.

5.4 Dichloromethane, pesticide quality or equivalent.

5.5 Acetone, pesticide quality or equivalent.

5.6 3% Ethanolic HCl. Prepared by adding 2.4 mL of Acetyl chloride (Instant Methanolic HCl Kit, Stock #18053A, Alltech Associates, Deerfield, IL) dropwise to 56 mL of absolute ethanol (Florida Distillers, Lake Alfred, FL). Sealed tightly in an amber glass bottle, this reagent may be stored at room temperature for at least two weeks.

5.7 Sulfuric acid, ACS grade. Carefully dilute with an equal volume of laboratory blank water to prepare 1+1 sulfuric acid for use.

5.8 Sodium hydroxide, ACS grade. Dissolve in laboratory blank water to prepare both 10 N NaOH and 1 N NaOH for use.

5.9 Ammonium hydroxide, ACS grade. Use as received.

5.10 Florisil, Selective Adsorbent for Gas Chromatography, 100/200 mesh. (Baker Analyzed Catalog #M369-07). Activate at 130 °C in a shallow tray overnight prior to use.

5.11 Alumina N (Neutral) Super I Activity. (Catalog #04580, Universal Scientific Inc., Atlanta, GA). Use as received.

5.12 Boiling Chips, 10/40 mesh, heat at 400 °C for 30 minutes before use to insure freedom from contamination.

5.13 Wide and narrow range of pH paper.

Standards

6.1 Concentrated stock solutions may be prepared by weighing the primary neat materials and dissolving in ethanol. Primary

standards may be secured from the following vendors.

6.1.1 PCR Incorporated, Gainesville, FL 1-800-331-6313

6.1.2 MTM Research Chemicals, Lancaster Synthesis, Windham, NH, 1-800-238-2324

6.1.3 ChemService, West Chester, PA 215-692-3026

6.2 Surrogate spike solution. Prepare a three-component surrogate spiking solution by diluting the concentrated stock of perfluorononanoic acid, perfluorodecanoic acid, and 11H-Eicosafluoroundecanoic acid. The final concentration of each surrogate should be 10 ug/mL in ethanol.

Note: Early work did not include the perfluorodecanoic acid surrogate.

6.3 Matrix spiking solution. Prepare an FC-143 spiking solution by diluting the concentrated stock to 10 ug/mL using ethanol.

6.4 Internal standard stock solution. Prepare a three-component internal standard solution by diluting concentrated stocks of 1,2-Dibromoethane, 1,2-Dichlorobenzene, and 1,2,4-Trichlorobenzene. The final concentration of each internal standard should be 2 ug/mL, 40 ug/mL, and 4 ug/mL respectively in hexane.

EXCEPTION - Recent work has discontinued 1,2-Dibromoethane as an internal standard and substituted Ethyl perfluoroheptanoate at 20 ug/mL in this solution. A concentrated stock solution of the ester may be prepared by weighing approximately 10 mg of the acid directly into a reactor vial and following the derivatizing procedure beginning at procedure step 7.22.

6.5 Instrument calibration working standards may be prepared from either the acids or the salt primary materials by processing known masses through the derivatization steps of the sample preparation.

Sample Extraction - Separatory Funnel Technique

7.1 Set up a different 2-L separatory funnel for each sample and the blank. Using a graduated 1-L Erlenmeyer flask place 1 liter of sample into the separatory funnel.

7.2 Fortify each sample and the blank with 0.1 mL of the surrogate spiking solution (see 6.2). Fortify any matrix or blank spike aliquots with both surrogate and 0.1 mL of matrix spiking solution (see 6.3).

7.3 Adjust the sample to pH >12 with 10 N NaOH. Test the pH with pH paper.

7.4 Add 100 mL of dichloromethane to the separatory funnel and shake for two minutes to reach equilibrium. Vent the funnel frequently especially for the first few seconds of shaking to release the vapor pressure which will be produced. Allow the liquid phases to separate and discard the lower organic phase. If a significant emulsion develops, use a centrifuge to separate the phases.

7.5 Adjust the sample to pH <1.5 with 1+1 sulfuric acid.

7.6 Rinse the 1-L Erlenmeyer flask used to aliquot the sample with 200 mL of ether before adding this ether to the separatory funnel.

7.7 Assemble a Kuderna-Danish (KD) apparatus by connecting a 10-mL concentrator tube to a 500-mL evaporative flask. Place two or three boiling chips into the unit.

7.8 Shake the separatory funnel for two minutes and allow the phases to separate before draining the sample (lower phase) back into its original Erlenmeyer. Drain the ether extract directly into the assembled KD apparatus.

7.9 Return the aqueous sample to its separatory funnel. Rinse the Erlenmeyer flask with 100 mL of fresh ether before adding the ether to the separatory funnel for the last extraction. Repeat step 7.8.

7.10 Add 4.0 mL of 1 N NaOH and approximately 20 mL of fresh ether to the "empty" separatory funnel and shake (roll around) for several seconds. Allow at least two minutes for the liquid to drain down to the stopcock before adding the base and the ether to the KD apparatus.

7.11 Attach a three-ball macro Snyder column to the KD flask.

7.12 Concentrate the combined extract on the steam bath until boiling slows considerably and the KD begins to get warm. Only the aqueous liquid should be present in the receiver with ether refluxing in the Snyder column.

7.13 With the KD still on the steam bath slowly add at least 5 mL of dichloromethane into the macro Snyder column. The dichloromethane should be added slowly to avoided superheating with any contact against the warm receiver region. Continue heating for approximately one minute to exchange most of the residual ether.

7.14 Remove the KD from the heat and cool for two minutes or more before pouring 5 mL of dichloromethane down the macro Snyder column. Remove the Snyder column and add two or three new boiling chips. Replace the Snyder column and return to the steam bath. Heat the KD until only the aqueous phase appears to be present in

the receiver and the KD begins to warm. This procedure should remove the dissolved ether without floating the aqueous keeper out of the receiver into the KD regions.

7.15 Remove the KD from the heat for the last time and allow it to cool. Replace the Snyder column with a 24/40 stopper. Disassemble the receiver and transfer all liquid to a 4-dram vial. Add three mL of 1 N NaOH and three mL of fresh dichloromethane to the receiver before reconnecting it to the KD flask. Gently rotate the apparatus to rinse the interior walls. Vapor pressure will be produced which should be released frequently at the stoppered joint. After several seconds of rotation, return the apparatus to the upright position and allow a few minutes for the liquids to drain into the receiver. Remove the receiver from the KD and transfer the rinse liquids to the 4-dram vial. Additional fresh dichloromethane may be required to establish equal volumes of the two liquid phases in the vial.

Note: The stopper, the KD, the Snyder, and the empty receiver should be rinsed with water without delay to avoid future problems with this reusable glassware. Do not allow the basic water to dry on the glass surfaces.

7.16 Secure the cap on the 4-dram vial. Vigorously shake the vial for 30 seconds to reach equilibrium. Remember to vent any pressure which may be produced. Briefly centrifuge the vial to secure a good phase separation. Use a disposable 5-mL pipet to remove the dichloromethane phase (lower) and discard it to waste.

7.17 Adjust the aqueous sample extract to pH <1.5 using 1+1 sulfuric acid. Verify the pH by placing a small drop of the adjusted sample onto pH indicator paper.

7.18 Place 5 mL of fresh ether into the vial containing the acidified aqueous extract. Recap the vial and shake for 30 seconds remembering to vent any pressure which may develop. After equilibration and phase separation, transfer the upper ether phase to a clean 10-mL receiver. **CAUTION:** Do not approach the liquid/liquid interface too closely. Severe problems may result from the transfer of <u>any</u> aqueous phase.

7.19 Repeat step 7.18 for the last extraction. To the combined ether extract add 0.1 mL of concentrated ammonium hydroxide. Add two or three boiling chips to the receiver and attach a micro two-ball Snyder column. Take the extract to the steam bath and concentrate it to approximately 0.5 mL volume. Remove the sample from the heat and allow it to cool.

7.20 Transfer the extract to a clean 5-mL reactor vial. Complete the transfer quantitatively using 0.5 mL of acetone.

7.21 Further concentrate the sample to dryness using a gentle

stream of clean nitrogen at room temperature. This process should take approximately one hour due to the small amount of water in the sample. Many samples will offer a visible residue at dryness. Each sample will require careful inspection to determine that all water has been removed. Some samples may have tiny droplets of water covered by a thin membrane of solid phase which can be broken by adjusting the stream of nitrogen. Experience will dictate the best way of judging the dryness of the sample. Nitrogen blowdown may be continued as long as one hour past dryness without loss of target compounds.

7.22 Add 1.0 mL of ethanolic HCl reagent to the dry sample residue. Secure the screw cap to the vial and shake it for several seconds. Place the sample into an oven at 65 °C for one hour to derivatize the compounds of interest to their respective ethyl esters.

7.23 Remove the samples from the oven and allow the reactor vials to cool. Remove the cap and add 1.0 mL of clean hexane and 1.0 mL of laboratory blank water. Recap and shake vigorously for several seconds. Allow the phases to separate and discard most of the lower liquid phase keeping the upper "hexane" phase.

7.24 Add Florisil to a clean empty 2-mL autosampler vial. The amount of Florisil should provide approximately 1 mm of depth in the vial. Transfer the "hexane" phase from the reactor vial in procedure step 7.23 to the vial containing Florisil. Be careful not to transfer any residual aqueous phase which may still be present. Shake the Florisil vigorously for a several seconds and then allow it to settle.
IMPORTANT NOTE: The extract is stable against the Florisil for less than one hour. Leaving the extract against Florisil for more than several minutes will result in gradual loss of the compounds of interest. The "hexane" phase from procedure step 7.23 contains a significant amount of ethanol and should not be diluted with pure hexane before performing the Florisil adsorption step. If any dilutions are needed, then they should be performed after removing the extract from the Florisil vial.

7.25 Transfer a measured portion of the final extract away from the Florisil and place it into an autosampler vial already containing internal standards. This final extract should be stable for at least forty days. The vial should be clearly labeled and is ready for analysis.

Sample Extraction - Continuous Extractor Technique

8.0 An alternative to separatory funnel extraction is continuous liquid/liquid extraction. FC-143 may be successfully extracted from an acidified water sample using a continuous extractor and dichloromethane solvent.

8.1 Set up a different 1-Liter continuous extractor unit for each sample and a blank. Place a few boiling chips in the round bottom flask supported snugly by the heating mantle. Attach the extraction reservoir to the flask with sufficient support for safe operation. Add approximately 300 mL of dichloromethane to the reservoir.

8.2 Use a graduated Erlenmeyer flask to measure 1 liter of the sample and transfer it into the continuous extractor reservoir. Fortify the sample with 0.1 mL of surrogate solution (see 6.2). Fortify any matrix spike or blank spike sample with both surrogate and 0.1 mL of matrix spiking solution (see 6.3).

8.3 Adjust the sample to pH <1.5 using 1+1 sulfuric acid.

8.4 Add extra dichloromethane to the reservoir if necessary to insure proper solvent circulation during the extraction. Attach the condenser to the reservoir and start cool water flow through the condenser jacket. Energize the heating mantle to maintain gentle solvent boiling for sixteen hours of continuous extraction.

8.5 When extraction time has expired, discontinuing heating and allow the unit to cool. Turn off the water to the condenser jacket. Disconnect the collection flask and transfer the extract directly to a KD apparatus using a small amount of acetone to complete the transfer quantitatively.

8.6 Concentrate the extract using the steam bath. When the apparent volume of the extract reaches 2 mL, remove the KD from the heat and allow it to cool.

8.7 Replace the 3-ball Snyder column with a 24/40 stopper. Disassemble the receiver and transfer the extract to a 4-dram vial. Add seven mL of 1 N NaOH and three mL of fresh dichloromethane to the receiver before reconnecting it to the KD flask. Gently rotate the apparatus to rinse the interior walls. Vapor pressure will be produced which should be released frequently at the stoppered joint. After several seconds of rotation, return the apparatus to the upright position and allow a few minutes for the liquids to drain into the receiver. Remove the receiver from the KD and transfer the rinse liquids to the 4-dram vial. Additional fresh dichloromethane may be required to establish equal volumes of the two liquid phases in the vial.

Note: The stopper, the KD, the Snyder, and the empty receiver should be rinsed with water without delay to avoid future problems with this reusable glassware. Do not allow the basic water to dry on the glass surfaces.

8.8 Finish the sample preparation in the same manner as that used for separatory funnel extractions. Continue at procedure step 7.16.

Instrument Calibration - Primary Analysis

9.1 Figure 1 through Figure 4 are example chromatograms with recommended instrument conditions included in the legends. Figure 1 is a typical instrument calibration standard. Figure 3 illustrates the multiple peak pattern of FC-143 using the primary analytical column.

9.2 Instrument calibration and sample analysis must be performed using multiple internal standards. The three internal standard compounds listed in section 6.4 are recommended to establish both relative retention times (RRT) and relative response factors (RRF). Internal standards appearing in a chromatogram will establish primary search windows for those target compounds nearby in the chromatogram. Relative retention times are calculated using equation 2.

$$RRT = RT^{target}/RT^{IS} \qquad \text{Eq.2}$$

The relative response factor is calculated as follows.

$$\text{Absolute Response Factor} = RF = \text{Amount/Area} \qquad \text{Eq.3}$$

$$\text{Relative Response Factor} = RRF = RF^{target}/RF^{IS} \qquad \text{Eq.4}$$

Note: <u>Amount</u> in equation 3 refers to the mass (e.g. ug) of compound mixed into the solution injected.

9.3 Prepare initial calibration standards at a minimum of three concentration levels for both FC-143 and the surrogates. Analyze each level of calibration standard. For each compound, tabulate the RRF at each level (see equation 4). If the RRF over the working range is constant enough (less than 20% relative standard deviation), then the midpoint value may be used for calculation. Alternatively, the calibration data may be used to construct a calibration curve of relative response against relative amounts.

9.4 Continuing calibration standards must be injected after every ten sample extracts and at the end of an analytical sequence. Relative response factors must be compared to the initial calibration values. Treat the continuing calibration standard as an unknown sample and compute the concentration injected. If the calculated concentration differs more than 20% from the true value, then initial calibration must be repeated and all affected samples must be reanalyzed.

Instrument Calibration - Confirmation Analysis

10.0 FC-143 tentatively identified by the primary analysis should be confirmed by at least one qualitative technique. Confirmation data may be supplied by analysis using a dissimilar analytical

column, or by gas chromatography with mass spectrometry detection (if the target concentration is adequate), or by a second derivatization forming a different ester. Confirmation analysis must be qualitative and may be quantitative.

10.1 GC Dissimilar Column Confirmation

10.1.1 Column: HP-5 (or equivalent) 5% phenyl methyl polysiloxane stationary phase, 25 meters in length, 0.32 mm inside diameter, 0.5 um film thickness.

10.1.2 Chromatographic conditions:
Oven Program: 50 °C for 2 minutes
to 90 °C at 5 °C/minute
to 280 °C at 10 °C/minute
(hold 2 minutes)
Injector Zone: 200 °C
Detector Zone: 320 °C
Helium carrier inlet pressure: 5.5 psig
1.0 uL splitless injection, split on at 0.5 minutes

10.1.3 The primary initial and continuing calibration standards and criteria are used for GC dissimilar column confirmation (sections 9.2 - 9.4)

10.1.4 The sample extracts requiring confirmation and the associated method blanks should be analyzed at the same dilutions as for the primary analysis.

10.2 GC/MS Confirmation

10.2.1 GC/MS analysis normally requires a minimum concentration of 10 ug/mL in the field sample extract. The sample extracts requiring confirmation and the associated method blanks must be analyzed. A reference standard for the target compound must also be analyzed by GC/MS. The concentration of the reference standard must be at a level that would demonstrate the ability to confirm the target.

10.2.2 To confirm the identification of FC-143, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions.

10.3 Confirmation by Analysis of an Alternate Ester

10.3.1 Both standards and sample extracts may be derivatized to an alternate ester before analysis according to the following reaction.

$$RCOOH + R'OH \longrightarrow RCOOR' + HOH$$

Choice of the alcohol to which acetyl chloride is added (section 5.6) will determine which ester is prepared at procedure step 7.22. The methyl, ethyl, n-propyl, and n-butyl esters of FC-143 have been prepared by using the corresponding alcoholic HCl. Only the ethyl ester has been processed through full method validation.

10.3.2 Before samples are confirmed by this technique, the laboratory must demonstrate the ability to successfully process and analyze representative standards.

10.4 Simultaneous Primary and Confirmation Analysis

10.4.1 A gas chromatograph using only one injection port, one column oven, and one detector can produce a chromatogram from two dissimilar columns installed in parallel. The resulting chromatogram is a superimposed (overlaid) composite of the separations achieved with each independent column. Each compound in the sample will produce two chromatographic peaks thus providing enhanced qualitative information for every injection.

10.4.2 The recommended primary and confirmation columns may be connected in parallel using two "Y" connectors (part number 20405, Restek Corporation, Bellefonte, PA) and short sections of uncoated deactivated fused silica tubing. Alternatively, a 2-hole ferrule may be used at the injection port and at the detector.

10.4.3 An example chromatogram is presented in Figure 4. The "#" symbol precedes the name of a chromatographic peak produced by the confirmation column. Named peaks produced by the primary column will not include the "#" symbol. Therefore, the FC-143 peak was produced by the Rtx-200 column, and the # FC-143 peak was produced by the HP-5 column.

10.4.4 When a 2-hole graphite ferrule is used at the detector, peak identification may be assisted by temporarily removing one column from the detector for selected injections.

Quality Control

11.1 Every laboratory must operate a formal quality control program which meets or exceeds the requirements of the data quality objectives.

11.2 Before using this method, laboratory capability must be demonstrated by analyzing spiked samples. Seven replicates spiked at sufficiently low FC-143 concentration must be analyzed to estimate the method detection limit (Table 1 and Table 2). Figure 2 is an example chromatogram of blank water spiked to estimate the method detection limit using the separatory funnel extraction

technique. Also four additional replicates must be spiked at the matrix spike level and analyzed to create additional precision and accuracy data available for audits (Table 3).

11.3 Ongoing performance evaluation will be accomplished by analyzing a matrix spike and a matrix spike duplicate for every twenty field samples. A control sample will be extracted and analyzed with every set of matrix spikes. Surrogates will be spiked into every field sample, matrix spike, matrix spike duplicate, control sample, and laboratory blank. Surrogate recoveries will be control charted to determine control limits for corrective action.

Gas Chromatography

12.1 The gas chromatographic conditions listed in Figure 1 are recommended for primary analysis. Peaks tentatively identified in a sample require confirmation analysis (section 10.0).

12.2 Qualitative Analysis.

12.2.1 Peak identification is based upon relative retention time comparison to calibration data. Library retention times are established on a daily basis. A nearby internal standard serves as a time reference for all chromatographic acquisitions. A target compound should be identified in a sample only if the chromatographic peak matches the predicted retention time within 0.05 minutes.

$$\text{Predicted RT} = (RRT^{library})(RT_{IS}^{sample}) \qquad \text{Eq.5}$$

12.2.2 Equation 5 should serve only as a recommended treatment for peak identification. The experienced analyst must consider other chromatographic features such as peak shape, resolution, distance from the time reference, and multiple peak pattern recognition.

12.3 Quantitative Analysis.

12.3.1 Sample extracts should be prepared for analysis by mixing 50 uL of internal standard solution (see 6.4) into 0.5 mL of the sample extract.

12.3.2 The concentration of FC-143 identified in a sample may be calculated using equation 6. It is recommended that the nearest internal standard not suffering from co-elution be used for calculation, but the discretion of the analyst should prevail.

$$PPB_x^{sample} = \frac{(ug_{IS}^{sample})(Area_x^{sample})(RRF_x)}{(Area_{IS}^{sample})(L\ extracted)(D.F.)} \qquad \text{Eq.6}$$

PPB_x^{sample} = concentration of target (in the sample)
ug_{IS}^{sample} = mass of material (I.S.) mixed with the solution injected.
$Area_x^{sample}$ = peak area of target (in the sample)
RRF_x = relative response factor (equation 4)
$Area_{IS}^{sample}$ = peak area of internal standard (in the sample)
L extracted = Total raw sample producing total extract
D.F. = dilution factor of the extract

12.3.3 The concentration of FC-143 identified in a sample may alternatively be calculated using the initial calibration curve.

TABLE 1
LOW LEVEL SPIKING STUDY - SEPARATORY FUNNEL

FC-143 Spiked	FC-143 Conc. Found (ug/L)	FC-143 % Recovery	ET-C9 SURR.	ET-C11 SURR.
0.0 ppb	ND	N/A	52%	47%
0.1 ppb	0.062	62%	51%	48%
0.1 ppb	0.052	58%	44%	41%
0.1 ppb	0.061	61%	49%	46%
0.1 ppb	0.059	59%	53%	47%
0.1 ppb	0.058	58%	50%	48%
0.1 ppb	0.062	62%	51%	47%
0.1 ppb	0.062	62%	54%	50%
0.2 ppb	0.11	55%	48%	48%
0.4 ppb	0.23	58%	53%	46%

ND - Not detected

Mean Concentration FC-143 (seven 0.1 ppb replicates) = 0.059 ppb
Standard Deviation FC-143 (seven 0.1 ppb replicates) = 0.0036 ppb
Three std. dev. FC-143 (seven 0.1 ppb replicates) = 0.011 ppb

One liter of blank water was spiked and extracted

TABLE 2
LOW LEVEL SPIKING STUDY - CONTINUOUS EXTRACTOR

FC-143 Spiked	FC-143 Conc. Found (ug/L)	FC-143 % Recovery	ET-C9 SURR.	ET-C11 SURR.
0.0 ppb	ND	N/A	50%	49%
0.0 ppb	ND	N/A	61%	64%
0.1 ppb	0.041	41%	64%	65%
0.1 ppb	0.039	39%	64%	58%
0.1 ppb	0.045	45%	69%	67%
0.1 ppb	0.042	42%	57%	50%
0.1 ppb	0.045	45%	67%	60%
0.1 ppb	0.043	43%	64%	64%
0.1 ppb	0.041	41%	67%	70%
0.2 ppb	0.076	38%	64%	68%
0.4 ppb	0.155	39%	60%	65%
1 ppb	0.670	67%	61%	68%
10 ppb	8.94	89%	81%	81%
100 ppb	96.3	96%	90%	83%

ND - Not detected

Mean Concentration FC-143 (seven 0.1 ppb replicates) = 0.042 ppb
Standard Deviation FC-143 (seven 0.1 ppb replicates) = 0.0022 ppb
Three std. dev. FC-143 (seven 0.1 ppb replicates) = 0.0066 ppb

One liter of blank water was spiked and extracted

TABLE 3
PRECISION & ACCURACY STUDY - SEPARATORY FUNNEL

FC-143 Spiked	FC-143 Conc. Found (ug/L)	FC-143 % Recovery	ET-C9 SURR.	ET-C11 SURR.
1 ppb	1.10	110%	100%	88%
1 ppb	0.88	88%	78%	69%
1 ppb	1.01	101%	94%	87%
1 ppb	1.03	103%	98%	92%
1 ppb	0.84	84%	78%	65%

One liter of blank water was spiked and extracted

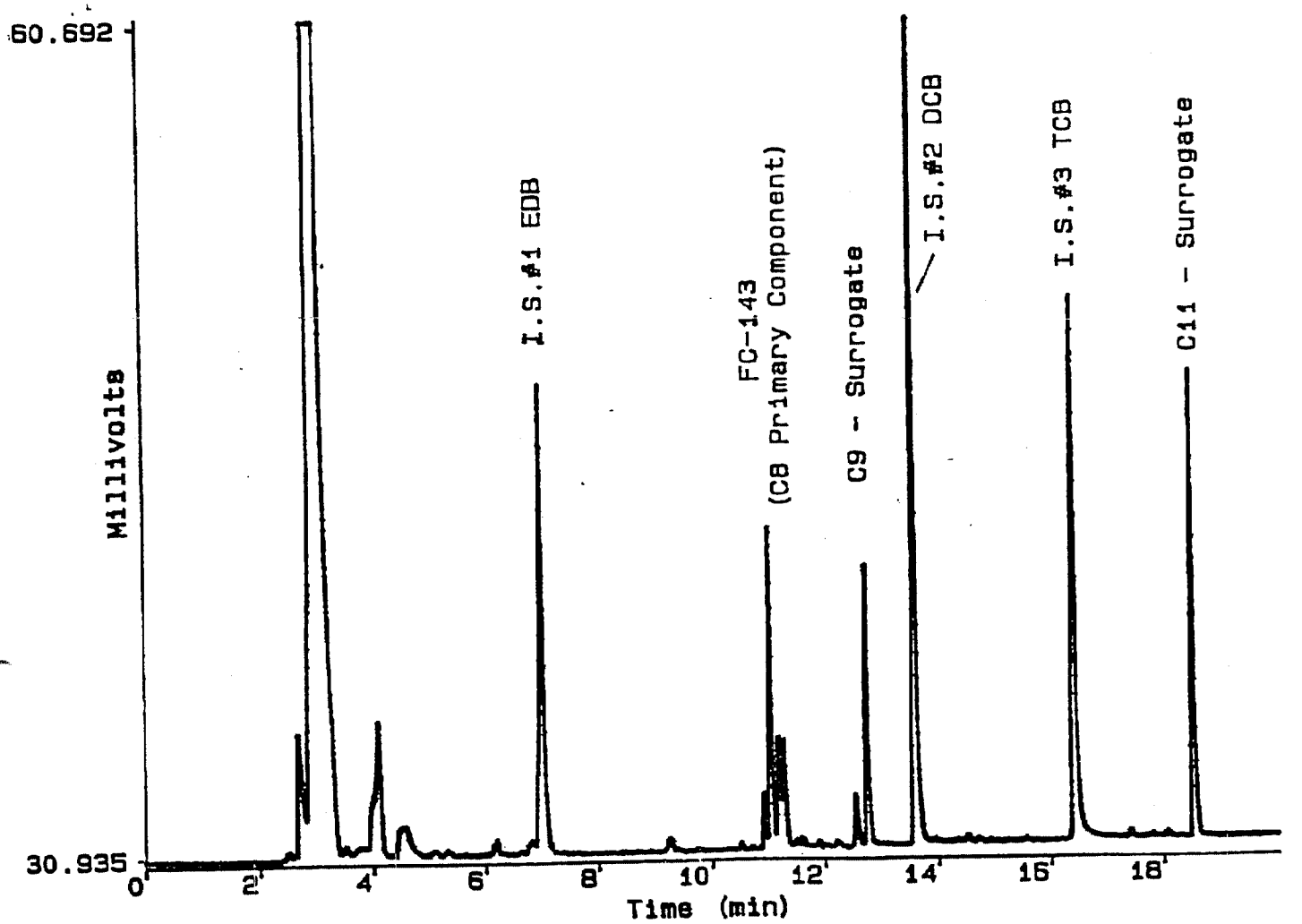

Figure 1. GC/ECD chromatogram of instrument standard at 1 ug/mL FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

