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Synthesis and Biological Screening of Novel Hybrid Fluorocarbon Hydrocarbon Compounds for Use as Artificial Blood Substitutes

Second Annual Report, July 1977—July 1978

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October 15, 1979

Prepared for

Division of Blood Diseases and Resources
National Heart, Lung and Blood Institute
National Institutes of Health

Through agreement with
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ABSTRACT

A series of hybrid fluorochemicals of general structure $R^1R^2R^3CR^4$, was prepared where the R^i's (i=1,2,3) is a saturated fluoroalkyl group of formula C_nF_{2n+1}, and R^4 is an alkyl group C_nH_{2n+1} or a related moiety containing amino, ether or ester functions but no CF bonds. Such compounds, except for a few isolated examples, have been unknown and none have been tested for toxicity or their physical properties systematically studied. We proposed that compounds of this class containing approximately eight to twenty carbons total would have physical properties suitable for use as the oxygen carrying phase of fluorochemical emulsion artificial blood, and that there was reason to hope for low toxicity. The program included the chemical synthesis, and physical and biological testing of pure single isomers of the proposed artificial blood candidate compounds.

Results of the work include: (a) the successful synthesis of several candidate compounds, i.e., compounds with acceptable physical properties; (b) preliminary toxicity results indicating that at least two of these are non-toxic, contrary to the previous belief that significant hydrogen content necessitates toxicity; and (c) the development of a method for predicting vapor pressure and oxygen solubility from chemical structure alone, to guide the synthetic efforts in the most fruitful direction. Much of the effort was devoted to synthesis of chemical intermediates and development of testing methodology.

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SECTION I

INTRODUCTION

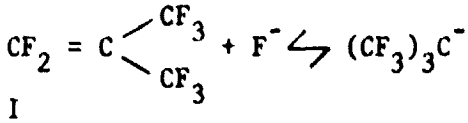
This report contains the results from the second year's work on Synthesis and Biological Screening of Novel Hybrid Fluorocarbon Hydrocarbon Compounds for Use as Artificial Blood Substitutes and summarizes and draws conclusions about the results of both years of the contract.

Previous work in this field relied entirely on perfluorinated organic materials, (in which all hydrogen has been replaced by fluorine) because of the high toxicity observed for the few partially fluorinated materials which had been tested. We had proposed that the separation of perfluoroalkyl groups, viz. $R_f(C_nF_{2n+1})$, and a hydrogen-containing group R by a quaternary carbon would eliminate or greatly reduce the toxicity hitherto associated with hydrogen and fluorine in the same molecule. Only a very few examples of the proposed class of hybrid fluorochemicals were known; none had been tested for biological properties, and none of the known ones appeared to have vapor pressures in the required range. The objective of our research was to demonstrate that the proposed hybrid compounds could in fact be synthesized, that they would be non-toxic, and that they would have physical properties, especially vapor pressures and oxygen solubilities, suitable for application in artificial blood.

A further hypothesis was that the toxicity of the new compounds could be evaluated via intraperitoneal testing in mice, avoiding the necessity of preparing adequately stable, fine-particle emulsions for intravenous administration. The literature contained indications that some materials were "toxic" when administered intravenously as coarse emulsions but "nontoxic" as fine emulsions, and the necessity of measuring the particle size distributions in each emulsion appeared to add greatly to the time and cost of biological testing.

In the realm of chemical synthesis, we clearly demonstrated that a substantial variety of the proposed "hybrid" fluorochemicals could be prepared and did possess acceptable physical properties. Though time did not permit a

careful study of initially unpromising synthetic reactions, indications were that alkylation of the intermediate carbanions is successful only when the carbanion is relatively unhindered. Thus, a series of hybrid alkanes and ethers derived from octafluoroisobutene (I) and F-2-metnyl-2-pentene (II) were both prepared in good yield, but no similar hybrid compounds could be prepared from the mixed trimers of hexafluoropropene (III).



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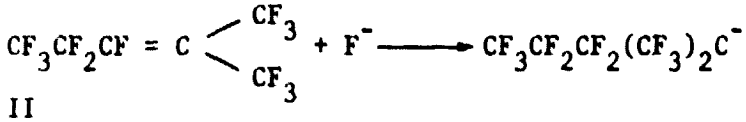
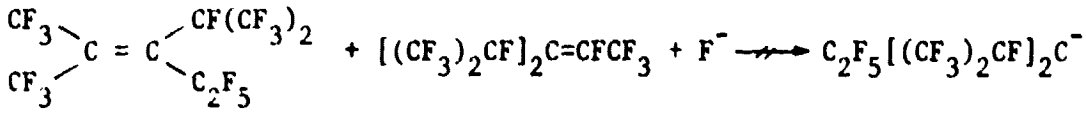


II



III

Because of the vital importance of emulsion technology to this program, work at JPL and the Rockwell Science Center was done to give a preliminary physical-chemical interpretation of the interactions of surfactants with fluorocarbons to provide a future basis for developing quantitative criteria for emulsion stability.

During this effort, computational procedures were developed to calculate the oxygen solubility and vapor pressure of candidate hybrid materials from just the structural chemical formulas. This allowed screening compounds before they were synthesized, so that they would come close to meeting the oxygen solubility and vapor pressure criteria, i.e., oxygen solubility of 35 cc or more for 100 cc of material and a vapor pressure below 50 mm at 37°C. From solution theory concepts it is estimated that the maximum oxygen solubility will not be more than about 63 cc O_2/100 cc for any organic liquid with a vapor pressure $\leq$ 50 mm. Copies of papers addressing this subject are included in Appendixes B and C.

This study demonstrates that the proposed intraperitoneal route of administration does in fact lead to rapid body absorption of fluorochemicals with molecular weights up to 666, provided that the fluorochemicals are administered as coarse emulsions rather than as neat liquids. When so administered, the fluorochemicals are rapidly absorbed and taken up by the liver and spleen, much like the absorption pattern previously found for intravenous administration. Subsequent elimination from the initial depots via expiration also appears very similar to previous observations based on intravenous administration, based on the limited data on excretion rate. The intraperitoneal test protocol now appears to be the method of choice for preliminary in vivo toxicity screening of new fluorochemicals.

On the key point of toxicity of the proposed hybrid fluorochemicals, the project has been a qualified success: out of the thirty-odd candidate compounds synthesized to date, three were found to be nontoxic in the mouse screen. Several others were nontoxic or of very low toxicity in a tissue culture test performed by Dr. R. P. Geyer, but failed in the mouse screen. The nontoxic compounds and examples of toxic ones are

$(CF_3)_3C(CH_2)_nC(CF_3)_3$; n = 2, AB - 14; n = 3, AB - 6

$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2C(CF_3)_2CF_2CF_2CF_3$, AB - 32

Toxic:

rapidly toxic $(CF_3)_3COH$

$CF_3CF_2CF_2(CF_3)_2COH$

$(CF_3)_3CCH_2CO_2C_2H_5$; $CF_3CF_2CF_2(CF_3)_2CCH_2CO_2C_2H_5$

Slowly toxic:

$CF_3CF_2CF_2(CF_3)_2C\text{-}OR$

$CF_3CF_2CF_2(CF_3)_2C\text{-}$

$R = C_nH_{2n+1}$; n = 1 - 4

Although the scope of this work did not include a search for possible metabolites, the pattern of toxicity suggests that AB-6, AB-14, and AB-32 all resist metabolic attack because the carbon-hydrogen bonds of the alkyl moiety are well-shielded by bulky perfluoroalkyl groups at both ends, whereas the toxic examples are either already in an oxidized state (the alcohol and es-

ters) or else possess a hydrocarbon chain relatively exposed to enzymatic attack. The hope that compounds such as $(CF_3)_3CCH_2CO_2C_2H_5$ might be nontoxic was initially based on reports that similar hydrocarbon acids, such as $(CH_3)_3CCOOH$, are not metabolized and are excreted unchanged, but in fact the high doses required for artificial blood applications may make comparisons with all such previous work invalid. A summary of salient results and the physical and biological properties of the three candidate blood substitute compounds AB-6, AB-14, and AB-32 follows.

SECTION II

TEST AND EVALUATION SUMMARIES FOR THREE BEST COMPOUNDS

A. INTRODUCTION

The three nontoxic compounds synthesized under this program were: AB-6, AB-14, and AB-32. To summarize, these three nontoxic compounds, considered as candidates for further testing, were found to have been readily absorbed from the peritoneum; they produced no gross longterm toxicity; they showed moderate histopathological changes, in most tissues, but usually marked changes in the spleen; and they were eliminated from the body at reasonable rates. Only limited pharmacokinetics could be done on these three FCCs due to the small amounts of available compounds. These three FCCs, along with JPL-AB-13, deserve further detailed investigation with continuation of pharmacokinetic studies and eventual exchange transfusions in rats.

On April 13, 1978, at Atlantic City, New Jersey, a group of representatives from the current fluorocarbon project met to discuss the best candidate compounds for continued production in a new initiative. Several members of the group felt that they were not ready at this time to declare specific compounds. Rather, time was spent in developing a list of criteria by which the best compounds would be judged. These criteria are as follows:

1. Oxygen solubility.

   Oxygen solubility should be in the range of 35 to 50 volume percent.

2. Toxicity.

   Toxicity should be judged by tissue culture and animal injection of the unmodified material (animal injection would be intraperitoneal). Intravenous replacement studies, in the form of an emulsion, may be done, but rigorous data with this type of testing would not be required.

3. Dwell time.

   No quantitative measures were agreed upon, but it was thought that a minimal residual amount should be present after one year.

4. The compound should not be metabolized.

5. Emulsion stability.

   a. Use of a nontoxic stabilizer should be possible.

   b. The material should form a nontoxic emulsion when given intravenously.

c. The emulsion should be stable on the shelf for one to two months and for a short period of time (several hours) when mixed with physiological solutions.

6. Vapor pressure.

The vapor pressure of the compound should be of such a value that it does not interfere with the respiratory function of the recipient.

7. The compound must have been successfully synthesized and large-scale production should be practical.

B. CANDIDATE HYBRID FLUOROCARBON ARTIFICAL BLOOD COMPOUND JPL-AB-6

1,3-bis(NONAFLUORO-t-BUTYL)PROPANE[1]

$(CF_3)_3C-(CH_2)_3-C(CF_3)_3$

$C_{11}H_6F_{18}$ (MW = 480.13)

Glass-like prism from methanol

Melting point 32° C

SUMMARY[2]

1. Oxygen Solubility

Calculated: 36.7 cm^3 0_2/100 cm^3 of JPL -AB-6 at 25° C

Found: 35.8 cm^3 0_2/100 cm^3 of JPL -AB-6 at 25° C

2. Toxicity

a. Tissue Culture. Studies by Professor Geyer at Harvard Medical School indicate that JPL-AB-6 was nontoxic in his tissue culture method.

b. Animal Studies. Following UBTL's Mouse Toxicity Test Protocol (see Appendix A), three mice were injected i.p. (intraperitoneally) with JPL-AB-6 emulsion (100 ml of 20% v/v emulsion/kg of animal weight) and showed no immediate effects that differed from the controls. One mouse was sacrificed at 24 hours for Absorption Verification Studies (AVS) and prepared for histopathology studies. Peritoneal recovery of FC from this mouse was 27%; histopathology studies showed essentially normal tissue patterns with only mild congestion. (The other two mice survived for one week and were then lost through a technician error.)

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[1] The IUPAC name is 2,2,6,6 - tetrakis(trifluoromethyl) 1,1,1,7,7,7-hexafluoroheptane.

[2] Data presented in the format suggested by NIH/NHLBI memo April 14, 1978 per meeting Atlantic City 4-15-78.

With these screening results, JPL-AB-6 was considered to be a promising candidate and following resynthesis, three more mice were studied. Again, one mouse was sacrificed at 24 hours for absorption verification and peritoneal wash. The other two mice were used for the two week Mouse Toxicity Study and survived without significant distress. One of these mice was sacrificed for histopathology studies which showed a "toxic" effect upon the liver with swollen vacuolated liver cord cells and clumped chromatin. The spleen showed focal areas of histiocyte response. The overall impression was that these changes were related to phagocytosis and the clearing mechanism of the body.

Fluoride ion was 2 to 3 ppm for the neat and 4 to 6 ppm for the sonicated emulsion.

In this study good stability was observed in the JPL-AB-6 material from the limited data available (see Table 2-1).

3. Dwell Time

Further resynthesis of JPL-AB-6 yielded 15.3 grams of JPL-AB-6 for more complete testing in a larger group of mice. A summary of the mice dosed with this FC (fluorochemical) is shown in Table 2-1, and a description of the table follows.

Three mice were injected intraperitoneally with the emulsion for the 24 hours Absorption Verification Study. These lived, but were sacrificed at 24 hours for G. C. assay of the peritoneal wash. Table 2-2 indicates that (through recovery) approximately 95% of the fluorocarbon was absorbed in 24 hours. This fast rate of absorption ensures that the mouse is exposed to the full dose physiologically.

Five mice were injected intraperitoneally with the emulsion for the two week Mouse Toxicity Screen (MTS). One mouse died on the second day, quiet and sick. The other four remained alive and well along with the controls and were sacrificed at two weeks according to the two-week Mouse Toxicity Screening Protocol. Two mice were preserved for a histopathology examination after their peritoneal wash. Recovery of JPL-AB-6 residues from the peritoneal washes and selected organ tissues of the remaining mice are shown in Table 2-3.

These data indicate that essentially all of the fluorocarbon has been absorbed and that approximately 80% of it has been eliminated from the body during the 14 days post-injection. This fluorocarbon compound appears to be similar to the FC-47 (perfluorotributylamine) in its initial distribution with

the spleen and liver scavenging most of the emulsion. These data are very encouraging because they indicate a reasonable rate of excretion of the compound. Its duration (in mice) is long enough to be effective as a blood replacement component, yet it appears to be excreted from the body in a reasonable period of time.

4. Metabolized Forms of JPL-AB-6

No metabolized form has been observed to date; further work should be done in this topic area.

5. Emulsion Stability

This compound exhibits relative stabiity as an emulsified system suitable for intraperitoneal injection. Pleuronic F-68 is apparently an adequate stabilizer exhibiting no obvious serious toxic effects. However, in-depth emulsification studies remain to be done and will become important when blood replacement studies are to be considered.

6. Shelf-Life Studies

During the screening studies done in the first year, a trend was noted with some compounds that suggested the possibility that some new FCs may become more toxic with aging. This was based on the observation that some severe reactions occurred in mice injected several days later with a given FC when compared to those initially dosed.

Table 2-1 shows that mice were still alive at about 1 month following the intra-peritoneal injection of both "aged" emulsion and "aged" neat. The neat was emulsified for injection after aging in that form.

One factor to consider with this series of JPL-AB-6 compared to last year's is that the FC was more carefully purified before testing.

The emulsion was aged for about 2 weeks and the neat for 4 weeks at 37°C, intermittently exposed to room air.

7. Vapor Pressure

The measured vapor pressure of solid JPL-AB-6 is 2.0 mm Hg at 30°C. The vapor pressure calculated for liquid AB-6 at 30° is 4.8 mm Hg. The equations used to predict vapor pressure are valid only for liquids and the discrepancy is in the correct direction.

Table 2-1. Fluorocarbon Tissue Distribution following Intraperitoneal Injection

SAMPLE	FC No. 6				FC No. 14				FC No. 32			
	24 Hours		14 Days		24 Hours		14 Days		24 Hours		14 Days	
	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected
Intra-peritoneal Wash	-	4.5	-	0.4	-	7.0	-	0.5	-	3.0	-	2.6
Liver	-	-	27.5	7.5	112.0	21.0	73.7	36.3	38.2	7.0	18.8	4.9
Spleen	-	-	87.9	9.2	166.0	4.6	87.9	12.6	159.0	2.8	82.4	5.4
Brain	-	-	0.05	0	3.1	0.2	0.5	0.02	6.0	0.4	0.6	0.1
Blood*	-	-	2.2	0.7	0	0	0	0	28.1	9.8	0	0
Kidney	-	-	-	-	26.7	1.4	2.7	0.4	25.4	2.2	2.6	0.4
Remainder of Body	-	-	1.7	4.7	0.1	14.7	0.02	0.1	0.4	1.2	0.2	0.4
TOTAL				22.5		48.9		49.9		26.4		13.8

*Blood volume of 10% of total body weight was assumed.

Table 2-2. Summary of Mice Dosed with New FCCs

FCC	Injection Form	Dose	Time Alive Post-Injection	Follow-up Studies	No. of Mice
JPL-AB-15					
	Emulsion	30 g/kg	Sacrificed-24 hours	Histopath (?)	1
			4 hours	----	2
	Neat	30 g/kg	4-6 days	----	2
			Sacrificed-5 weeks	----	1
	Reduced Dose Emulsion	3 g/kg	22 hours	----	3
		0.3 g/kg	4 days	----	1
			Alive - 1 month	----	2
		(All controls alive and well)			
JPL-AB-16					
	Emulsion	30 g/kg	2-3 days	----	3
			Sacrificed-24 hours	Perit. Wash, Tissue Levels, Histopath	6
	Neat	30 g/kg	Sacrificed-24 hours	Perit. Wash, Tissue Levels, Histopath	3
			Sacrificed-3 weeks	Perit. Wash, Tissue Levels, Histopath	3
	Reduced Dose Emulsion	3 g/kg	2 days	----	3
		0.3 g/kg	25 days	----	1
			Alive - 1 month	----	2
		(All controls alive and well)			
JPL-AB-20					
	Emulsion	30 g/kg	4-20 hours	----	3
	Neat	30 g/kg	2 days	----	1
		(All controls alive and well)			
JPL-AB-21					
	Emulsion	30 g/kg	21 hours	----	3
	Neat	30 g/kg	1-1/2 days	----	3
		(All controls alive and well)			

Table 2-3. 24-hour Absorption of AB-6

Mouse Number	Amount Recovered I.P.	% of Injected
125	29.79 mg	3.9%
126	25.41 mg	3.4%
127	51.45 mg	6.3%

Table 2-3. Tissue Content of AB-6 After 14 days

Tissue	Average mg/g tissue	Average mg/ organ	% of Injected
Peritoneum*	---	3.0	0.4
Liver	27.5	60.0	7.5
Brain	0.05	0.02	0
Blood**	2.2	5.4 (estimated)	0.7
Spleen	87.9	76.8	9.2
Remainder of Body (inc. Blood)	1.7	38.9	4.7
TOTAL		178.7	21.8

* Average of 4 mice. The remainder of the tissue averages were done on 2 mice.

** Figures shown for "Blood" are also included in "Remainder of Body" and are not repeated as part of the "Total."

8. Synthesis

The synthesis of JPL-AB-6 is a two-step process as follows:

(1) $CF_3CF=CF_2 \xrightarrow{750°} (CF_3)_2C=CF_2$

(2) $2(CF_3)_2C=CF_2 + ICH_2CH_2CH_2I \xrightarrow[\text{DMF}]{\text{KF}}$ at RT

$(CF_3)_3CCH_2CH_2CH_2C(CF_3)_3$

The experimental details can be found in the first annual report (JPL Document 77-80).

C. CANDIDATE HYBRID FLUOROCARBON ARTIFICIAL BLOOD COMPOUND JPL-AB-14

1,2-Bis(F-t-butyl)ethane[1]

$(CF_3)_3C\ CH_2CH_2(CF_3)_3$

$C_{10}H_4F_{18}$ (MW = 466.11)

Granular white solid from methanol

Melting Point 60 to 61°C

SUMMARY

1. Oxygen Solubility

Calculated 40.2 $cm^3/100\ cm^3$ of JPL-AB-14 at 25°C

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[1]The IUPAC name is 2,2,5,5,-tetrakis(trifluoromethyl)-1,1,1,6,6,6,-hexafluorohexane.

2. Toxicity

a. JPL AB-14. Two mice were injected intraperitoneally for the 24-hour Absorption Verification Study. The mice lived and were sacrified at the end of 24 hours. The spleen was very large, approximately two times normal size, and was whitish in color. The peritoneal recovery study indicated 7% remaining after 24 hours. Organ tissues were assayed for FCC levels in one mouse and the other underwent histopathological studies.

Because of the encouraging results of the Absorption Verification Study, two more mice were injected for the two-week Mouse Toxicity Screening. These mice were sacrificed at the end of the two-week period and FCC assays were done on peritoneal washes and tissues. All three had peritoneal washes performed, but only two were assayed for tissue levels, and the third was reserved for histopathological studies.