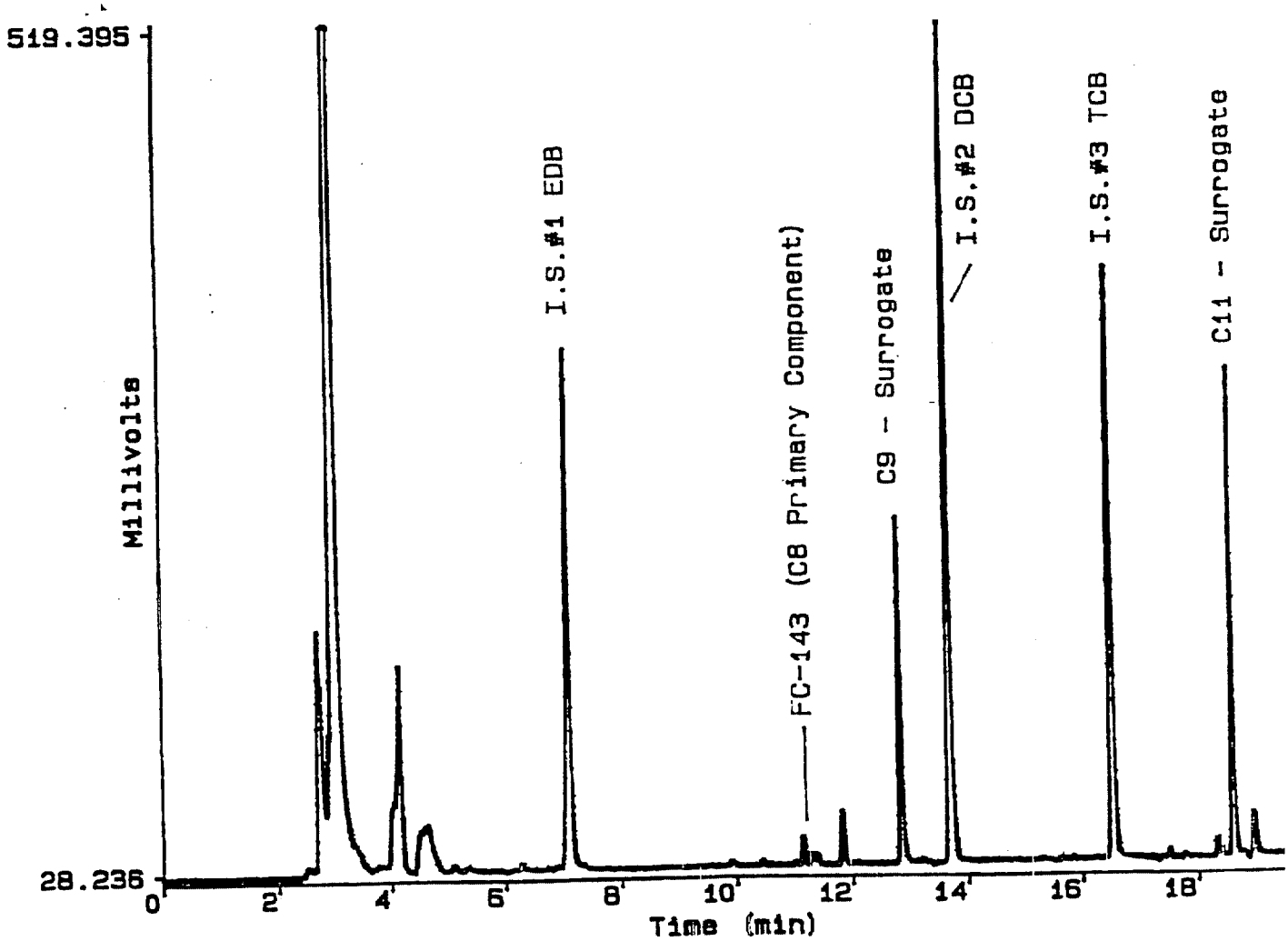

Figure 2. GC/ECD chromatogram of blank water (extracted by separatory funnel) spiked at 0.1 ug/L FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

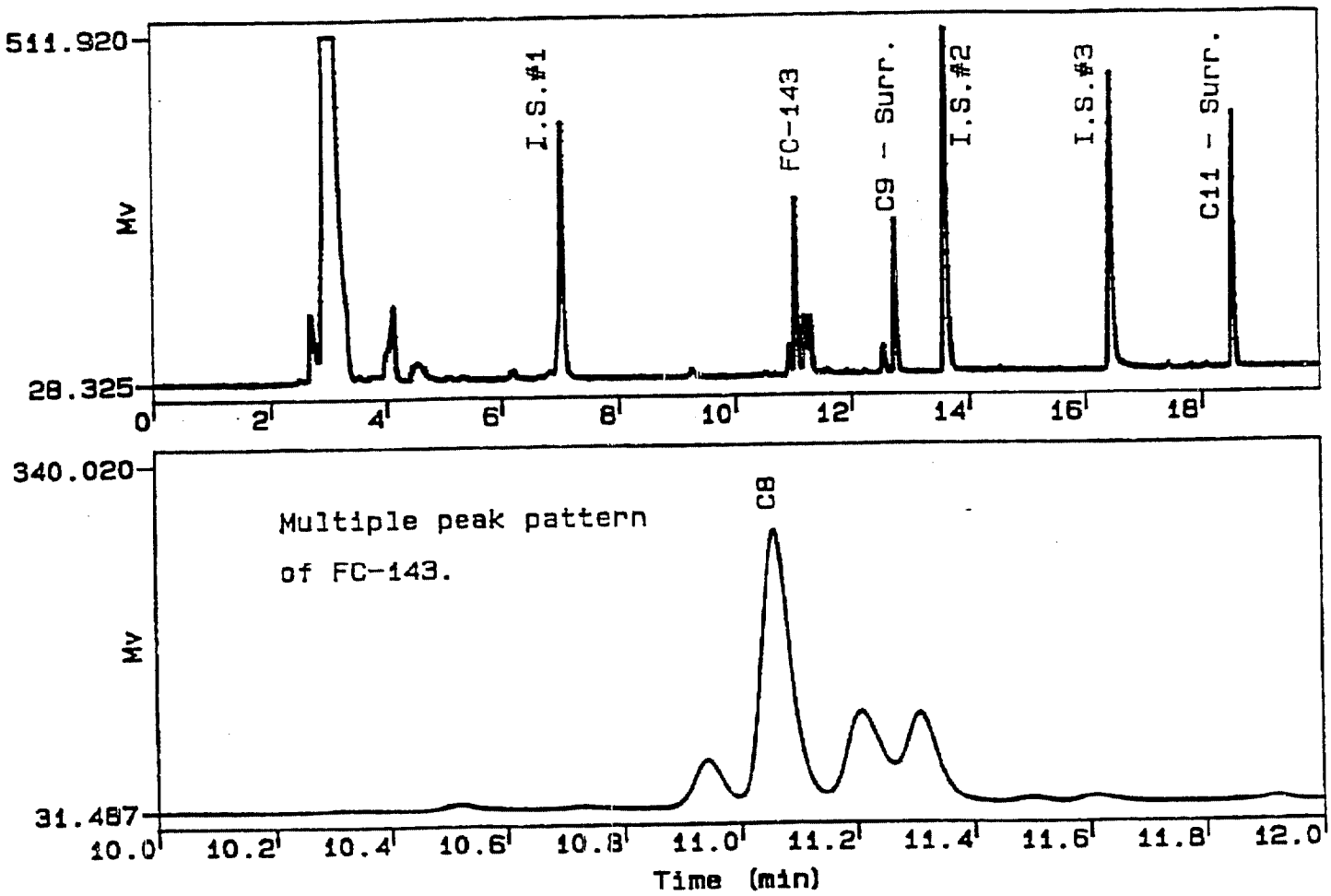

Figure 3. GC/ECD chromatogram of instrument standard at 1 ug/mL and multiple peak pattern of FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

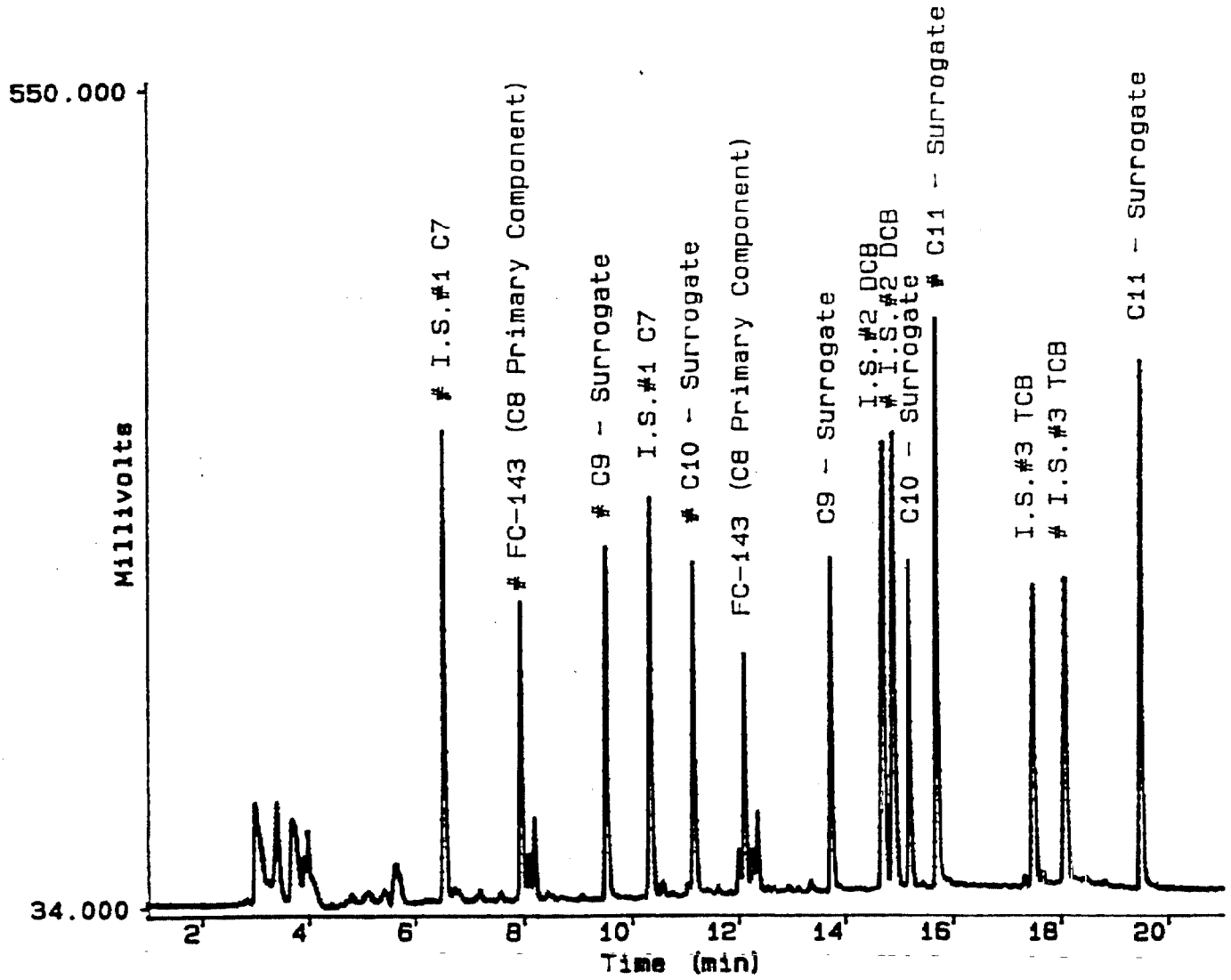

Figure 4. GC/ECD chromatogram of instrument standard at 1 ug/mL FC-143. Dual (parallel) capillary columns: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. HP-5 (Hewlett Packard), 25m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold for 4 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes. Peak naming convention: The "#" symbol preceding the compound name indicates a peak produced by the HP-5 capillary column.

AR 226 - 2728

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Scientists

September 9, 1991

LMG32550.MD

Mr. Michael McClusky
E.I. Dupont de Nemours
P.O. Box 1217
Parkersburg, West Virginia 26102

RE: Progress Report for FC-143 Method Development.

Dear Sir:

Since the beginning of work we have talked by telephone frequently concerning the FC-143 method development in progress at our laboratory. In brief summary, we have developed an analytical method capable of measuring FC-143 at levels below 0.1 ppb in water matrix. Further method refinement and ruggedness testing continue on schedule. Development costs to date are approximately $16K and are below projected estimates. The final costs are not expected to exceed plan. Details of the technical progress will be described below followed by a breakdown of the current expenses.

TECHNICAL PROGRESS

The analytical method uses liquid/liquid partitioning to extract FC-143 from a one-liter acidified water sample into an organic solvent. Method validation has already been completed using separatory funnel technique with diethyl ether as the solvent. We are hopeful that the approach can be expanded to include continuous extractors using dichloromethane solvent. Continuous extractors are less likely to produce emulsions with difficult samples, and dichloromethane is a less hazardous solvent. Solid state extraction using granular activated carbon was briefly investigated but failed to offer early success.

Sample extracts are concentrated by boiling off the solvent using standard Kadurna Danish technique on a steam bath. A few milliliters of 1 N NaOH is mixed into the sample as a keeper before concentration. We have not yet tested the use of nitrogen blowdown to complete this procedure step where a large volume of solvent must be removed. There is some concern that the boiling action may be necessary to rapidly convert the acid form of the analyte to its salt and avoid volatility losses. Our laboratory is equipped with Zymark nitrogen blowdown units to test this option.

The NaOH concentrate is transferred to a 4-dram screw cap vial and washed with dichloromethane to remove neutral and basic interferences. A centrifuge may be necessary to separate the liquid phases before discarding the organic phase. Sulfuric acid

is then added to the aqueous concentrate to convert the analyte from the salt to the associated acid before performing a last mini-extraction into ether.

Early work attempted to dry the etherial extract after the final extraction using drying salts. Anhydrous potassium carbonate was tested for speedy drying. Deactivated anhydrous sodium sulfate was also tested. Both drying salts were found to produce measurable loss of analyte and were therefor removed from the procedure and replaced by concentration to dryness described below.

One hundred microliters of concentrated ammonium hydroxide is added to the final "wet" etherial extract immediately before micro-concentration on a steam bath. The final extract is quickly concentrated from approximately 10 mL to approximately 0.5 mL. Ether is used to quantitatively transfer the extract to a 5-mL reaction vial. Gentle nitrogen blowdown is used to finish concentrating the sample to dryness.

Acetyl chloride is carefully mixed with absolute ethanol to prepare a <u>very dry</u> 3% ethanolic HCl solution. The sample residue is dissolved in 1.0 mL of the ethanolic HCl solution and heated at 60°C for 30 minutes to form the ethyl ester.

Two final cleanups are performed before the esters are analyzed. After the reaction vials have cooled, 1.0 mL of hexane and 1.0 mL of blank water is mixed into the sample with vigorous shaking. The ester will partition into the top liquid phase. Most of the floating phase is transferred to a 2-mL autosampler vial already containing approximately 1 mm of Florisil. After shaking the vial for a few seconds, the extract is ready for analysis by gas chromatography with electron capture detection (GC/ECD).

Two surrogates (perfluorononanoic acid and 11H-Eicosafluoroundecanoic acid) are spiked into every sample at the beginning of sample preparation to monitor the success of that sample prep. Three internal standards are mixed into a measured portion of the final extract to monitor the success of data acquisition. FC-143 and the surrogates are derivatized to form esters because neither the acid nor the salt forms are amenable to gas chromatography.

How should the instrument be calibrated, and how should sample results be calculated? These are issues which have several options. If pure and accurate ester standards were available for FC-143 and the surrogates, then these could be diluted and used for instrument calibration directly. Several standards were secured from three sources: PCR Research Chemicals, MTM research Chemicals of Lancaster Synthesis Inc., and FC-118 was provided by Dupont. For the perfluorooctanoic acid both the technical and the pure

grades were purchased from PCR. Disturbing discrepancies were observed when various standards were compared.

If calibration standards were to be prepared through the entire lengthy procedure for every batch of samples, one would worry about the variability of calibration from batch to batch. A reasonable alternative would be to prepare a calibration standard using only minimum preparation to reduce variability. This approach was taken for the validation work. The FC-118 supplied by Dupont was blown to dryness and assumed pure as a primary standard for FC-143 determination. A mixture of this FC-143 and the surrogate acids was introduced to the procedure at the derivatization step and carried from that point to the end of the preparation. Instrument calibration was performed by diluting the final preparation to create five concentrations injected into the instrument. Calibration curves were constructed to calculate FC-143 and surrogate recoveries for all subsequent samples. To test the linearity of the derivatization itself, the first five samples analyzed were five discrete samples at variable masses of FC-143 taken only through the derivatization and final cleanup steps. These five discrete partial preparations were virtually identical to dilutions of the one partial preparation diluted for calibration. Linearity of samples processed through the entire preparation is still under investigation.

Ten samples were processed through the entire analytical method and are summarized in the table below.

FC-143 SPIKED	FC-143 FOUND	ET-C9 SURR.	ET-C11 SURR.
0.0 ppb	0.000 ppb	52%	47%
0.1 ppb	0.062 ppb	51%	48%
0.1 ppb	0.052 ppb	44%	41%
0.1 ppb	0.061 ppb	49%	46%
0.1 ppb	0.059 ppb	53%	47%
0.1 ppb	0.058 ppb	50%	48%
0.1 ppb	0.062 ppb	51%	47%
0.1 ppb	0.062 ppb	54%	50%
0.2 ppb	0.11 ppb	48%	48%
0.4 ppb	0.23 ppb	53%	46%

These samples used laboratory blank water as the matrix. A significant bias is observed in the FC-143 data and is reflected in the surrogate recoveries. Analytical precision was good. Three standard deviations of the seven 0.1 ppb replicates was 0.011 ppb. Typical chromatograms are enclosed as well as the calibration curve described in this text.

Confirmation analysis may be accomplished by using a dissimilar analytical column. Chromatographic conditions have been developed for two dissimilar columns. A second approach to confirmation is possible by derivatizing the acids to a different ester. The methyl, ethyl, n-propyl, and n-butyl esters have been successfully prepared.

CURRENT PROJECT COSTS

The table below offers a current breakdown of costs. Some figures are estimated since shipping costs or effective discounts are not yet known.

Labor - Consulting Chemist.....	(129Hr)($71.07/Hr)	= $9168.03
Labor - Senior Chemist.....	(2Hr)($48.93/Hr)	= $ 97.86
Labor - Extraction Supervisor..	(4Hr)($50.00/Hr)	= $ 200.00
Labor - Associate Chemist.....	(24.7Hr)($35.33/Hr)	= $ 872.65
Instrument Time - GC.....	(40.75Hr)($85.00/Hr)	= $3463.75
Instrument Time - GC/MS.....	(0Hr)($150.00/Hr)	= $ 0.00
Reagents and supplies		
Analytical Standards Ordered 7/31.....		$ 274.00
RTX-200 Capillary Column Ordered 8/1.....		$ 450.00
Reaction Vials Ordered 8/1.....		$ 78.92
Ethanol Reagent.....		$ 150.00
24/40 Stoppers.....		$ 126.88
Acetyl Chloride.....		$ 100.00
Analytical Standards.....		$ 75.00
Reaction Vial Septa.....		$ 130.24
Miscellaneous Supplies.....		$ 100.00
Communication.....		$ 200.00
Total.....		$ 15487.33

The next written report will accompany the final analytical method as well as summarize complete costs for the project.

Sincerely,

Jewell W. Smiley

Jewell W. Smiley
Consulting Chemist
Gas Chromatography Supervisor

cc: Craig Vinson/LMG
Herb Kelly/LMG
David Smith/LMG

Analysis of Perfluoro-Octanoic Acid Samples

Dave DuBois, Betsy McCord and Liz Lozada 10/2/2000

Summary and background:

We received 4 samples from M. Kaiser to analyze for chemical structure. We analyzed them by ^{19}F and ^{1}H NMR. One sample was also analyzed by ^{13}C NMR.

Samples:

Notebook	LIMS	Description
88570-8-1	68425	3M FC-118 20% PFOA
88570-8-2	71402	Miteni RM 350T 20% PFOA
88570-8-3	71404	MDF Recovered C-8 20% PFOA
88570-8-4	71405	Dynan 30% PFOA GUS-NR-69641

Results:

Sample 8-1 was typical of the 4 samples. It contains the following groups:

$CF_3(CF_2)_x$
$(CF_3)_2CF(CF_2)_x$
3 types of $CF_3(CF_2)_xCF(CF_3)(CF_2)_y$

Thus there are 4 types of CF_3CF (-71 to -75 ppm; -184 to –188 ppm) groups and 4 additional types of $-CF_3$ groups (–82 to –85 ppm), as shown in the about structures. There are a large number of signals in the –110 to –130 range which are CF_2 groups.

There was an unidentified signal at –63 ppm which might be $-CF_2Cl$, CF_2I, CF_2Br, CH_3ORf, or possibly a vinylidene CF_3 or a tBu CF_3.

Sample 8-3 was almost identical to 8-1 except that it contained a small amount of $CF_3CFHCOOH$ (-76, -196 ppm). This material had sharper NMR lines. The 1H NMR showed a doublet of quartets at about 5.2 ppm that supports this assignment. The j values observed agreed with that of an authentic spectrum of A. Sievert.

Samples 8-2 and 8-4 were almost identical to 8-1.

Quantitative Results, relative mol%:

Sample	$CF_3(CF_2)_x$	$(CF_3)_2CF(CF_2)_x$	$CF_3(CF_2)_xCF(CF_3)(CF_2)_y$			CF_3CFHCO_2H	pentad
(ppm)	-83.5	-73.9	A, -71.7	B, -71.9	C, -72.3	-75.8,-195.5	-63.3
8_1	78.9	8.6	4.3	3.3	4.4	0.0	0.7
8_2	79.0	7.9	3.9	3.5	4.7	0.0	1.0
8_3	72.4	10.2	5.1	4.4	5.7	1.2	1.0
8_4	83.0	7.4	3.3	2.3	3.6	0.0	0.5

Experimental:

^{19}F NMR spectra were obtained on a 400 MHz Varian Inova spectrometer on a 5 mm probe on samples run neat with a D_2O containing capillary for lock. Spectra were externally referenced to a capillary containing F-11 (0 ppm). Spectra were acquired at 30 °C using an rf pulse of 2 usec (pw90 = 14 usec), a spectral width of 90 kHz, an acquisition time of 1.28 sec and a recycle delay of 20 sec. A total of 256 transients were acquired. ^{1}H (with water suppression) and ^{13}C (10 mm) spectra were obtained on the same instrument using standard conditions.

Acknowledgement:

We would like to thank A. Sievert for his help and advice with the ^{19}F NMR spectra.