Pathology studies showed gross enlargement of the liver and especially the spleen at 24 hours and at 14 days. The spleen exhibited a gray-white cast. Histopathology studies on the 24-hour mouse showed "lipid-laden" macrophages in the spleen and other minor changes, but these changes are not as marked as seen in other test animals. Tissues from other organs showed only minimal changes. In the two-week mouse, lipid-laden macrophages were seen in the liver and spleen with fairly marked tissue reaction (swollen cells) throughout all other tissues examined.

b. Status. This FCC appears to be similar to JPL-AB-6, a promising compound studied in the first year. It is recovered for further study and more complete evaluation.

3. Dwell time

The FCC assay levels are shown in Table 2-1 and indicate that the FCC was readily absorbed from the peritoneum (93% in 24 hours and 99.5% in 14 days), that high concentrations are observed in the liver and spleen at 24 hours, that these concentrations in the liver and spleen drop by about one-half in 4 days, and that the FCC is about 50% eliminated at the end of 24 hours. We must emphasize, however, that Table 2 data was obtained from individual animals in some cases and an average of 2 or 3 animals at most. This accounts for some discrepancies in specific comparisons, yet the general trends are similar for all three FCCS.

4. Metabolized Form of JPL-AB-14

No metabolized form has been observed to date; further work should be done in this topic area.

5. Emulsion Stability

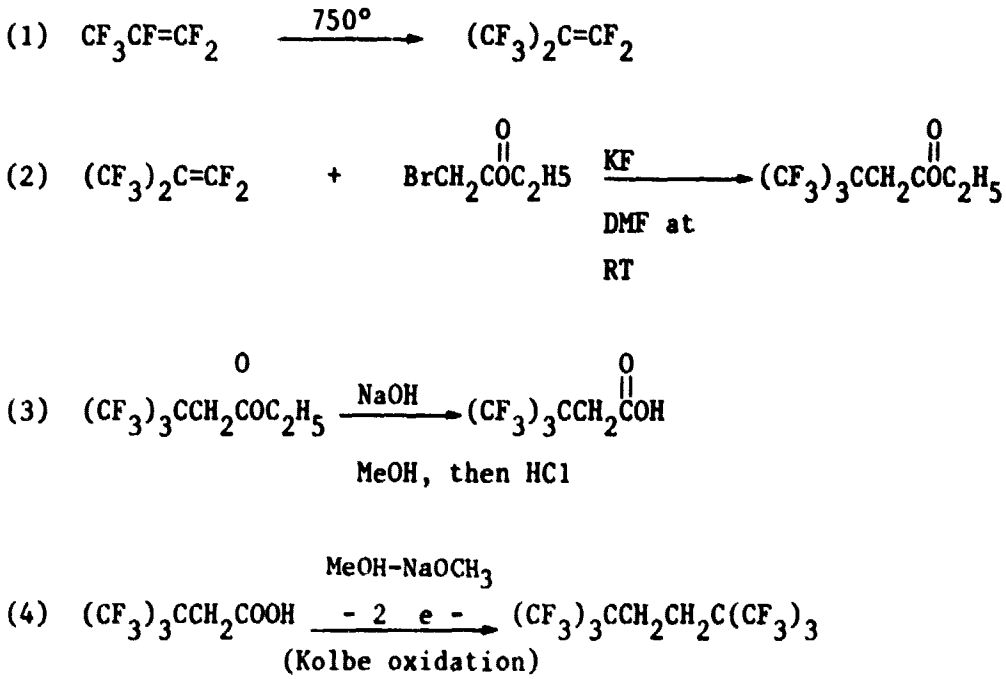
This compound exhibits relative stability as an emulsified system suitable for intraperitoneal injection. Pleuronic F-68 is apparently an adequate stabilizer exhibiting no obvious serious toxic effects. However, indepth emulsification studies remain to be done and will become important when blood replacement studies are to be considered.

6. Vapor Pressure

The calculated vapor pressure is about 13.9 mm. This value is well below the 40 to 50 mm upper limit suggested by Geyer and Clark.

7. Synthesis

The synthesis of JPL-AB-14 is a four-step process as follows:



D. CANDIDATE HYBRID FLUOROCARBON ARTIFICIAL BLOOD COMPOUND JPL-AB-32

4,4,7,7-tetrakis(trifluoromethyl)-1,1,1,2,2,3,3,8,8,9,9,-10,10,10-tetradecafluorodecane or
5-H,5-H,6-H-perfluoro-4,4,7,7-tetramethyldecane
$CF_3CF_2CF_2C(CF_3)_2CH_2CH_2C(CF_3)_2CF_2CF_{2}CF3$
$C_{14}H_4F_{26}$ (MW = 666.17)
Oily Liquid

SUMMARY

1. Oxygen Solubility

   Calculated 36.3 cm^3 0_2/100 cm^3 of JPL-AB-32

2. Toxicity

   a. JPL-AB-32. Two mice survived the 24-hour AVS study. One mouse was sacrificed for histopathological studies and one for FCC tissue assay. Two additional mice survived the two-week MTS study and were sacrificed for histopathological and tissue assay studies. The histopathological studies are pending.

   b. Status. This FCC is similar to JPL-AB-6 and -14 and classified as a promising compound.

3. Dwell Time

   Table 2-1 shows the FCC levels for this FCC. The data generally parallels that seen with JPL-AB-14. The FCC was readily absorbed and there were rather high concentrations in the spleen (liver moderate) at 24 hours dropping to about half in these organs in 14 days. About 74% of the total FCC injected was eliminated in 24 hours and 86% in 14 days. Of note is the relatively high concentrations in the blood at 24 hours, with a drop to zero at 14 days.

   This data, like that for JPL-AB-6 and -14, was obtained from single animals in some cases and the statistical validity is thus impaired. However, the trends are similar for all three FCCs.

4. Metabolized Forms of JPL-AB-32

   None have been observed to date; further work should be done.

5. Emulsion Stability

This compound exhibits relative stability as an emulsified system suitable for intraperitoneal injection. Pluronic F-68 is apparently an adequate stabilizer exhibiting no obvious serious toxic effects. However, indepth emulsification studies remain to be done and will become important when blood replacement studies are to be considered.

6. Vapor Pressure

The calculated vapor pressure is 1.4 mm. This is well below the 40 to 50mm upper limit suggested by Geyer and Clark.

7. Synthesis

The synthesis of JPL-AB-32 is a three step process as follows:

(1) $(CF_3)_2C{=}CFCF_2CF_3 + BrCH_2CO_2C_2H_5 \xrightarrow[DMA]{KF} CF_3CF_2CF_2C(CF_3)_2CH_2CO_2C_2H_5$

(2) $CF_3CF_2CF_2C(CF_3)_2CH_2CO_2C_2H_5 \xrightarrow[(2)\ HCl]{(1)\ KOH,\ ETOH} CF_3CF_2CF_2C(CF_3)_2CH_2CO_2H$

(3) $2CF_3CF_2CF_2C(CF_3)_2CH_2COOH \xrightarrow[2e-]{MeONa\ /\ MeOH} [CF_3CF_2CF_1C(CF_3)_2CH_2\text{-}]_2$

Step 3 is a KOLBE electrochemical oxidation reaction. The experimental details can be found in paragraph III C.

SECTION III

SYNTHESIS

A. INTRODUCTION

The synthesis of a series of novel fluorochemicals was carried out, followed by physical testing to verify structure and purity and to measure properties related to their performance in fluorochemical emulsion artifical blood.

The synthesis plan was guided by three basic concepts:

1. Convergent Synthesis. "Convergent" describes a synthetic design in which relatively large prefabricated subunits, which may or may not be identical, are assembled to make the final product, i.e.:

Convergent: $A + B \rightarrow AB$; $C + D \rightarrow CD$; $AB + CD \rightarrow ABCD$
(or 2 AB ABAB)

Non-convergent: $A + B \rightarrow AB$; $AB + C \rightarrow ABC$; $ABC + D \rightarrow ABCD$

A successful convergent synthesis leads to a readily purified final compound, since the individual subunits typically differ greatly in physical properties from the product of their combination. Routes to highly fluorinated materials which introduce many fluorines at one time, even though the reaction may be carried out in a single reactor as a single operation, involve a great many intermediate steps and often lead to hard-to-separate mixtures of closely related products.

2. Use of readily available fluoropolymer monomers as the subunits containing most of the fluorine. Technology for the commercial production of C_2-C_4 perfluoroolefins is highly developed, and almost certainly offers the cheapest route to highly fluorinated organic compounds.

3. Preparation of partially fluorinated candidate compounds composed of nonfluorinated and completely fluorinated (i.e., all C-H bonds replaced by C-F) subunits. This concept was based on the conviction that inclusion of nonfluorinated fragments can lead to synthetic and economic advantages via

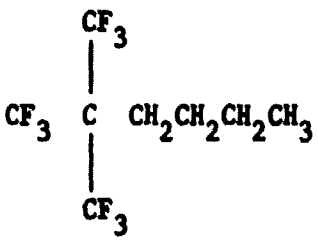
concepts (1) and (2), that the physical properties of the partially fluorinated materials will fall in the acceptable range, and that an understanding of the chemical-structural factors which contribute to toxicity, emulsion properties, circulatory lifetime and ultimate bodily disposition of fluorochemicals is still so fragmentary that examination of such structures is still warranted.

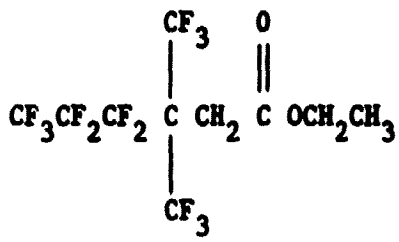
It should be pointed out here that the advantages potentially derivable from concepts (1) and (2) are not dependent on the validity of concept (3).

B. CHEMICAL APPROACH

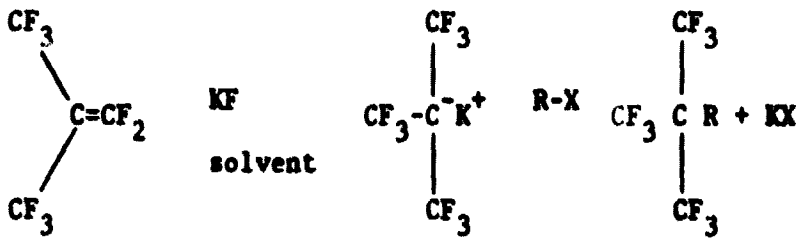
1. Introduction

The primary objective of the chemical synthesis carried out at JPL was to produce low cost fluorine containing compounds with oxygen solubilities of at least 30 ml of dissolved O_2/100 ml of pure compound and vapor pressures of less than 40 torr at 37.5°C. Specifically, we have synthesized fluorocarbon-hydrocarbon hydrid compounds where the fluorocarbon segment is linked to the hydrocarbon portion of the molecule via a tertiary carbon in the fluorocarbon moiety, e.g.:



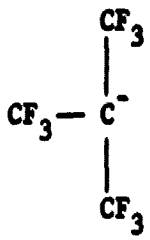


This was accomplished by the generation from suitable fluoroolefins and KF or CsF in dipolar-aprotic solvents of tertiary fluorocarbanions. These are then alkylated with hydrocarbon alkyl halides:

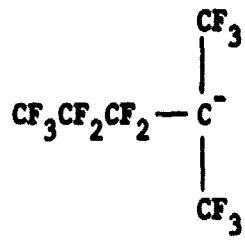


R-X = alkyl halide

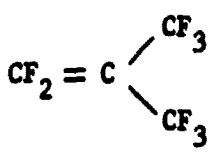
In the second year explorations were performed principally in regard to the reactivity of carbanions (a) and (b) formed from octafluoroisobutene (c) and F-2-methyl-2-pentene (d) respectively (below).



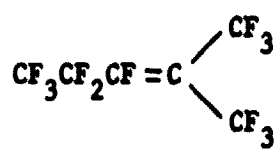
(a)



(b)



(c)



(d)

Near the end of the first year success was attained in producing efficiently, reproducibly, and in kilogram quantities, the thermodynamically stable dimer of hexafluoropropene, F-2-methyl-2-pentene.

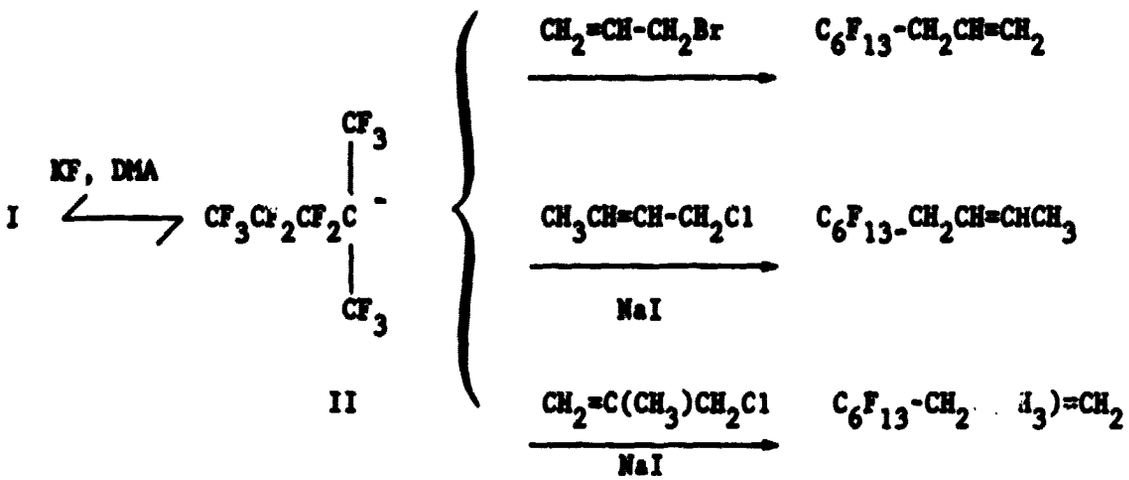
$$2\ CF_3CF = CF_2 \xrightarrow[DMA]{KF} CF_3CF_2CF = C(CF_3)_2 \quad (I)$$

Exploitation of the chemistry of this dimer was begun and conditions were discovered for converting it by simple chemistry, in good yield and purity, to the hybrid fluorochemicals originally proposed.

2. Direct alkylation of the tertiary center.

When F-2-methyl-2-pentene is stirred at room temperature with dry, powdered, potassium fluoride suspended in dimethylacetamide, the intermediate

tertiary carbanion is not detectable by ^{19}F NMR, but its reaction products with allylic halides are slowly formed in high yield, without any discernable side-reactions.



Allylic chlorides alone do not react at an acceptable rate, but do react in the presence of a catalytic amount of sodium iodide, which generates an equilibrium concentration of allylic iodide by nucleophilic displacement.

While success was not attained last year in hydrogenating $(CF_3)_3CCH_2CH=CH_2$ in solution over the usual catalysts at low hydrogen pressure, it was found that each of the above olefins may be reduced very cleanly in the gas phase by passing the olefin vapor in a stream of excess hydrogen through a column packed with palladized firebrick (see paragraph III C for the procedures). The yields of the saturated perfluoroalkylalkanes appear to be quantitative except for small handling losses; no HF or any by-products indicative of carbon-fluorine or carbon-carbon cleavage have been detected.

3. Synthesis and alkylation of perfluoro-2-methyl-2-pentanol

The reaction of carbanion II above with a nitrosating agent gives the corresponding nitroso compound, which may be oxidized <u>in situ</u> or isolated and oxidized in a separate step to the nitrite, which is in turn quantitatively hydrolyzed to the alcohol.

II $C_6F_{13}I \xrightarrow[NOCl]{XS\ N_2O_4} C_6F_{13}NO$ or $C_6F_{13}I \xrightarrow[O_2]{N_2O_4} C_6F_{13}ONO$

or

$$C_6F_{13}ONO \xrightarrow{KOH} C_6F_{13}OK \xrightarrow{H_2SO_4} C_6F_{13}OH$$

The tertiary alcohol is so acidic that it is readily soluble in aqueous potassium hydroxide and easily separated from small amounts of accompanying neutral byproducts (still being identified).

The potassium salt may be prepared directly in dimethyl sulfoxide solution by reaction of the alcohol with solid KOH, and alkylated in situ in high yield with a primary alkyl iodide or sulfonate.

$$C_6F_{13}\text{-}OH \xrightarrow[RI]{KOH,\ DMSO} C_6F_{13}OR$$

By this method the methyl, ethyl, n-propyl, ethers, isopropyl and n-butyl were prepared.

4. Other Chemistry:

Reduction of $C_6F_{13}NO$ has been found so far to give the corresponding hydroxylamine, but other conditions will be explored in the hope of obtaining the amine, $C_6F_{13}NH_2$ from the readily-prepared nitroso compound.

$$C_6F_{13}NO \xrightarrow[CF_3COOH,\ r.t]{Zn(Hg),HCl} C_6F_{13}NHOH$$

It was discovered that the perfluoro-tert-hexyl and perfluoro-tert-butyl-carbanions can be coupled with p-nitrobenzenediazonium fluoborate in dimethyl-acetamide, although previous attempts to use that diazonium salt in dimethyl-formamide gave only decomposition products.

$$(CF_3)_2C{=}CF_2 + KF + p\text{-}NO_2C_6H_4\text{-}N_2^+ BF_4^- \xrightarrow[-20°]{DMA} (CF_3)_3C\text{-}N{=}N\text{-}C_6H_4\text{-}p\text{-}NO_2$$

Reduction of the $(CF_3)_3C$- derivative with amalgamated zinc in acetic acid gives the corresponding amine. The same reaction has been performed with the C_6F_{13}- analog and is reported under paragraph C.

An experimental procedure follows for each of the new compounds which was isolated in pure form during this effort.

C. EXPERIMENTAL

1. Synthetic Methods

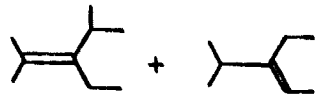
The following is a compilation of the synthetic procedures for pertinent starting materials and final test compounds synthesized at JPL during this contract year.

All synthetic procedures up to and including alkaline or aqueous washes of crude FC products were carried out in a good chemical fume hood, and further procedures were performed in the hood so far as practicable. F-olefins, especially octafluoroisobutene (OFIB) are known to be toxic, and other new substances were considered toxic until shown not to be. Procedures involving N_2O_4, a strong oxidant, or glass vessels under vacuum or pressure were conducted behind a safety shield. Especially hazardous procedures are so noted.

The infrared and NMR spectra are presented with each compound description. The NMR spectra were obtained on a Varian T-60 or HR-60 system. The infrared spectra were run on a Perkin-Elmer Infrared Model 137. Elemental analysis was done at California Institute of Technology Analytical Laboratory.

Table 3-1 includes a summary of compounds synthesized and tested, (along with their designation code number) during the first year of the program (Reference 20). Table 3-2 summarizes compounds prepared during the second year.

Table 3-1. Fluorochemical Compounds Synthesized and Submitted for Testing to UBTL

Designation	Structure
JPL-AB-1	$(CF_3)_3C\text{-}CH_2\text{-}CH{=}CH_2$
JPL-AB-2	(C_9F_{18}) 
JPL-AB-3	$(CF_3)_3C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$
JPL-AB-4	$(CF_3)_3C\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}CH_2\text{-}CH_3$
JPL-B-5	$(CF_3)_3CNH_2$
JPL-AB-6	$(CF_3)_3C\text{-}(CH_2)_3\text{-}C(CF_3)_3$
JPL-AB-7	$(CF_3)_3COH$*
JPL-AB-8	$(CF_3)_3CCFClCFClCF_3$
JPL-AB-9	$(CF_3)_3C\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}C(CF_3)_3$
JPL-AB-10	$(CF_3)_3C(CH_2)_3I$
JPL-AB-11	$CF_3CF_2CF_2(CF_3)_2CCH_2CH{=}CH_2$
JPL-AB-12	$(CF_3)_3CCH_2CO_2^-\ Na^+$
JPL-AB-13	$(CF_3)_3C(CH_2)_5C(CF_3)_3$
JPL-AB-14	$(CF_3)_3C(CH_2)_2C(CF_3)_3$

*A commercial material obtained from PCR, Gainesville, FL.