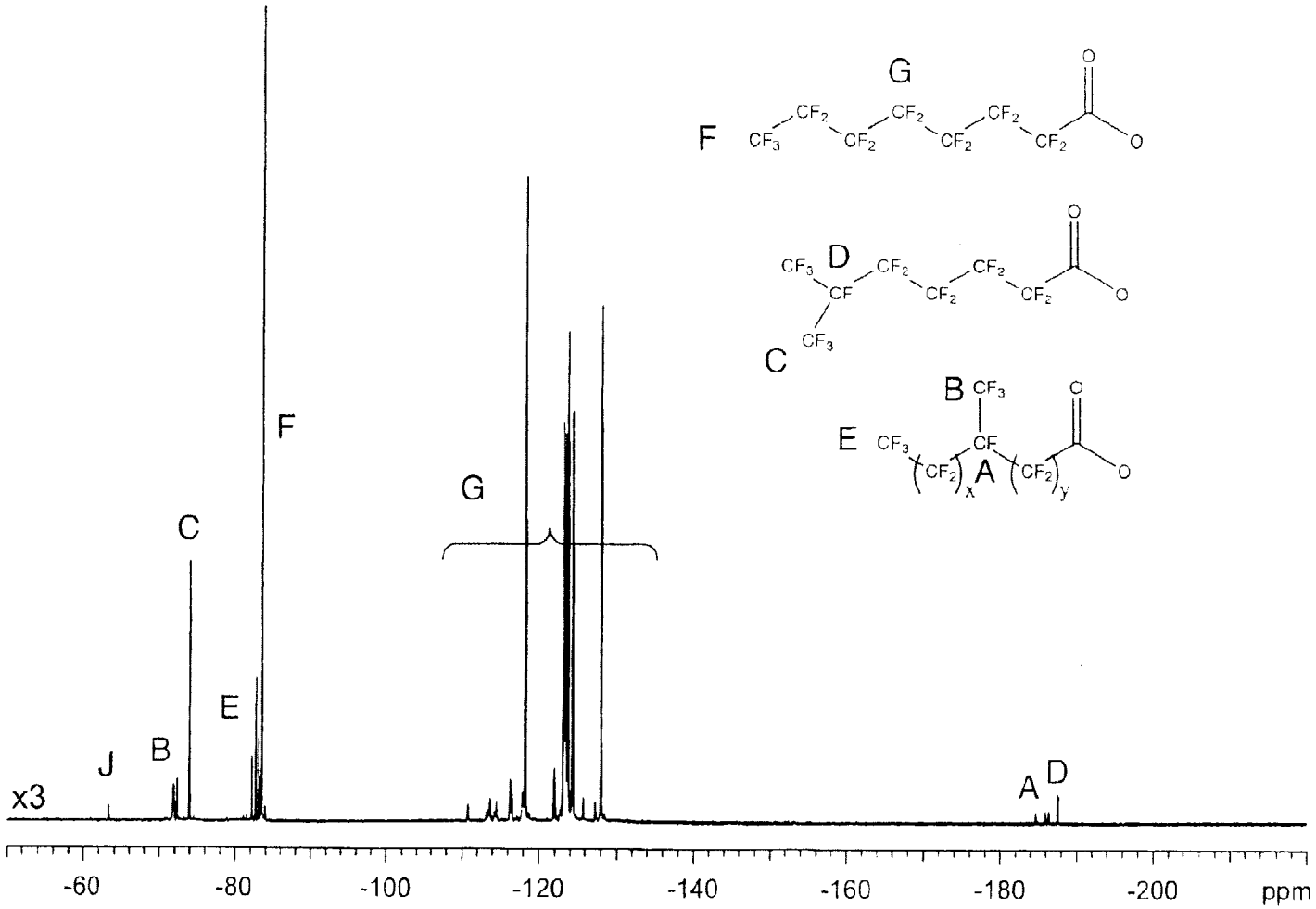


Figure 1. ^{19}F NMR of Sample 88570-8-1, 3M FC-118 20% PFOA

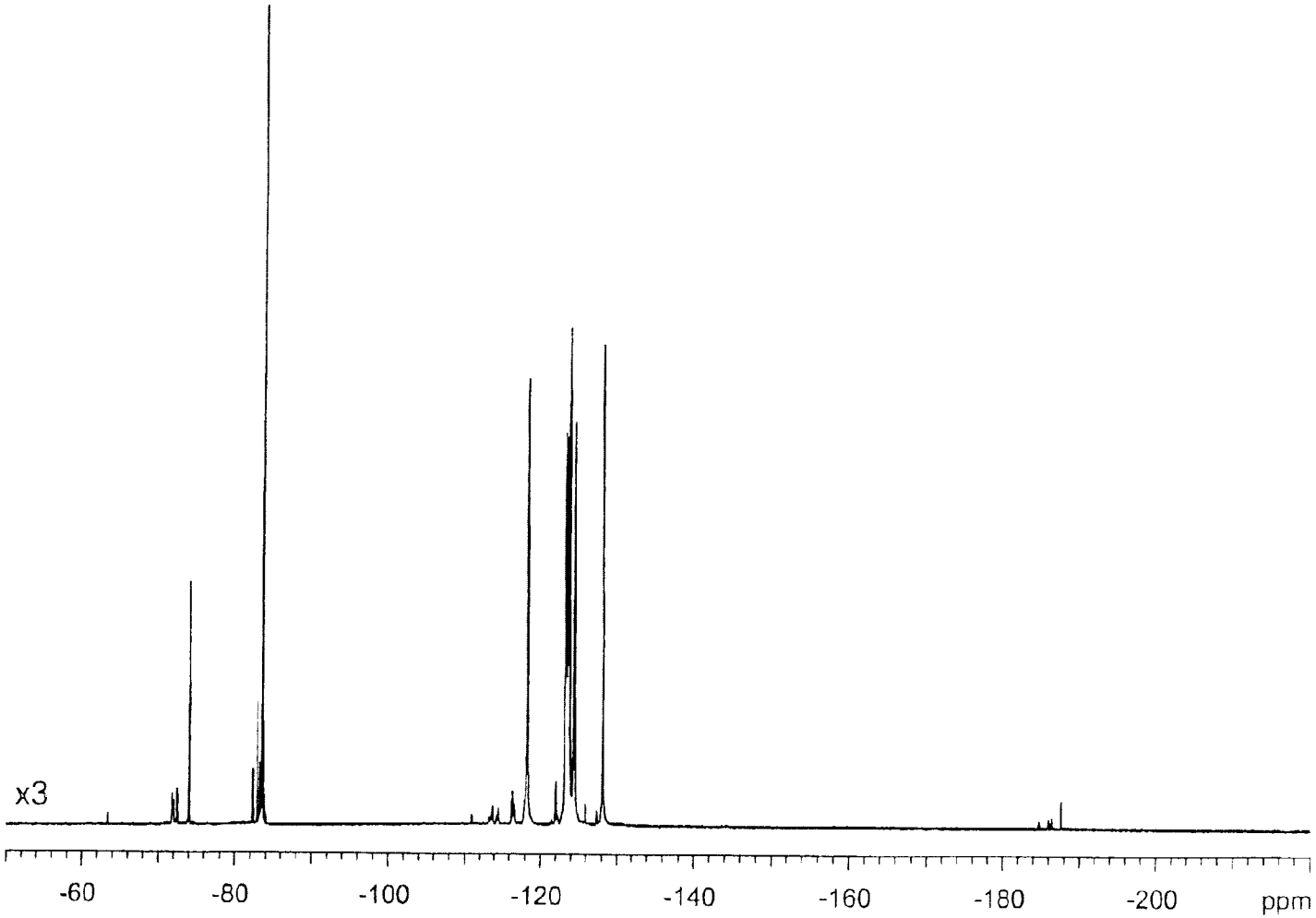


Figure 2. ^{19}F NMR of Sample 88570-8-2, Miteni RM 350T 20% PFOA

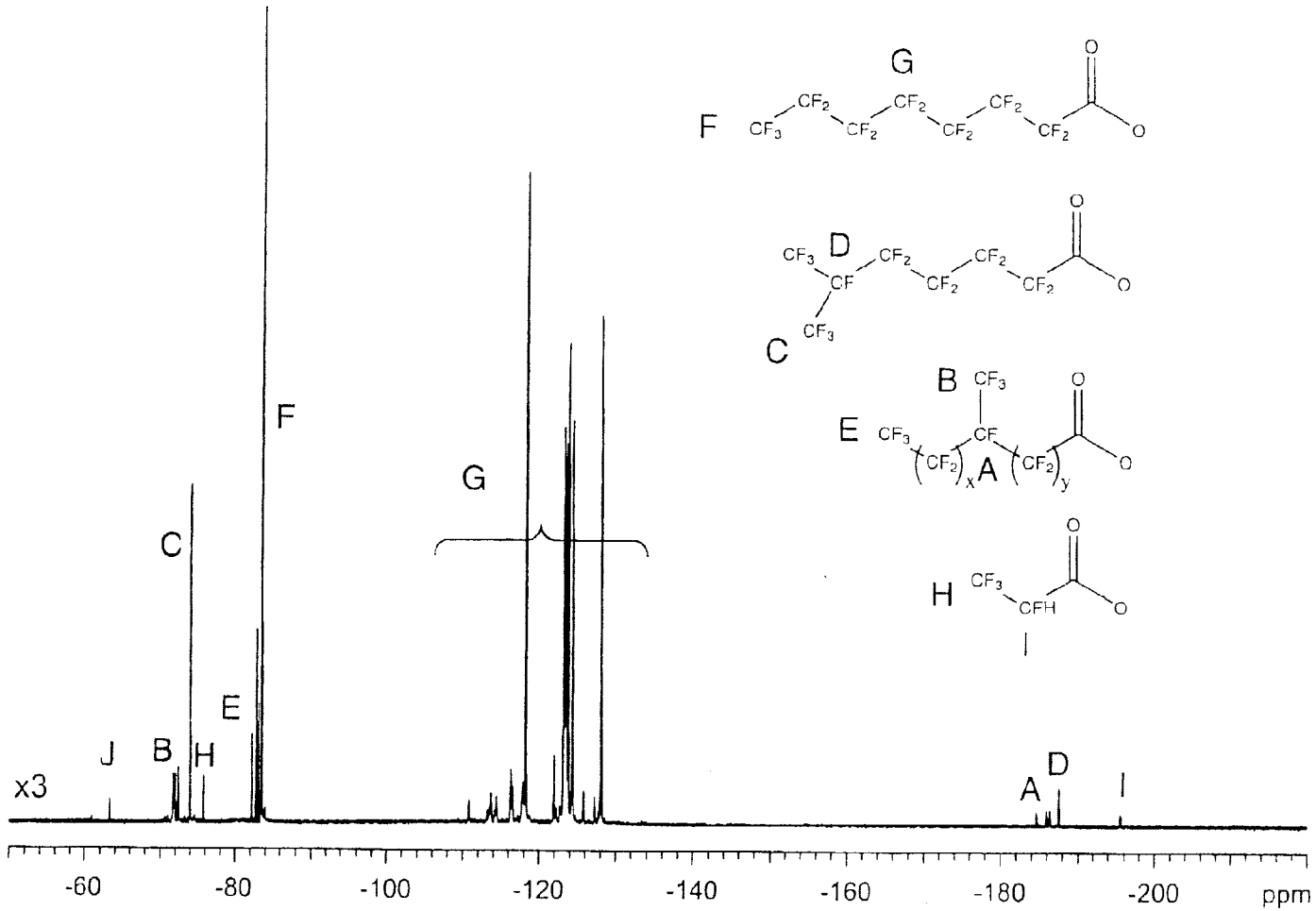


Figure 3. ^{19}F NMR of Sample 88570-8-3, MDF Recovered C-8 20% PFOA

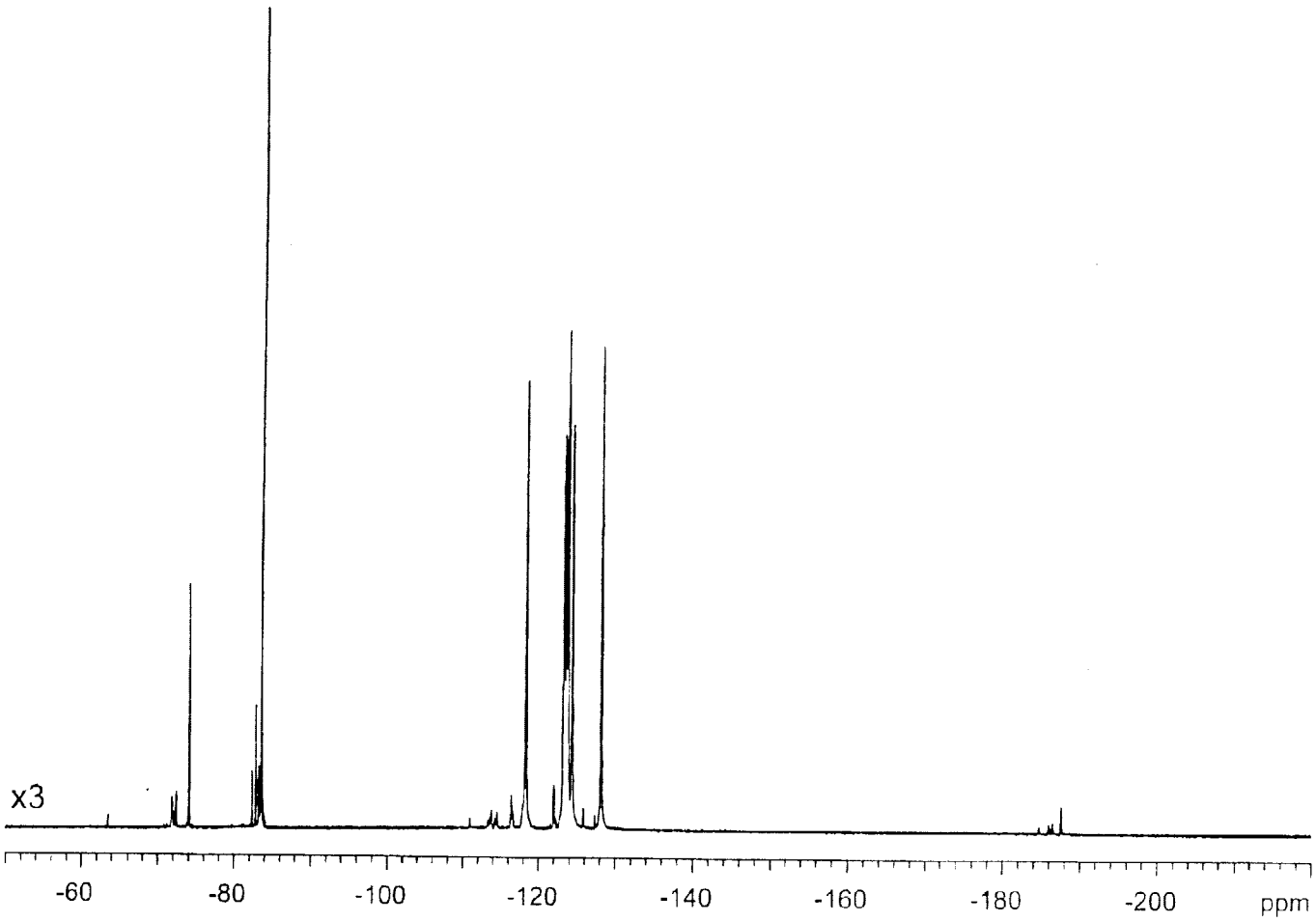


Figure 4. ^{19}F NMR of Sample 88570-8-4, Dynan 30% PFOA GUS-NR-69641

Analysis of Perfluoro-Octanoic Acid Samples

Dave DuBois, Betsy McCord and Liz Lozada 10/2/2000

Summary and background:

PFOS chemicals are used in a wide range of products from fire-fighting foams, coatings for fabrics, leather, and some paper products, to industrial uses such as mist suppressants in acid baths. DuPont produces fluorochemicals based on the perfluoroctanyl iodide chemistry such as Telomer B alcohol. If DuPont can replace materials made with 3M's PFOS with materials based on DuPont's telomer chemistry, there would be a large potential replacement market for us. Our knowledge of persistence, bioaccumulation, and toxicity (PBT) of DuPont materials is incomplete based on the possible applications in the marketplace. CTO Dr. Joseph Miller established a team to quickly address the issues. The team's charter is to provide the science and technology basis to proactively address SHE concerns related to our products. Subteams on Toxicology, Exposure, Environmental Fate and Effects, and PFOA alternatives are working on solutions. Key to each of these teams is the Analytical subteam, which will need to provide product analysis via existing or newly developed methods.

We received 4 samples from M. Kaiser to analyze for chemical structure. We analyzed them by ^{19}F and ^{1}H NMR. One sample was also analyzed by ^{13}C NMR.

Samples:

Notebook	LIMS	Description
88570-8-1	68425	3M FC-118 20% PFOA
88570-8-2	71402	Miteni RM 350T 20% PFOA
88570-8-3	71404	MDF Recovered C-8 20% PFOA
88570-8-4	71405	Dynan 30% PFOA GUS-NR-69641

Results:

Sample 8-1 was typical of the 4 samples. It contains the following groups:

$CF_3(CF_2)_x$
$(CF_3)_2CF(CF_2)_x$
3 types of $CF_3(CF_2)_xCF(CF_3)(CF_2)_y$
$(CF_3)_3C(CF_2)_x$

Thus there are 4 types of CF_3CF (-71 to -75 ppm; -184 to –188 ppm) groups and 4 additional types of $-CF_3$ groups (–82 to –85 ppm), as shown in the about structures. There are a large number of signals in the –110 to –130 range which are CF_2 groups.

There was a signal at –63 ppm which was assigned to $(CF_3)_3C(CF_2)_3$ group. Its chemical shift and multiplicity correspond to values found in the literature.

Sample 8-3 was almost identical to 8-1 except that it contained a small amount of $CF_3CFHCOOH$ (-76, -196 ppm). This material had sharper NMR lines. The 1H NMR showed a doublet of quartets at about 5.2 ppm that supports this assignment. The j values observed agreed with that of an authentic spectrum of A. Sievert.

Samples 8-2 and 8-4 were almost identical to 8-1. The ^1H NMR spectrum of 8-4 showed a singlet at 3.4 ppm, which may be methanol; spiking experiments are underway to verify this.

Quantitative Results, relative mol%:

Sample	$CF_3(CF_2)_x$	$(CF_3)_2CF(CF_2)_x$	$CF_3(CF_2)_xCF(CF_3)(CF_2)_y$			CF_3CFHCO_2H	$(CF_3)_3C(CF_2)_3$
(ppm)	-83.5	-73.9	A, -71.7	B. -71.9	C, -72.3	-75.8,-195.5	-63.3
8_1	78.9	8.6	4.3	3.3	4.4	0.0	0.2
8_2	79.0	7.9	3.9	3.5	4.7	0.0	0.3
8_3	72.4	10.2	5.1	4.4	5.7	1.2	0.3
8_4	83.0	7.4	3.3	2.3	3.6	0.0	0.2

Experimental:

^{19}F NMR spectra were obtained on a 400 MHz Varian Inova spectrometer on a 5 mm probe on samples run neat with a D_2O containing capillary for lock. Spectra were externally referenced to a capillary containing F-11 (0 ppm). Spectra were acquired at 30 °C using an rf pulse of 2 usec (pw90 = 14 usec), a spectral width of 90 kHz, an acquisition time of 1.28 sec and a recycle delay of 20 sec. A total of 256 transients were acquired. ^{1}H (with water suppression) and ^{13}C (10 mm) spectra were obtained on the same instrument using standard conditions.

Acknowledgement:

We would like to thank A. Sievert for his help and advice with the ^{19}F NMR spectra.

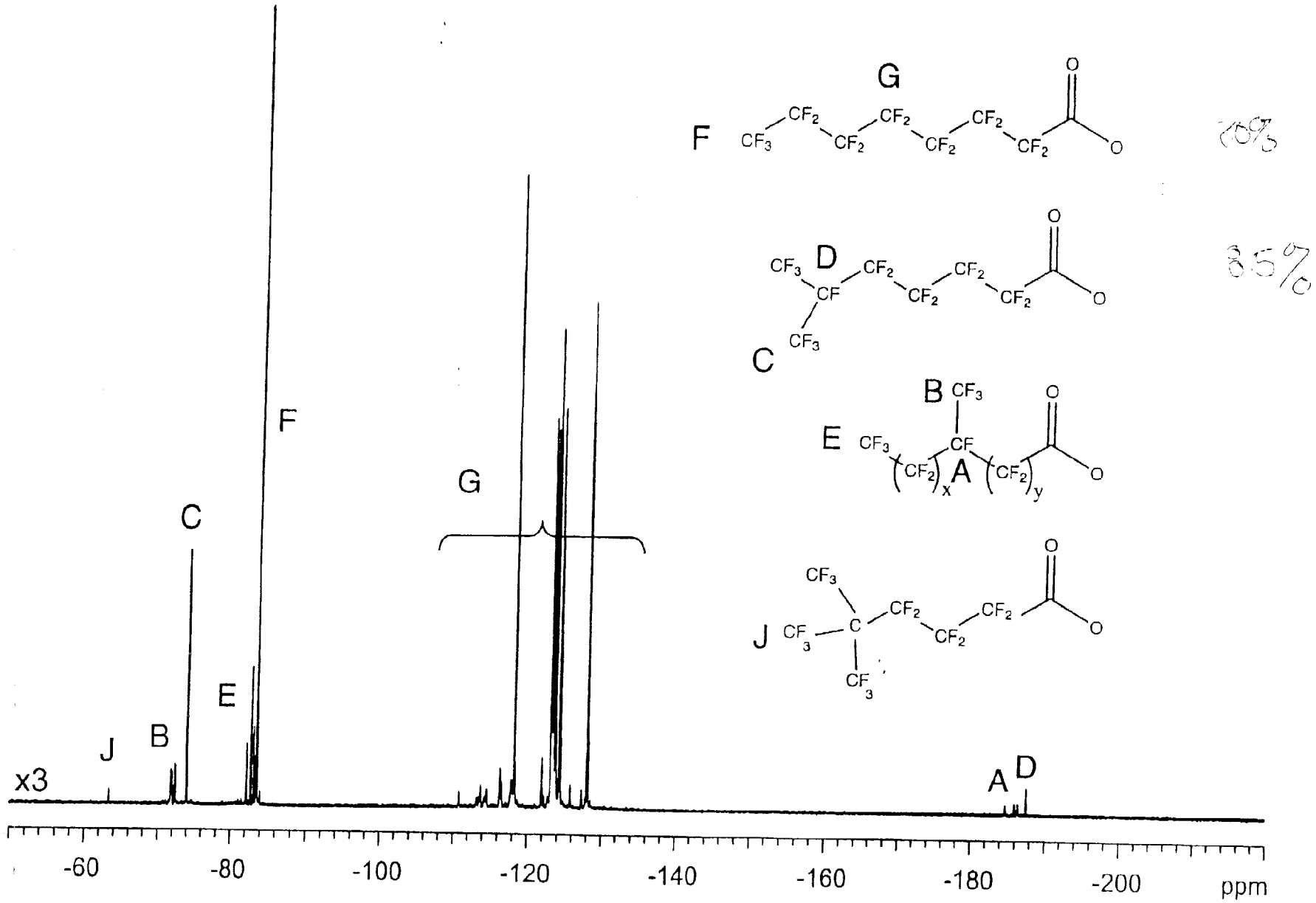


Figure 1. ^{19}F NMR of Sample 88570-8-1, 3M FC-118 20% PFOA

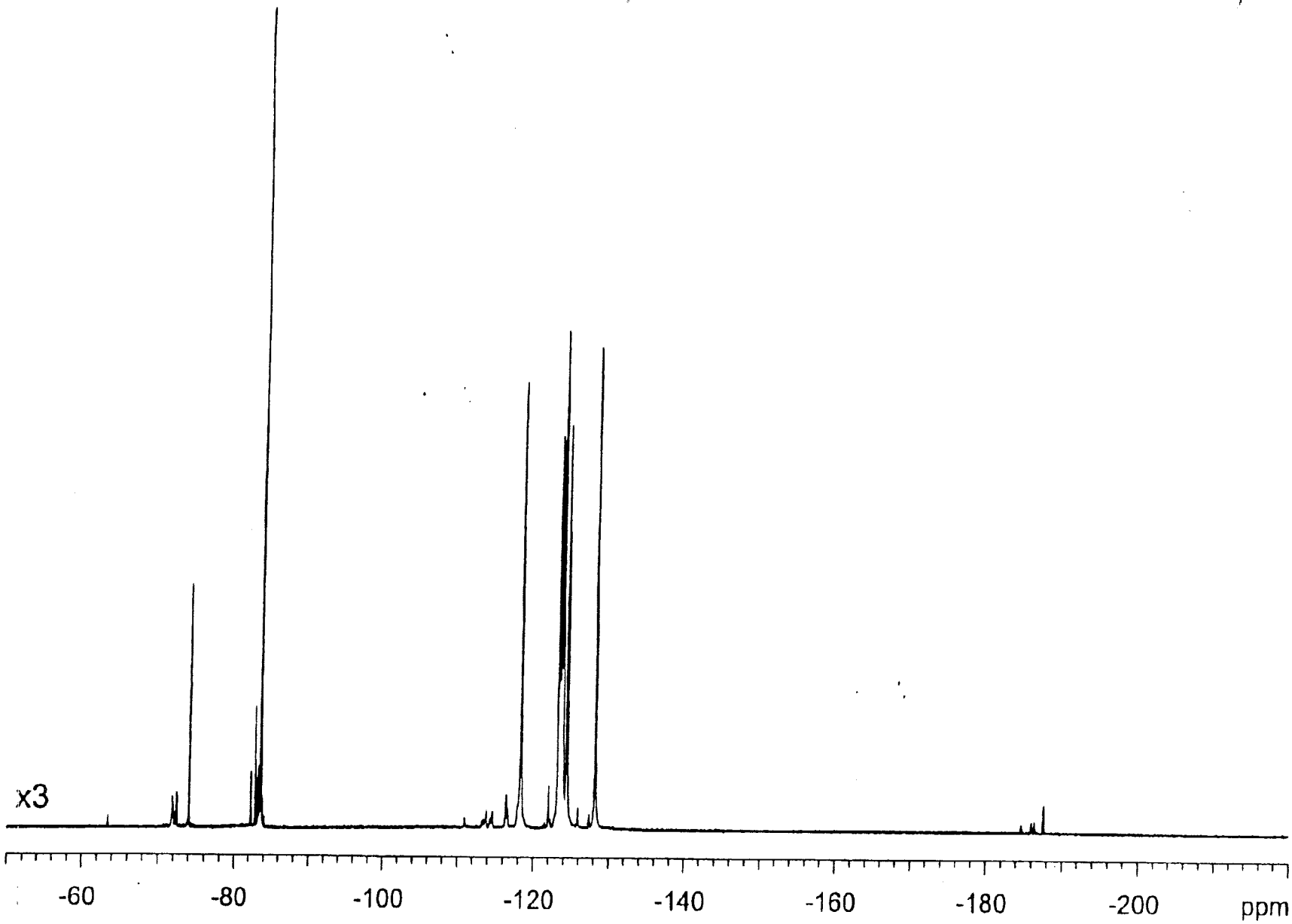


Figure 2. ^{19}F NMR of Sample 88570-8-2, Miteni RM 350T 20% PFOA

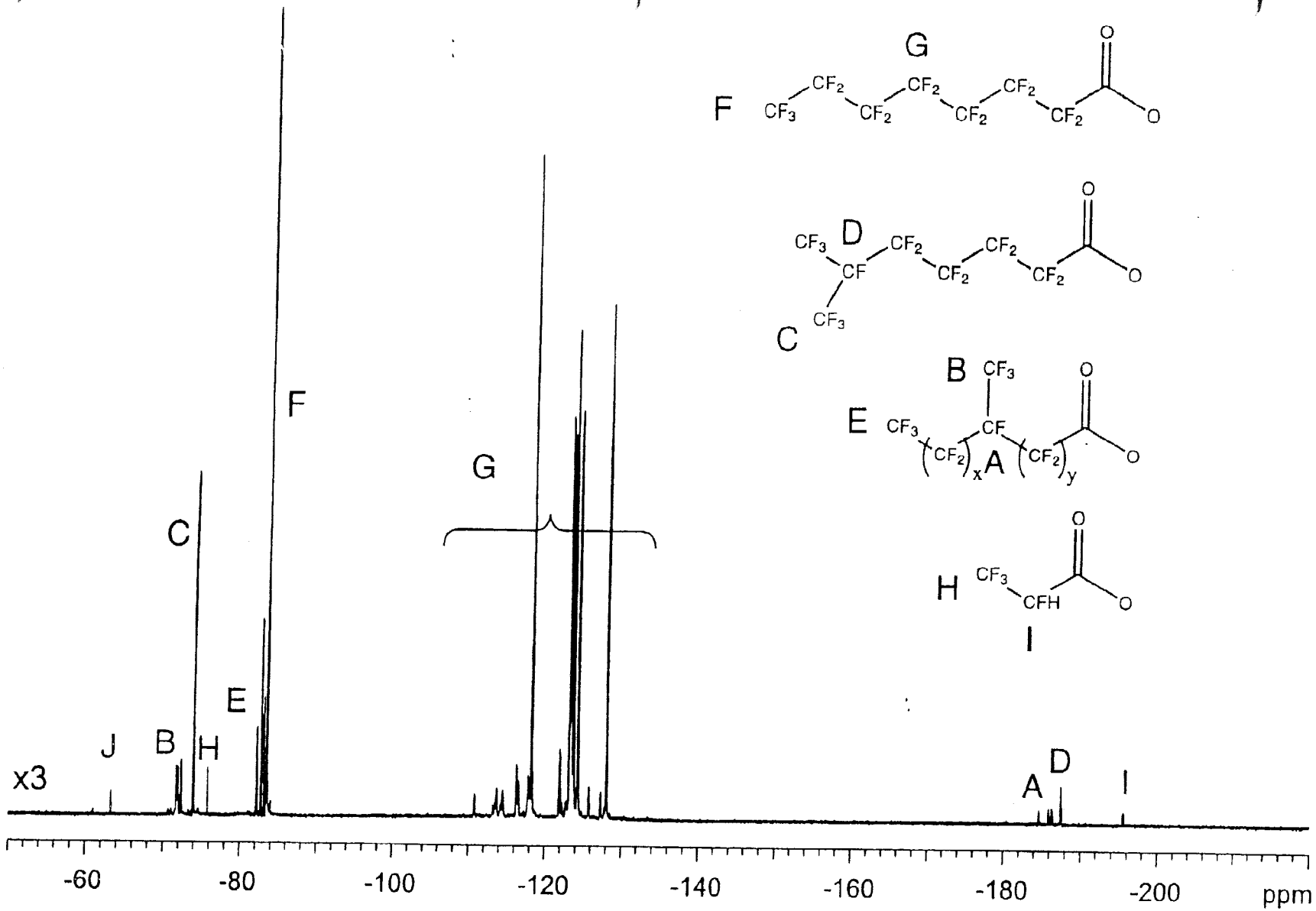


Figure 3. ^{19}F NMR of Sample 88570-8-3, MDF Recovered C-8 20% PFOA

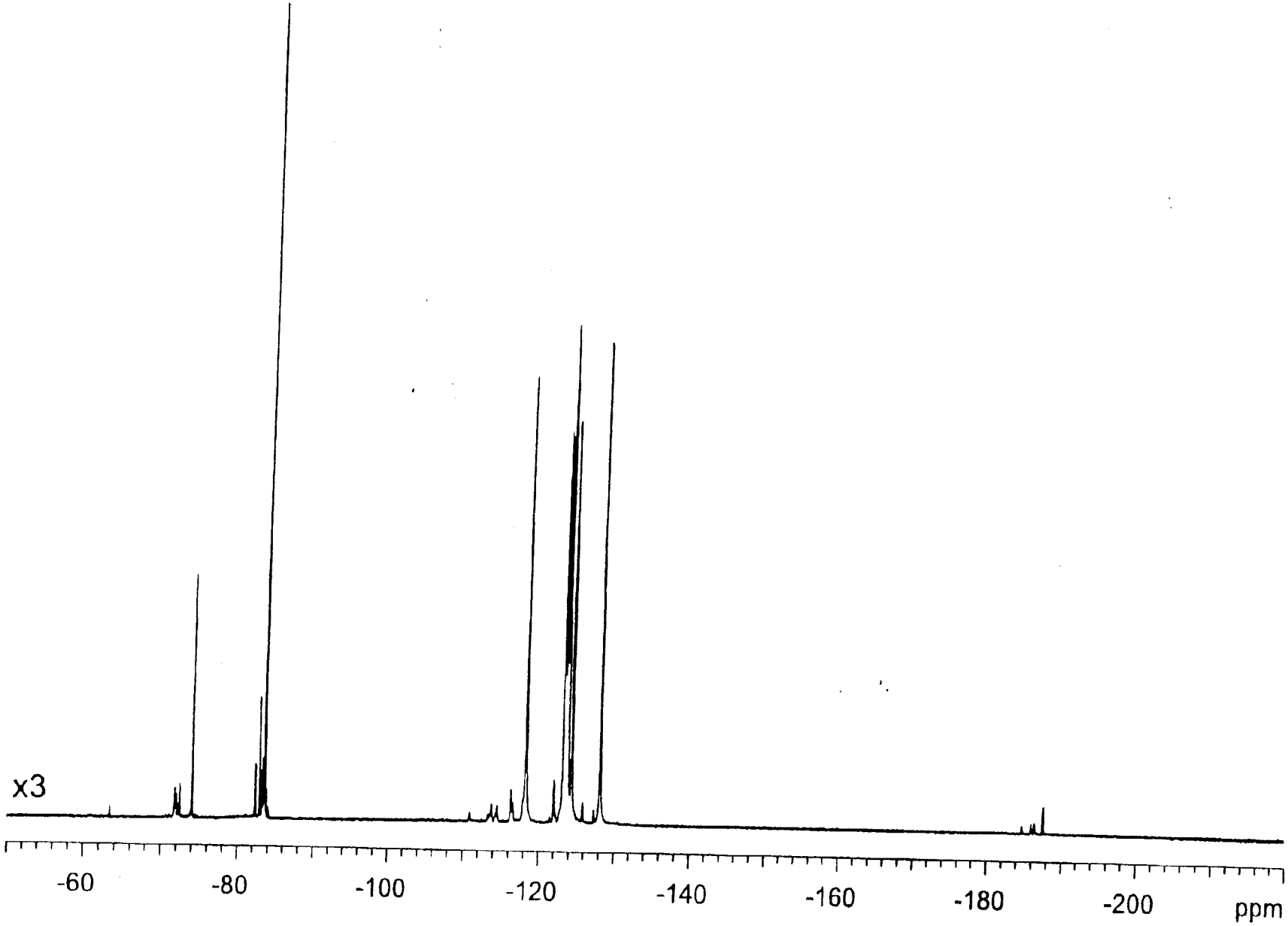
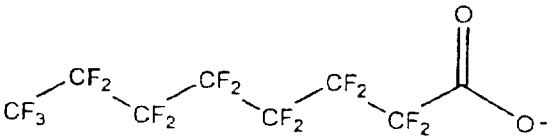
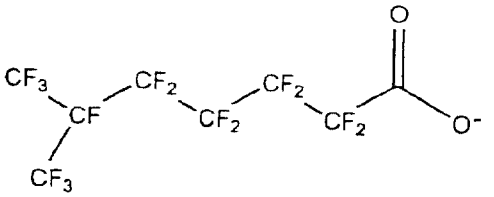
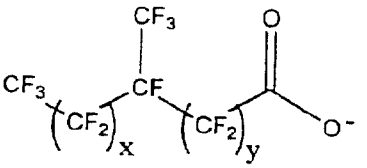
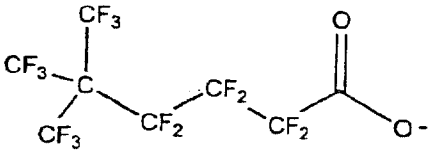


Figure 4. ^{19}F NMR of Sample 88570-8-4, Dynan 30% PFOA GUS-NR-69641

For Sample 88570-8-1

Compound:	NMR mol%	3M Fax mol%
	78.9	80%
	8.6	8.5
	12.0	11.3
	0.2	0.3

E. I. du Pont de Nemours & Co. (Inc.)
Polymer Products Department
Experimental Station Laboratory

Determination of Perfluorooctanoic Acid in Water

Gas Chromatographic Method

I. Applicability

This method has been developed for the determination of ammonium perfluorooctanoate (C_8-APFC) and perfluorooctanoic acid in aqueous solution, at concentrations from 0.01-10 µg/mL. The procedure described here is for the analysis of aqueous solutions from air impingers. The method has also been used for determination of C_8 in blood samples and, depending on specific interferences, could be applied to other aqueous systems. A modification which eliminates the drying step can be used for appropriate solid samples (such as Nuclepore filters used in air sampling) and for methanol solutions.

II. Unusual Safety Considerations

The 3M Company has found in preliminary studies that C_8 caused birth defects when fed to rats in a laboratory experiment. Female employees of child bearing capability should be restricted from any portion of this procedure which offers significant chance for exposure. Although no health problems are known for workers exposed to C_8, it has been found in blood samples and may be very slowly eliminated from the body (Reference 3). Standard laboratory safety practices for handling toxic, emoroyotoxic, etc. materials and for corrosives should be used in working with these perfluorinated acids and their solutions.

The electron caputre detector contains a radioactive source (^{63}Ni), and the manufacturer's instructions for safe operation must be observed.

Care should be taken to avoid contact in working with the low temperature baths used for freeze-drying.

III. Principle

Freeze-drying (lyophilization) is used to remove the water from sample aliquots and permit derivatization. Addition of methanolic HCl to the dried residue, along with perfluorodecanoic acid (C_{10}) internal standard, converts the acids to more volatile methyl esters. These are extracted from the reaction mixture into hexane solution for GC analysis. An electron capture detector (ECD) is used for sensitivity and selectivity.

IV. Fundamental Equations

$$C_7F_{15}COO^-M^+ + CH_3OH \xrightarrow{3\%\ HCl} C_7F_{15}COOCH_3 + MOH$$

$$(M^+ = NH_4^+,\ Na^+,\ H^+)$$

(acid not amenable to GC analysis) ———> (non-polar, volatile ester for GC)

V. Interferences

As in any GC analysis, compounds with the same retention time as the compound of interest will not be distinguished from it. These can include impurities present in the reagents or other components in the samples themselves.

Interfering peaks from solvents, derivatizing reagent, etc., are not generally observed in this laboratory with the reagents specified below (Note 1), but each new batch should be checked and a blank included in every analysis. A small peak at the C_8 ester position is found in reagent blanks containing perfluorodecanoic acid internal standard however, probably from C_8 present as an impurity in that material. This will give an intercept slightly greater than zero in the calibration plot, but is a significant contribution to the C_8 peak area only at the lowest concentrations (ca 1/3 of the total for 0.01 ppm C_8, when ca 1 ppm C_{10} is added).

In the air impinger samples examined to date, no obvious interference or contribution from compounds other than C_8 has been observed. Preliminary analyses of FEP "in-situ" dispersing agent indicate that while one component of that mixture would co-elute with C_8, it should be accompanied by other nearby peaks.

VI. Sensitivity, Precision and Accuracy

The method has been used over the concentration range 0.01-10 µg/mL C_8. Adjustments in the volumes of sample or reagents could probably be made to extend this somewhat in either direction, if necessary. Due to the relatively narrow linear range of the ECD, however, the calibration curve must always cover the region of interest.

From the limited data available at this time, precision is estimated to be ± 10% relative, with quantitative recovery of spikes within this uncertainty. Accuracy will also be affected by the choice of C_8 standard, which should correspond to the fluorosurfactant composition of the samples (Note 2).

VII. Apparatus

Instruments and equipment used in this laboratory are specified here; equivalent apparatus can be substituted.

1. Gas Chromatograph and Supplies

Hewlett-Packard 5830 GC with HP 18803 Electron Capture Detector, equipped for on-column injection with glass packed columns

10 ft x 2 mm id glass columns (HP configuration 5 with ECD adapter), packed with 10% OV-210 on 70/80 mesh Chromosorb W.AW.DMCS. and conditioned at 200°C

Hamilton 701N 10 µL syringe

2. Lyophilizer

Labconco No. 75352 bench-top freeze dryer (12-port, dry ice cooled)

Labconco No. 75406 (150 mL) or No. 75408 (300 mL) Fast-Freeze flasks, with No. 75476 stainless steel adapters

Vacuum pump with auxiliary cold trap

McLeod gage (or electronic vacuum gage) reading in the 5-0.005 Torr range.

3. Thermostated Reaction Block

Pierce No. 18802 Reacti-Therm Heating Module, with Reacti-Block to hold 2-dram vials and thermometer (Block C, No. 18803, has 12 holes but they must be enlarged to ca 19 mm to accomodate the 2-dram vials. Block B, No. 18802, can be used as is, but will hold only 9 vials and provides poorer thermal contact).

4. Vials and Septum Caps

Wheaton No. 224884 or Pierce No. 13028 2-dram (ca 7 mL) screw cap septum vials (borosilicate glass)

Pierce No. 12713 Teflon*-Silicone Septa and No. 13216 open top screw caps

5. Pipets and Dispensers

Gilson P200 Pipetman, micropipet for quantitative delivery of 50 µL aliquots

1 and 2 mL volumetric pipets for measuring sample and reagent aliquots. (Brinkman Dispensette Bottle-Top Dispensers are a great convenience for repetitive delivery of solvents.)

6. Analytical Balance

7. Common laboratory equipment

VIII. Reagents

1. C_8 standard, FC-143 ammonium perfluorooctanoate (3M) or material in use in the area to be monitored (Note 2)

2. Perfluorodecanoic acid, PCR Research Chemicals

3. Methanol, Fisher HPLC (A-452) or Fisher Certified ACS (A-412) (Note 1)

* Reg. U.S. Pat. & Tm. Off.

4. Derivatization Reagent, 3% HCl in methanol, prepared from Applied Science No. 18053 Instant Methanolic HCl Kit, but substituting Fisher HPLC or ACS Methanol for the Lipopure Methanol provided (Note 1).

   The reagent should be stored in the refrigerator, and can be kept for ca 1 month.

5. Hexane, Phillips Spectrograde or Applied Science Lipopure (Note 1)

6. Water, distilled or deionized

7. Sodium hydroxide, 0.05 M aqueous solution

IX. Procedure

A. Preparation of Standard Solutions

Only final solution concentrations are given here without detailed preparation procedures, since amounts needed, concentrations of interest, etc., will vary between laboratories. Preparations should be such that concentrations can be calculated to three significant figures.

1. Perfluorodecanoic acid in methanol: ca 20 µg/mL

2. Ammonium perfluorooctanoate in water: At least four solutions should be prepared which cover the concentration range of interest. For the example in the chromatograms and calculations below (0.1-1.0 ppm), standard solutions were prepared containing 0.1, 0.25, 0.5, 0.75, and 1.0 µg/mL FC-143.

B. Preparation of Samples and Standards for Analysis (Note 3)

For each sample or standard solution, pipet a 1 mL aliquot into a 2-dram vial. Prepare a blank similarly, using distilled water. Add 50 µL 0.05 M NaOH to each and mix gently, keeping the solution in the bottom of the vial to avoid loss to the cap, sides, etc..

C. Freeze-Drying

1. A schematic diagram of the feeeze-drying apparatus is shown in Figure 1. Each of the 12 ports has a separate valve, so that flasks can be attached or removed while the system is under vacuum. The apparatus should be pumped down to an acceptable vacuum (Note 4) and the cold traps filled before attaching the drying flasks.

2. To prevent bumping, the samples must be frozen before they are placed under vacuum. To reduce the chance of contamination or sample loss, cover each vial with a light filter beforehand; a small piece of Kimwipe held in place with a rubber band works well. Freeze the solution in each (Note 5), and keep the vials at dry ice temperature until all of them are ready.

3. Place up to four vials in each drying flask and attach to the drying chamber. The pressure should drop below 0.5 Torr again within a few minutes if no leaks are present, and the samples should remain frozen as sublimation takes place. Allow them to dry for ca 4 hours, or until no ice is left in any of the vials.

D. Derivatization

1. To the dried residue in each vial, add 1 mL methanolic HCl derivatization reagent and 50 µL C_{10} standard solution (ca 20 µg/mL in methanol). Close with a septum screw cap and shake well.

2. Thermostat for 1 hour at 65°C.

   (At this point, the samples can be cooled to room temperature and left overnight to analyze the next day.)

3. Cool to room temperature, add 1 mL each hexane and distilled water, and shake for ca 2 minutes.

4. After the phases separate, the upper hexane layer can be sampled directly for GC analysis. The solutions are stable in this form for at least several days, although hexane will gradually evaporate after the septum has been pierced.

E. GC Analysis

1. GC Conditions - Instrument and column as above.

Temperatures:	Injection port	200°C
	Detector	325°C
	Column oven	100°C, isothermal
Carrier Gas:	90% argon/10% methane (or nitrogen if recommended for the instrument) flow ca 30 mL/min	
Injection Volume:	2 µL	
Sensitivity:	attenuation as needed to keep peak height measurable, 2^5 - 2^{11} on HP 5830.	
Run Time:	11-20 minutes, depending on sensitivity (Note 6)	

After installing the column and establishing the conditions above, check the ECD base frequency and noise level according to the manufacturer's instructions, to insure that they are stable and within acceptable limits.

2. Each solution should be run twice (or duplicates run once each), bracketing samples with standards of similar concentration in the second series of injections.

   Representative chromatograms are shown in Figure 2 for blanks, standards and samples. Either peak height or peak area can be measured for quantitation.

X. Calculations (Note 7)

1. Normalized C_8 Peak Values

A tabulation of peak heights for analysis of a series of five standards and two samples (each prepared in duplicate) is shown in Figure 3.

To correct for any variations in injection volume, reagent volumes, etc., the raw C_8 peak heights (or areas) are first normalized relative to the internal standard average

$$C_8 \text{ (corrected)} = C_8 \times \frac{C_{10} \text{ average}}{C_{10}}$$

(Calculation of the C_{10} average also provides a measure of the precision in sample preparation and GC analysis).

2. Calibration Plot

A calibration curve obtained by plotting corrected C_8 peak height vs. concentration of the standards is also shown in Figure 3.

For non-linear plots such as this, a smooth curve should be drawn through the data points. In some cases, a straight line can be fit through several points, and a linear least squares calculation made for the slope and intercept in that region.

3. Calculation of Sample Concentrations

Using the corrected C_8 peak heights (or areas) for the samples, corresponding concentrations can be read directly from the calibration plot as shown in Figure 3. Average the values from duplicates for each sample.

XI. Notes

1. The solvents specified in Section VIII were found preferable to several tried for this analysis, but others may also be satisfactory. Even with the same reagents, the appearance of the blank may depend on the condition of the detector. With the ECD used here, contamination results in a significant decrease in selectivity and increased reagent interference.

2. The FC-143 samples examined to date appear to be a mixture of ca 70-75% straight chain material with several secondary components (including branched isomers) from 1-9%. (Determined by NMR and capillary GC with FID peak area). Under the GC conditions used here, this results in a group of poorly resolved peaks at the C_8 ester position, as seen in Figure 2. In contrast, commercial perfluoro-n-octanoic acid (PCR) and some other ammonium salts contain $\gtrsim$95% straight chain C_8, and appear more nearly as a single peak in the analysis. The response factor determined relative to the C_{10} internal standard also differs for the two types of material, so it is important to choose the standard most similar to the samples to be analyzed. All of the aqueous samples examined thus far closely resemble FC-143 standards, but for chromatograms differing greatly in appearance it may be more appropriate to substitute a straight-chain standard.

3. At least for initial analyses using this procedure, duplicates should be prepared as a check on precision.

4. For this freeze-drying application pressures less than 0.5 Torr seem to be satisfactory, although 0.025-0.05 may be achieved. Readings will also depend on the type of gage used, since the McLeod gage does not read the pressure of water vapor as electronic gages do.

5. A small dry ice/acetone bath or the center cold well of the drying chamber can be used for rapid freezing of the solutions in the bottom of the vials. As noted in Section II, care must be taken to avoid contact with the cold bath.

6. As seen in the chromatograms in Figure 2, some small, broad peaks appear late in the run. (These appear to come from the C_{10} standard, and might be eliminated in purified material). When running at high sensitivity for low C_8 concentrations, these can interfere with the following run and must be allowed to elute before the next injection.

7. From the calibration plot in Figure 3, it can be seen that the ECD response is not linear with concentration over this range. Linear plots may be obtained over other ranges, but still with concentration-dependent response factors. The calculations described here thus make use of a calibration curve, where the internal standard is used to normalize raw peak values before plotting.

   Calculations could be done by a general internal standard procedure instead for an ECD which shows a more linear response and constant relative response factors. (A calibration plot of this sort has been observed with a Varian 3700 GC under similar conditions).

XII. References

1. Research Notebooks E20029, E22306, E22424 (S. Stafford)

2. J. Belisle and D. F. Hagen, "A Method for Determination of Perfluorooctanoic Acid in Blood and other Biological Samples", Analytical Biochemistry 101, 369 (1980)

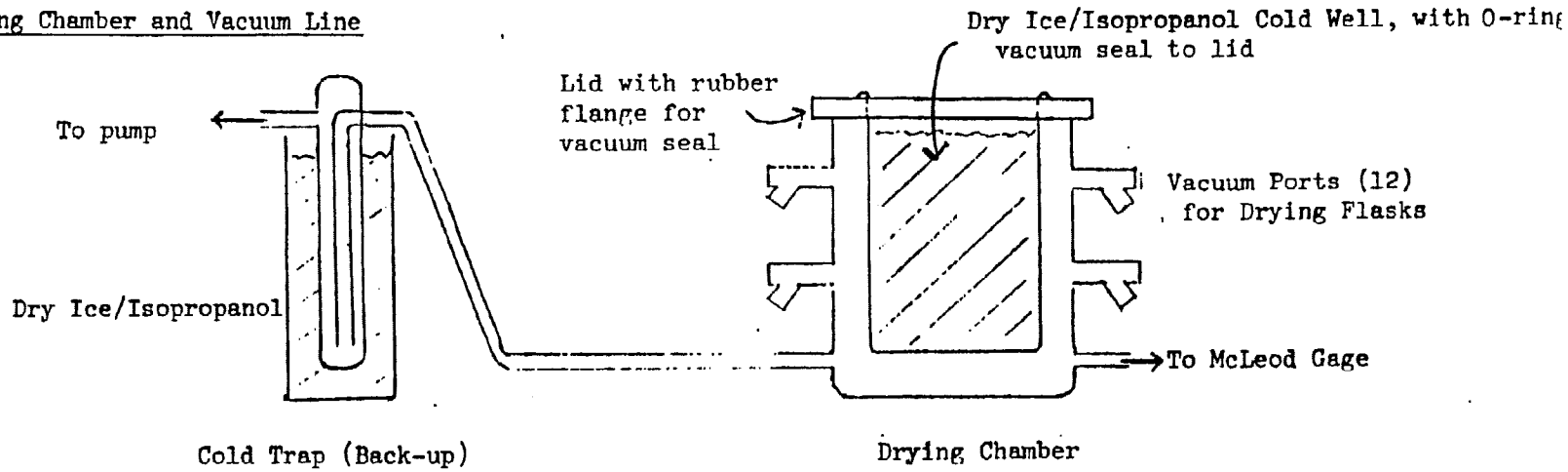
3. F. A. Ubel, S. D. Sorenson, & D. E. Roach, "Health Status of Plant Workers Exposed to Fluorochemicals - A Preliminary Report" American Industrial Hygiene Association Journal 41, 584 (1980)

Prepared by S. S. Stafford, 8/8/80
Revised by S. S. Stafford 4/3/81

Approved by L. J. Papa

FIGURE 1 - APPARATUS FOR FREEZE-DRYING SAMPLES

(a) Drying Chamber and Vacuum Line



(b) Vacuum Port, Drying Flask, and Samples

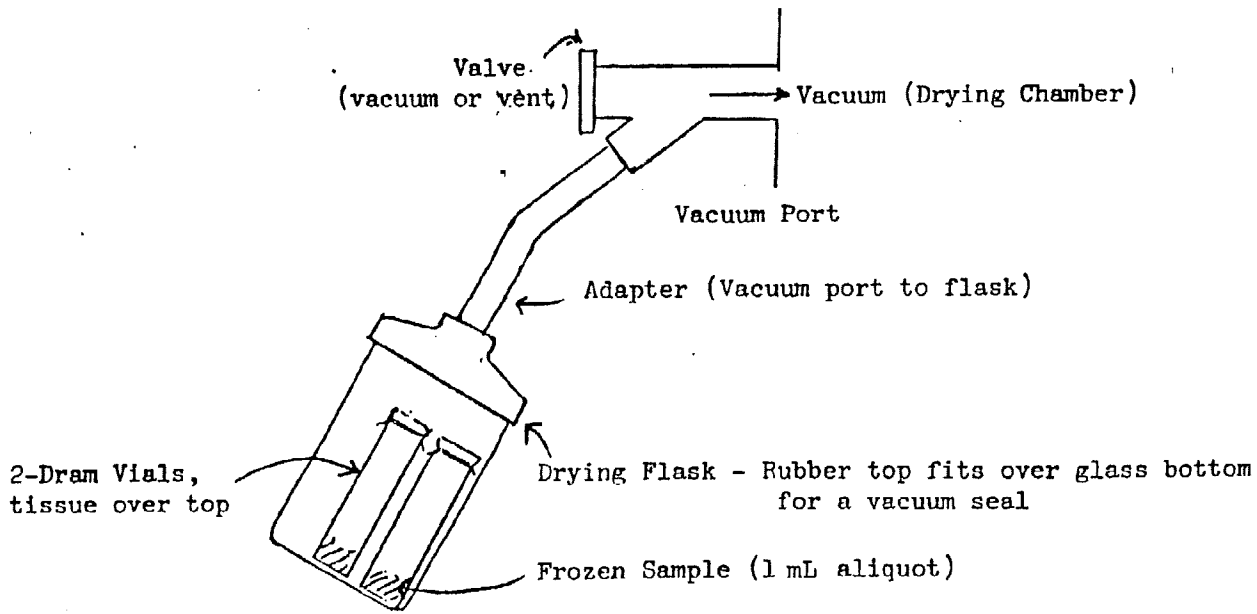
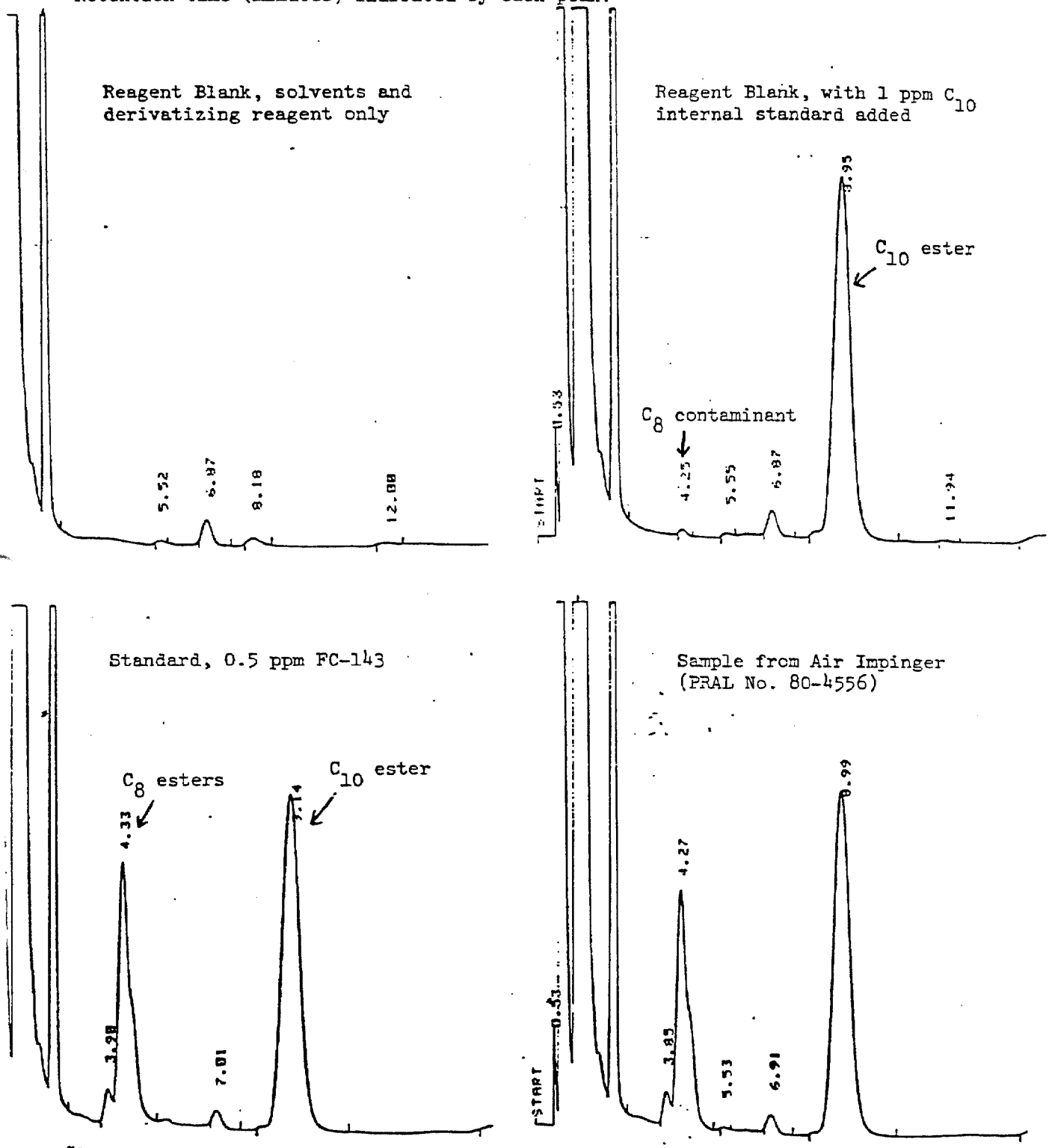


FIGURE 2 - CHROMATOGRAMS FOR C_8 DETERMINATION *

GC conditions as on p. 5. Attenuation 2^7, chart speed 1 cm/min.
Retention time (minutes) indicated by each peak.



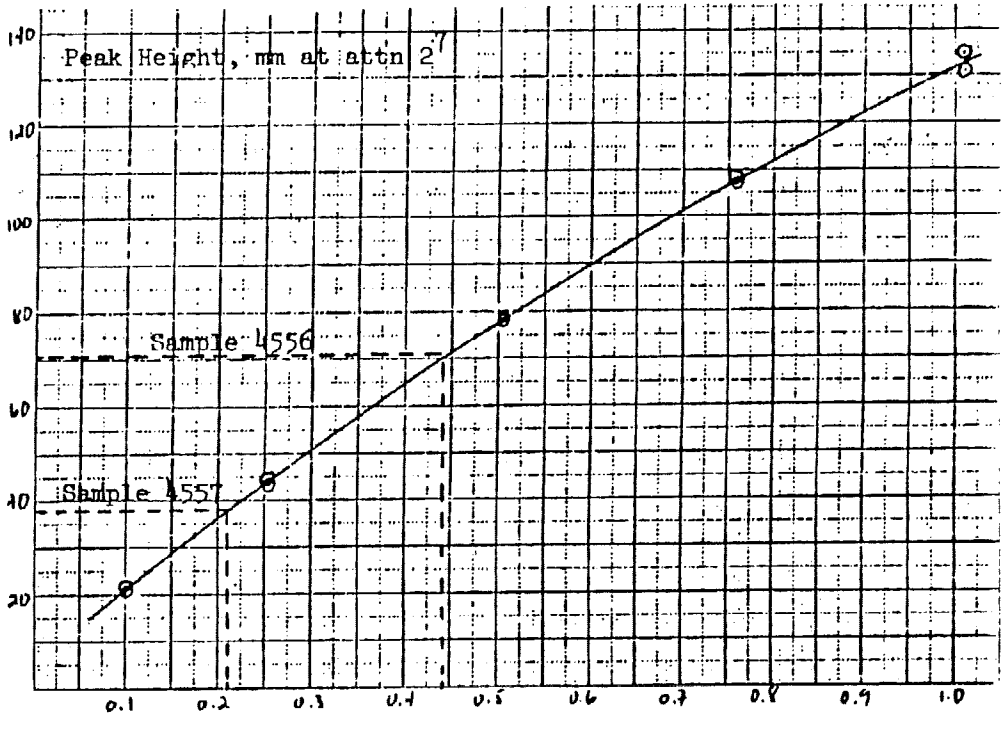
*The results shown here are for solutions prepared using 2 mL each derivatizing reagent, hexane, and water, rather than 1 mL as in the method. Relative peak heights will not be affected, but attenuation is 2x lower.