Table 3-2. Fluorochemical Compounds Synthesized During the Second Year

DESIGNATION	STRUCTURE
JPL-AB-15	$CF_3CF_2CF_2(CF_3)_2C(CH_2)_2CH_3$
JPL-AB-16	$CF_3CF_2CF_2(CF_3)_2C(CH_2)_3CH_3$
JPL-AB-17	$CF_3CF_2CF_2(CF_3)_2CH_2CH(CH_3)_2$
JPL-AB-18	$CF_3CF_2CF_2(CF_3)_2COH$
JPL-AB-19	$CF_3CF_2CF_2(CF_3)_2COCH_3$
JPL-AB-20	$CF_3CF_2CF_2(CF_3)_2COCH_2CH_3$
JPL-AB-21	$CF_3CF_2CF_2(CF_3)_2COCH_2CH_2CH_3$
JPL-AB-22	$CF_3CF_2CF_2(CF_3)_2CCH_2OCH_2CH_3$
JPL-AB-23	$CF_3CF_2CF_2(CF_3)_2CNO$
JPL-AB-24	$CF_3CF_2CF_2(CF_3)_2CCH_2CO_2CH_2CH_3$
JPL-AB-25	$(CF_3)_3CCH_2CH_2OCH_2CH_3$
JPL-AB-26	$(CF_3)_3CCH_2CH_2OCH_2CH_2C(CF_3)_3$
JPL-AB-27	$CF_3CF_2CF_2(CF_3)_2COCH(CH_3)_2$
JPL-AB-28	$CF_3CF_2CF_2(CF_3)_2CO(CH_2)_3CH_3$
JPL-AB-29	$CF_3CF_2CF_2(CF_3)_2CCH_3$
JPL-AB-30	$CF_3CF_2CF_2(CF_3)_2CNHOH$
JPL-AB-31	$CF_3CF_2CF_2(CF_3)_2CCH_2CH_3$
JPL-AB-32	$[CF_3CF_2CF_2(CF_3)_2CCH_2-]_2$
JPL-AB-33	$(CF_3)_3CO(CH_2)_3CH_3$
JPL-AB-34	$(CF_3)_3CO(CH_2)_3OC(CF_3)_3$
JPL-AB-35	$(CH_3)_3C(CH_2)_2C(CH_3)_3$
JPL-AB-36	$CF_3CF_2CF_2(CF_3)_2CNH_2$

Table 3-3. Fluorochemical Compounds Synthesized During the Second Year (Continuation)

DESIGNATION	STRUCTURE
JPL-AB-37	$CF_3CF_2CF_2(CF_3)_2COC(CH_3)_3$*
JPL-AB-38	$CF_3CF_2CF_2(CF_3)_2COCH_2CO_2C_2H_5$*
JPL-AB-39	$CF_3CF_2CF_2(CF_3)_2CCH_2COOH$
JPL-AB-40	$CF_3CF_2CF_2(CF_3)_2CCH_2CH{=}CHCH_3$
JPL-AB-41	$CF_3CF_2CF_2(CF_3)_2CCH_2(CH_3)C{=}CH_2$
JPL-AB-42	$pNO_2C_6H_4N{=}NC(CF_3)_2CF_2CF_2CF_3$

* Synthesis attempted

2. Vapor-phase Catalytic Hydrogenation: General Procedure

Thirty g of Chromosorb P (40 mesh) was slurried in 10% aqueous hydrochloric acid and heated on the steam bath for ca. one hour, then washed by decantation with distilled water. This procedure was repeated twice. Then 50 ml of concentrated hydrochloric acid and 1.0 g of $PdCl_2$ was added and the mixture was taken to dryness on the rotary evaporator under aspirator pressure, using a steam bath as the heat source.

Twelve point six (12.6)g of the $PdCl_2$ -treated support was packed into the inner coil of a reflux condenser. The coil was heated by steam from a flask of boiling water while hydrogen was passed through at 100 - 150 cc/min until the color of Chromosorb turned from orange to dark gray (about 0.5 hour).

For reduction of a volatile liquid substrate, the column was preceded by an evaporator having a glass frit for the hydrogen to bubble through, a thermometer well to monitor the temperature of the liquid, and a heat source. Following the column was a trap cooled in a dry ice-isopropanol bath and a soap-bubble flowmeter (See Figure 3-1).

With C_{10} olefins, the evaporator was held at 80 - 85° and the hydrogen flow set at about 135 cc/min. Throughput was approximately one gram/hour. When unreacted olefin appeared in the condensate, as determined by PMR, the catalyst was replaced. One charge was sufficient to reduce at least 100 g of distilled olefin. The apparatus was set up in a hood.

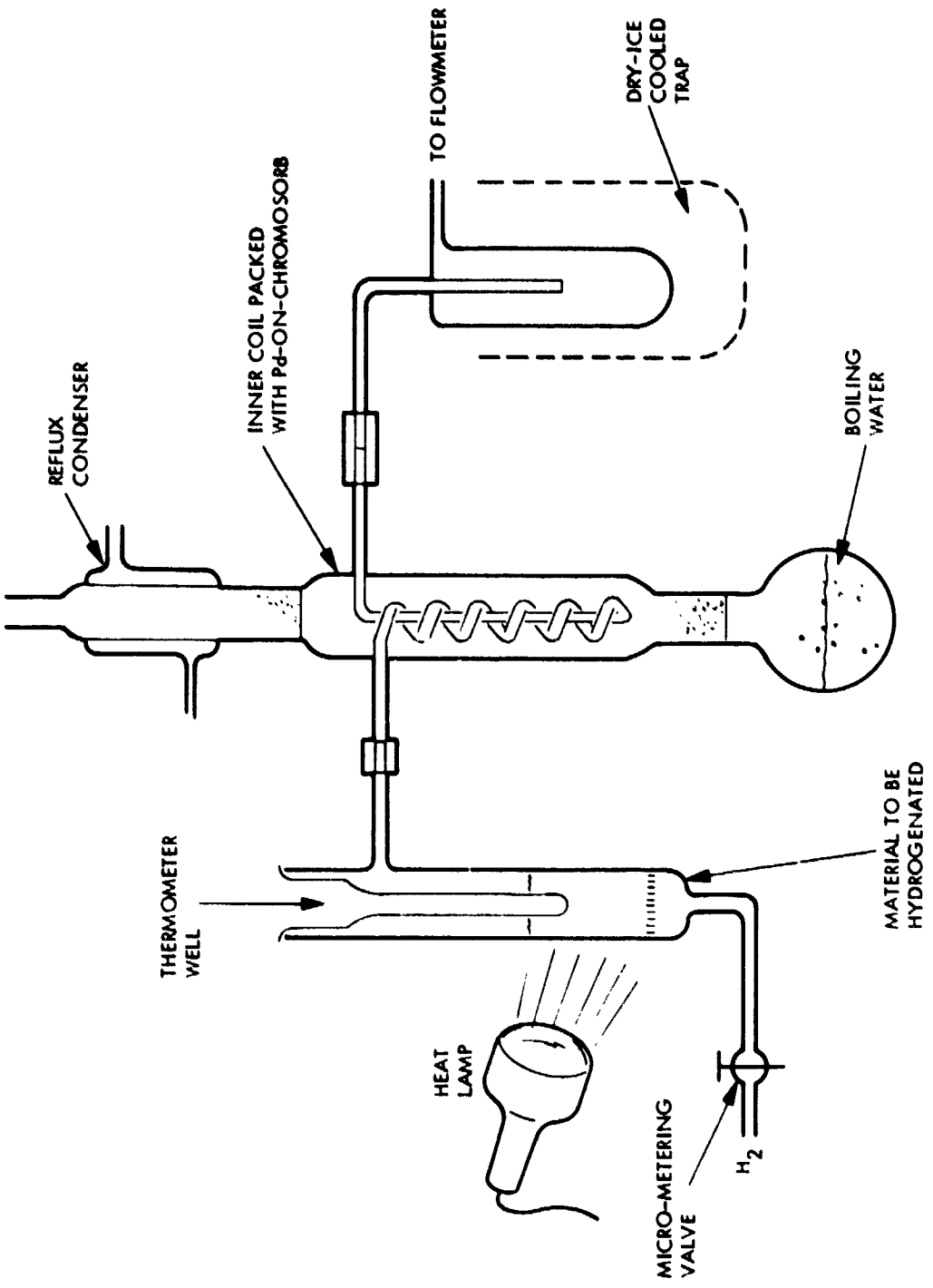


Figure 3-1. Vapor Phase Hydrogenation Apparatus

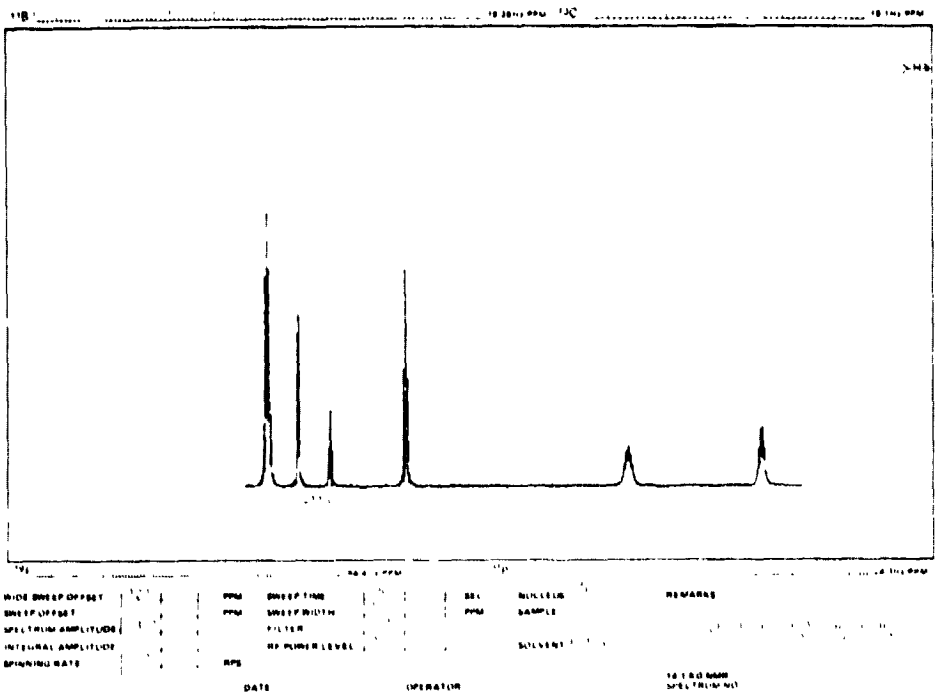
3. 4,4-Bis(trifluoromethyl)-1,1,1,2,2,3,3,-heptafluoroheptane (JPL-AB-15)

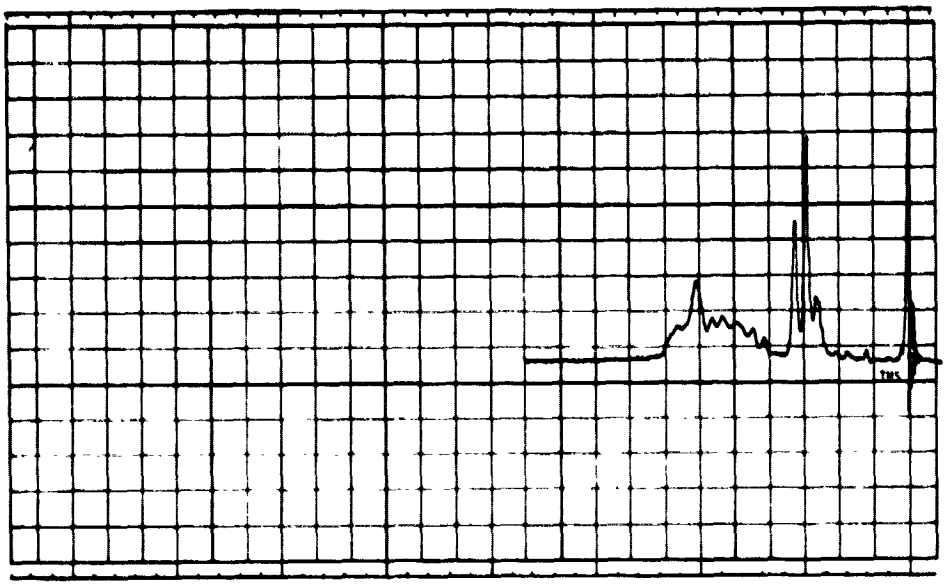
$$CF_3CF_2CF_2C(CF_3)_2CH_2CH{=}CH_2 \xrightarrow[\text{Cat.}]{H_2} CF_3CF_2CF_2C(CF_3)_2CH_2CH_2CH_2$$

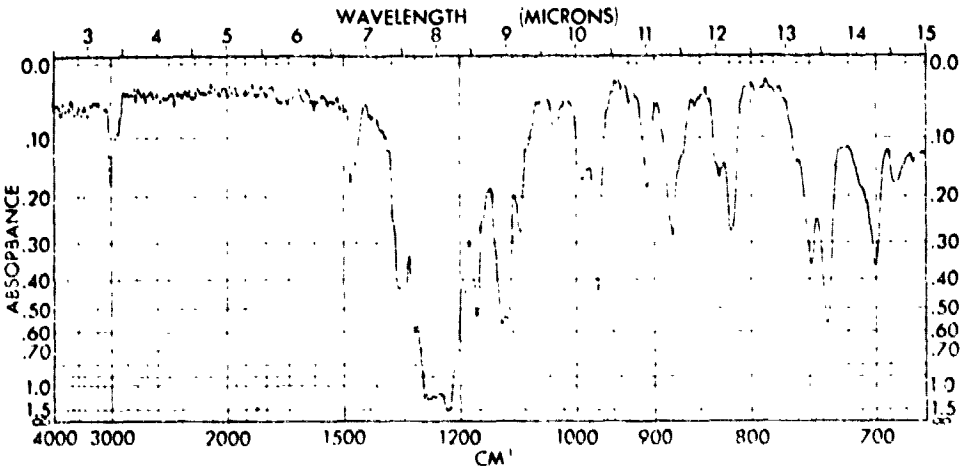
A total of 72.8 g of crude 4,4-bis-(trifluoromethyl)-1,1,1,2,2,3,3-heptafluoro-6-heptene (JPL-AB-11)was reduced by the general procedure for vapor phase hydrogenation, except in this first example an earlier version of the apparatus without the thermometer well and flowmeter was used. Sixty-two point nine (62.9) g of colorless saturated product was collected, 86%, and 3.9 g of tarry residue remained in the evaporator. The condensate was stirred for two days with 96% H_2SO_4, separated, dried over solid potassium hydroxide, and distilled at aspirator pressure. Yield, 56 g of water-white product showing no detectable olefinic hydrogen by PMR. Boiling point 121/719 mm, D^{24} 1.516 g/cm^3. JPL-AB-15 is a new compound.

Calculated for $C_9F_{13}H_7$ (362.13): C, 29.85%; H, 1.95%; F, 68.20%.

Found: C, 29.73%; H, 1.97%.







SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

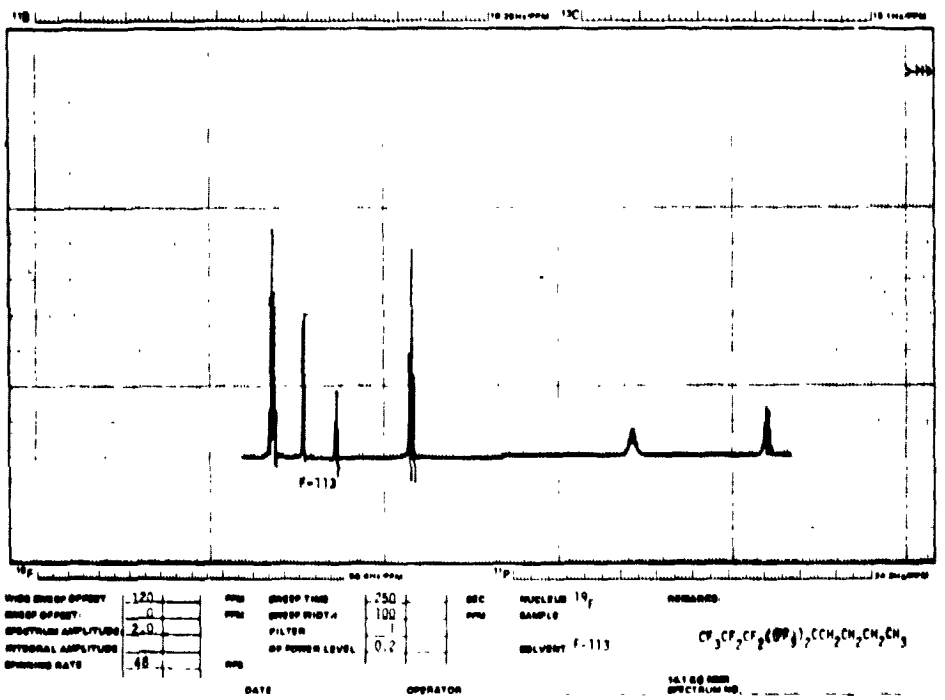
4. 4,4Bis(trifluoromethyl)-1,1,1,2,2,3,3,-heptafluorooctane (JPL-AB-16)

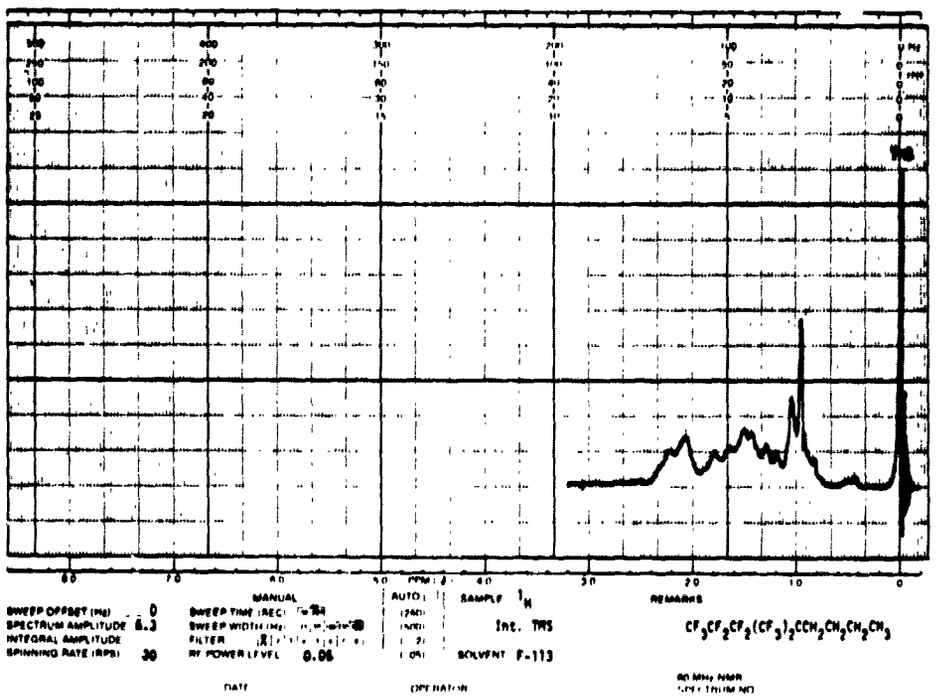
$$CF_3CF_2CF_2C(CF_3)_2CH_2CH{=}CHCH_3 \xrightarrow[\text{cat.}]{H2} CF_3CF_2CF_2C(CF_3)_2CH_2CH_2CH_2CH_3$$

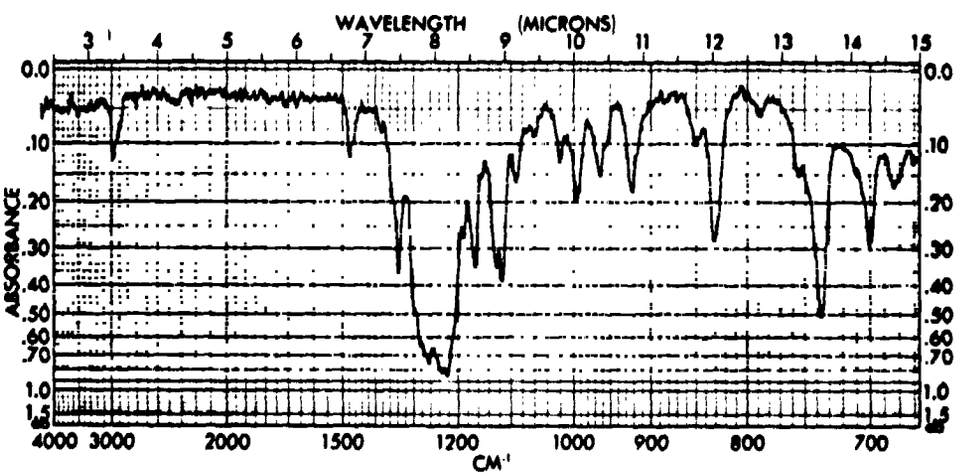
Olefin $CF_3CF_2CF_2C(CF_3)_2CH_2CH{=}CHCH_3$ (JPL-AB-40), 78.7 g, was hydrogenated by the general procedure for vapor-phase hydrogenation. The crude product was dried over solid KOH and redistilled. Yield 77.2 g, 97.6%. Boiling Point 137.5°C/730mm D^{24} 1.466 g/cm^2. JPL-AB-16 is a new compound.