FIGURE 3 - CALCULATION OF CORRECTED PEAK HEIGHTS AND CALIBRATION PLOT *

(a) Peak Height values for runs of ten standard and four sample solutions (duplicate preparations at each concentration)

Solution	[FC-143], µg/ml Added	Run #	Peak Height, mm at 2⁷ Raw Values C_8	C_{10}	Corrected $C_8 \times \frac{102}{C_{10}}$
Std A-1	1.01	7	138	104	135
" A-2	"	20	131	102.2	131
" B-1	0.758	8	107.2	101.5	108
" B-2	"	19	106	101.5	107
" C-1	0.505	9	99	103	78.2
" C-2	"	16	77.5	100.8	78.4
" D-1	0.253	10	44	103.5	43.4
" D-2	"	13	44.2	101.2	44.5
" E-1	0.101	11	22	102.5	21.9
" E-2	"	12	21.5	106	20.7
** Sample	Calculated				
80-4556-1	0.440	17	70	102	70.0
80-4556-2	0.445	18	70	101	70.7
80-4557-1	0.210	14	38.5	103.5	37.9
80-4557-2	0.200	15	37.8	105	36.7
				ave = 102 ± 2	

(b) Calibration Plot, Corrected C_8 peak height vs. concentration



[FC-143], µg/mL

** Calculated concentrations for duplicate sample preparations read from the curve

*The results shown here are for solutions prepared using 2 mL each derivatizing reagent, hexane, and water, rather than 1 mL as in the method. Relative peak heights will not be affected, but attenuation is 2x lower.

AR226_2730

Study Title

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF ZONYL®([redacted]) (OECD 209)

Test Guideline(s)

OECD (1984) Guideline for Testing of Chemicals, Section 2, No. 209: "Activated Sludge, Respiration Inhibition Test", adopted April 4, 1984

Author(s)

William R. Berti, Ph.D.

Study Completion Date

30-August-2004

Test Facility

E. I. du Pont de Nemours and Company
Environmental and Microbiological Sciences & Engineering
Biochemical Science and Engineering
Central Research & Development
Glasgow, Building 300, P.O. Box 6101
Newark, DE 19714-6101

Submitter

E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
USA

EMSER Study / Project Number

97-03/ 4842

Report Number

EMSER 97-03

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report, with the exception of the items listed below was conducted in compliance with the following GLP Standards:

- United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160 (effective October 16, 1989)

which are consistent with:

- The OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM/98(17), OECD, Paris, 1998
- MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283).

The following items are exceptions to the GLP Standards. These items do not impact the validity of the study.

The test chemical was not characterized under GLP. It is a commercially available material that was provided by the submitter. The certificate of analysis (COA) was provided by the manufacturer and the accuracy of the data is considered sufficient for the purposes of this study.

The reference chemical was not characterized under GLP. It is a commercially available material. The COA was provided by the supplier and the accuracy of the data is considered sufficient for the purposes of this study.

The Study Protocol was not signed by the study director prior to the start of the study. The study director gave approval to start the study by an electronic message.

Analysis to confirm concentration and uniformity of the test solutions was not performed. This is believed not to affect the validity of the study because the test chemical was accurately measured and added to the test vessels.

Study Director

William R. Berti, Ph.D.
Senior Research Biologist
DuPont Environmental and Microbiological Sciences & Engineering

30-Aug-2004
Date

Submitter

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QUALITY ASSURANCE STATEMENT

Study Number
97-03/4842

Study Title
Activated Sludge Respiration Inhibition Test of Zonyl®[redacted](OECD 209)

The conduct of this study was subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below:

Study Phase Inspected	Inspection/Audit Dates	Dates Findings Reported to Study Director	Dates Findings Reported to Management
Study Conduct	14 Nov 2003	19 Nov 2003	19 Nov 2003
Report	07-08 Jul 2004	12 Jul 2004	12 Jul 2004

Maryanne B. Oster
Staff Quality Assurance Auditor
DuPont Haskell Laboratory for Health & Environmental Services

27 August 2004
Date

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CERTIFICATION OF AUTHENTICITY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF ZONYL®([redacted]) (OECD 209)

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

William R. Berti, Ph.D.
Senior Research Biologist

30-Aug-2004
Date

Approved by:

John T. Gannon, Ph.D.
Research Manager

08-30-04
Date

Study Initiation Date:
17-Nov-2003

Date Study Completed:
30-August-2004

Submitter:
E. I. du Pont de Nemours and Company
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U.S.A.

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ACTIVATED SLUDGE RESPIRATION INHIBITION TEST OF ZONYL®(FCC) (OECD 209)

1.0 SUMMARY

Test System:

Zonyl®(FCC) was tested for toxicity towards activated sludge according to OECD Guideline 209 in the version dated 4-April-1984. For the determination of the toxic behavior of the test substance, activated sludge from the aeration tank of a municipal sewage treatment plant was exposed to the test chemical over a range of 10 to 1000 mg L^{-1}. After 30 minutes of incubation, the respiration rate of the activated sludge was determined and compared to the respiration rate of the controls.

Findings:

Under the conditions of the test, the highest test concentration of the test substance, 1000 mg L^{-1}, exhibited <20% inhibition of the respiration rate (the detection limit of the system).

Conclusions:

The EC_{50} of the test chemical could not be quantified because the highest nominal test concentration of 1000 mg/L resulted in less than 20% inhibition after 30 m of contact time. The EC_{50} of the test chemical is estimated to be greater than 1000 mg L^{-1}.

2.0 GENERAL STUDY INFORMATION

Study Objectives

The aim of this study was the determination of the acute toxic behaviour of Zonyl®([redacted]) towards the microorganisms of activated sludge according to OECD guideline 209 in the version of April 4, 1984. The objectives of this study were to:

- Determine the effect of the test substance, Zonyl®([redacted]) on microorganisms from municipal sewage sludge and
- Assess the potential of the test chemical to adversely affect aerobic municipal sewage treatment plants

The testing protocol used a microbial inoculum derived from a municipal wastewater treatment plant and an artificial sewage feed. The respiration rate of the test system was determined after a 30 m time period under controlled laboratory conditions.

Test System Justification

The test system is outlined by the OECD guideline 209 and was requested by the submitter.

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Study Personnel

E. I. du Pont de Nemours and Company
Environmental and Microbiological Sciences & Engineering

Management:	John T. Gannon, Ph.D.	
Study Director:	William R. Berti, Ph.D. E. I. du Pont de Nemours and Company Environmental and Microbiological Sciences & Engineering Central Research & Development, Glasgow Building 300 P.O. Box 6101 Newark, DE 19714-6101 USA	
Principle Investigator:	William R. Berti, Ph.D. Same as above	Study Phase(s): All
Technical Personnel:	Lynn Ann Dekleva Patrick W. Folsom Mark Starr Lisa M. Sulecki	

Study Execution Dates

Study Initiation Date:	17-Nov-2003
Experimental Start Date:	13-Nov-2003
Experimental Termination Date:	14-Nov-2003
Study Completion Date:	30-Aug-2004

3.0 MATERIALS AND METHODS

3.1 Test Guidelines

The purpose of the procedure is to provide a rapid screening method whereby substances that may adversely affect aerobic microorganisms from municipal sewage treatment plants can be identified and to indicate the suitable non-inhibitory concentrations of test substances to be used in biodegradability tests. The test uses a microbial inoculum and a synthetic sewage feed. The respiration rate of the test system is measured after an exposure period of 30 minutes under controlled laboratory conditions. The inhibitory effect of the test chemical at the particular concentrations is expressed as percentage of the mean respiration rate of the controls.

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3.2 *Test System*

3.2.1 *Chemical System*

3.2.1.1 *Test Item*

Name:	Zonyl®([redacted])
Synonyms:	NA
Active substance:	Zonyl®([redacted])
CAS Name:	NA
CAS Number:	NA
EMSE Sample Number:	CRD12005
Supplier	Scott Krenzel DuPont Haskell Laboratory Newark, DE
Submitter Sample:	H-24616
Lot Number:	436

3.2.1.2 *Reference Item*

Toxic Reference

Name:	3,5-dichlorophenol
Active substance(s):	3,5-dichlorophenol
CAS Number(s):	591-35-5
Product Number:	Aldrich D70600
Lot Number:	15809KI
EMSE Sample No.	M57657
Concentration of a.s., nominal:	97%
Concentration of a.s., analyzed:	99.7%
Certificate of Analysis Date:	August 2000
Expiry Date:	August 2005
Date Received:	January 2002
Appearance/Color:	White Powder
Storage Conditions:	Store in a cool dry place; keep tightly closed
Safety Precautions:	Irritant; wear suitable gloves and eye/face protection.
Manufacturer:	Aldrich Chemical Co., Inc. 1001 West St. Paul Milwaukee, WI 53233 USA

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3.2.1.3 *Test Vehicle*

No test vehicle was used in this study for the test substance. The test chemical was mixed with synthetic sewage feed, water, and inoculum before addition to the test vessel.

3.2.1.4 *Application Information*

The test chemical was added to the test vessels from a stock solution at the following concentrations: 1000, 320, 100, 32 and 10 mg L^{-1}.

3.2.2 *Biological System*

Secondary activated sludge from Cecil County Fletchwood Facility, Maryland (USA) Publically Owned Treatment Works (POTW) was used as the microbial inoculum. The activated sludge was aerated and used within 24 h of collection. The solids were separated by coarse filtration, centrifuged at 10,000 m s^{-2} for approximately 10 minutes, washed twice, and resuspended in tap water. The solids concentration was determined by dry weight and adjusted to yield an inoculum concentration equivalent to about 4 g per liter (on dry weight basis).

3.2.3 *Physical System*

3.2.3.1 *Test Units*

The test vessels used in the incubation period were glass flasks of approximately 1-liter. For the measurement of the respiration rate, samples were transferred to 300-mL Biological Oxygen Demand (BOD) glass bottles.

3.2.3.2 *Test Conditions*

Test solutions were aerated for a 30-minute contact period at room temperature.

3.3 *Test Conduct*

All test vessels were 1-liter glass flasks and contained a final volume of 500 mL. The test solution contained 16 mL of synthetic sewage feed, 200 mL microbial inoculum (containing approximately 4 g dry weight/L), the test chemical at the appropriate test solution concentration, and tap water to a final test volume of 500 mL. The test was initiated with the first positive control by combining the sewage feed, tap water, and inoculum in a 500 mL graduated cylinder and transferring it to the appropriately labeled test vessel. Aeration at 0.5 to 1.0 liter per minute initiated the test. The test vessel was continuously stirred using a stir bar and stir plate. The procedure was repeated at approximately 15-minute intervals with the reference chemical (at three concentrations) and then the test chemical to give a series of test vessels containing different concentrations of the test substance. A negative (abiotic) control at the highest test concentration was initiated by combining sewage feed, tap water, and test substance stock solution at the highest concentration in a 500 mL graduated cylinder. The solution was transferred to the test vessel to begin aeration. The final flask was a second positive control, prepared exactly as the first. The test was conducted so that the reference and test substances were bracketed by the two controls:

1. first positive control
2. reference chemical at 3 concentrations
3. test chemical at 5 concentrations

4. negative (abiotic) control at highest concentration of test substance
5. second positive control

3.4 *Parameters Observed*

3.4.1 *Analysis of Test Systems*

The respiration rate of a well-mixed sample of each treatment was determined after exactly 30 minutes of incubation time with aeration. The oxygen concentration was measured with an oxygen electrode and recorded over a period of up to about 10 minutes. During measurement, the samples were continuously stirred with a stirrer built into the oxygen electrode and measurements were recorded using an attached printer. The rate of oxygen consumption (in mg O_2 L^{-1} h^{-1}) was determined from the most linear part of the respiration curve.

The temperature was measured in the positive control flasks at the start and the end of the incubation period.

3.5 *Result Analysis*

The respiration rate was calculated from the recorder output as mg O_2 L^{-1} h^{-1} over a period of approximately 10 minutes or less. The portion of the respiration curve over which the respiration rate was determined should be linear.

The inhibitory effects were determined by comparing the rates at each concentration of either test or reference chemical to the rates in the controls. The results are expressed as percentage of the mean value of the respiration rates of the two controls according to:

$$\left[1 - \frac{2R_S}{R_{c1} + R_{c2}}\right] * 100 = \text{Percent inhibition} \qquad (1)$$

where

Rs = oxygen consumption rate at tested concentration of test substance

R_{c1} = oxygen consumption rate of positive control 1

R_{c2} = oxygen consumption rate of positive control 2

The percent inhibition was calculated at each test concentration as above.

A linear regression analysis was performed using the Regression Analysis Tool in Microsoft® Excel 2000. Percent inhibition was used as the input y-values. Log10 of the test or reference chemical concentration was used as the input x-values. From this analysis, an equation relating chemical concentration to percent inhibition was determined at the 95% confidence level (p = 0.05)

Percent Inhibition = A * [log10 (EC)] + B (2)

where:

A = X Variable 1, determined by the regression analysis

EC = Effective Concentration of the Test or Reference Chemical, in mg/L

B = Intercept, determined by the regression analysis

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To calculate an EC_{50}, equation (2) was solved for [$\log_{10}(EC)$]. Percent Inhibition was then set equal to 50.

$$\text{Log}_{10}(EC_{50}) = (\text{Percent Inhibition} - B)/ A \qquad (3)$$

For A = 50.933 and B = -5.404

$$\text{Log}_{10}(EC_{50}) = (50 - (-5.404))/ 50.933)$$

$$= 1.088$$

$$EC_{50} = 10^{1.088} = 12 \text{ mg/L}$$

Similarly, an EC20 and EC80 can be calculated by using a Percent Inhibition equal to 20 or 80, respectively, in equation (3). For example:

$$\text{Log}_{10}(EC_{20}) = (20 - (-5.404))/ 50.933)$$

$$= 0.4988$$

$$EC_{20} = 10^{0.4988} = 3 \text{ mg/L}$$

The percent inhibition also was plotted against concentration as a log-normal (or log-probability) graph, from which an EC_{50} value can be derived graphically.

3.6 *Validity Criteria of the Study*

The test results are valid if:

- The two positive control respiration rates (PCRR) rates are within 15 percent of each other.
- The EC_{50} of reference chemical 3,5-dichlorophenol is in the accepted range of 5 to 30 mg L^{-1}.

4.0 RESULTS AND DISCUSSION

- The test chemical showed no acute toxic effect towards the microbial inoculum (activated sludge) at the highest concentration of 1000 mg L^{-1} that was tested.
- EC_{50} of the reference chemical was 12 mg 3,5-dichlorophenol per liter, which was within the acceptable range of from 5 to 30 mg L^{-1}.
- The respiration rates of the two positive controls were within 4%.

5.0 CONCLUSIONS

- The EC_{50} of the test chemical Zonyl®([redacted]s estimated to be greater than 1000 mg L^{-1}.
- The EC_{50} of the reference chemical 3,5-dichlorophenol is 12 mg L^{-1}.
- The respiration rates of the two positive controls were within 15% of each other.
- The test is valid.

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6.0 RETENTION OF RECORDS

For the periods demanded by GLP guidelines and specific country requirements, study documents and materials will be stored in the archives of the DuPont CR&D Environmental & Microbiological Sciences and Engineering (EMSE) archives and/or Iron Mountain Records Management, Wilmington, Delaware 19802 USA, including but not limited to:

- study protocol;
- any protocol and/or report amendments or addenda or SOP deviations;
- all raw data;
- one original signed copy of the final report;
- all documentation generated by the Quality Assurance Unit (to be archived by the Quality Assurance Unit, separate from study records);
- laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator, and/or freezer raw data.

Documents and materials are archived according to the principles of Good Laboratory Practice in the organization of the testing facility.

7.0 DISPOSAL OF TEST ITEM

After issuance of the final report, the remaining test chemical will be stored at the DuPont EMSE laboratory until its expiration date and then destroyed by burning, unless other arrangements are made between the submitter and the Test Facility.

8.0 REFERENCES

1. OECD (1984) Guideline for Testing of Chemicals, Section 2, No. 209: "Activated Sludge, Respiration Inhibition Test", adopted April 04, 1984
2. International Standard ISO 8192: "Water quality – Test for inhibition of oxygen consumption by activated sludge". First edition 1986-07-15. Ref. No. ISO 8192-1986 (E).

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TABLE 1:
INFLUENCE OF TEST CHEMICAL ZONYL®([redacted]) AND REFERENCE CHEMICAL 3, 5-DICHLOROPHENOL ON THE O_2 CONSUMPTION OF ACTIVATED SLUDGE

No.	Treatment	Concentration	O_2 Concentration†		O_2 Consumption	Inhibition‡
			Start	End		
		mg/L	*mg O_2/L*		*mg O_2/L/h*	%
1	Positive Control 1	0	6.32	2.72	44.0	NA¶
11	Positive Control 2	0	4.37	2.61	42.6	NA
	Mean				43.3	
	Standard deviation				1.02	
2	3,5-dicholorophenol	3.2	6.26	3.19	33.4	22.9
3	3,5-dicholorophenol	10	6.50	3.54	23.5	45.7
4	3,5-dicholorophenol	32	8.00	6.89	12.2	71.9
5	Zonyl®([redacted])	10	4.98	2.53	41.8	3.58
6	Zonyl®([redacted])	32	5.18	2.74	41.9	3.31
7	Zonyl®([redacted])	100	6.52	2.64	42.1	2.73
8	Zonyl®([redacted])	320	6.38	2.52	41.9	3.27
9	Zonyl®([redacted])	1000	6.35	2.38	39.7	8.25
10	Zonyl(R([redacted]) (negative control)	1000	8.69	8.71	-0.11	100

†The readings of the Dissolved O_2 meter at start and end of linear portion during the period over which respiration rate was calculated between approximately 6.5 and 2.5 mg O_2/L or over a 10 min period when the repiration rate was low.

‡Inhibition of the reference and test substances was calculated using the average value of the positive control solutions. A negative value indicates that respiration was enhanced, not inhibited.

¶ NA = Not Applicable.

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FIGURE 1:
INFLUENCE OF THE REFERENCE CHEMICAL 3,5-DICHLOROPHENOL ON THE RESPIRATION RATE OF AEROBIC WASTEWATER MICROORGANISMS AFTER 30 MINUTES OF EXPOSURE

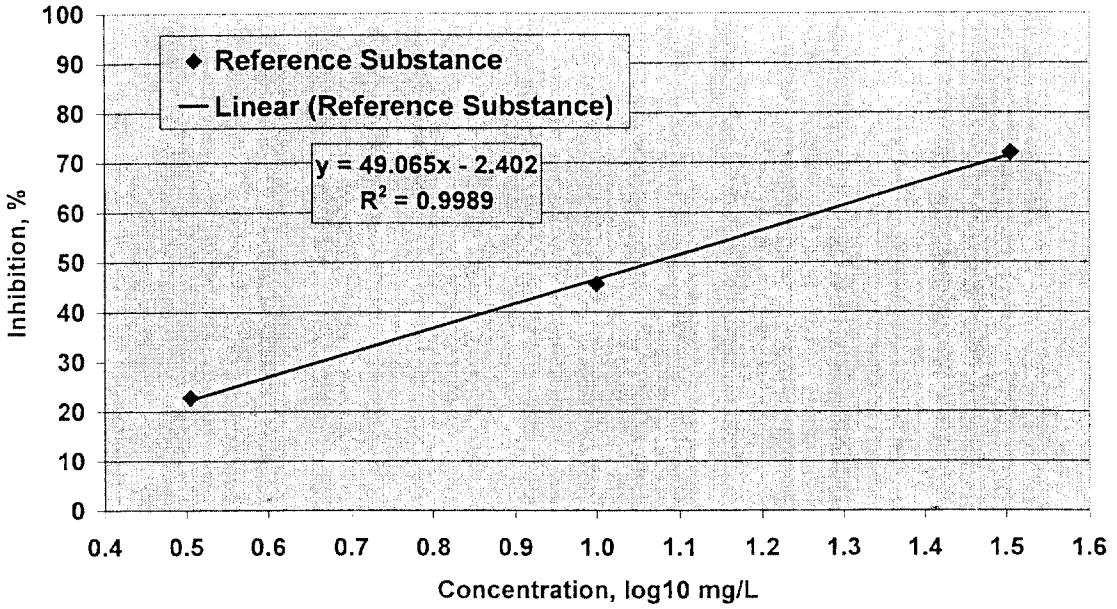

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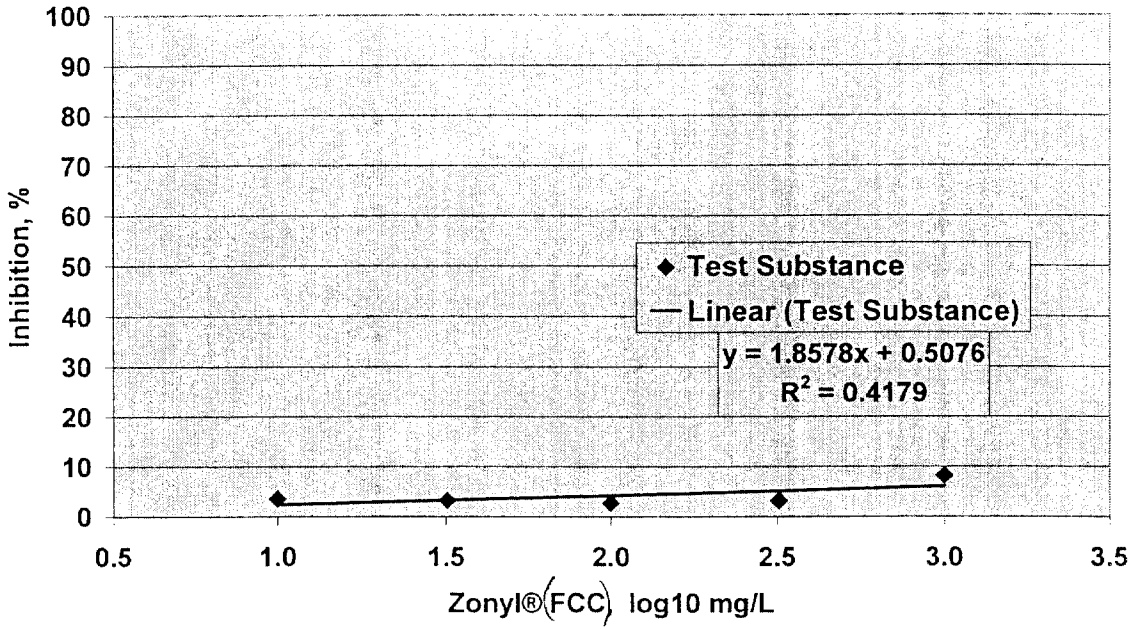
FIGURE 2:
INFLUENCE OF THE TEST CHEMICAL ZONYL®([redacted]) ON THE RESPIRATION RATE OF AEROBIC WASTEWATER MICROORGANISMS AFTER 30 MINUTES OF EXPOSURE



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APPENDIX A:
DATA TABLES AND CALCULATIONS FOR POSITIVE CONTROLS, REFERENCE AND TEST CHEMICALS

Positive Controls

Total Time	Positive Control 1	Positive Control 2
h	*Dissolved Oxygen, mg/L*	
0.00	8.96	8.94
0.01	6.69	4.48
0.02	6.32	4.37
0.03	5.97	4.04
0.03	5.63	3.64
0.04	5.31	3.29
0.05	4.68	2.95
0.06	4.34	2.61
0.07	4.02	2.24
0.08	3.70	1.91
0.08	3.35	1.50
0.09	3.06	1.20
0.10	2.72	0.87
0.11	2.40	
0.12	2.06	
0.13	1.77	

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Reference Chemical: 3,5-dichlorophenol

Total Time	Reference 1: 3.2 mg/L	Reference 2: 10 mg/L	Reference 3: 32 mg/L
h	*Dissolved Oxygen, mg/L*		
0.00	9.07	9.36	8.09
0.01	7.89	6.50	8.00
0.02	7.65	6.28	7.91
0.03	7.36	6.07	7.78
0.03	7.16	5.88	7.68
0.04	6.83	5.68	7.59
0.05	6.55	5.48	7.50
0.06	6.26	5.27	7.39
0.07	5.96	5.08	7.30
0.08	5.68	4.87	7.19
0.08	5.38	4.71	7.07
0.09	5.10	4.50	6.98
0.10	4.83	4.32	6.89
0.11	4.56	4.12	
0.12	4.29	3.93	
0.13	4.01	3.73	
0.13	3.71	3.54	
0.14	3.46		
0.15	3.19		
0.16			

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Test Chemical: Zonyl®[redacted]

Total Time	Test Chemical, 10 mg/L	Test Chemical, 32 mg/L	Test Chemical, 100 mg/L	Test Chemical, 320 mg/L	Test Chemical, 1000 mg/L	Abiotic Control, 1000 mg/L
h	*Dissolved Oxygen, mg/L*					
0.00	8.58	8.05	8.20	8.84	8.64	9.03
0.01	4.98	5.18	6.52	6.71	7.00	8.69
0.02	4.60	4.83	6.13	6.38	6.68	8.71
0.03	4.22	4.49	5.81	6.03	6.35	8.72
0.03	3.90	4.15	5.44	5.66	6.04	8.74
0.04	3.55	3.79	5.09	5.26	5.70	8.74
0.05	3.21	3.45	4.74	4.97	5.36	8.72
0.06	2.86	3.08	4.37	4.62	5.04	8.77
0.07	2.53	2.74	4.05	4.26	4.71	8.73
0.08	2.20	2.39	3.68	3.94	4.39	8.73
0.08	1.88	2.09	3.37	3.56	4.01	8.76
0.09	1.47	1.75	2.98	3.22	3.71	8.72
0.10	1.14	1.36	2.64	2.88	3.39	8.72
0.11	0.83	1.03	2.31	2.52	3.07	8.73
0.12			2.00	2.19	2.71	8.71
0.13			1.58	1.85	2.38	
0.13			1.25	1.48		
0.14				1.11		

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APFO CONTAINMENT
AT
WASHINGTON WORKS

Part II - Process Water

Charts Prepared for Meetings at Shimizu

Jan. 20-22, 2003

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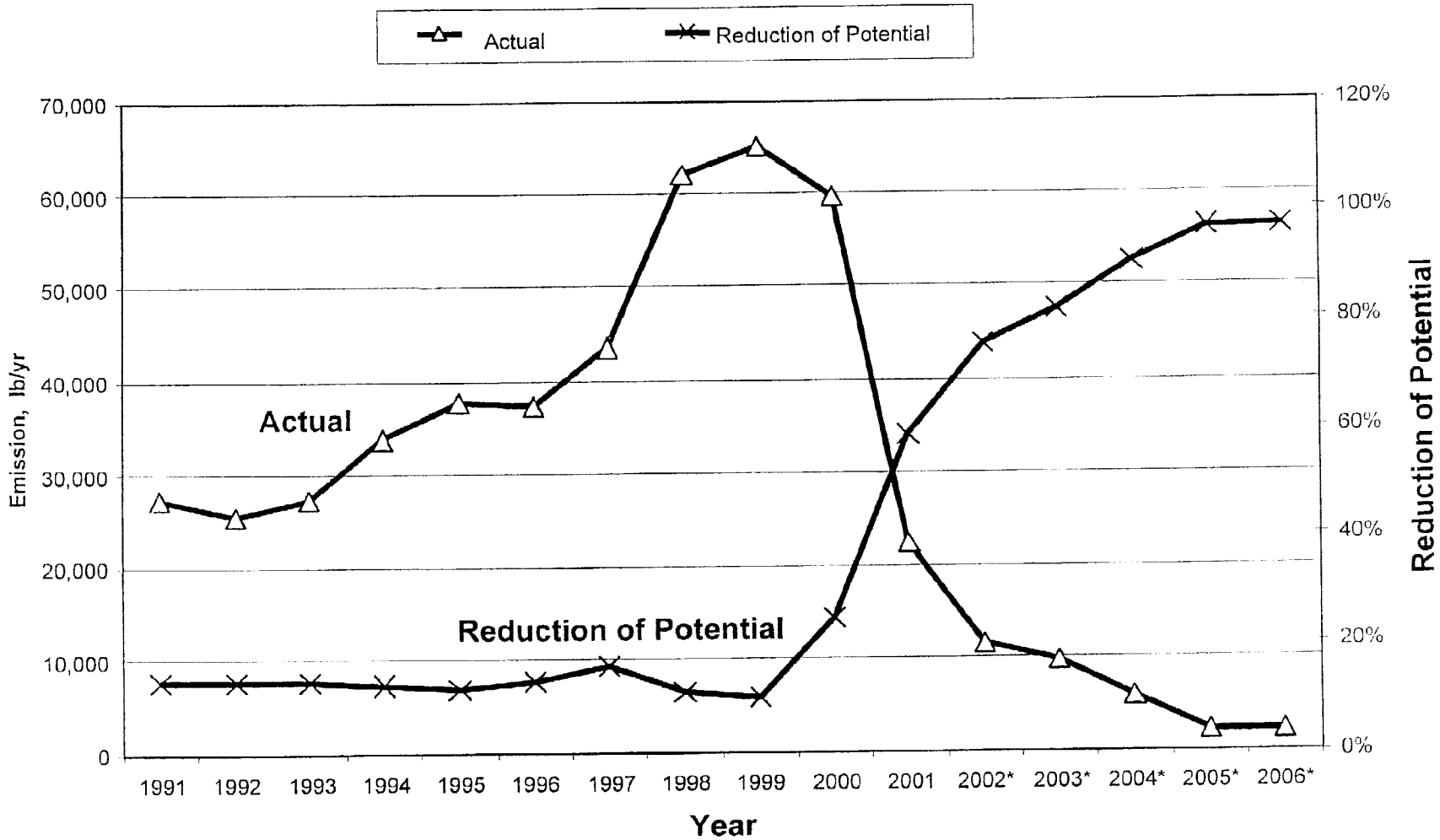
AR226-2731

Water Emission (WW)



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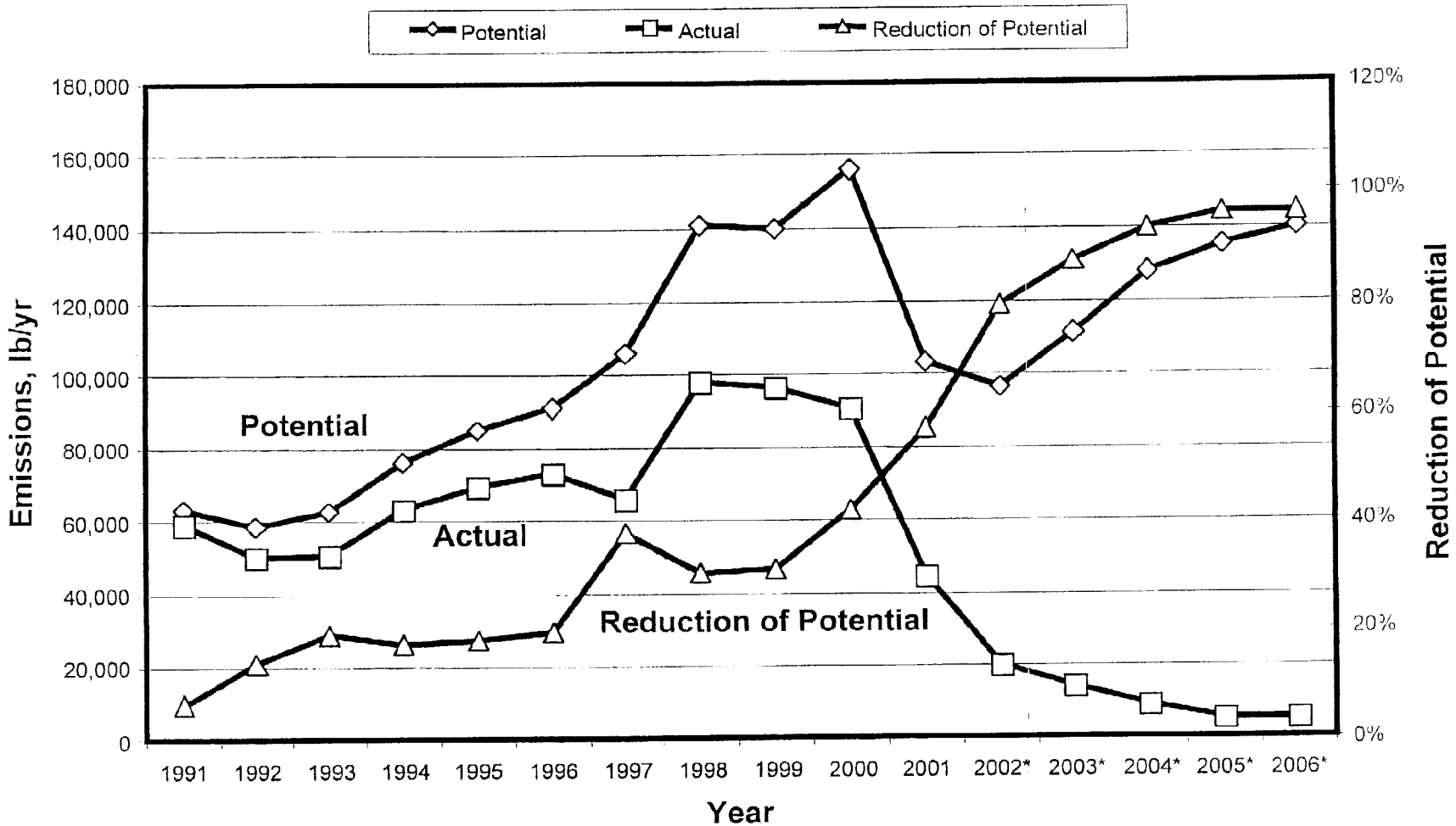
Total Emission (WW)



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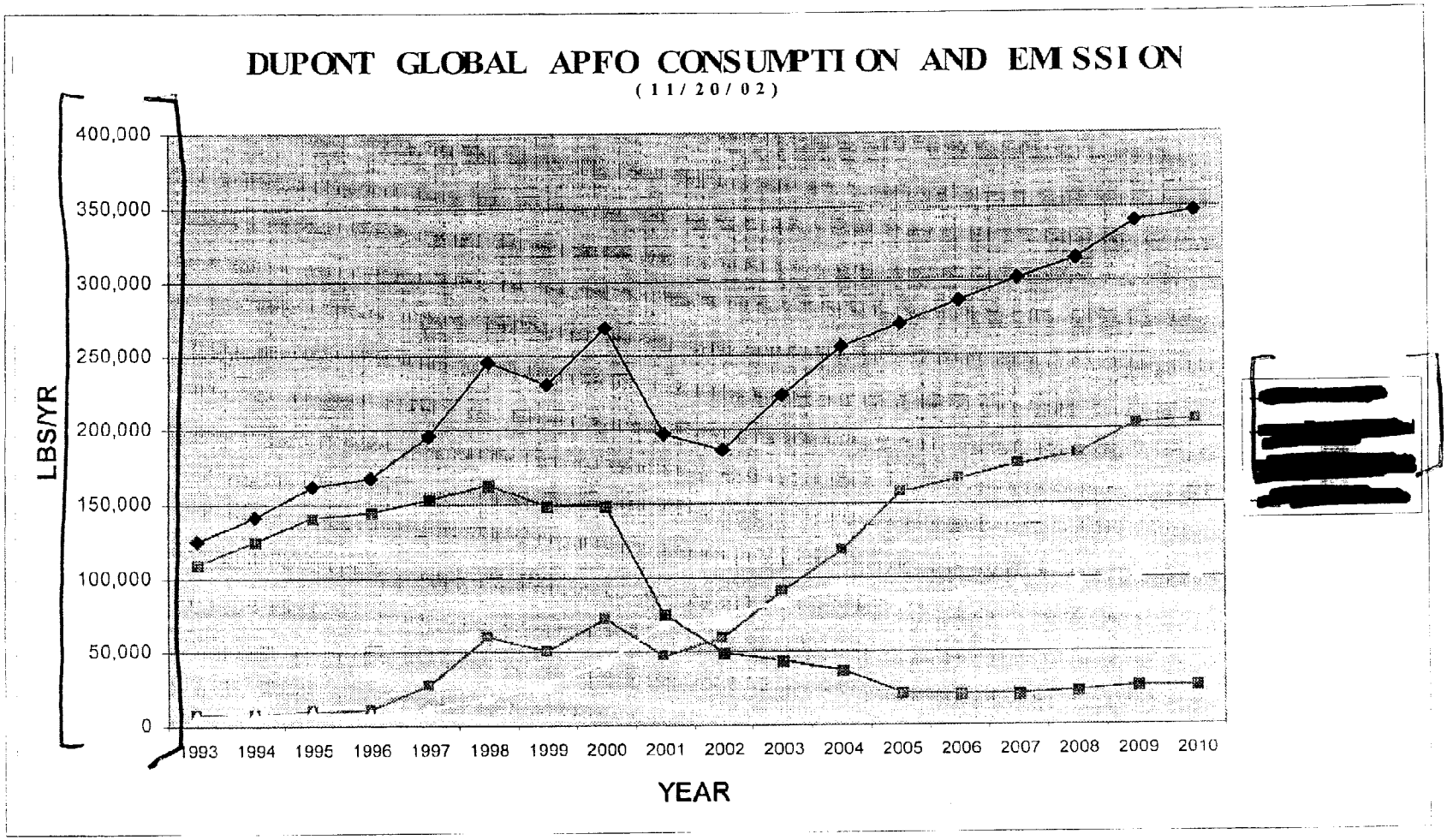

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C-8 Losses to Environment (Snap shot at end of 2002)

(10-01-02)

Source	Parkersburg Emission lb/yr	Total Emission lb/yr
FP Scrubber (95%)	1261	1261
Copolymers Scrubber (90%)	2167	2167
Oberlin Downtime (2%)	523	523
Carbon Bed Efficiency (94%)	1721	1721
FP Coagulation Water	2193	2193
Aborted Batches (From FP)	1500	1500
Granular Mother Liquor	488	488
Waste Supernate, Water to CW		5683
Landfill, CW		2436
Landfill, Emelle		0
Total Emitted		17972
To Customers		7790

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	AIR EMISSIONS			WATER EMISSIONS		
	Potential, lbs	Actual, lbs	Reduction,%	Potential, lbs	Actual, lbs	Reduction,%
1999	67340	31209	54	72334	65101	10
2000	76788	31120	59	78908	59556	25
2001	49301	22167	55	53983	22381	59
2002*	46892	8221	82	39555	10440	74
2003*	55734	4101	93	46321	9097	80
2004*	65657	2824	96	52731	5569	89
2005*	71713	2956	96	53467	1874	96
2006*	72506	3010	96	57235	1959	97

* Based on Facility Planning forecast updated in Sept. 2002.

(10/04/02)

AR 226_2732

The miracles of science

Study Title

MODELING RELEASES OF AMMONIUM PERFLUOROOCTANOATE INTO THE OHIO RIVER

Author

William R. Berti

Report Completed on

May 22, 2000

Performing Laboratory

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Report No. EMSE-054-00

CCER
CORPORATE CENTER FOR ENGINEERING RESEARCH

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GENERAL INFORMATION

Chemical Studied: Ammonium perfluorooctanoate

Synonyms/Codes: APFO, C-8, FC-143

DuPont Notebook No.: Not Applicable

Sponsor; Robert Pinchot
E. I. du Pont de Nemours and Company
BARLML CRP-711/2210-B

Study Initiated/Completed: September 1999 to May 2000

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MODELING RELEASES OF AMMONIUM PERFLUOROOCTANOATE INTO THE OHIO RIVER

SUMMARY

PDM indicated that APFO concentrations of 1.0 µg APFO/L would be exceeded about 50% of the time during the year. APFO concentrations of in the river would exceed 0.1 µg APFO/L 90% of the time during the year and 10 µg APFO/L about 2.2% of the time during the year.

Average annual APFO concentrations in the Ohio River calculated by using a Microsoft® Excel spreadsheet was 0.423 µg APFO/L. Modeled APFO concentrations in the river ranged from a low of 0.199 µg APFO/L in March to a high of 0.965 µg APFO/L in September, which correspond to high and low river flows, respectively. Average Ohio River flows and volume data calculated from the US Geological Survey was collected at the Belleville Dam and used in the spreadsheet model. The Belleville Dam is on the Ohio River 13 miles downstream of the Washington Works Plant. This river flow data is the closest location downstream from the plant where this type of information is available.

Study Conducted and
Report Prepared by: _____

William R. Berti (date)
Senior Research Biologist

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INTRODUCTION/PURPOSE

APFO (ammonium perfluoroocatanoate) is a fluorinated surfactant used in the manufacturing of Teflon® at Washington Works. A portion the APFO (18,416 kg in 1996) was released to the Ohio River as part of an NPDES permitted release for the facility (WV0001279). The molecular weight of APFO is 431.098 g/mole. Other properties of APFO are listed below.

- Made by 3M Company
- Health rating of 2
- Exposure limits 0.01 mg/m^3 skin 8 hr
- LD_{50} acute oral rat = 680 mg/kg
- BOD_{20} = nil
- Biodegradation = nil
- BCF = 1.8
- Molecular formula $CF_3(CF_2)_6COO^-NH_4^+$
- pH ~ 5 (0.5% aqueous)
- pKa = 2.8 (-COOH)
- Melting point = 56-58°C (-COOH)
- COD = 700 mg/kg
- Koc = 25
- Water solubility > 1000 mg APFO/L
- Vapor pressure (at 22C) = $7.1 * 10^{-05}$ mm Hg

Our objective was to calculate the concentrations of APFO that could reasonably be expected to occur in the Ohio River downstream of the NPDES permitted outfall at the DuPont Washington Works.

MATERIALS/METHOD

Releases of ammonium perfluorooctanoate (APFO) to the Ohio River from the DuPont Washington Works Plant were modeled using the Probabilistic Dilution Model (PDM Beta Version 4.0 Beta June 11, 1999, US EPA Office of Pollution Prevention and Toxics) and a constructed Microsoft® Excel spreadsheet model. APFO release data for 1996 were used in both modeling exercises.

PDM inputs included the NPDES number of WV0001279. This corresponds to a reach number of 05030202039, a mean streamflow of 4.75E+04 million liters per day (MLD), a low streamflow of 9980.47 MLD and an effluent flow of 216.75 MLD. We assumed that APFO was released 335 days/year, the loading was 55.138 kg/day, and the wastewater treatment efficiency was 0%. We then varied the APFO concentration as the Concentration of Concern (COC) from 0.1 to 50 µg APFO/L to determine the COC percent of year exceedence.

Microsoft® Excel spreadsheet used river flow data from the US Geological Survey (USGS: <http://waterdata.usgs.gov/nwis-w/WV/data.components/hist.cgi?statnum=03159530>). This is the mean daily river flow in cubic feet/sec measured at the Belleville Dam from 10/1/1974

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to 9/30/1985. The Belleville Dam is on the Ohio River 13 miles downstream of the Washington Works Plant. This river flow data is the closest location downstream from the plant where this type of information is available. This river flow data compares well with Ohio River monthly average flows at Pittsburgh, PA, Huntington, WV, and Cincinnati, OH from the US Army Corps of Engineers (ftp://www.lrd-wc.usace.army.mil/Monthly_htm/). The average daily river flow for the month was calculated from available data between those two dates. This was then converted to the average measured flow per month. We assumed the APFO was discharged to the river at a constant rate except for the month of October when production at Washington Works is usually suspended for annual maintenance. We assumed that the total discharge of was 18,416 kg APFO (40,600 lbs APFO) divided by 11 months. Table shows the results of this spreadsheet model.

RESULTS/DISCUSSION

PDM indicated that APFO concentrations of 1.0 µg APFO/L would be exceeded about 50% of the time during the year. APFO concentrations of in the river would exceed 0.1 µg APFO/L 90% of the time during the year and 10 µg APFO/L about 2.2% of the time during the year (Table 1). Figure 1 shows model input/output.

Average annual APFO concentrations in the Ohio River calculated by constructing and using a Microsoft® Excel spreadsheet model was 0.423 µg APFO/L (Table 2). Modeled APFO concentrations in the river ranged from a low of 0.199 µg APFO/L in March to a high of 0.965 µg APFO/L in September, which correspond to high and low river flows, respectively. Average Ohio River flows and volume data calculated from the US Geological Survey was collected at the Belleville Dam and used in the spreadsheet model. The Belleville Dam on the Ohio River 13 miles downstream of the Washington Works Plant. This river flow data is the closest location downstream from the plant where this type of information is available.

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TABLE 1: Probabilistic Dilution Model (PDM) results of APFO concentrations and percent of year that the concentration of APFO will be exceeded in the Ohio River.

Concentration of APFO	Percent of year concentration exceeded
ug/L	%
0.1	90.9
0.5	71.6
1	51.0
3	17.8
6	6.1
10	2.2
25	0.2
50	0.0

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Table 2. Microsoft Excel spreadsheet calculations of modeled APFO concentrations in the Ohio River.

Month	Measured flow	Measured flow	APFO discharge to Ohio River in 1996	Estimated APFO concentration in Ohio River
	ft³/sec	*L/mo*	*kg*	*µg/L*
January	64,001	4.69E+12	1,674	0.357
February	88,909	6.74E+12	1,674	0.248
March	111,236	8.43E+12	1,674	0.199
April	94,017	6.90E+12	1,674	0.243
May	67,107	5.09E+12	1,674	0.329
June	47,379	3.48E+12	1,674	0.482
July	30,989	2.35E+12	1,674	0.713
August	30,502	2.31E+12	1,674	0.724
September	25,339	1.73E+12	1,674	0.965
October	33,030	2.50E+12	0	0.000
November	42,330	3.11E+12	1,674	0.539
December	78,986	5.99E+12	1,674	0.280
Average		**4.44E+12**		**0.423**
Total	**713,824**	**5.33E+13**	**18,416**	**0.345**

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ADSORPTION-DESORPTION STUDIES OF AMMONIUM PERFLUOROOCTANOATE

FIGURE 1: Probabilistic Dilution Model (PDM) Beta Version 4.0 input and output screen.

PDM Site Specific Screen

Chemical ID: C-8
Scenario #: 1
Reach number: 05030202039
Gaging station:
Period of record: 1996
# of observations: NA
Mean streamflow: 4.75e+04 MLD
Low streamflow: 9980.47 MLD
Effluent flow: 216.75 MLD

Facility name: E.I.DU PONT DE NEMOURS & CO.
Facility location: PARKERSBURG, WV 26102
Receiving stream name: OHIO R
NPDES Number: WV0001279 (List) WV0001279
☑ Facility on reach?

Discharge type: ☑ Direct ☐ Indirect
Release activity: ☐ MFG ☐ PRO ☐ IND USE ☐ COMM USE ☐ CONS USE
Data source: FacSrch - Direct

INPUT DATA:
Release days: 335 days/year
Loading: 55.138 kg/site/day
Concentration of Concern (COC): 10 ug/L
Wastewater treatment efficiency: 0 %

Results (COC Exceedence)
Percent of year: 2.24 %
Days per year: 8.17 days/year

Results History

COC (ug/L)	% YEAR COC EXCEEDED	#DAYS COC EXCEEDED	RELEASE DAYS	LOADING (kg/site/day)	WASTE WATER TREATMENT
0.1	90.9	331.6	335.	55.1	0.
0.5	71.6	261.5	335.	55.1	0.
1.	51.	186.	335.	55.1	0.
5.	8.4	30.6	335.	55.1	0.
10.	2.2	8.2	335.	55.1	0.

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AR 226- 2733

The miracles of science

Study Title

ADSORPTION-DESORPTION SCREENING STUDIES OF AMMONIUM PERFLUOROOCTANOATE

Author

William R. Berti

Report Completed

May 2000

Performing Laboratory

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Environmental and Microbiological Sciences & Engineering
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Newark, DE 19714-6101

Report No. EMSE-053-00

CORPORATE CENTER FOR ENGINEERING RESEARCH

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GENERAL INFORMATION

Material Tested: Ammonium perfluorooctanoate

Synonyms/Codes: APFO, C-8, FC-143

DuPont Notebook No.: E98312

Sponsor: Robert Pinchot
E. I. du Pont de Nemours and Company
BARLML CRP-711/2210-B

Study Initiated / Completed: September 1999 / May 2000

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ADSORPTION-DESORPTION SCREENING STUDIES OF AMMONIUM PERFLUOROOCTANOATE

SUMMARY

Five of nine materials appeared to adsorb a significant amount (greater than 25% of APFO added) at one or more concentrations of APFO. For four of the five materials to which APFO significantly adsorbed, most of the adsorbed APFO appeared not to desorb after 2 washings at one or more concentrations of added APFO.

Study Conducted by: _____
Kristi Barnett (date)
Research Technician, EMS&E

Study Conducted by: _____
Jack DeCarolis (date)
Staff Biologist, EMS&E

Study Conducted by: _____
George Fisher (date)
Research Technician

Study Conducted by: _____
Bogdan Szostek, Ph.D. (date)
Research Scientist, Haskell Lab

Report Prepared by: _____
William R. Berti, Ph.D. (date)
Senior Research Biologist, EMS&E

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INTRODUCTION AND PURPOSE

APFO adsorption/desorption screening studies were performed on several test materials, including two clays, a washed sand, peat moss, and an agricultural soil from Delaware, USA. The adsorption/desorption screening studies were also performed using two surface soils and two sediments from the Ohio River collected in the vicinity of the DuPont Washington Works plant near Parkersburg, West Virginia.

The adsorption/desorption procedure used in this study was adapted from a method developed by the Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data (1). Our objectives were to develop an adsorption/desorption protocol and analytical procedure for APFO. As part of this objective, we screened a variety of test materials for their APFO adsorption/desorption characteristics.

MATERIALS AND METHODS

Test Materials

The Kaolin (KGa-2) and Montmorillonite (SAz-1) are Clay Mineral Society "Source Clays" and were obtained from the curator of the sample collection in the Department of Geology at the University of Missouri, Columbia. Additional information on these and other Source Clays has been published (2). The Quikrete® All-Purpose sand and Scotts® sphagnum peat moss were purchased locally at a builders' supply store (Home Depot®). The Sassafras soil is a surface (0 to 15 cm) soil collected from an agricultural field in Delaware, USA. The Garden Area soil and East Wood soil were surface soils collected from the DuPont Washington Works plant. The Upstream sediment was obtained from the bank of the Ohio River upstream of the DuPont Washington Works NPDES permitted outfall, and the Downstream sediment was from downstream of the outfall. The two soil and sediment sample from the vicinity Washington Works were collected in the fall of 1999.

Physical and chemical properties of the less than 2-mm size fractions of the soils, sediments, clays, sand, and peat moss used in this study are listed in Tables 1 and 2. Except for the clays, all samples were screened through a 2-mm (10 mesh) stainless steel sieve in preparation for the absorption/desorption screening test. Furthermore, the sand was washed using DI water through 2-mm and 50-um stainless steel sieves. Material retained on the 50 um sieve was used in the study. The sand, soils, and sediments used in the studies were air-dried prior to use in the studies. Data calculations, however, were based on oven-dry weights of the samples. Oven-dry weights were determined after drying a subsample of material in an oven at 105°C for a minimum of 24 h.

The pH of the sample were determined in a 1:1 test material to water. Percent organic matter was determined using the loss on ignition method.

Screening Test: Adsorption Studies

The adsorption test was performed in duplicate on each material. A blank containing 0.01M $CaCl_2$ solution with test materials and no APFO and a single control at each APFO concentration but no material were also included. Each material was equilibrated with the aqueous phase of a solution of the test chemical at 5.0, 0.5 and 0.05 mg L^{-1}. prepared in 0.01M $CaCl_2$

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Sterile 50 mL Corning polypropylene centrifuge tubes were used as the test vessels. One part air-dried material (4.0 g) was weighed and 5 part test solution (20 mL) was decanted into a centrifuge tube, except the control tube. The centrifuge tubes were secured on an end-over-end mixer and agitated at about 30 rpm for 24 hours. Samples were centrifuged for 20 minutes using a RT6000B Sorvall centrifuge at 4000 x g and then filtered through a 0.2 µm nylon syringe filter into a new centrifuge tube. The volume of aqueous supernatant was measured and refrigerated at about 5°C until analyzed for the parent compound using an LC-MS. The blank and the control tubes were subjected to the same steps as the test systems, including filtering.

Screening Test: Desorption Studies

To each solid phase (pellet) was added a fresh volume of 0.01M $CaCl_2$ solution without the test chemical equal to that added in the adsorption test, 20 mL. The sample was mixed, centrifuged, filtered, stored, and analyses as was done in the adsorption studies. This desorption step was repeated a second time, resulting in two washings that were analyzed separately.

Calculations

Data calculations included:

1. The percent of APFO adsorbed, A:
   $A = (G - Ce \times Vo)/G \times 100 = x/G \times 100$
2. The percent of APFO which is desorbed, D:
   $D = [(C1 + C2)V - (Vo - V)Ce]/x \times 100$
3. The percent of APFO which is not desorbed, R:
   $R = [G - (Ce + C1 + C2)V]/x \times 100$
4. The adsorption coefficient, K':
   $K' = (x/m)/Ce$
5. The adsorption coefficient calculated as a function of the organic carbon content of the test material, K'oc:
   $K'oc = K\ (100/\text{percent organic carbon})$

Where:

1. m = dry weight of soil employed (g)
2. Ce = concentration of APFO remaining in solution (V) in the adsorption step (µg L^{-1})
3. C1 = concentration of APFO in solution in the first wash (µg L^{-1})
4. C2 = concentration of APFO in solution in the second wash (µg L^{-1})
5. Vo = original volume of solution employed (mL)
6. V = volume of solution obtained after the adsorption step (mL)
7. G = quantity of material recovered from the soilless control (µg)

RESULTS AND DISCUSSION

Of the nine materials tested, five of them (peat moss, Upstream sediment, East Wood soil, Garden Area soil, and Kaolin Clay) exhibited significant adsorption (greater than 25% of APFO added) at one or more of the added concentrations of APFO. Peat moss alone, however, adsorbed a significant amount at all three concentrations of added APFO; 50, 500, and 5,000 µg APFO/L. Kaolin clay adsorbed a significant amount of APFO at 500 and 5,000 µg APFO/L. The Garden Area soil collected at Washington Works also adsorbed significant amounts of APFO at 500 µg APFO/L. Upstream sediments from the Ohio River and East Wood soil adsorbed a

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significant amount of APFO at 50 µg APFO/L only. Sand, Montmorillonite clay, Downstream sediment, and Sassafras soil did not adsorb a significant amount of APFO at any concentration of the added APFO.