Calculated for $C_{10}H_9F_{13}$ (376.16): C, 31.93%; H, 2.41%; F, 65.66%;

Found: C, 31.92%; H, 2.80%.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2CH_2CH_3$	PURITY	2.	
	PHASE Liq/Vap	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

5. 4,4-Bis(trifluoromethyl)-1,1,1,2,2,3,3-heptafluoro-6-methylheptane (JPL-AB-17)

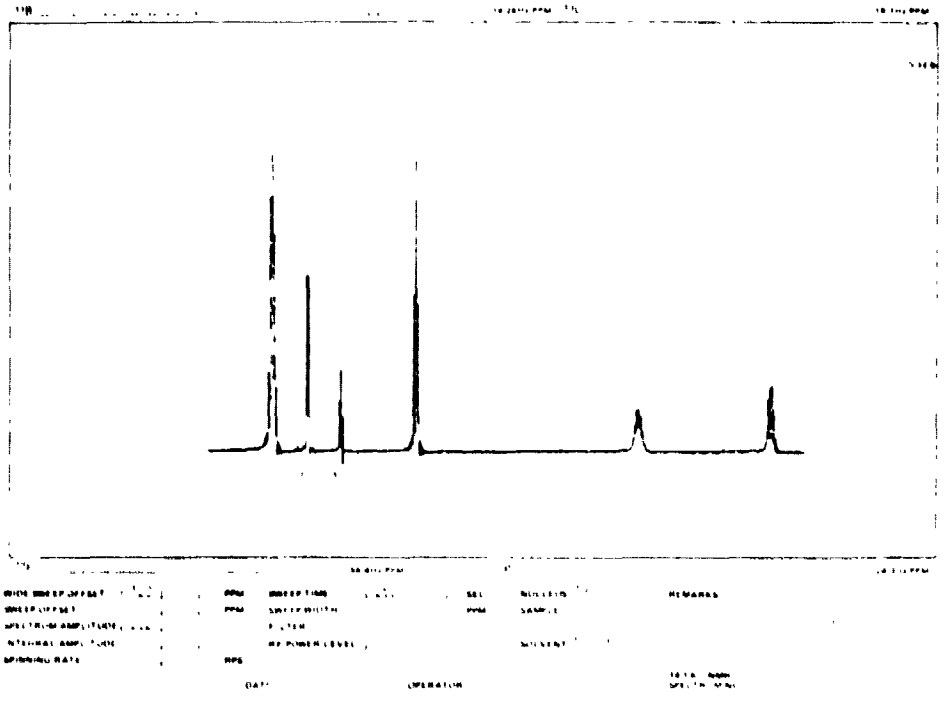
$$CF_3CF_2CF_2C(CF_3)_2CH_2C(CH_3)=CH_2 \xrightarrow[\text{Cat.}]{H_2} CF_3CF_2CF_2C(CF_3)_2CH_2CH(CH_3)_2$$

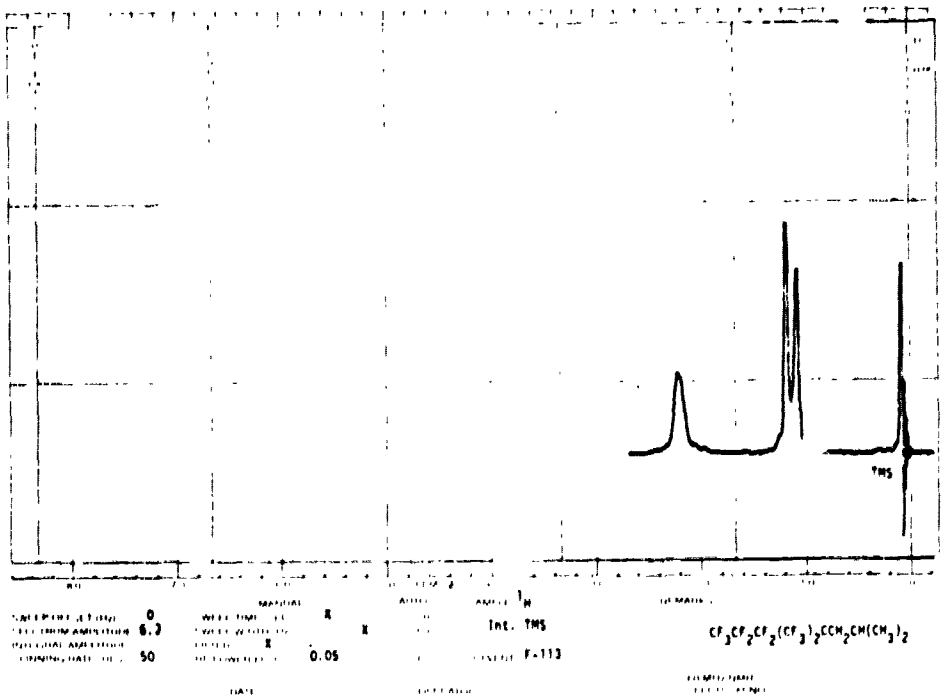
Olefin $CF_3CF_3CF_2C(CF_3)_2CH_2C(CH_3)=CH_2$ (JPL-AB-41), 103.1 g, was reduced by the general procedure for vapor-phase hydrogenation. Samples of product were withdrawn for spectroscopy and preparative gc purification and the balance distilled from solid KOH. Boiling Point 132°C/721 mm, D^{24} 1.453 g/cm^3. JPL-AB-17 is a new compound

Calculated for $C_{10}H_9F_{13}$ (376.16): C, 31.93%; H 2.41%; F, 65.66%.

Found: C, 31.95%; H, 2.44%.

C, 31.99%; H, 2.53%.





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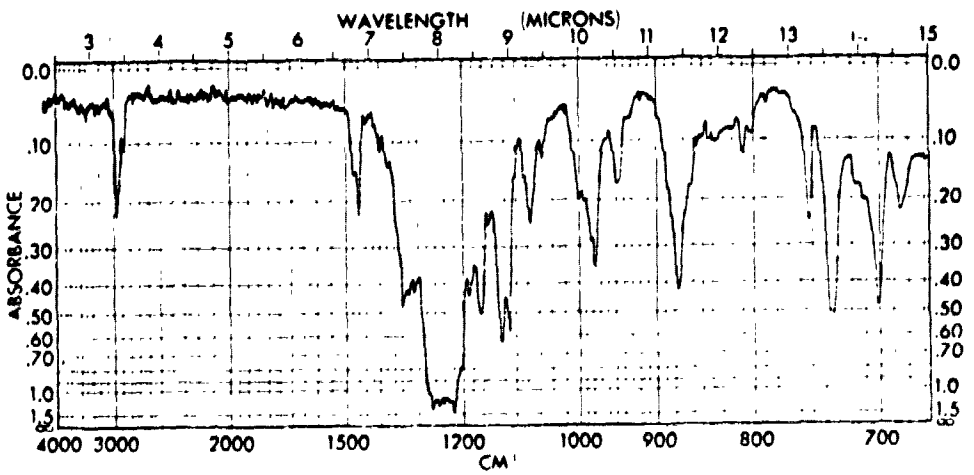


SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2CH(CH_3)_2$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

6. Perfluoro-2-methyl-2-pentanol (JPL-AB-18)

$$(CF_3)_2C{=}CFCF_2CF_2CF_3 + N_2O_4 \xrightarrow[DMA]{KF} [C_5F_{13}NO] \rightarrow CF_3CF_2CF_2C(CF_3)_2OH$$

The apparatus was set up with a hood and safety shield.

Dry KF, 50g; dimethylacetamide, 500 ml, and perfluoro-2-methyl-2-pentene, 171.3 g (0.571 mol) were combined in a one-liter 3-neck flask and stirred at 4 - 7° (external ice-salt bath) while 109 g N_2O_4 (1.18 moles) was slowly distilled in a slight flow of O_2. A -28°C (boiling CF_2Cl_2) reflux condenser was used to minimize escape of NO_2. The addition was slightly exothermic and was interrupted whenever the pot temperature reached +7°. Immediately after the addition the reaction mixture was deep blue; FMR examination of the lower fluorochemical phase showed complete consumption of starting olefin and a single predominate product, subsequently shown to be $CF_3CF_2CF_2C(CF_3)_2NO$. The reaction mixture was let warm slowly (the ice-salt bath left in place) to room temperature with stirring. After 18 hours the blue color had disappeared and the lower phase was light yellow. After ca. 48 hours additional, most of the fluorochemical seemed to be dissolved in the dimethylacetamide, and much white solid was present. Water, ca. 400 ml, and 6.2 g H_3BO_3 (0.1 mol) were added; the lower phase re-formed and most of the solid dissolved. Sulfamic acid, H_2NSO_3H, was added in small portions to destroy the nitrous acid present, and the reaction mixture was distilled at atmospheric pressure until the condensate was clear. The distillate consisted of a lower fluorochemical phase and an upper aqueous phase, both almost colorless. Potassium hydroxide, 37.4 g (0.66 mol), was added to the distillate, whereupon most of the lower phase dissolved in the aqueous layer. A small amount of neutral material was separated by vacuum distillation into a -78° trap, then the clear aqueous KOH solution was acidified with H_2SO_4 and distilled at aspirator pressure into a dry ice cooled receiver. Yield 108.9 g of the perfluoro alcohol as a dense, water-white liquid, 56.7%. Boiling Point 93/728 mm D^{24} 1.770 g/cm^3. (Note: see preparations of JPL-AB-23 for nitroso compound). JPL-AB-18 is a new compound.

Calculated for $C_6F_{13}OH$ (33 0%): C, 21.45%; H, 0.30%; 0, 4.76%; F, 73.49..

Found: C, 21.19%; h, 0.49%.

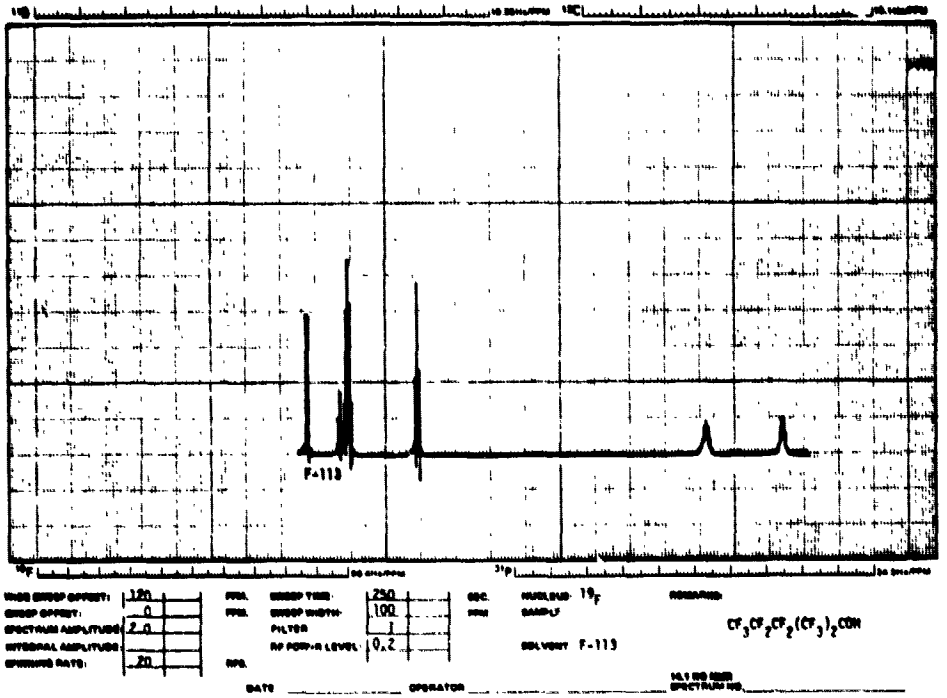
FMR: 0.9 ppm, 6F, tt; 8.8 ppm, 3F, t; 41.9 ppm, 2F, m; 50.9 ppm, 2F, m, all upfield of the t of $CF_2ClCFCl_2$.

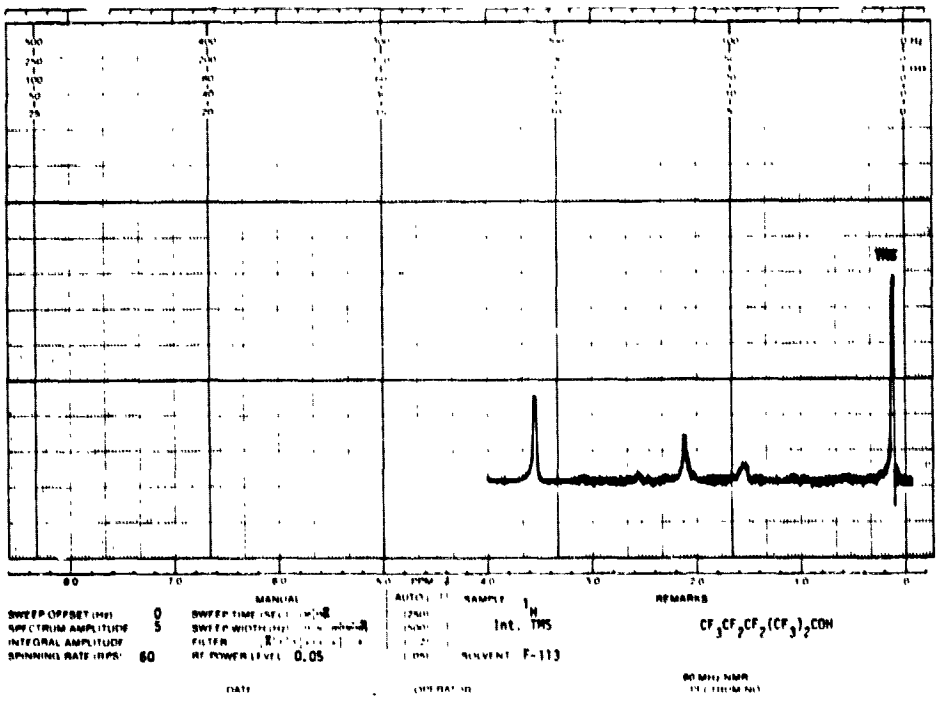
The neutral fraction weighed 17.5 g (approximately 9% yield) and consisted of a mixture of two still unidentified $CF_3CF_2CF_2C(CF_3)_2X$ products, possibly one with X = NO_2, the other X = ONO_2. The $C(CF_3)_2$ tt of the major ~60%) component appears 4.1 ppm downfield of the d of $CF_2ClCFCl_2$; the $C(CF_3)_2$ tt of the minor component at 0.4 ppm downfield.

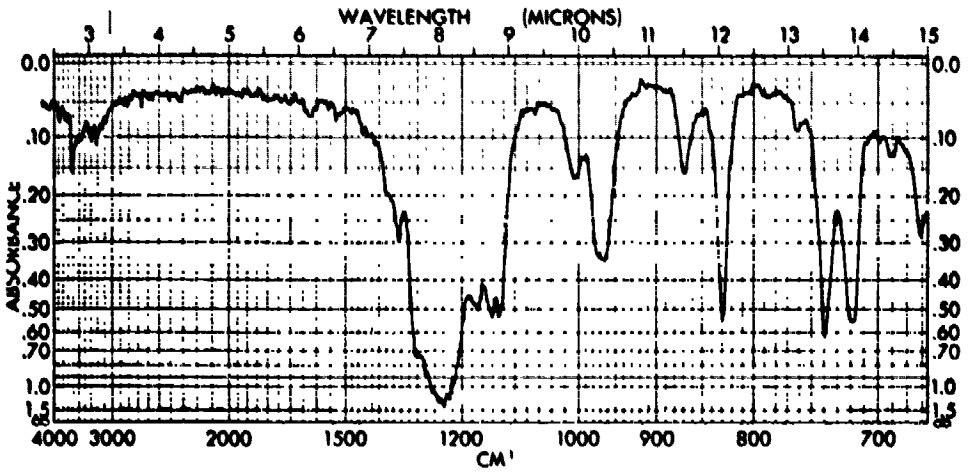
7. Improved Preparation of Perfluoro-2-methyl-2-pentanol (JPL-AB-18)

The apparatus was set up with a hood and safety shield.

Dried potassium fluoride, 58 g(1.0 mol); 550 ml dimethylacetamide; and 201.3 g (0.67 mol) perfluoro-2-methyl-2-pentene were combined and stirred magnetically in a one-liter 3 N flask equipped with cold finger condenser, thermometer well, and drying tube. The mixture was cooled to -20° in a dry ice-isopropanol bath, and nitrosyl chloride was passed in from a lecture bottle, keeping the temperature below -5°, until a green color persisted (the reaction product forms a clear blue lower layer, and the presence of excess yellow-brown NOCl causes the mixture to appear green). An fmr spectrum of the blue lower layer showed complete consumption of starting material. The reaction mixture was poured into a 2-liter separatory funnel containing 30 g of boric acid in ca. one liter of ice water. The blue lower layer was drawn off and without further purification, placed in a 250 ml 3 N flask equipped with a cold finger condenser and oxidized with a slow stream of oxygen. The blue color turned green, then yellow within 30 minutes. The oxidation is exothermic. The crude yellow nitrite ester was poured into 300 ml of water and stirred until gas evolution ceased (NO + NO_2), then made strongly basic by addition of 60 g KOH pellets. Insoluble material (40.8 g) was removed by direct steam distillation; distillation was stopped when the distillate ran clear of insoluble material. The residual aqueous solution was cooled and acidified with sulfuric acid. The lower layer which formed was separated, mixed with 75 ml of 96% H_2SO_4, and the mixture distilled at aspirator pressure into a dry ice-cooled receiver. Yield 145.5 g (64.5% of colorless product, pure by fmr. JPL-AB-18 is a new compound.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2CF_2(CF_3)_2COH$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

8. 2-Methoxy-perfluoro-2-methylpentane (JPL-AB-19)

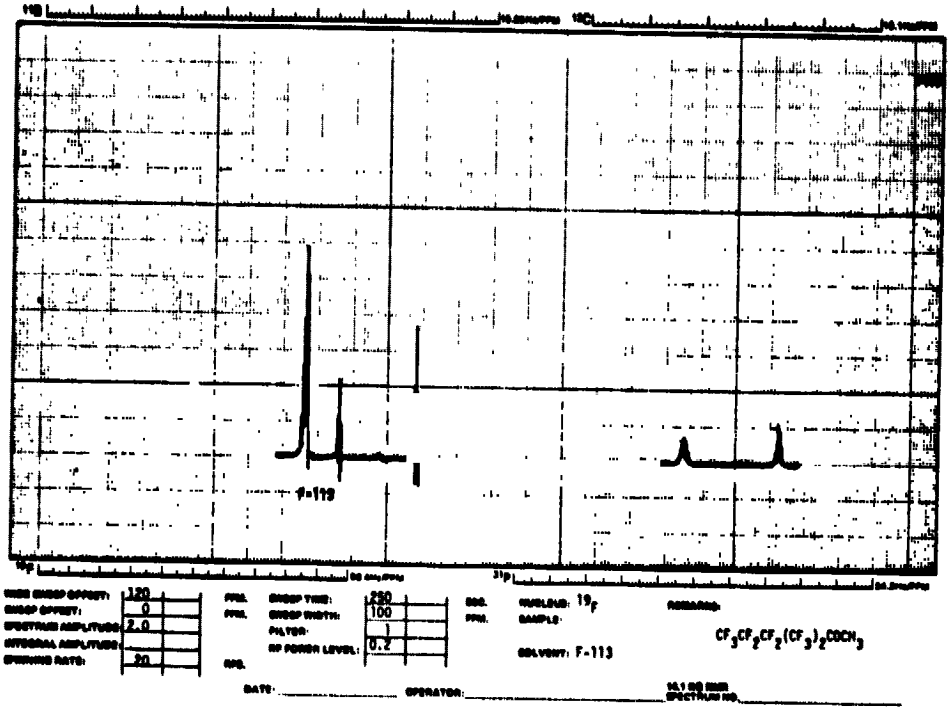
$$CF_3CF_2CF_2C(CF_3)_2OH + KOH \xrightarrow[DMSO]{} C_6F_{13}OK$$