Once adsorbed on peat moss, most of the APFO (>76%) did not desorb after two washings with a solution of 0.01M $CaCl_2$. For the Kaolin clay, most the APFO adsorbed when added at 5,000 µg APFO/L desorbed with washing (>60%). At APFO added at 500 µg APFO/L, however, an average of 98% did not desorb from the Kaolin clay after two washings. The East Wood soil, which adsorbed 46% of the APFO added at 50 µg APFO/L desorbed only about 2%. For the Garden Area soil 57% of the APFO desorbed when adsorbed from the 500 µg APFO/L solution. No measurable desorption of APFO from the Upstream sediment after two washings was observed when the APFO was added at 50 µg APFO/L.

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ADSORPTION-DESORPTION SCREENING STUDIES OF AMMONIUM PERFLUOROOCTANOATE

TABLE 1. Physical properties of test materials.

Test material	Moisture content†	Test Material Dry Wt., m	Sand	Silt	Clay	Organic Matter	Organic Carbon‡
	%	g	%	%	%	%	%
Sand	0.24	4.0	100	0	0	ND	ND
Peat moss	47	2.1	ND§	ND	ND	ND	ND
Kaolin clay	0.78	4.0	0	0	100	ND	ND
Montmorillonite	7.4	3.7	0	0	100	ND	ND
Sassafrass soil	1.4	3.9	71	19	10	1	0.58
East Wood soil	14	3.5	45	40	15	1.6	0.93
Garden Area soil	9.4	3.7	37	42	21	2.9	1.68
Upstream sediment	13	3.5	59	30	11	1.5	0.87
Downstream sediment	9.0	3.7	69	21	10	0.6	0.35

† Based on drying at 105C for a minimum of 24 h.
‡ Estimated from percent organic matter.
§ ND is not determined.

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TABLE 2. Chemical properties of test materials.

Test material	pH†	CEC‡	Exchangeable cations, meq/100g§				
		meq/100g	*Ca*	*Mg*	*Na*	*K*	*H*
Sand	6.6	ND¶	ND	ND	ND	ND	ND
Peat moss	3.7	ND	ND	ND	ND	ND	ND
Kaolin clay	3.8	3.3	ND	ND	ND	ND	ND
Montmorillonite	7.6	120	ND	ND	ND	ND	ND
Sassafrass soil	6.3	2.8	2.10	0.51	0.01	0.21	0.00
East Wood soil	5.6	10	4.19	0.61	0.04	0.15	5.01
Garden Area soil	4.7	17.2	5.85	1.15	0.01	0.18	10.01
Upstream sediment	7.1	7.4	6.08	0.86	0.27	0.15	0.04
Downstream sediment	6.9	5.9	4.54	1.09	0.20	0.09	0.00

† 1:1 test material to water.
‡ Cation Exchange Capacity.
§ Exchangeable cations in milliequivalents/100 g test material determined by a modified ammonium acetate method.
¶ ND is not determined.

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TABLE 3. Absorption/desorption screening test data.

Test material	Nominal APFO concentration	Original solution volume, Vo	Volume of solution after adsorption step, V	APFO in solution after adsorption step, Ce	APFO in first wash, C1	APFO in second wash, C2
	$\mu g\ L^{-1}$	mL	mL	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$
Sand	0.00	20.0	18.0	2.79E+01	ND	ND
	0.05	20.0	18.0	1.39E+02	ND	ND
	0.5	20.0	18.0	4.98E+02	ND	ND
	5	20.0	18.0	3.80E+03	ND	ND
Peat moss	0.00	20.0	18.0	4.00E+01	0.00E+00	0.00E+00
	0.05	20.0	18.0	7.98E+00	0.00E+00	0.00E+00
	0.5	20.0	18.0	6.55E+01	4.07E+01	2.62E+01
	5	20.0	18.0	9.62E+02	5.87E+02	3.37E+02
Kaolin clay	0.00	20.0	18.0	1.65E+00	0.00E+00	0.00E+00
	0.05	20.0	18.0	3.83E+01	NA	NA
	0.5	20.0	18.0	3.27E+02	3.31E+01	7.15E+00
	5	20.0	18.0	3.02E+03	4.52E+02	3.82E+02
Montmorillonite	0.00	20.0	18.0	4.45E+01	0.00E+00	0.00E+00
	0.05	20.0	18.0	4.68E+01	ND	ND
	0.5	20.0	18.0	3.80E+02	6.25E+01	8.00E+00
	5	20.0	18.0	3.69E+03	ND	ND

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TABLE 3 (cont'd)

Test material	Nominal APFO concentration	Original solution volume, Vo	Volume of solution after adsorption step, V	APFO in solution after adsorption step, Ce	APFO in first wash, C1	APFO in second wash, C2
	µg L^{-1}	mL	mL	µg L^{-1}	µg L^{-1}	µg L^{-1}
Sassafrass soil	0.00	20.0	18.0	0.00E+00	ND	ND
	0.05	20.0	18.0	4.33E+01	ND	ND
	0.5	20.0	18.0	4.35E+02	ND	ND
	5	20.0	18.0	3.63E+03	ND	ND
East Wood soil	0.00	20.0	18.0	7.50E-02	0.00E+00	0.00E+00
	0.05	20.0	18.0	1.18E+01	1.63E+00	0.00E+00
	0.5	20.0	17.8	3.98E+02	7.53E+01	1.98E+01
	5	20.0	18.0	4.78E+03	7.44E+02	1.43E+02
Garden Area soil	0.00	20.0	18.0	1.15E+01	6.93E+00	2.60E+00
	0.05	20.0	18.0	2.57E+01	1.24E+01	3.50E+00
	0.5	20.0	18.5	2.76E+02	1.19E+02	5.03E+01
	5	20.0	17.0	3.68E+03	1.09E+03	3.79E+02
Upstream sediment	0	20.0	18.0	0.00E+00	0.00E+00	0.00E+00
	50	20.0	18.0	1.59E+01	8.50E-01	0.00E+00
	500	20.0	18.5	4.12E+02	7.11E+01	2.10E+01
	5000	20.0	18.0	4.36E+03	7.71E+02	1.79E+02

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TABLE 3 (cont'd)

Test material	Nominal APFO concentration	Original solution volume, Vo	Volume of solution after adsorption step, V	APFO in solution after adsorption step, Ce	APFO in first wash, C1	APFO in second wash, C2
	$\mu g\ L^{-1}$	mL	mL	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$
Downstream sediment	0	20.0	18.5	0.00E+00	0.00E+00	0.00E+00
	50	20.0	18.0	1.99E+01	0.00E+00	0.00E+00
	500	20.0	18.0	4.58E+02	4.63E+01	9.83E+00
	5000	20.0	17.8	5.54E+03	5.72E+02	6.64E+01
Sassafrass soil	0.00	20.0	18.5	0.00E+00	0.00E+00	0.00E+00
	0.05	20.0	18.5	2.04E+01	4.50E-01	0.00E+00
	0.5	20.0	19.0	4.94E+02	5.32E+01	1.10E+01
	5	20.0	16.3	5.25E+03	8.40E+02	9.64E+01

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TABLE 4. Adsorption/desorption screening test calculations.

Test material	Nominal APFO concentration	APFO recovered from the soilless control, G	x	x/m or Cs	Material Adsorbed, A	Adsorbed material desorbed, D	Adsorbed material not desorbed, R	Adsorption coefficient, K'	Adsorption coefficient as a function of organic carbon, K'oc
	$\mu g\ L^{-1}$	μg	μg	$\mu g/g$	%	%	%		
Sand	0.00	0	-5.57E-01	-1.40E-01	0	ND	ND	-5.01E-03	ND†
	0.05	0.896	-1.88E+00	-4.71E-01	-210	ND	ND	-2.93E-03	ND
	0.5	9.99	3.50E-02	8.77E-03	0	ND	ND	2.36E-05	ND
	5	83	6.96E+00	1.55E+00	7	ND	ND	4.04E-04	ND
Peat moss	0.00	0	-7.99E-01	-3.77E-01	#VALUE!	#VALUE!	#VALUE!	#VALUE!	ND
	0.05	0.896	7.37E-01	3.47E-01	82	-2	102	4.36E-02	ND
	0.5	9.99	8.68E+00	4.09E+00	87	12	88	6.91E-02	ND
	5	83	6.38E+01	3.01E+01	77	23	77	3.28E-02	ND
Kaolin Clay	0.00	0	-3.30E-02	-8.31E-03	#VALUE!	0	#VALUE!	#VALUE!	ND
	0.05	0.896	1.30E-01	3.26E-02	15	ND	#VALUE!	8.61E-04	ND
	0.5	9.99	3.45E+00	8.68E-01	35	2	98	2.71E-03	ND
	5	83	2.26E+01	5.70E+00	27	61	39	1.90E-03	ND
Montmorillonite	0.00	0	-8.90E-01	-2.40E-01	#VALUE!	#VALUE!	#VALUE!	#VALUE!	ND
	0.05	0.896	-3.90E-02	-1.05E-02	-4	#VALUE!	#VALUE!	-2.10E-04	ND
	0.5	9.99	2.38E+00	6.44E-01	24	21	79	1.70E-03	ND
	5	83	9.15E+00	2.47E+00	11	#VALUE!	#VALUE!	6.75E-04	ND
Sassafrass Soil	0.00	0	0.00E+00	0.00E+00	#VALUE!	ND	ND	#DIV/0!	#DIV/0!
	0.05	0.896	2.95E-02	7.48E-03	3	ND	ND	1.76E-04	3.03E-02
	0.5	9.99	1.30E+00	3.28E-01	13	ND	ND	7.70E-04	1.33E-01
	5	83	1.04E+01	2.63E+00	12	ND	ND	7.26E-04	1.25E-01

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TABLE 4 (cont'd)

Test material	Nominal APFO concentration	APFO recovered from the soilless control, G	x	x/m or Cs	Material Adsorbed, A	Adsorbed material desorbed, D	Adsorbed material not desorbed, R	Adsorption coefficient, K'	Adsorption coefficient as a function of organic carbon, K'oc
	$\mu g\ L^{-1}$	μg	μg	$\mu g/g$	%	%	%		
East Wood Soil	0.00	0	-1.50E-03	-4.26E-04	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	0.05	0.422	1.86E-01	6.35E-02	46	2	87	5.39E-03	5.81E-01
	0.5	10.1	2.14E+00	7.93E-01	19	29	39	2.00E-03	2.15E-01
	5	96.8	1.17E+00	1.12E+00	-6	162	-315	2.34E-04	2.52E-02
Garden Area Soil	0.00	0	-2.31E-01	-6.30E-02	#DIV/0!	-64	164	-5.47E-03	-3.25E-01
	0.05	0.422	-9.15E-02	-7.15E-03	-18	-2142	2726	-2.70E-04	-1.60E-02
	0.5	10.1	4.58E+00	1.30E+00	44	57	34	4.72E-03	2.81E-01
	5	96.8	2.32E+01	7.50E+00	18	51	9	2.04E-03	1.21E-01
Upstream Sediment	0	0	0.00E+00	0.00E+00	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	50	0.422	1.05E-01	4.25E-02	27	-11	90	0.00E+00	0.00E+00
	500	10.1	1.86E+00	6.27E-01	16	56	17	0.00E+00	0.00E+00
	5000	96.8	9.52E+00	3.23E+00	3	84	-82	7.64E-04	8.78E-02
Downstream Sediment	0	0	0.00E+00	0.00E+00	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	50	0.422	2.35E-02	2.11E-02	9	-81	100	1.15E-03	3.30E-01
	500	10.1	9.36E-01	4.40E-01	7	5	34	9.75E-04	2.80E-01
	5000	96.8	-1.40E+01	-2.29E+00	-23	13	259	-4.11E-04	-1.18E-01
Sassafrass Soil	0.00	0	0.00E+00	0.00E+00	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
	0.05	0.422	1.35E-02	1.46E-02	6	-44	84	7.35E-04	1.27E-01
	0.5	10.1	2.17E-01	1.19E-01	0	180	-201	2.43E-04	4.19E-02
	5	96.8	-8.28E+00	1.20E+00	-17	-107	-2450	2.24E-04	3.86E-02

† ND = Not determined.

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FIGURE 1. Adsorption isoline of APFO onto Sand, Peat moss, Kaolin clay, Montmorillonite clay, and Sassafras soil.

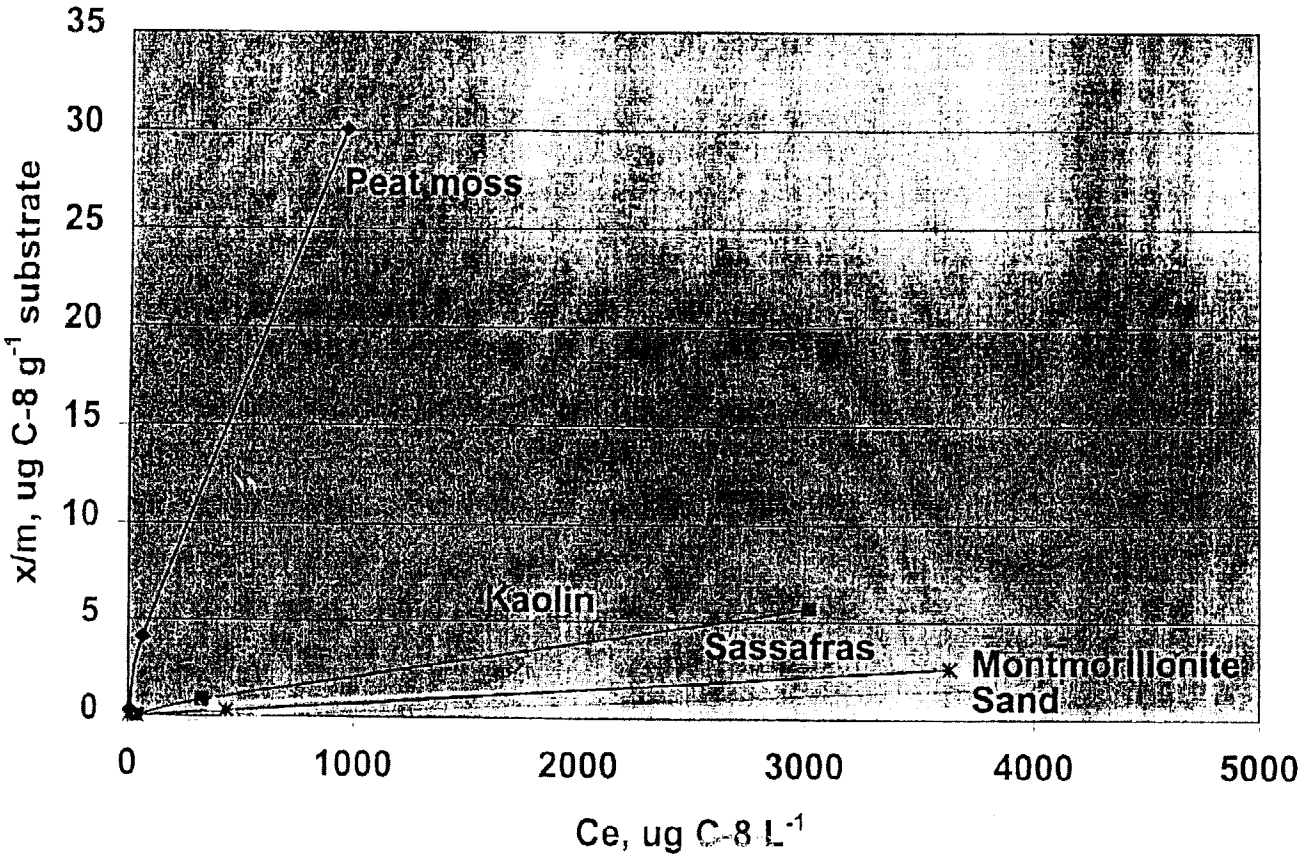

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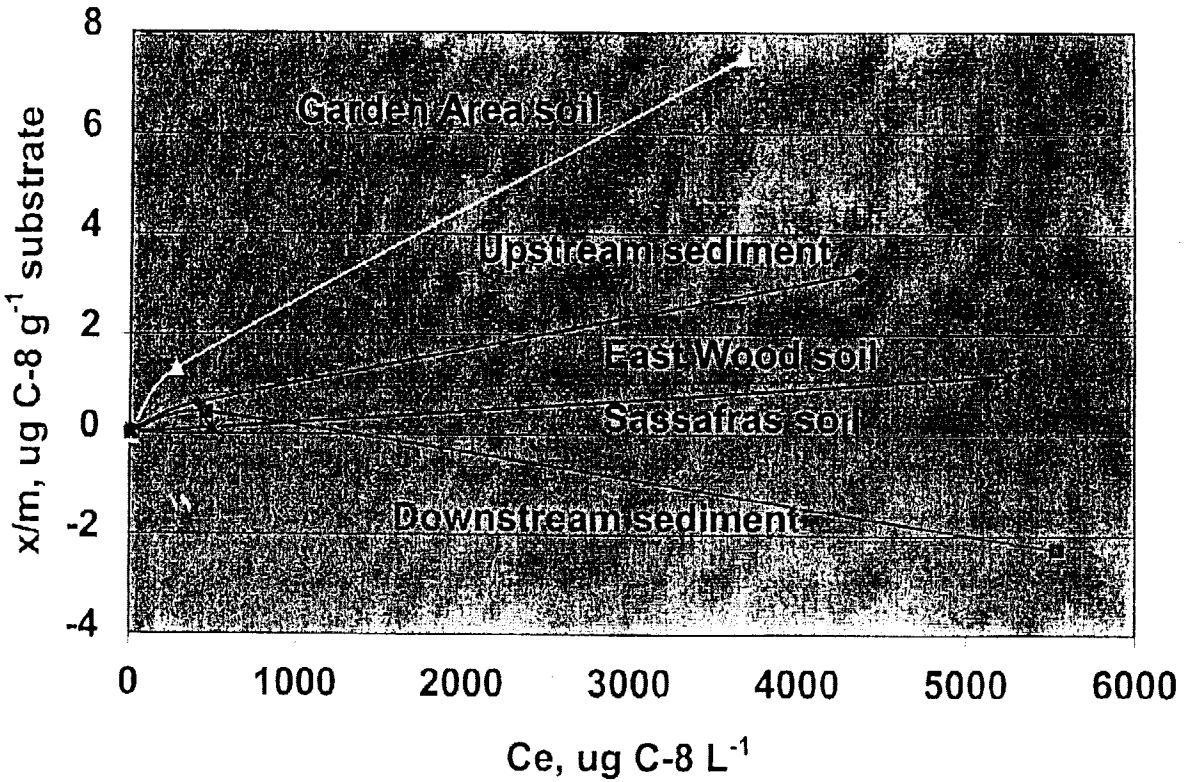
FIGURE 2: Adsorption isoline of APFO onto East Wood soil, Garden Area soil, Upstream sediment, and downstream sediments.



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Reference:

1 US EPA. 1998. Fate, transport and transformation test guidelines: OPPTS 835.1220 sediment and soil adsorption/desorption isotherm. United State Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances. EPA712-C-98-048. January 1998

2 Olphen, H.V., and J.J. Fripiat (eds.). 1979. Data handbook for clay materials and other non-metallic minerals Pergamon Press. New York 346 pp.

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AR 226_ 2734

CLOSING REPORT

ADDENDUM #1

PRODUCT AREA: DUPONT [redacted] FINE POWDER / DISPERSION

[redacted]	AUTHOR TL MAYLE - B168, WW SCION - CRD P14-1286 ~~PP CR DIST~~

TA NO. T-4655 REV. 2 PAGE 1 OF 12 ST ACCOUNTING: MTE ☒ MDV ☐
OPERATION: [redacted] COST OF TEST: N/A
PRODUCT: PROCESS WASTE DISPOSAL AMT OF PRODUCT: N/A
ISSUE DATE: 9/26/97 REF. TA'S: N/A

PURPOSE: TO SEPARATE AND PROPERLY DISPOSE OF C-8 CONTAMINATED PROCESS WASTE AND NON-C-8 WASTE.

REASON FOR ADDENDUM: IN THE INITIAL CLOSING REPORT THE RECOMMENDATIONS WERE INADVERTENTLY LISTED AS CONCLUSIONS. THIS ADDENDUM NOW INCORPORATES THE PROPER CONCLUSIONS.

SUMMARY: ATTACHED.

DISPOSITION OF PRODUCT: N/A

PSM/FOS FOLLOW-UP: (Ref. FQS Manual, Procedure 11, Att. 1, "Checklist for Managing Changes")

Are any of the recommendations from this TA being commercially implemented?
☐ Yes ☒ No
If yes applies to only *SOME* of the recommendations, list them below for tracking purposes.

RESPONSIBILITY FOR FOLLOW-UP: N/A

Date TA Completed: 2/01/97

Approvals: [redacted]

Date CR Written: 4/16/97

CR Submitted By: C.K. DILLON. C.K. Dillon 9/18/97

Authorization: [redacted] 9/18/97

CR4655R2-ADD1.DOC

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CONCLUSIONS: SEE ATTACHED.

RECOMMENDATION(S): SEE ATTACHED.

DISCUSSION: SEE ATTACHED.

ANALYSIS OF CHANGE: SEE ATTACHED.

Environmental Impact (Optional): N/A

Waste Impact (Optional): N/A

Effect on Advertising Claims (Optional): N/A

Energy Effect/Utilities Effect (Optional): N/A

Cost Accounting (Optional): N/A

Patent Situation (Optional): N/A

Attachments (Optional): N/A

SUMMARY:

On January 1, 1996, waste disposal ceased at the DuPont Letart Landfill. This landfill was the primary waste disposal point for the [redacted]BTO non-RCRA waste. This waste was generally made up of waste containing C-8 and waste not containing C-8. [redacted] Corporate Management elected to dispose of C-8 contaminated waste in a contained landfill. A hazardous waste landfill in Emelle, Alabama was selected as the disposal point for the C-8 contaminated waste. Waste not contaminated with C-8 would be disposed in the DuPont Dry Run Landfill. In order to effectively implement these new landfill requirements, a team was developed which was made up of representatives from the various [redacted] Areas. The primary products of the team were

- Determining what waste in each area needed to be sent to the Emelle Landfill and what waste was acceptable for the Dry Run Landfill,
- Developing procedures/practices for packaging C-8 contaminated waste and packaging waste not contaminated with C-8, and
- Developing a system for tracking each areas' costs associated with solid waste disposal at the Emelle Landfill.

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Various samples of process waste were tested and evaluated for C-8 levels to determine which waste needed to be disposed at the Emelle Landfill and which waste could be disposed at the Dry Run Landfill. Tables for each area were constructed which listed the waste and the required disposal point. This information was then incorporated into the BTO Waste Disposal Manual.

A general procedure was written for all areas for packaging C-8 contaminated waste and for packaging waste not contaminated with C-8. Any area specific requirements were included in the procedures for the specific area. Initially the areas were not diligent in complying with the procedures; therefore, the procedures were modified to require drum inspection and inspection sign-off within each area.

Cost accounting for waste sent to the Emelle Landfill is based on a user-pay system. Loadsheets break-down shipments by building number/area, and these loadsheets are then used to distribute the invoiced disposal costs for each load. In 1996, [redacted] disposal charges for the Emelle Landfill were approximately [redacted] Fine Powder/Dispersion's portion of this was approximately [redacted].

The ground water and surface water at the Dry Run Landfill are tested for C-8 levels on an annual basis. Testing has been completed only once since [redacted] began using this landfill; therefore, no conclusions have been drawn from this test data.

CONCLUSIONS:

- The drum packaging procedures and/or practices developed in this TA do not ensure that all drums from all areas will be at least 90% full and have no free liquid when they arrive at the Emelle Landfill.

- The accounting system developed for tracking Emelle Landfill costs on an area-by-area basis is acceptable.

RECOMMENDATIONS:

- Continue to use the waste packaging/inspection process outlined in this TA and incorporate the procedures into the Fine Powder/Dispersion operating procedures. Resp. [redacted]

- Include as part of the procedures the requirement to wear a Comfo II respirator with GMAH cartridge when adding absorbent to the waste drums. [redacted] by: 12/31/97

- Change Waste Disposal Manual for Fine Powder/Dispersion wastes to include all required drum labeling information for Emelle and Dry Run Drums. Also verify with the Site Environmental Group that empty Triton containers can be disposed in Dry Run Landfill. [redacted] 12/31/97

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DISCUSSION:

[redacted] Corporate Management elected to dispose of C-8 contaminated wastes in contained landfills. For [redacted] Washington Works, the Emelle Treatment Facility in Emelle, Alabama was chosen as the disposal location for C-8 contaminated wastes. The landfill at the Emelle Facility has a double liner and leachate collection system. The facility is a permitted secure hazardous waste treatment, storage and disposal facility.

To determine the wastes that needed to be sent to Emelle for disposal (C-8 contaminated wastes), sample testing and process experience were utilized. The Washington Works Environmental Group had no defined quantitative limit for C-8 levels in waste to be disposed at Dry Run. The Environmental Group and [redacted] Representatives did define process "dividing points" for C-8 versus non C-8 material. Attachment No. 1 summarizes test data and defines process dividing points. Since C-8 is used extensively in the Fine Powder/Dispersion process, no process scrap from this area will be disposed in the Dry Run Landfill without conducting C-8 testing and obtaining approval from the Environmental Group.

Using this information, waste tables were generated for each area which list the disposal point for the area's wastes. The waste tables for Fine Powder/Dispersion are in Attachment No. 2. This information was incorporated into the BTO Waste Disposal Manual No. 30 in mid-1996. Review of this information for Fine Powder Dispersion shows that the tables do not contain all of the drum labeling requirements for Emelle and Dry Run wastes. In addition, the manual indicates that empty Triton containers can be disposed at Dry Run, whereas the TA waste tables indicate that these containers must be disposed at the Emelle Landfill.

In order to implement the change in disposal practices, procedures were written for packaging and labeling of Emelle and Dry Run wastes. Properly packaged drums (i.e. at least 90% full and no free liquids) is a requirement in order for the drums to be landfilled. The Emelle Facility inspects each drum, and if the drum is not properly filled, the deficiency is corrected at Emelle for an additional charge. Initially, the procedures were written such that the areas packaged and labeled the waste, and the warehouse personnel inspected the drums for packaging/labeling deficiencies. This approach was unsuccessful as some areas were not diligent in following the procedures. As a result, the waste drum inventory destined for Emelle occasionally built to over 500 drums as the drums were held waiting on deficiency corrections.

In an effort to improve the packaging/labeling process, the procedures were modified to require inspection by the areas. Prior to moving material to the drum storage area, each area was required to inspect individual drums for proper packaging and labeling. Once this was completed, a date inspected label was attached to the drum and initialed. If drums were moved to the storage area without the label, the warehouse notified the area or moved the drums back to the area until the problem was corrected. This appears to have been only partially successful; some drums still contain free liquid and are not at least 90% full when they arrive at the Emelle Landfill. It is believed that drums less than 90% full can be attributed to settling during transit and/or inadequate filling of absorbent. Drums with free liquid may be partially attributed to separation during transit, however, limited dewatering capabilities in the areas has been cited as the more likely cause. Gravity separation has generally been used to dewater the waste, and it is recognized that this process is not always effective for some types of waste. The number of drums that needed additional filling/dewatering at Emelle in 1996 was typically less than 20% (18 drums) of each shipment. For the first 3 months of 1997, this number increased to 25%-30% (22-26 drums). No recommendations have been made regarding improvements to the dewatering/packaging facilities used within the areas. Each area will have the ability to monitor Emelle disposal costs and the area can make improvements to their processes if viable. For Fine Powder/Dispersion, drums prepared for Emelle should be periodically audited to ensure that drums are being packaged according to the procedures.

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Review of the drum preparation process has also shown that respiratory protection will be required for packaging the Emelle waste. It has been noted by area operators that the absorbent used to top-off drums generates dust when poured into the drums. In order to provide adequate protection from the dust components, a Comfo II (or approved equivalent) with GMAH cartridges will be required to be worn by the operator when filling drums with absorbent. This requirement will be included as part of the operating direction update.

The cost accounting system for the Emelle Landfill costs was developed based on a "user pay" philosophy. Warehouse personnel tabulate drum information on loadsheets as the trailer is loaded for shipment to Emelle (Attachment No. 3). This loadsheet is forwarded to the Accounting Group and is used to distribute the Emelle Landfill invoice costs for the shipment. A typical Emelle invoice is shown in Attachment 4. The drum and cost data are tabulated by the Accounting Group on a spreadsheet (Attachment No. 5).

The current disposal costs (per unit) for the Emelle Landfill are listed in Attachment No. 6. The base disposal cost per drum is [redacted] This is the cost that is charged if the drummed waste is properly packaged (i.e. no free liquids and at least 90% full). If a drum has free liquid, the waste in the drum is "solidified" at Emelle at a cost of $165/drum. If a drum is less than 90% full, absorbent is added at a cost of [redacted] per drum. During 1996, Fine Powder/Dispersion disposed of [redacted] drums and [redacted] paper rolls at Emelle for approximately [redacted] It is estimated that the paper rolls accounted for [redacted] of this total [redacted]

As noted previously in this closing report, [redacted] non-C-8 solid waste disposal in the Dry Run Landfill began in January, 1996. The C-8 levels at the Dry Run Landfill are monitored in the ground water and surface water (leachate) on a yearly basis. Ground water tests completed in April 1996 showed C-8 levels of -10 ppb. Likewise, tests completed in June of 1995 showed C-8 levels of 9-10 ppb. Studies have shown that ground water moves only about 2 feet per year at Dry Run; therefore, ground water testing for C-8 levels is likely to be inconclusive for many years. On the other hand, surface water tests should be able to provide some indication within a few years as to whether or not the [redacted] waste is significantly increasing the C-8 levels at Dry Run. In April of 1996, surface water testing showed C-8 levels of 86 ppb, compared to levels of 53 ppb shown in June of 1995. It is unknown if this change in surface water C-8 levels is statistically significant. The Washington Works Environmental Group does not believe these results currently warrant any changes in the waste flow stream to Dry Run Landfill. As such, no recommendations have been made at this time regarding [redacted] waste disposal practices at the Dry Run Landfill.

Analysis of Change:

As noted in the discussion of this closing report, additional respiratory protection will be required when adding absorbent to the drums. No other new safety or health issues were observed that were a result of running this TA.

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ATTACHMENT 1

INTEROFFICE MEMORANDUM

Date: 05-Dec-1995 11:29am
From: CRAIG K DILLON
DILLONCK
Dept: Fluoroproducts
Tel No: 304-863-4972

TO: Distribution List

Subject: Meeting Notes - C-8 at Dry Run

A meeting was held on November 30 to discuss the acceptable C-8 levels for [redacted] waste that is proposed to be landfilled at Dry Run. Those in attendance were [redacted]

[redacted] presented data on various [redacted] waste samples that were extracted by [redacted] for C-8 levels. Details of the samples extracted are recorded in book No. E56538 page 123 [redacted]. [redacted] also provided as reference extraction data furnished by Quality Analytical Lab on bio-cake. Summary of the data is as follows:

SAMPLE	DESCRIPTION	POLYMER C-8 LEVEL PPM	WATER EXTRACTION EXTRACTED C-8 LEVEL PPM**
150-1	Fine Powder Trench Scrap - undried	59	0.44
150-2	Granular Vacuum System Scrap - dry	2	0.08
150-3	PFA from Process K test dryer - dry	11	0.04
150-4	FEP-4100 from Line 3 - dry	7	0.04
150-5	1 ppm blank	-	1.04
NA	Bio-cake sample (Quality Analytical)	0.5-1.0	0.004

[redacted] noted that the extraction method used by Quality Analytical differed from the method used by Doughty. Based on the extraction of the bio-cake, Weber indicated that approximately 7 pounds per year of C-8 is currently landfilled at Dry Run due to bio-cake.

Based on the test results, the group agreed that Granular scrap can be landfilled at Dry Run. In addition, any melted scrap PFA or FEP can be landfilled at Dry Run. For PFA, this would be scrap material generated at the exit side of the extruder and beyond (generally cubes). For FEP, this would be scrap material generated at the exit side of the humid heat treater and beyond (generally slabs, shred, cubes). It was the consensus of the group that the potential landfill amounts of these scrap materials would not significantly impact C-8 levels at Dry Run.

For Fine Powder/Dispersion, no process scrap will be allowed at Dry Run. This was based on the test results and also on process configuration. There is concern that the process configuration would not allow for distinct

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separation of C-8 and non-C-8 scrap. If a large amount of finished Fine Powder/Dispersion must be disposed, then this material should be tested for C-8 and handled on an individual basis. It was also the consensus of the group that [redacted] continue to drive the sale of all scrap to reduce the need for landfilling.

Concern was also raised regarding a list of materials that [redacted] intends to landfill at Dry Run. This list was submitted to the Environmental Group for review. A meeting is planned for December 6 between Environmental/[redacted] to discuss the list. A copy of this list (for discussion) will be made available to the group after the meeting.

Please let me know if I have left out any items or if I have misinterpreted anything discussed at the meeting. Thank-you for participating.

Craig

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** Detection limit of test is 0.1-1.0 ppm

ATTACHMENT 2

FINE POWDER/DISPERSION WASTE TABLE
ACCEPTABLE WASTE FOR DRY RUN LANDFILL

WASTE DESCRIPTION	EST. YEARLY QUANTITY	SPECIAL PREPARATION REQUIREMENTS	CONTAINER FOR DISPOSAL
GENERAL PAPER TRASH, CARDBOARD, PPE	[redacted]	NO GLASS	GENERAL PLANT TRASH DUMPSTER
[redacted]	[redacted]	WATER WASH ANY RAW MATERIAL LEVERPAKS. EMPTY LEVERPAKS SHOULD THEN BE MOVED TO THE [redacted]3164 PAD.	ROUTINE PICK-UP WILL BE MADE BY P&S. P&S WILL CRUSH PRIOR TO LANDFILLING

FINE POWDER/DISPERSION WASTE TABLE
WASTE FOR EMELLE LANDFILL

WASTE DESCRIPTION	EST. YEARLY QUANTITY	SPECIAL PREPARATION REQUIREMENTS	CONTAINER FOR DISPOSAL
SCRAP WAX AND POLYMER BLEND TANKS WASTE	[redacted]	NO FREE LIQUIDS	RED 55-GALLON STEEL DRUM
WAX DECANTER WASTE	[redacted]	NO FREE LIQUIDS	RED 55-GALLON STEEL DRUM
SCRAP POLYMER FROM COAGULATORS, SUMPS, SCREENS	[redacted]	NO FREE LIQUIDS	PLASTIC LINED RED 55-GALLON STEEL DRUM
SCRAP POLYMER FROM DRYERS, PAPER BREAKS, CLEANINGS	[redacted]	NONE	RED 55-GALLON STEEL DRUM
EMPTY TRITON CONTAINERS	[redacted]	NONE	RED 55-GALLON STEEL DRUM
SUPERNATE TANK BOTTOMS	[redacted]	NO FREE LIQUIDS	RED 55-GALLON STEEL DRUM
DISPERSION COAGULUM, PACKOUT FILTERS, DECANTER CLEANINGS, TANK CLEANINGS	[redacted]	NO FREE LIQUIDS	RED 55-GALLON STEEL DRUM
USED FP DRYER PAPER	[redacted]	NONE	LOAD ROLLS ON BOX TRAILER
H&V SYSTEM FILTERS	[redacted]	NONE	BULK FIBRE PACKS OR RED 55-GALLON DRUMS
ONE-WAY TOTES	[redacted]	WATER WASH TO SUPERNATE SUMP TO REMOVE HEELS. TOTES MUST THEN BE CRUSHED	PALLETIZED AND STRAPPED
EMPTY/FULL SAMPLE BOTTLES/BAGS CONTAINING POLYMER/DISPERSION	[redacted]	NONE	RED 55-GALLON STEEL DRUMS
DISPERSION DRUM LINERS	[redacted]	REMOVE LINER FROM LEVERPAKS. NO FREE LIQUIDS	RED 55-GALLON STEEL DRUMS

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ATTACHMENT 3

EMELLE LOADSHEET
(Example only)

LOADING DATE: _____ **. ORDER #:** _____ **SEAL #:** _____

Building & AREA	NUMBER OF DRUMS
B22 RESEARCH LAB	
B23 BEAD FACILITY	
B184 [redacted]	
B162 FINE POWDER/DISPERSION (DRUMS)	
B162 FINE POWDER/DISPERSION (#OF PAPER ROLLS)	
B163 FEP	
B164 GRANULAR	
B177, B162M MONOMERS	
B180 TELOMERS	
B212 [redacted]	

Note: FORWARD COMPLETED SHEET TO THE PRODUCT ANALYST IN B170

Form Approval: _____ Date: _____

*DuPont Registered Trademark

ATTACHMENT 4

901711-1614

[redacted]

INVOICE

[redacted]

SERVICE PROVIDED BY:
EMELLE DISPOSAL FACILITY

DUPONT (PARKERSBURG WV)
..ATTN ACCOUNTS PAYABLE
PO BOX 4909

BEAUMONT TX 77704-4909

CUSTOMER ACCOUNT NUMBER: 696 695 5006479
INVOICE NUMBER: 69573844 INVOICE DATE: 03/19/97 PAGE: 1

004396504

970319

REFERENCE NO.	PROFILE	DESC / UNIT	QUANTITY	RATE	AMOUNT
000082997I-01	EME-X12494	FLUOROPOLYMER INDUSTRIAL WASTE		SVC DATE: 03/18/97	
	DRUM SOLID	NH 55G DRM	[redacted]	[redacted]	[redacted]
	OTHER SERVICES	DRMS < 90%	[redacted]	[redacted]	[redacted]
	SOLIDIFIED DRUMS	NH 55G DRM	[redacted]	[redacted]	[redacted]
	BULK SOLID	NH BOXES	[redacted]	[redacted]	[redacted]
	PO# OR CONTRACT#	LPW-288219			
	NON-HAZARDOUS FEE	WV NONHAZ	[redacted]	[redacted]	[redacted]
	ADEM MONITORING FEE	WV TONS	[redacted]	[redacted]	[redacted]
	SUMTER COUNTY FEE	WV 55 GAL	[redacted]	[redacted]	[redacted]
				SUBTOTAL	[redacted] *

ACCOUNTS PAYABLE PAYMENT CHECK ENTERED
MAR 26 1997
PAID
RATE
TIME PERIOD
REMIT TO
FREIGHT TERMS
NOT EXCEED
RELEASE OF LIENS
APPROVAL

BEAUMONT PAYMENT CENTER RECEIVED MAR 24 1997

REMIT TO ADDRESS ▶ CHEMICAL WASTE MANAGEMENT, INC
P.O. BOX 840606
DALLAS, TX 75284-0606

PLEASE PAY THIS AMOUNT ▶ [redacted]

ORIGINAL INVOICE

WE APPRECIATE YOUR BUSINESS!

AR 226-2735

TRADE SECRET

Study Title

EVALUATION OF THE BIODEGRADABILITY OF C-8 USING THE MODIFIED STURM TEST (OECD 301 B)

Author

John T. Gannon Jr., Ph.D.

Study Completed on

3/11/97

Performing Laboratory

E. I. du Pont de Nemours and Company
Central Research & Development
Environmental Biotechnology
Glasgow Business Community 301, P.O. Box 6101
Newark, DE 19714-6101

AEM Laboratory Report No. 24-97

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GENERAL INFORMATION

Material Tested: C-8

Synonyms/Codes: None

Submitter's Notebook No.: N/A

Sponsor: Roger Zipfel
FLPR
Washington Works

Experimental Start & Completion: 1/21/97 to 3/11/97

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EVALUATION OF THE BIODEGRADABILITY OF C-8 USING THE MODIFIED STURM TEST (OECD 301 B)

SUMMARY

The test chemical - **C-8** - was tested for biodegradability using the Modified Sturm Test. Within the 28-day test period, **C-8** reached a peak of 13 % at Day 28. Since the *"PASS"* criteria for the Modified Sturm Test is 60 % biodegradability in a 10 day window within the 28 day test period, it may be concluded that **C-8 did not demonstrate "Ready Biodegradability".**

Please note that failure to pass a "Ready Biodegradability Test" does not imply that the test material will persist in the environment for an extended period of time. Furthermore, evaluation of the individual components of the formulation would yield more specific information on the biodegradability potential of **C-8**.

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Introduction/Purpose

The Modified Sturm Test was performed to determine the biodegradability of the test material, C-8.

The Modified Sturm Test can be used for either water-soluble or water-insoluble and non-volatile chemicals. In this test, biodegradability is measured as CO_2 evolution. When bacteria biodegrade an organic chemical in an aerobic system, the end product of the metabolism is carbon dioxide. Therefore, carbon dioxide evolution is indicative of a chemical being biodegraded. If CO_2 evolution is greater than 60 %, then Ready Biodegradability has been demonstrated and no further testing is necessary. This "PASS" value must be reached in a 10-day window within the 28 day period of the test. The 10 day window begins when the degree of biodegradation has reached 10 % $ThCO_2$, and must end before day 28 of the Modified Sturm Test.

Materials/Methods

The Modified Sturm Test determination was conducted in accordance with AEM SOP MI056-P: Modified Sturm Test. See Table 1 for materials tested. Activated sludge collected from the City of Wilmington, DE POTW was used as the inoculum.

Results/Discussion

In the Modified Sturm Test, C-8 reached a peak of 13 % at Day 28 (Table 2 and Figure 1). Therefore, it may be concluded that "Ready Biodegradability" was not demonstrated.

Please note that failure to pass a "Ready Biodegradability Test" does not imply that the test material will persist in the environment for an extended period of time. Furthermore, evaluation of the individual components of the formulation would yield more specific information on the biodegradability potential of C-8.

The reference chemical, sodium benzoate, exceeded 60 % biodegradability within 14 days, therefore the biodegradability test was considered valid (Table 2 and Figure 1). The Theoretical CO_2 Produced was 0.24 mg CO_2 per mg of 30 % C-8.

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The toxicity test (which includes both the test chemical and the reference chemical in the same flask) yielded less than 25 % biodegradation within 14 days, therefore C-8 was considered as inhibitory to microorganisms in the inoculum.

Path Forward Discussion

If it can be assumed that the 30 % C-8 solution was essentially pure with only trace levels of impurities, then there may be a possibility that the terminal carbon (COO^-) may have been biodegraded by the microorganisms. As a path forward, we will repeat the experiment to determine if we have reproducible results. The Modified Sturm Test is used to determine biodegradability of organic chemicals by measuring the amount of CO_2 evolved without using ^{14}C labelled carbon. Therefore, the analysis is non-specific and it is not possible to conclusively determine which carbon was attacked by the microorganisms.

If reproducible results are observed upon repeating the Modified Sturm Test, then it is recommended that a follow-up biodegradability test should be conducted with ^{14}C labelled carbon on the terminal C (COO^-) of C-8. This will allow for the conclusive determination as to whether the terminal carbon of C-8 is biodegraded.

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TABLE 1

Description of Test and Control Substances

Test Substance Description
C-8 Sodium Benzoate

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TABLE 2: MODIFIED STURM TEST

TIME	C-8 % BIODEGRADABILITY	Sodium Benzoate % BIODEGRADABILITY
0 Days	0	0
3 Days	2	13
7 Days	5	24
10 Days	7	39
14 Days	10	63
20 Days	12	
24 Days	13	
28 Days	13	

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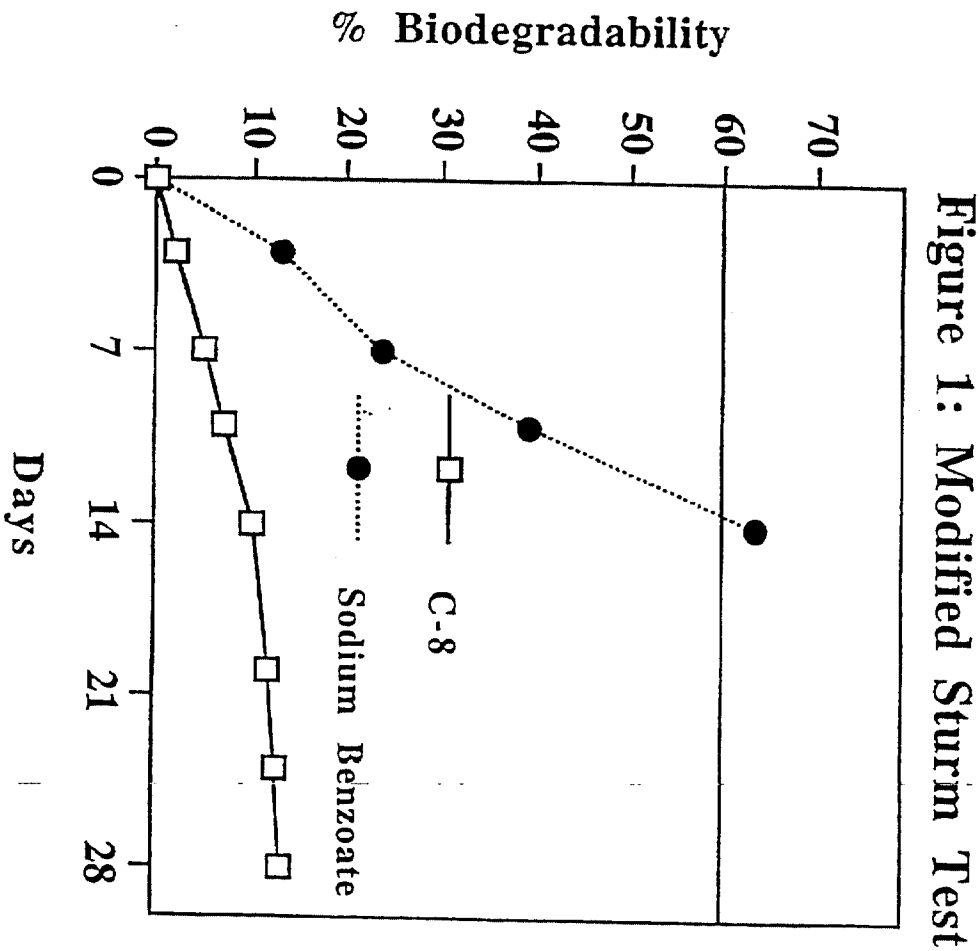

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EVALUATION OF SURFACTANT C-8 RELATIVE TO CHAMBERS WORKS

SUMMARY:

Washington Works is currently discharging the surfactant C-8, (Ammonium Perfluorooctonate) in a wastewater generated from one of its processes, to the Ohio River. Washington Works would like to decrease the amount of C-8 being discharged into the Ohio River. An alternative method of disposal would be to send additional wastewater containing C-8 to Chambers Works for treatment. Currently Washington Works is sending 12,000 lbs./year of C-8 mixed with wastewater by rail cars to Chambers Works and would like to increase it to 24,000 lbs./year.

The surfactant C-8 is not degradable by biological systems. Thus Washington Works has requested Engineering to determine the fate of C-8 in the Chambers Works wastewater treatment system and the Delaware River at the proposed loading of 24,000 lbs./year.

It appears that the increase of C-8 to 24,000 lbs/year to Chambers Works will not affect their NPDES permit. Also the point at which Chambers Works discharges into the Delaware River is brackish water thus not suitable for drinking water. Specific comments, conclusions, and recommendations are presented below:

- Currently no data exists on whether C-8 will bioaccumalate in fish tissue. However, an estimation of the octanol-water partition coefficient predicted that C-8 will not accumulate to large concentrations in the fatty tissues of organisms.

- Washington Works would like to concentrate the wastewater going to Chambers Works to reduce the amount of wastewater that needs to be sent. Therefore an evaluation of the Chambers Works ability to handle the increased concentration of pollutants form Washington Works should be completed. An E/BOD Test to determine if the increased concentrations will effect the PACT system should be done by Chambers Works (Stan Temple), Engineering, thru the Engineering Test Center or Washington Works.

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- The percent removals of C-8 across the PACT system should be determined. Currently a bench scale test is planned by Washington Works with Chambers Works activated carbon to determine the amount of removal of C-8 by the PACT system.

- It is recommended that Chambers Works equalize the Washington Works wastewater as much as possible to reduce concentrations within the PACT system and the discharge outfall, if this is not already being done.

DISCUSSION:

In review of the proposal to increase the amount of the surfactant C-8 to be treated at Chambers Works from 12,000 lbs/year to 24,000 lbs/year the following was evaluated to determine the fate and impact of C-8 within the Chambers Works WWTP and the Delaware River:

- Chambers Works NPDES permit

- Concentrations of C-8 at outfall 661, outfall 001, edge of the zone of initial dilution, and the edge of the mixing zone.

- The disposal of the activated carbon in relation to C-8

Attachment 1 outlines some human health and aquatic toxicity information on C-8 relative to Chambers Works NPDES permit. In relation to aquatic toxicity testing and the Ames test , this information shows that the surfactant C-8 will not negatively impact the permit. The Chambers permit does have a testing requirement for surfactants; however, it is only a monitor and report requirement. The Delaware River at the point which Chambers Works discharges is brackish, thus drinking waters concerns are not present.

The concentrations of C-8 were calculated at the following points; outfall 661, outfall 001, edge of the zone of initial dilution, and the edge of the mixing zone (Figure 1). Table 1 shows the current and proposed loading concentrations at the points identified above. Note, these concentrations were based on the assumption that no treatment occurs in the Chambers Works PACT system.

If the surfactant C-8 is attaching/coating to the activated carbon, the C-8 would be removed with the carbon once wasted. Chambers Works is currently disposing of waste PACT carbon to an onsite landfill. However, in December 1991 Chambers Works plans to regenerate the wasted carbon to reuse within the PACT system. The carbon regeneration system is a multiple hearth furnace with counter current flow. First the water in the carbon fines will evaporated at 212 F(100 C), then the poralysis of the carbon fines will occur at 1000 F(573 C) and lastly the PACT carbon will be regenerated. The surfactant C-8 volatilizes at 392 F(200 C) and decomposes at 752 F

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(400 C) to CO2 and HF. Therefore any C-8 removed by the PACT carbon will be destroyed during the regeneration process.

An evaluation was performed by Tony Rogers of Michigan Technological University on C-8 to estimate the octanol-water partition coefficient (kow) to predict the ability of C-8 to accumulate in the fatty tissue of organisms. His evaluation is in attachment 2. Based on his evaluation it was predicted that very little of the target chemical will partition into a lipid phase and therefore will not accumulate to large concentrations on the fatty tissues of organisms.

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TABLE 1

CONCENTRATIONS OF C-8 AT DIFFERENT LOCATIONS AT CHAMBERS WORKS
(mg/l)

	OUTFALL 662	OUTFALL 001	EDGE OF ZONE OF INTIAL DILUTION	EDGE OF MIXING ZONE
1) Current "C8"loading of 92.3 lbs./day over 130 days. No treatment of "C8".	0.58	0.269	0.048	0.023
2) Current "C8" loading of 12.8 lbs./day over 365 days. No treatment of "C8".	0.2	0.096	0.017	0.008
3) Future "C8" loading of 145 lbs./day over 165 days. No treatment of "C8".	0.92	0.41	0.075	0.036
4) Future "C8" loading of 65.7 lbs./day over 365 days. No treatment of "C8".	0.42	0.192	0.034	0.016

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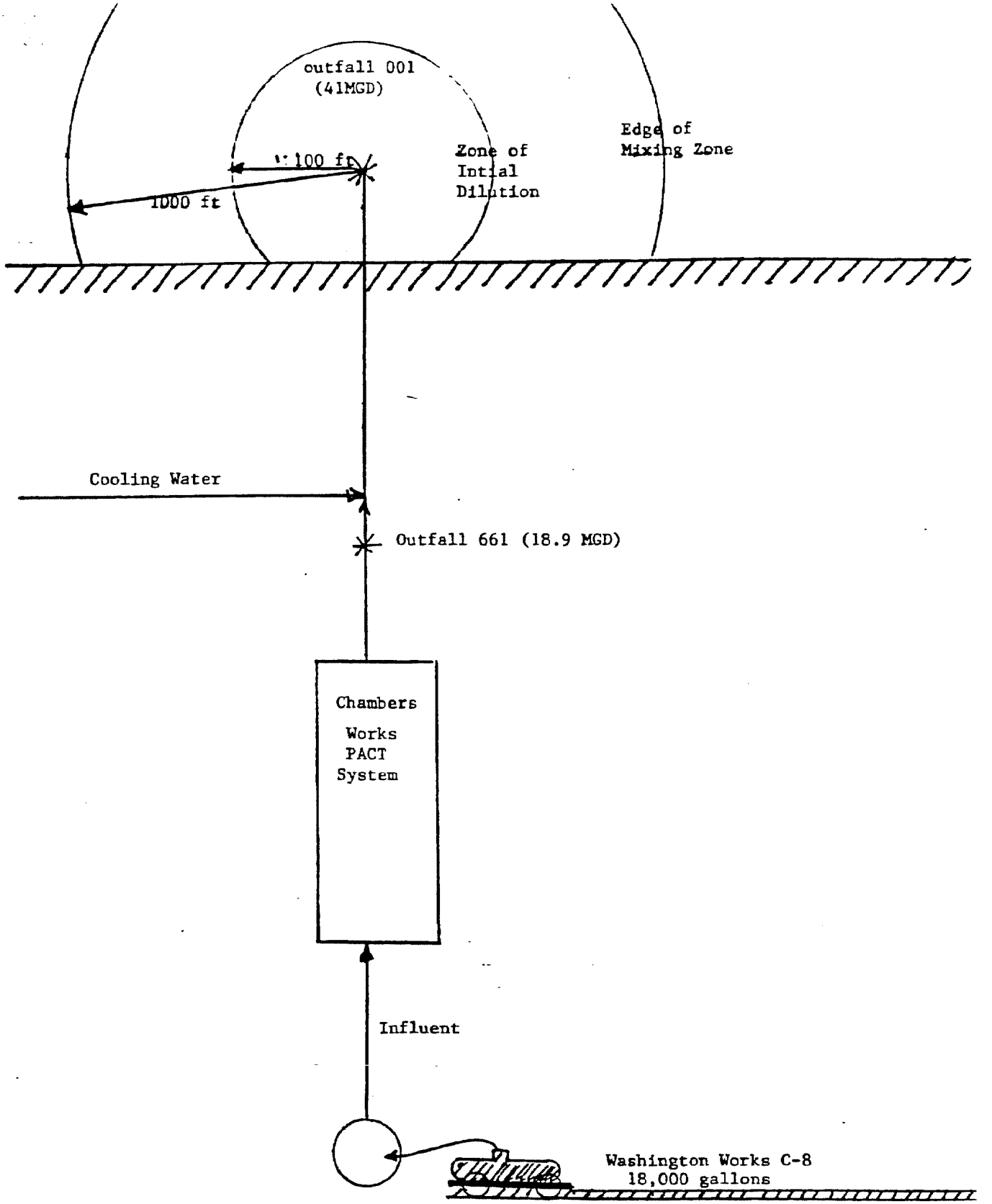

Figure 1

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