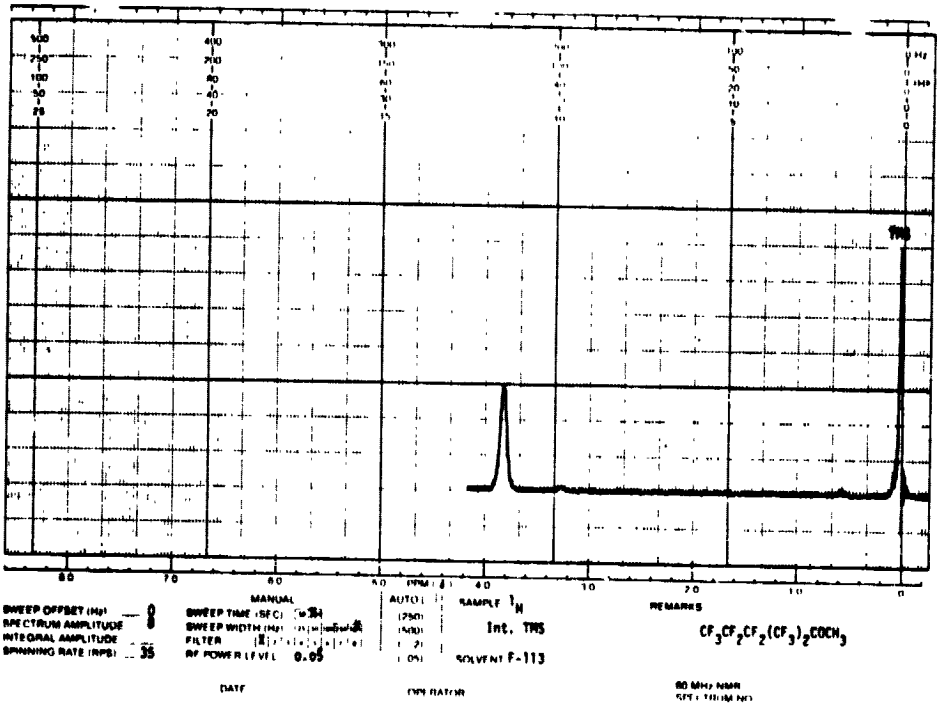
$$C_6F_{13}OK + (CH_3O)_2SO_2 \longrightarrow CF_3CF_2CF_2C(CF_3)_2OCH_3$$

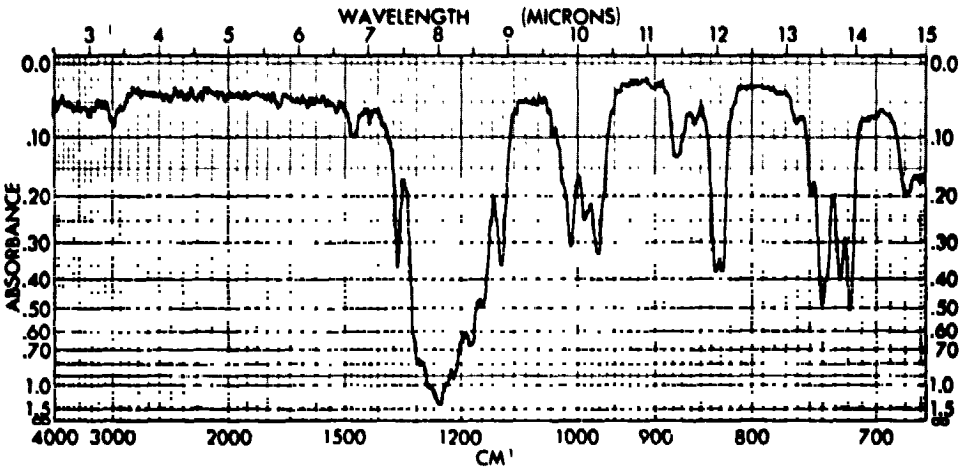
The apparatus was set up with hood and safety shield.

Perfluoro-2-methyl-2-pentanol, 25.0 g (0.0744) mol) was mixed with 125 g of dimethylsulfoxide. A single phase was present. Solid KOH (85% pellets, 7.7 g, 0.12 mol) was added with stirring; the KOH partly dissolved and the $C(CF_3)_2$ m shifted about 2.6 ppm upfield relative to the $-CF_2CF_3$ m. Dimethyl sulfate, 12.6 g (0.10 mol) was added; the solution immediately became turbid and within a few minutes a lower phase separated. The mixture was stirred 5 hours at room temperature and poured into water; the lower phase was separated, washed with dilute HCl, treated with solid KOH, distilled at aspirator pressure into a dry ice-cooled receiver, washed with 95% H_2SO_4, distilled again, and again treated with solid KOH. Yield 19.6 g, 75.3%, Boiling Point 97°C/728 mm, D^{24} 1.617. JPL-AB-19 is a new compound.

Calculated for $C_7H_3OF_{13}$ (350.07): C, 24.02%; H, 0.86%; O, 4.57%; F, 70.55%.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2COCH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR tfi	

SPECTRUM NO. SAMPLE

9. 2-Ethoxy-perfluoro-2-methylpentane (JPL-AB-20)

$$CF_3CF_2CF_2C(CF_3)_2OH + KOH \xrightarrow[DMSO]{} C_6F_{13}OK$$

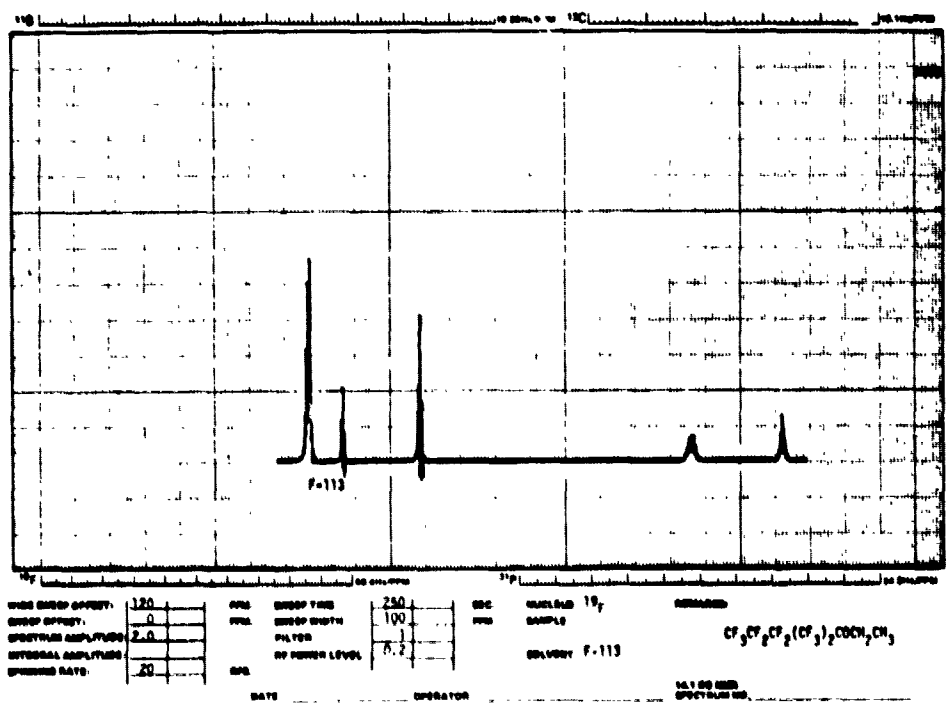
$$C_4F_{13}OK + CH_3CH_2I \longrightarrow CF_3CF_2CF_2C(CF_3)_2OCH_2CH_3$$

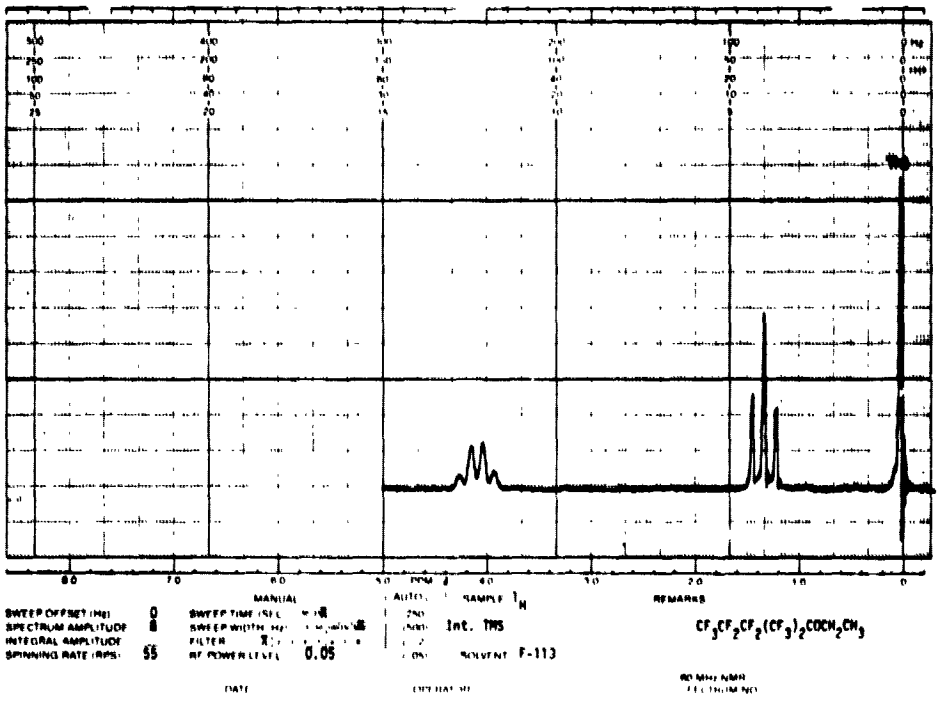
The apparatus was set up with a hood and safety shield.

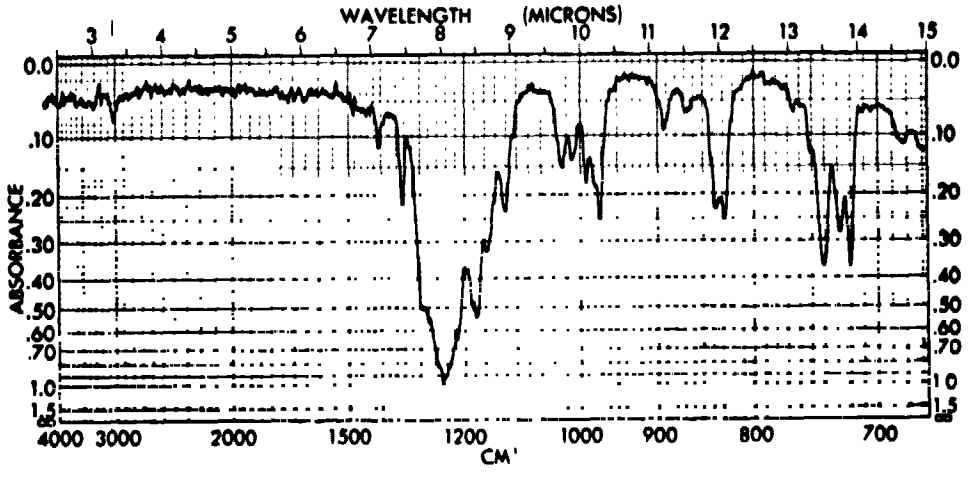
This ether was prepared as in the preceding example from 25.2 g alcohol, 8.2 g KOH and 16.4 g CH_3CH_2I (.105 mol). After 41 hours the reaction was worked up as in JPL-AB-19. The crude product was purified by one bulb-to-bulb vacuum distillation from solid KOH and treated with 5A molecular sieve. Yield 23.0 g, 84.2%. Boiling Point 109°C/728 mm, D^{24} 1.571 g/cm^3. JPL-AB-20 is a new compound.

Calculated for $C_8H_5OF_{13}$ (364.10): C, 26.39%; H, 1.38%; O, 4.39%; F, 67.83%.

Found: C, 25.96%; H, 1.56%.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2COCH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFI	

SPECTRUM NO. ____ SAMPLE ____

10. 2-n-Propoxy-perfluoro-2-methylpentane (JPL-AB-21)

$$CF_3CF_2CF_2C(CF_3)_2OH + KOH \xrightarrow[DMSO]{} C_6F_{13}OK$$

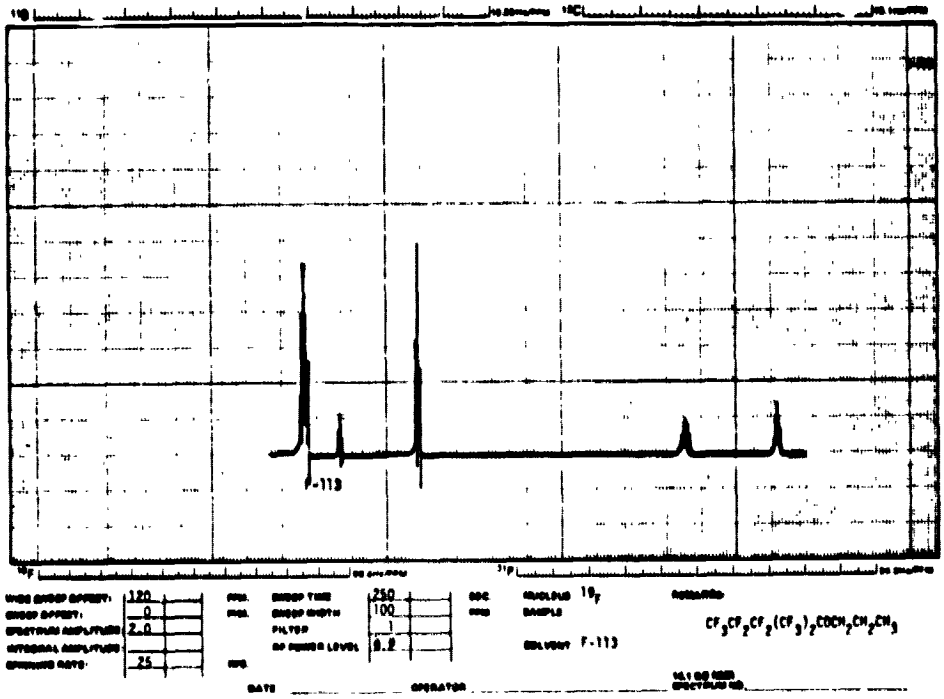
$$C_6F_{13}OK + CH_3CH_2CH_2I \longrightarrow CF_3CF_2CF_2C(CF_3)_2OCH_2CH_2CH_3$$

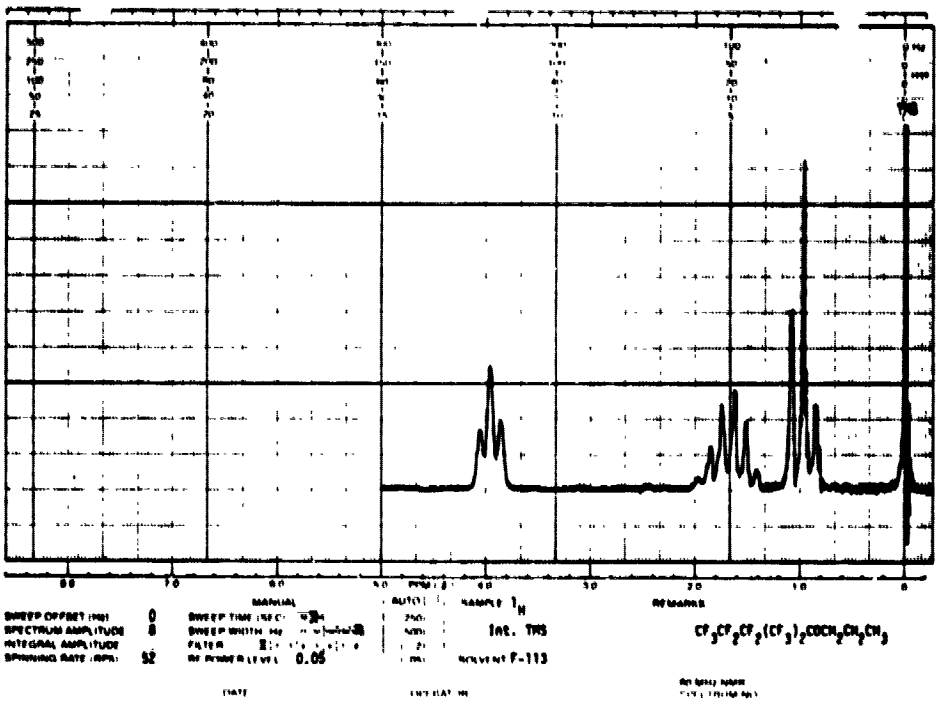
The apparatus was set up with a hood and safety shield.

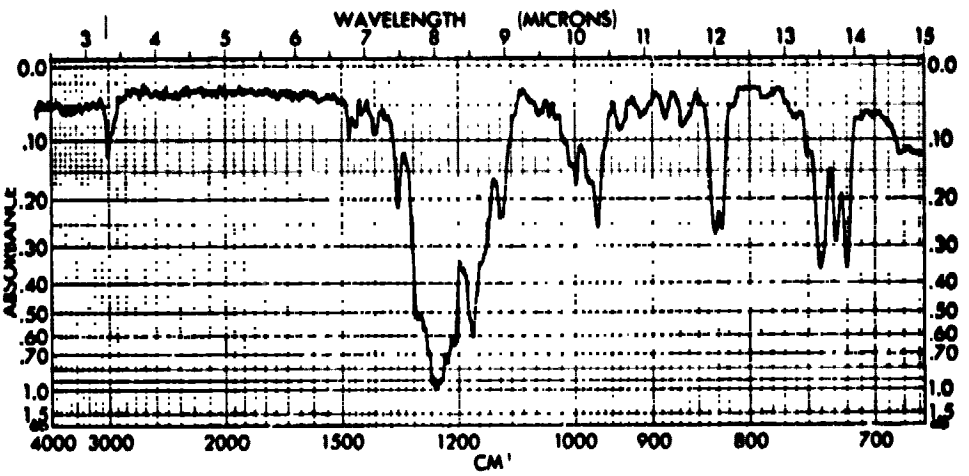
This ether was prepared as in the previous example from 25.0 g alcohol, 8.5 g KOH and 17.0 g n-propyl iodide (0.10 mol). The reaction mixture was clear initially but became turbid within five minutes. After 65.5 hours the mixture was worked up as before, and the crude product purified by a single bulb-to-bulb vacuum distillation from solid KOH. Yield 22.1 g, 78.6%. Boiling Point: 125°C/728 mm, D24: 1.607 g/cm^3. JPL-AB-21 is a new compound.

Calculated for $C_9H_7OF_{13}$ (378.13): C, 28.59%; H, 1.87%; O, 4.23%; F, 65.31%.

Found: C, 28.50%; H, 2.03%.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2COCH_2CH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

THE PERKIN ELMER CORPORATION, NORWALK CONN

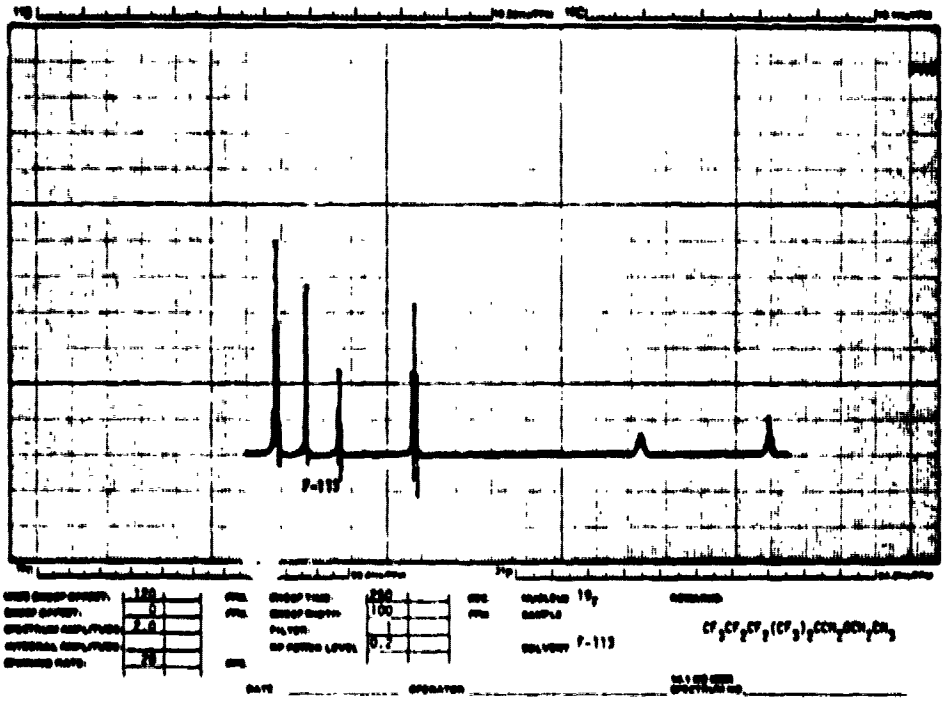
11. 4,4-Bis(trifluoromethyl)-5-ethoxy-1,1,1,2,2,3,3-heptafluoropentane (JPL-AB-22)

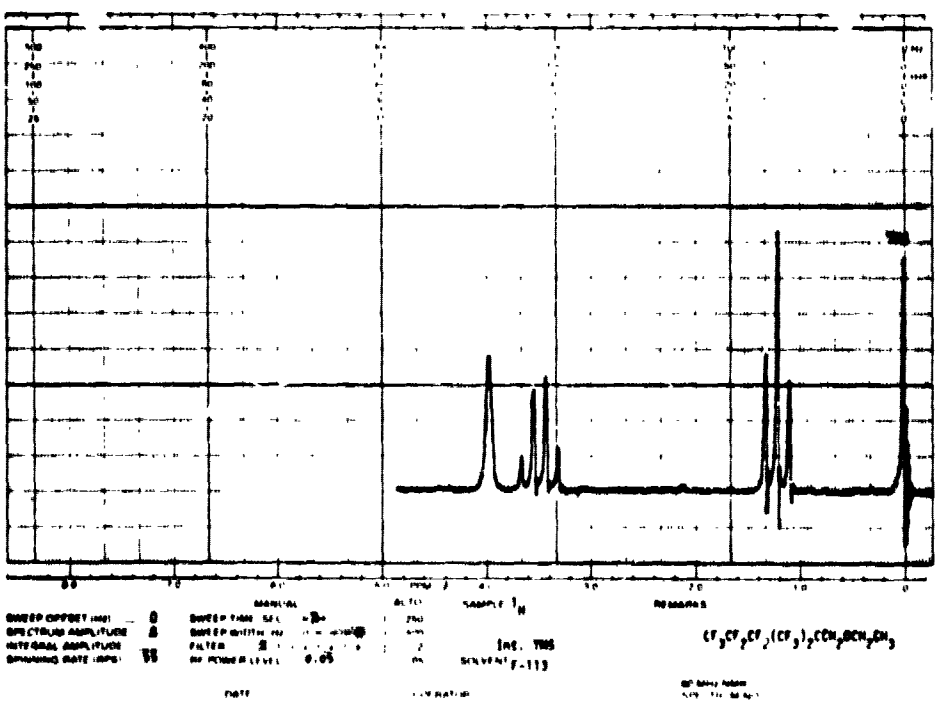
$$(CF_3)_2C{=}CFCF_2CF_3 + ClCH_2OCH_2CH_3 \xrightarrow[\text{Cat.}\ \text{THF}]{\text{KF}} CF_3CF_2CF_2(CF_3)_2CCH_2OCH_2CH_3$$

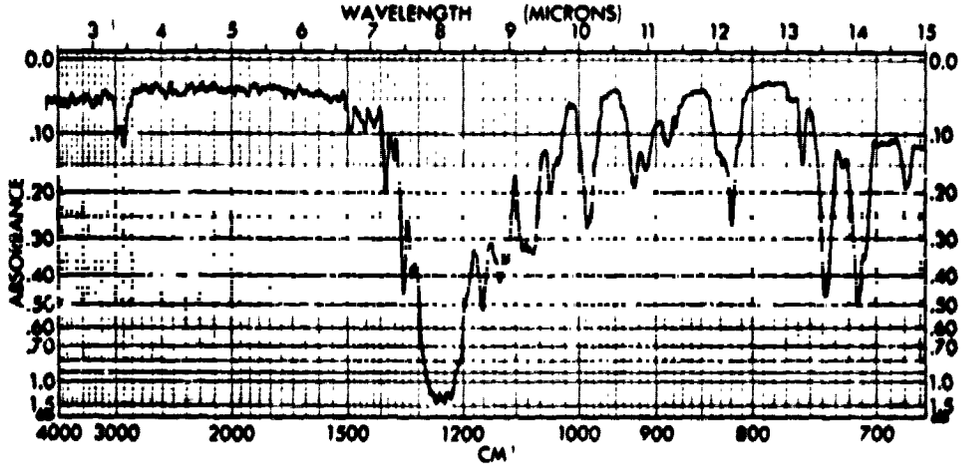
Apparatus was set up with hood and safety shield because of toxicity. Dry KF, 20 g; tetrahydrofuran, 250 ml; perfluoro-2-methyl-2-pentene, 46.6 g (0.155 mol) and chloromethyl ethyl ether, 22.2 g (0.235 mol) were combined and stirred at room temperature with exclusion of moisture. After 17 h, no reaction could be detected by FMR, and 5.0 g of 18-crown-6 ether-acetonitrile complex was added. After 20.5 h additional stirring, about 40% product was judged present by FMR analysis. The reaction was stirred a further 240 hours, 30 ml morpholine was added, and 24 h later the reaction was worked up by pouring the mixture into ca 1.5 liter water. The lower layer was washed with 3M HCl-0.5 M H_3BO_3, dried over solid KOH and distilled in vacuum. The crude product showed appreciable amounts of several impurities by FMR and PMR; successive treatment with strong aqueous KOH, morpholine and concentrated sulfuric acid, and distillation in vacuum from solid KOH gave 18.8 g, 32% of material with PMR and FMR spectra consistant with the assigned structure. Boiling Point 133°C/718 mm, D^{24} 1.530 g/cm^3. JPL-AB-22 is a new compound.

Calculated for $C_9H_7OF_{13}$ (378.13): C, 28.59%; H, 1.87%; O, 4.23%, F, 65.31%.

Found: C, 28.49%; H, 2.06%.







SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_2)_6CCH_2OCH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

THE PERKIN ELMER CORPORATION NORWALK CONN

12. Perfluoro-2-nitroso-2-methylpentane (JPL-AB-23)

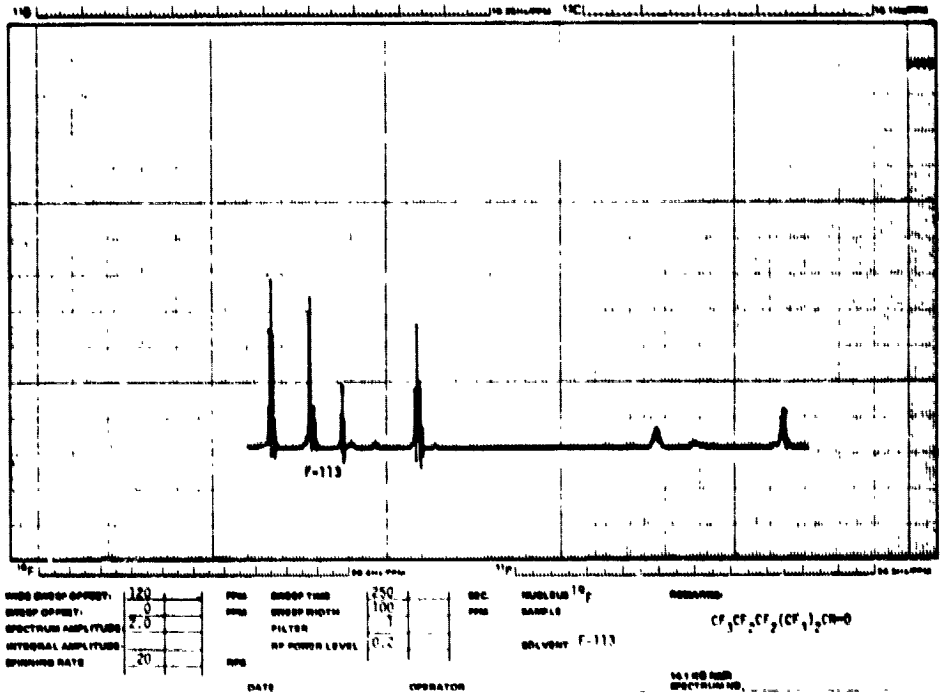
$$(CF_3)_2C{=}CFCF_2CF_3 + NOCl \xrightarrow[DMA]{KF} CF_3CF_2CF_2(CF_3)_2CNO$$

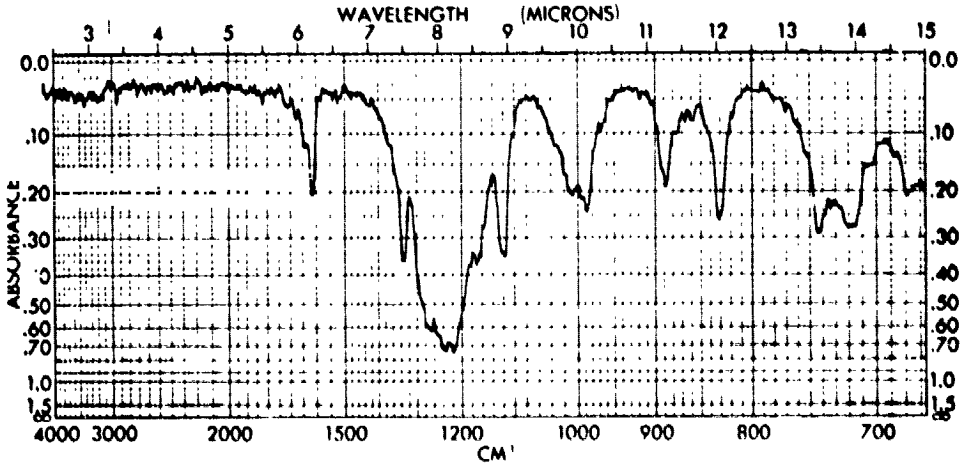
Apparatus was set up with hood and safety shield due to high toxicity.

Dry KF, 30g; dimethylacetamide, 250 ml; and perfluoro-2-methyl-2-pentene, 100.7 g(0.336 mol), were combined in a 500 ml 3 necked flask fitted with a -28 (boiling CF_2Cl_2) cold finger and magnetic stirring. The mixture was cooled to 3° in an ice bath and nitrosyl chloride (NOCl) slowly passed in from a cylinder until an excess was present (green color). The reaction mixture was then poured into ca. one liter of ice water in a separatory funnel. The lower blue phase was separated, dried quickly over solid KOH (some reaction) and distilled quickly at aspirator pressure into a dry ice-cooled receiver. The distillate was stored over 4A molecular sieve over a weekend in the freezer and again distilled as above. Yield 92.0 g (79%) of royal blue, mobile liquid, 80-90% pure by FMR. Boiling point 73°/729 D^{24} 1.702. JPL-AB-23 is a new compound.

Calculated for $C_6F_{13}NO$ (349.05): C, 20.65%, F, 70.76%; N, 4.01%; O, 4.58%.

Found: C, 19.47%.





SPECTRUM NO.	ORIGIN JPI	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CH=O$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

13. Ethyl-3,3-bis(trifluoromethyl)-4,4,5,5,6,6,6-heptafluorohexanoate (JPL-AB-24)

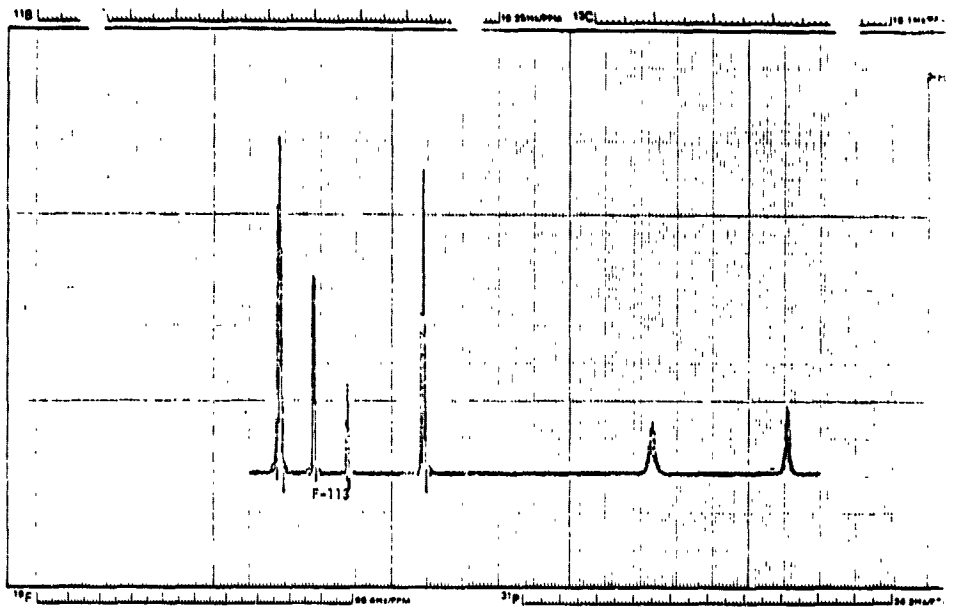
$$(CF_3)_2C{=}CFCF_2CF_3 + BrCH_2CO_2C_2H_5 \xrightarrow[DMA]{KF} CF_3CF_2CF_2C(CF_3)_2CH_2CO_2C_2H_5$$

Dry, finely-ground potassium fluoride (30 g, 0.52 mol) was transferred inside a dry box into a one liter round bottom flask containing a magnetic stirring bar. Dry, freshly distilled dimethylacetamide (500 ml) was added, followed by 106.7 g (0.356 mol) perfluoro-2-methyl-2-pentene and 81.8 g (0.49 mol) ethyl bromoacetate. The flask was closed with a drying tube and stirred at room temperature (30°C). When the FMR spectrum of the reaction mixture was taken 18 hours later, the reaction was found to be complete. The entire mixture was filtered by suction into a 2 liter separatory funnel, diluted with ice water and the lower layer drawn off. An excess of morpholine was mixed with the crude product and the mixtures stirred overnight, then poured into 3M HCl containing 0.5 M H_3BO_3. The lower layer was again drawn off. The yield of crude ester, essentially pure by FMR but showing some minor impurities in the PMR spectrum, was 116.7 g; short-path distillation under aspirator vacuum left a small residue of red tar and gave 103 g of light yellow mobile liquid, 71%. The product has a boiling point of 163 - 164°C at 732 torr and a density of 1.599 grams/ml at 22°C. JPI-AB-24 is a new compound.

An analytical sample was obtained by preparative gas chromatography.

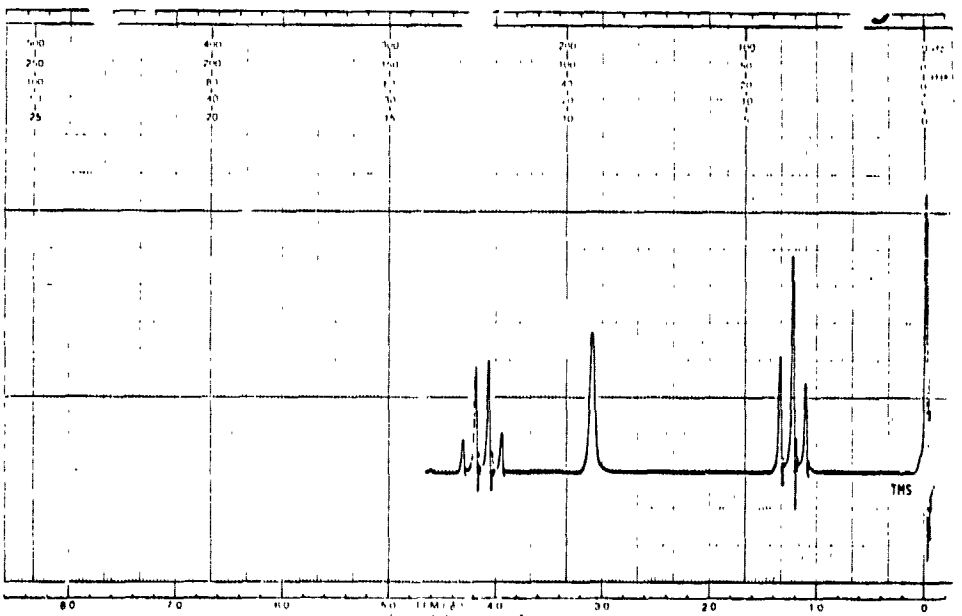
Calculated for: $C_{10}H_7F_{13}O_2$ (406.14): C, 29.57%; H, 1.74%; F, 60.81%; O, 7.88%.

Found: C, 29.84%; H, 1.92%.



WIDE SWEEP OFFSET: 120 PPM. SWEEP TIME: 250 SEC. NUCLEUS: ^{19}F REMARKS:
SWEEP OFFSET: 0 PPM. SWEEP WIDTH: 100 PPM SAMPLE: $CF_3CF_2CF_2(CF_3)_2CCH_2CO_2CH_2CH_3$
SPECTRUM AMPLITUDE: 3.2 FILTER 1
INTEGRAL AMPLITUDE RF POWER LEVEL: 0.2 SOLVENT: F-113
SPINNING RATE: 30 RPS.

DATE: OPERATOR: 94.1 KG NMR SPECTRUM NO.



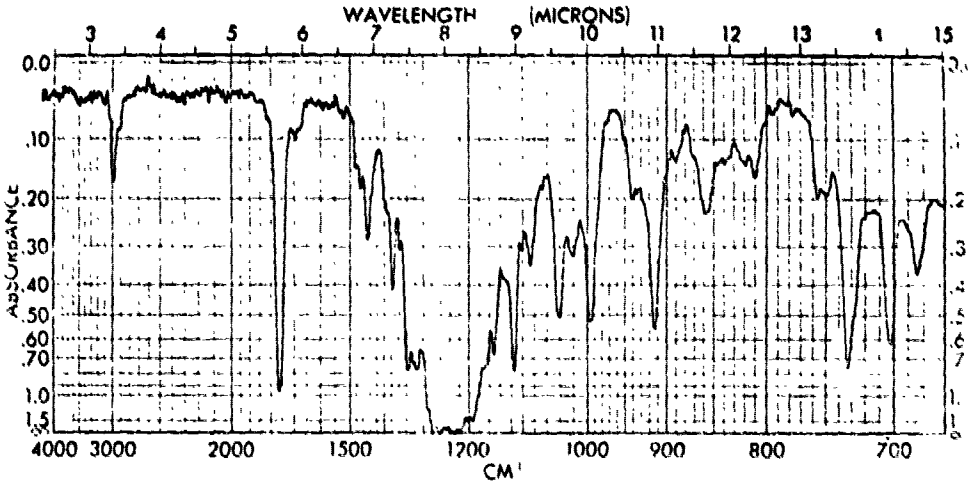
MANUAL AUTO SAMPLE ^1H REMARKS

SWEEP OFFSET (Hz) 0
SPECTRUM AMPLITUDE 3.2
INTEGRAL AMPLITUDE
SPINNING RATE (RPS) 35
FILTER % 0.05
RF POWER LEVEL

Int TMS $CF_3CF_2CF_2(CF_3)_2CCH_2CO_2CH_2CH_3$

SOLVENT F-113

DATE OPERATOR 60 MHz NMR SPECTRUM NO.



SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2CO_2CH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

14. 4-ethoxy-2,2-bis(trifluoromethyl)-1,1,1-trifluorobutane (JPL-AB-25)

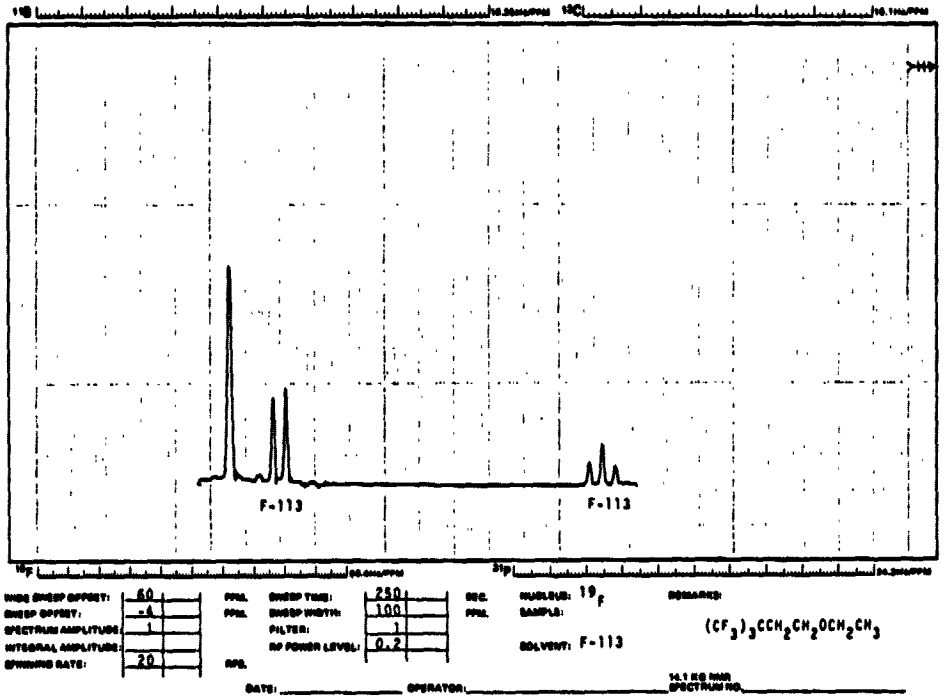
$$(CF_3)_2C{=}CF_2 + BrCH_2CH_2OCH_2CH_3 \xrightarrow[DMA,\ RT]{KF} (CF_3)_3CCH_2CH_2OCH_2CH_3$$

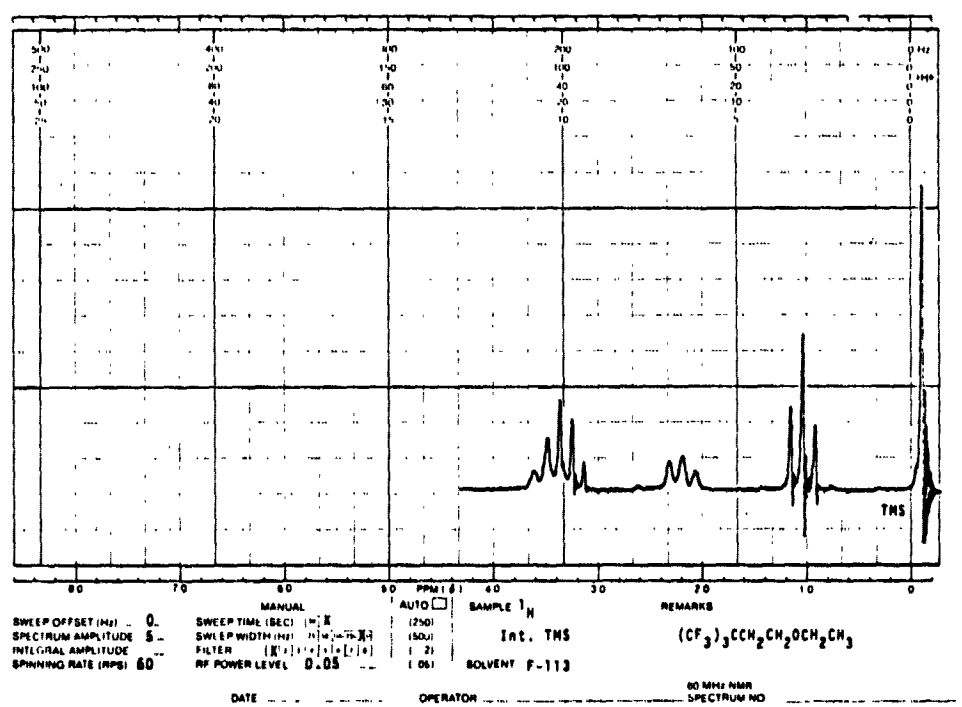
Apparatus was set up with hood and safety shield.

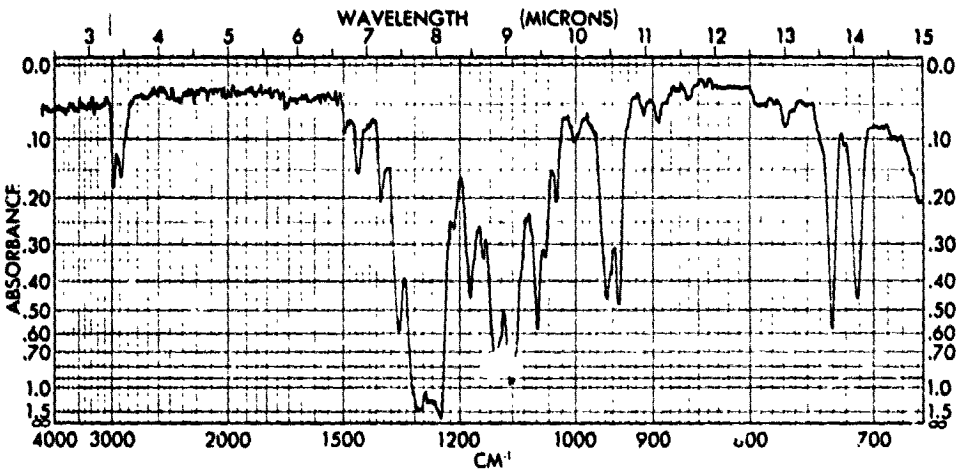
Thirty point six (30.6) grams (0.200 mol) of 2-bromoethyl ethyl ether was added to 100 ml of dry dimethylacetamide (DMA) in a 250 ml Fischer-Porter pressure bottle equipped for magnetic stirring. Fifty (50) grams (0.25 mol) of octafluoroisobutene (OFIB) was condensed by a -78°C cold finger into the bottle which was kept at -10°C by a dry ice/isopropanol bath throughout the addition. Fourteen point six (14.6) grams (0.252 mol) of dry potassium fluoride was then added and the vessel was closed. The reaction mixture was allowed to warm to room temperature with stirring. After stirring for two days, the pressure gauge in the reaction vessel was zero. The mixture was allowed to stir for a total of seven days and then placed in a separatory funnel and washed with 500 ml of water. Fifty nine (59) g of a crude lower layer was collected. Gas chromatography on a 20% FFAP column at 150°C showed product, starting alkyl halide, HFP-OFIB codimer and one other impurity. ^{19}F NMR showed a singlet for the product and the characteristic resonances for the co-dimer. The material was then distilled through a 5 cm vacuum jacketed Vigreux column. Twenty-one (21)g of material boiling between 110 - 120°C was collected. The material was then purified by gas chromatography on a 1 x 150 cm, 20% didecylphthalate column at 90°C with a He flow rate of 100 ml/min. Fifteen (15)g (0.051 mol, 26%) of pure product was collected. Boiling point = 115°C @ 730 m. Density = 1.458 at 23°C. JPL-AB-25 is a new compound.

Calculated for: $C_8H_9OF_9$, (292.14); C, 32.89; H, 3.11; O, 5.48; F, 58.53.

Found: C, 31.94%; H, 2.69%; C, 32.06%; H, 2.84%.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$(CF_3)_3CCH_2CH_2OCH_2CH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR TFT	

SPECTRUM NO. SAMPLE

15. β,β'-(nonafluoro-t-butyl-ethyl) ether (JPL-AB-26)

$$(CF_3)_2C{=}CF_2 + (ClCH_2CH_2)_2O \xrightarrow[DMA,\ RT]{NaI,\ KF} [(CF_3)_3CCH_2CH_2]_2O$$

The apparatus was set up with hood and safety shield because of high toxicity.

Twenty one point four (21.4)g (0.15 mol) of β,β'-chloroethyl ether, 2.25 g of sodium iodide and 100 ml of dry dimethylacetamide were placed in a 250 ml Fischer-Porter pressure bottle equipped with magnetic stirring and a dry ice/isopropanol bath held at -10°C. One hundred twenty (120) g (0.60 mol) of OFIB were condensed from a -78°C cold finger into the pot. Twenty-six point two (26.2) g (0.45 mol) of dry KF were then added and the top placed on the pressure bottle. The pot was allowed to warm to room temperature and stirred for 14 days. The pot contents were then washed with 1 liter of water in a 2-liter separatory funnel. Upon addition of the water, vigorous hydrolysis occurred accompanied by a slight rise in solution temperature. Ninety six point seven (96.7) g of a crude brown lower fluorocarbon layer was collected and dried with $MgSO_4$. ^{19}F NMR showed the crude phase to have 2 singlets of equal intensity downfield of the F113 doublet. Gas chromatography on a 1 x 150 cm, 20% didecylphthalate column at 90°C with a He flow rate of 100 ml/min showed the crude mixture to have 12 components. No product was isolated.

16. 2-Isopropoxy-perfluoro-2-methylpentane (JPL-AB-27)

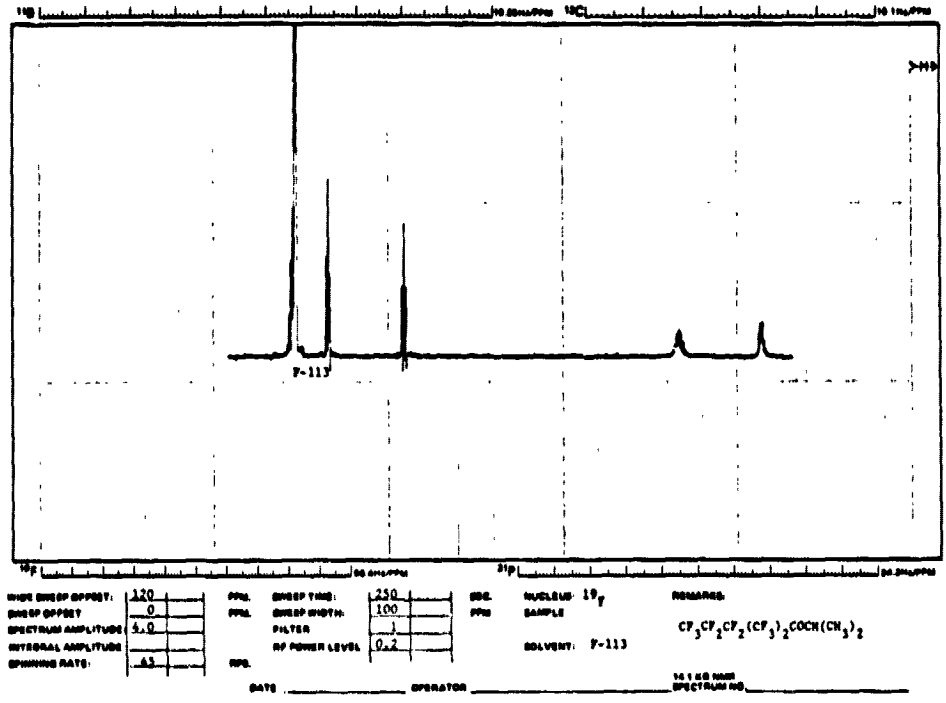
$$CF_3CF_2CF_2(CF_3)_2COH + ICH(CH_3)_2 \xrightarrow[DMSO]{KOH} CF_3CF_2CF_2(CF_3)_2COCH(CH_3)_2$$

Twenty five point zero (25.0) g of perfluoro-2-methyl-2-pentanol (0.0744 mol), 8.6 g of KOH pellets (0.13 mol), and 125 ml of dimethylsulfoxide were combined and stirred at room temperature. After 45 minutes, 18.7 g (0.110) of 2-iodopropane was added; the mixture did not become turbid within 10 min, as did the same reaction with primary iodides, but turbidity was present after 25 min. Some pressure buildup and olefin smell were evident, indicating competing elimination of HI and formation of propene. After twenty hours, an additional 5.3 g of iodide (0.031 mol) and 3.1 g KOH were added. After 113 hours total reaction time, the mixture was poured into 300 ml of water, and the lower layer separated and dried over solid KOH. The alkaline aqueous supernatent was acidified with concentrated HCl, whereupon 9.1 g (crude) perfluoroalcohol separated; it was identified by its FMR spectrum.

Short path distillation of the crude ether gave a colorless product. An analytical sample was obtained by preparative gas chromatography. Overall yield of 16.2 grams (58%), bp = 121°C at 732 torr, density = 1.531 at 21°C. JPL-AB-27 is a new compound.

Calculated for $C_9H_7F_{13}O$ (378.13); C, 28.59%; H, 1.87%; O, 4.12%; F, 65.31%.

Found: C, 28.42%; H, 1.89%.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INTEGRAL AMPLITUDE
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SWEEP WIDTH: 100
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RF POWER LEVEL 0.2
NUCLEUS 19F
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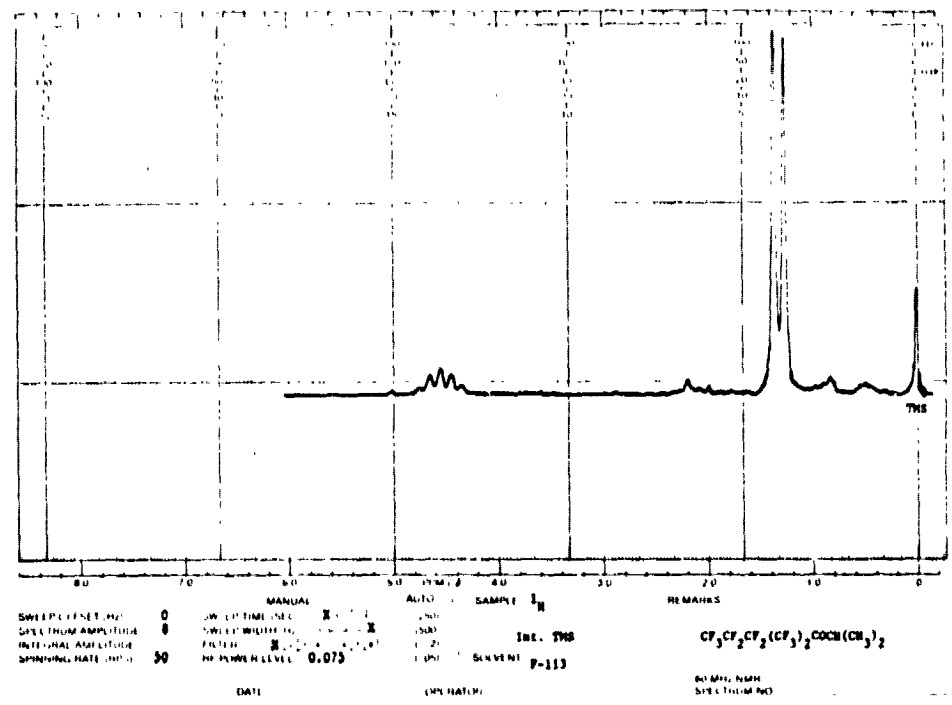
SOLVENT: F-113
REMARKS
CF3CF2CF2(CF3)2COCH(CH3)2
DATE
OPERATOR
SPECTRUM NO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Int. TMS
SOLVENT F-113
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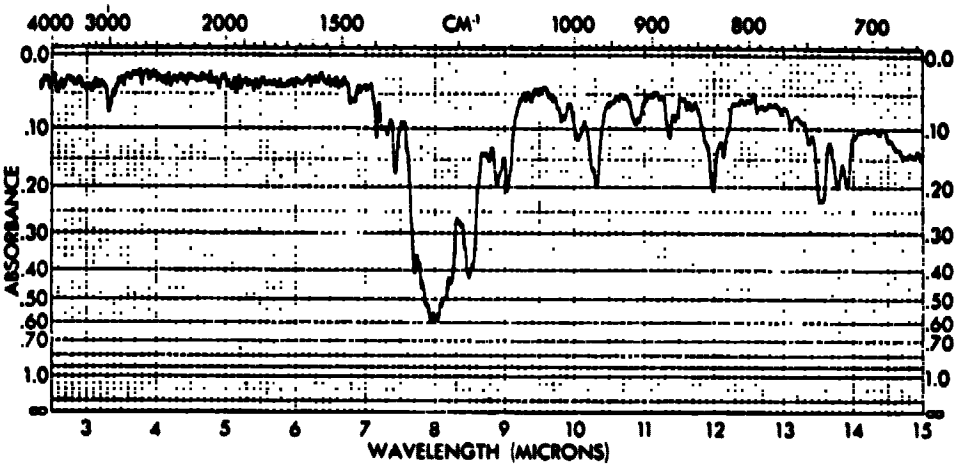
DATE
OPERATOR
SPECTRUM NO



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_2)_3COCH(CH_3)_2$	PURITY	2.	
	PHASE LIQUID FILM	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

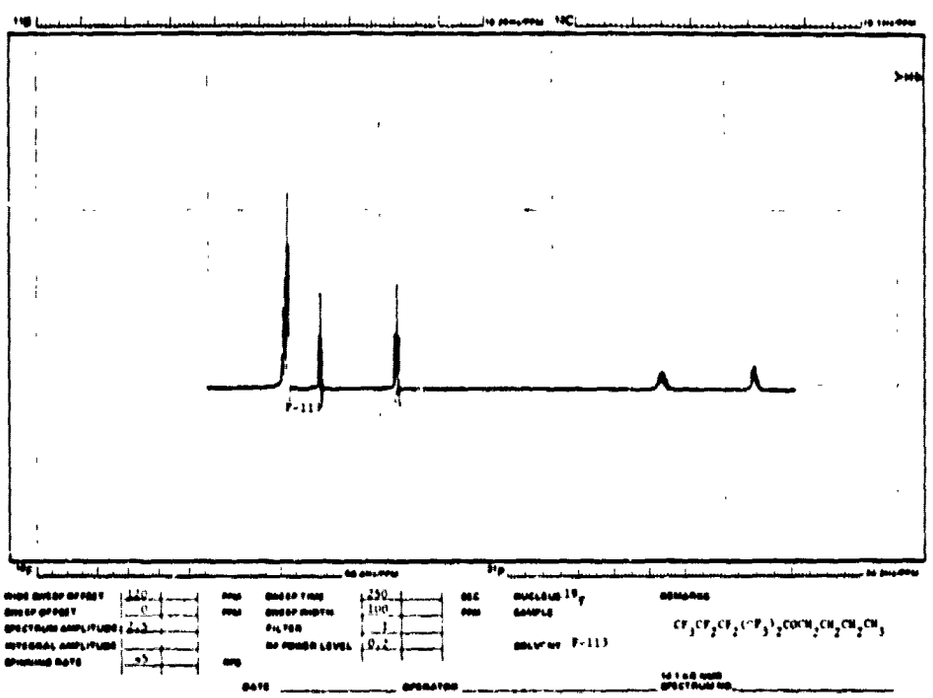
17. 2-n-Butoxy-perfluoro-2-methylpentane (JPL-AB-28)

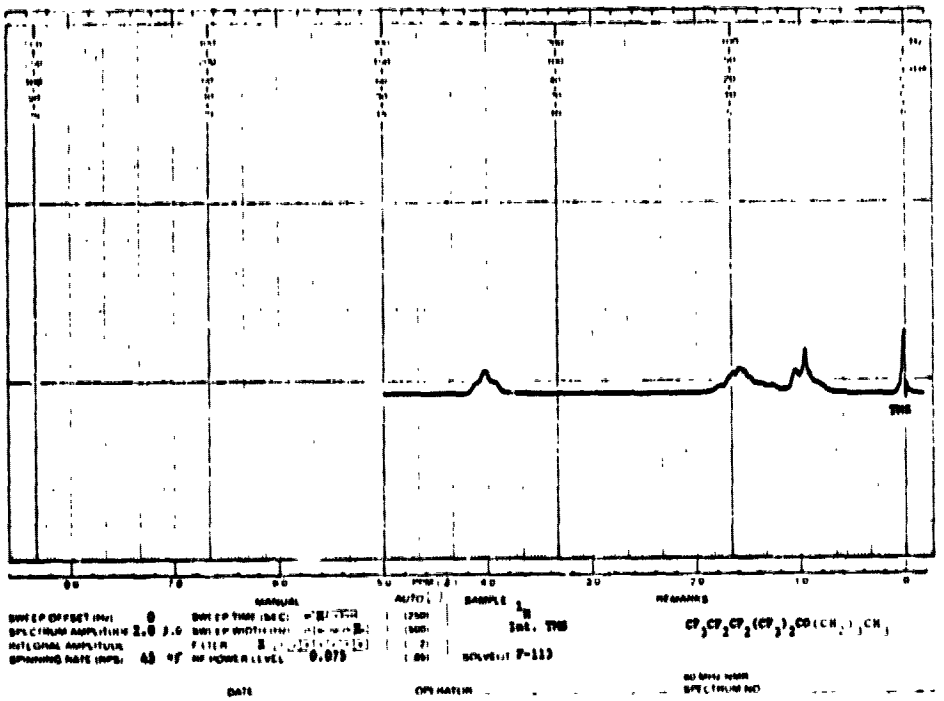
$$CF_3CF_2CF_2(CF_3)_3COH + ICH_2CH_2CH_2CH_3 \xrightarrow[DMSO]{KOH} CF_3CF_2CF_2(CF_3)_2COCH_2CH_2CH_2CH_3$$

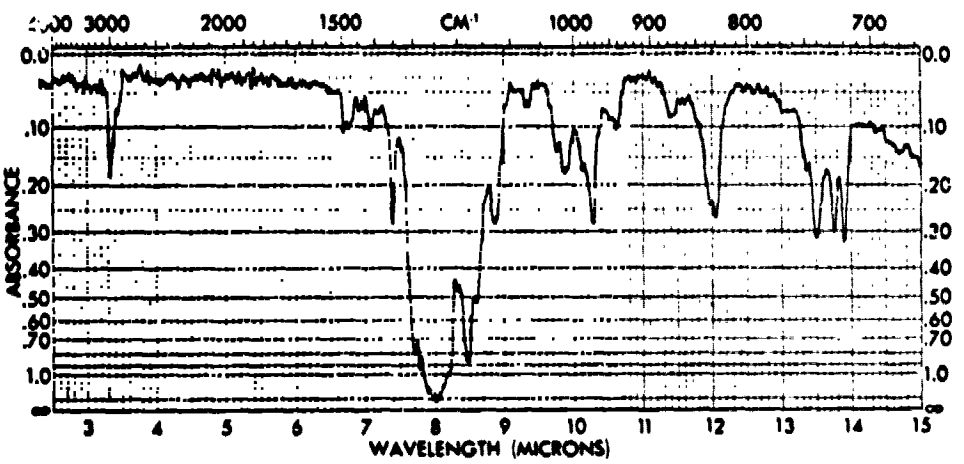
Twenty five point zero (25.0)g of perfluoro-2-methyl-2-pentanol (0.0744 mol), 125 g of dimethysulfoxide, 9.9 g of KOH pellets (0.115 mol), and 18.4 g of 1-iodobutane (0.100 mol) were combined and stirred at room temperature for three days. At the end of the reaction period the mixture was poured into ca. 300 ml of water and the crude lower layer drawn off and dried over solid KOH. Distillation of the crude product at aspirator pressure into a dry ice-cooled receiver gave a colorless liquid. An analytical sample was obtained by preparative gas chromatography. Overall yield 24.8 grams (84%), bp = 138°C at 732 torr, density = 1.472 at 21°C. JPL-AB-28 is a new compound.

Calculated for $C_{10}H_9OF_{13}$ (392.16): C, 30.63%; H, 2.31%; 0, 4.08%; F, 62.98%.

Found: C, 30.47%; H, 2.25%.







SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2CH_2CH_3$	PURITY	2.	
	PHASE LIQUID FILM	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. ____ SAMPLE ____

18. 2,2-Bis-trifluoromethyl-3,3,4,4,5,5,5-heptafluoropentane (JPL-AB-29)

$$(CF_3)_2C{=}CFCF_2CF_3 + (CH_3O)_2SO_2 \xrightarrow[DMA]{KF} CF_3CF_2CF_2(CF_3)_2CCH_3$$

Apparatus was set up with hood and safety shield.

Potassium fluoride, 95 g (1.64 mol; oven-dried and powdered), 500 ml of dimethylacetamide and 301 g (1.00 mole) of perfluoro-2-methyl-2-pentene were combined and stirred magnetically in a one liter flask, while 168 g (1.33 mol) of dimethyl sulfate was added in portions. The first portions caused no perceptible temperature rise (touch) but as the addition continued over about 0.5 hour the temperature rose gradually until the fluorochemical components refluxed gently. After the mixture cooled, ^{19}FMR examination showed consumption of starting material and the presence of two major components (vide infra). The mixture was poured into water and the lower layer examined by FMR: multiplets similar to that of the previously prepared $CF_3CF_2CF_2C(CF_3)_2R$ compounds was present (41.5%), along with $CF_3CF_2CF_2C(CF_3)_2H$ (12.1%) and a previously unobserved compound tentatively identified as $(CF_3)_2HCHCOCF_2CF_3$ (46.2%), presumably arising from attack of the solvent or the $CH_3OSO_3^-$ ion on the starting olefin. The relative amounts were determined by integration of the geminal CF_3 multiplets. The crude lower layer was stirred with a solution of KOH in isopropanol until the exothermic reaction subsided, then the mixture was distilled until no more fluorochemical co-distilled with the isopropanol. The crude product was washed with water, dried over KOH pellets and redistilled at aspirator pressure to give 111.2 g (33.3%) colorless liquid. Some discoloration occurred when the distilled product was redried over solid KOH, so diethyl amine was added to destroy the reactive impurities present. After 24 hours, the mixture was washed with dilute aq. acid, again dried over solid KOH, and redistilled. Overall yield 99.2 grams (30%), bp = 86°C at 731 torr, density = 1.667 at 21°C. JPL-AB-29 is a new compound.

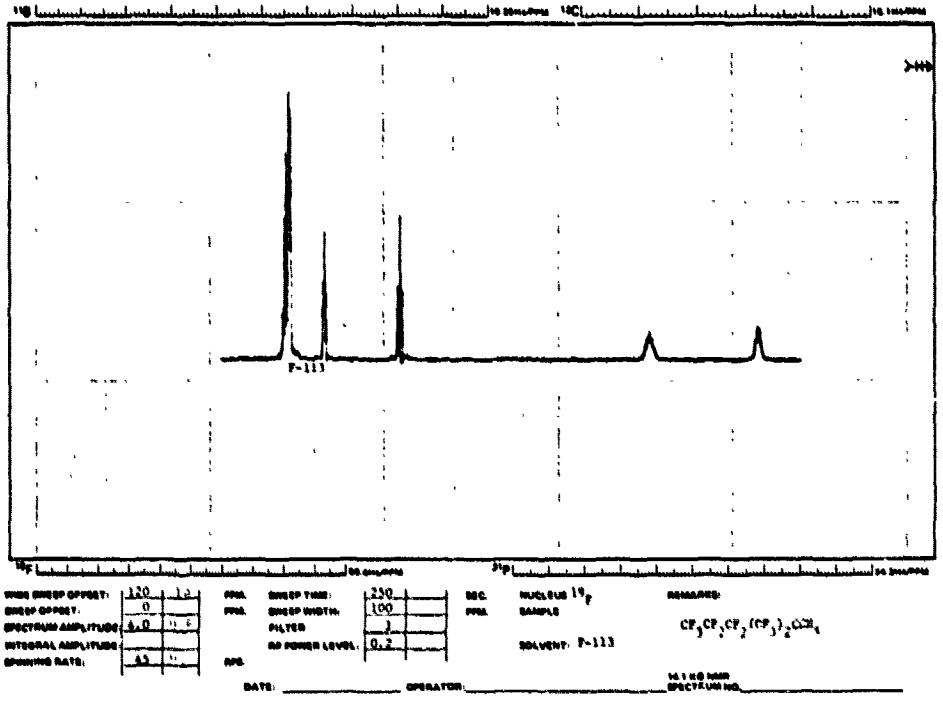
Calculated for $C_7H_3F_{13}$ (334.08): C, 25.17%; H, 0.91; F, 73.93.

Found: C, 24.29%; H, 0.90%.

C, 24.32%; H, 1.01%.

$$CF_3CF_2CF_2(CF_3)_2CCH_3 + Br_2 \xrightarrow{h\upsilon} CF_3CF_2CF_2(CF_3)_2CCH_2Br$$

Attempted bromination: 21.6 g (0.065 mol) of the KOH-isopropanol washed product above was placed in a Pyrex flask with 11.2 g Br_2 (0.070 mol). The two-phase mixture was heated and illuminated by a 25-W Tensor light placed against the flask and shielded with foil; after about 16 hours at reflux, the mixture was examined by PMR; only the starting material was present.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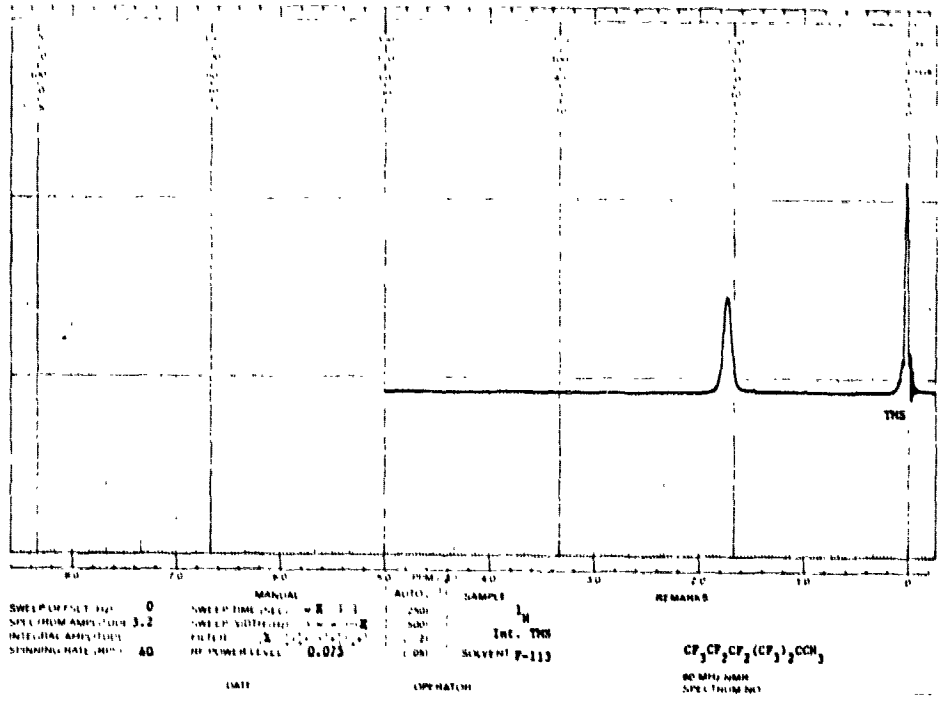
REMARKS:
$CF_3CF_2CF_2(CF_3)_2CCN_3$
DATE:
OPERATOR:
SPECTRUM NO.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REMARKS
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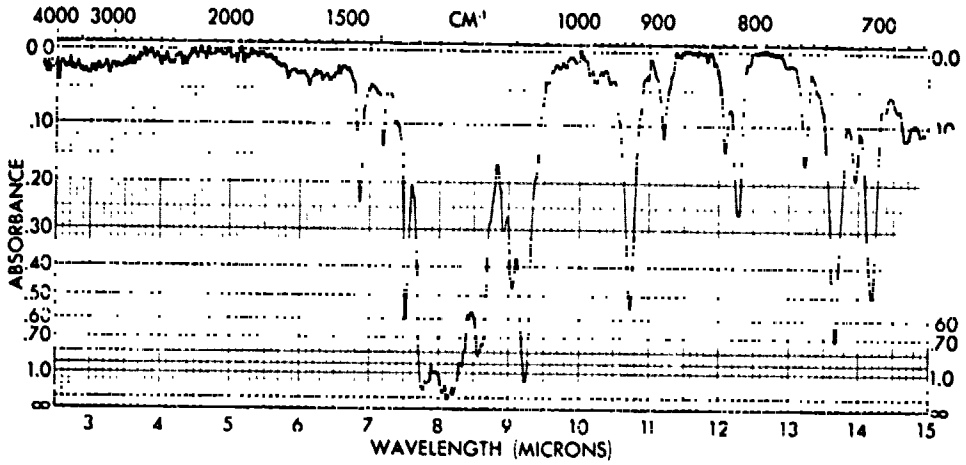


SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_3$	PURITY	2.	
	PHASE LIQUID FILM	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

19. Perfluoro-2-methyl-2-pentylhydroxylamine (JPL-AB-30)

$$CF_3CF_2CF_2(CF_3)_2CN{=}O \xrightarrow[TFA]{Zn/Hg,\ HCl} CF_3CF_2CF_2(CF_3)_2CNHOH$$

Apparatus was set up with hood and safety shield.

Ten point seven (10.7) g of perfluoro-2-nitroso-2-methylpentane (0.0307 mol) was dissolved in 42 ml of trifluoracetic acid. The solution was cooled to -15° and saturated with HCl gas, then 5.3 g of granulated amalgamated zinc (about 2.5 equivalents) were added. The solution was stirred and let warm slowly to room temperature. At the end of one hour the mixture was still blue, but after stirring overnight a white precipitate was present and the solution was colorless. Twenty-five (25) ml of 96% H_2SO_4 were added, and most of the trifluoroacetic acid was distilled off at aspirator pressure. The distillate was faintly blue. The distillation residue was diluted with water; a lower phase formed which was steam-distilled out. The lower product phase of the distillate was separated: yield 5.34 g (50%) of a colorless liquid identified by nmr spectroscopy as $CF_3CF_2CF_2C(CF_3)_2NHOH$. The proton nmr spectrum of the crude product showed two fairly sharp singlets at 5.80 and 1.82 ppm downfield of external TMS (ratio 1:1.44; vs expected 1:1, perhaps due to a trace of water exchanging with the -NHOH protons). The fmr spectrum in Freon 113 shows only a single t of t at 66.52 ∅ for the $CF_3CF_2CF_2C(CF_3)NHOH$ fluorines. When the F-113 solution of the product was treated with aqueous HONO, the solution became blue, and the 66.52 t of t in the fmr spectrum disappeared and was replaced by the quintet of $CF_3CF_2CF_2C(CF_3)_2NO$ at 63.64 ∅. These observations are consistent only with the reduction product being the hydroxylamine, rather than the amine $CF_3CF_2CF_2C(CF_3)_2NH_2$. A larger-scale preparation of the hydroxylamine is in progress. JPL-AB-30 is a new compound.

20. 3,3-Bis(Trifluoromethyl)-4,4,5,5,6,6,6-Heptafluorohexane (JPL-AB-31)

$$(CF_3)_2C{=}CFCF_2CF_3 \xrightarrow[CH_3CH_2I]{KF,\ DMA} CF_3CF_2CF_2(CF_3)_2CCH_2CH_3$$

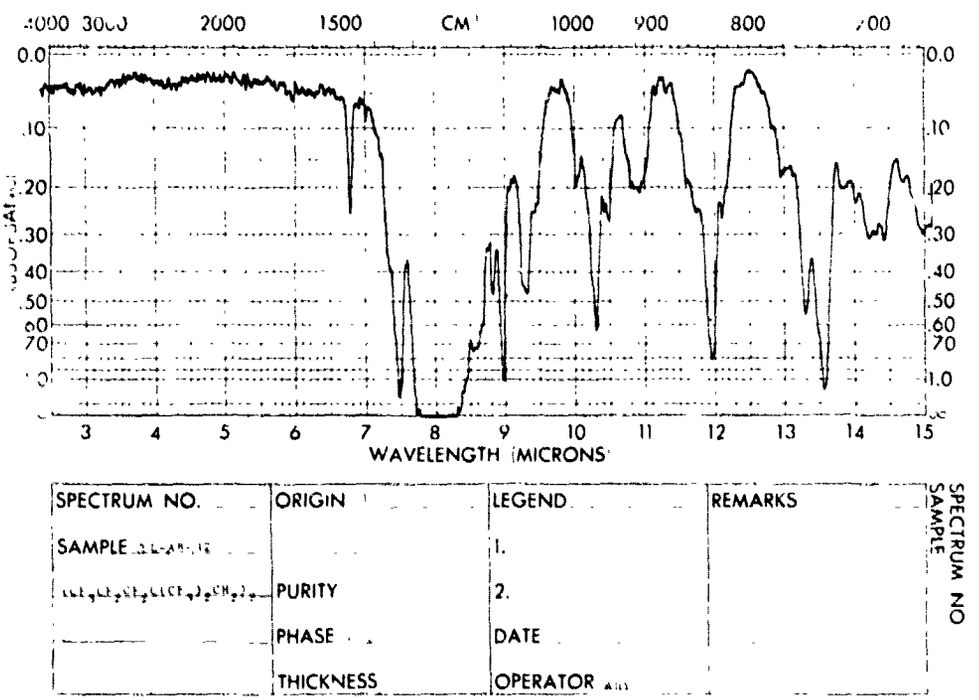
113 g (0.337 mol) perfluoro-2-methyl-2-pentene, 41.3 g (0.711 mol) dry potassium fluoride, 250 ml dry dimethylacetamide, and 72 g (0.462 mol) ethyl iodide were combined and stirred at room temperature. After 59 days, sometime during which the stirrer broke down, the mixture was poured into 1.5 l of water. Examination of the crude straw-colored lower layer by fmr spectroscopy showed complete reaction. The crude product was drawn off and mixed with 60 ml of diethylamine; the mixture darkened and became warm. After 48 hours that mixture was poured into water and the lower layer was separated and stirred overnight with 60 ml of 96% H_2SO_4. The product was distilled in vacuum directly from the H_2SO_4, redistilled in vacuum from solid KOH, dried over $CaCl_2$ and filtered to give 97.8 g of water-white mobile liquid, 74.6%. JPL-AB-31 is a new compound.

The product was redistilled at atmospheric pressure to obtain a sample for testing and analysis.

21. 5,5,6,6-Tetra-H-perfluoro-4,4,7,7-tetramethyldecane (JPL-AB-32)

$$\underset{\text{(A)}}{CF_3CF_2CF_2C(CF_3)_2CH_2COOH} \xrightarrow[-2e^-]{NaOMe,MeOH} \underset{\text{(B)}}{(CF_3CF_2CF_2C(CF_3)_2\text{-}CH_2)_2}$$

The Kolbe oxidation of acid (AB-39, p 3-64) was conducted in the apparatus used in the synthesis of JPL-AB-14. Sodium metal, 0.2 g (0.0086 moles) was added to 120 ml of anhydrous methanol placed in the electrolysis flask. (For a description of the apparatus see Reference 20). After the sodium reacted, 18.0 g (0.0476 moles) of acid A, were added. The product is immiscible, co-distills with methanol, and was collected in a Dean-Stark water separator. The end of the electrolysis was determined in the reaction by the change of pH to basic. The yield of crude product was 13 g, 82.2%. The product was purified by a short path distillation, B.P., 82°C at 14 mm. JPL-AB-32 is a new compound.



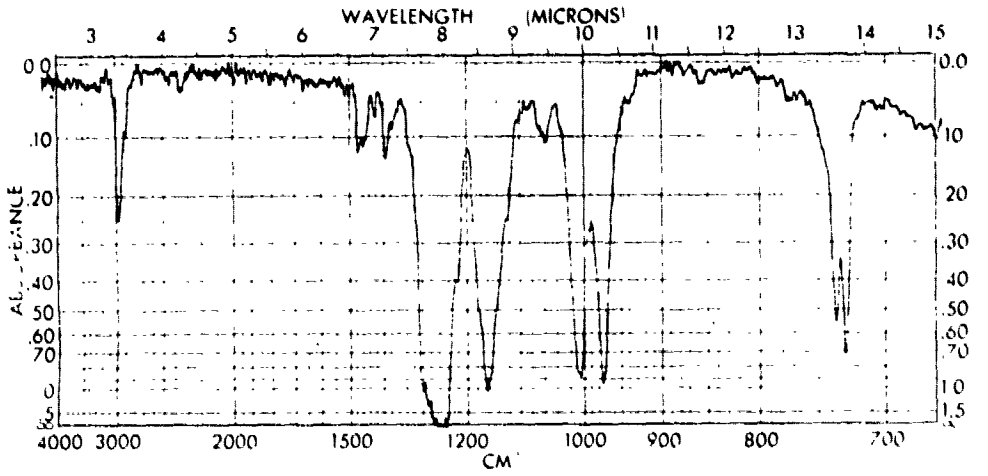
22. 1-(Perfluoro-t-butoxy)butane (JPL-AB-33)

$$(CF_3)_3COH + KOH \xrightarrow[DMSO]{} (CF_3)_3COK + CH_3CH_2CH_2CH_2I \xrightarrow[DMSO]{} (CF_3)_3COCH_2CH_2CH_2CH_3$$

Solid KOH (85% pellets, 10 g, 0.15 mol) was added to 125 ml of DMSO with stirring. After 30 minutes at room temperature the mixture was cooled to 10°C and perfluoro-t-butanol (25 g, 0.105 mol) was added. The reaction mixture was stirred for another 30 minutes and n-butyl iodide (18.4 g, 0.1 mol) was added. After 20 days of stirring at room temperature the product was isolated as in preceding examples. Yield 22 g, 71%. The product was purified by preparative GC. Boiling point 86°C at 733 mm; D = 1.35 g/cm^3. JPL-AB-33 is a new compound.

Calculated For: $C_8H_9F_9O$ (292.14): C, 32.89%; H, 3.11%; O, 5.48%; F, 58.53%.

Found: C, 32.67%; H, 3.18%.
C, 32.56%; H, 3.00%.



1,3-Bis(perfluoro-t-butoxy)propane (JPL-AB-34)

$$(CF_3)_3COH + KOH \xrightarrow[DMSO]{} (CF_3)_3COK + ICH_2CH_2CH_2I \xrightarrow[DMSO]{} (CF_3)_3COCH_2CH_2CH_2OC(CF_3)_3$$

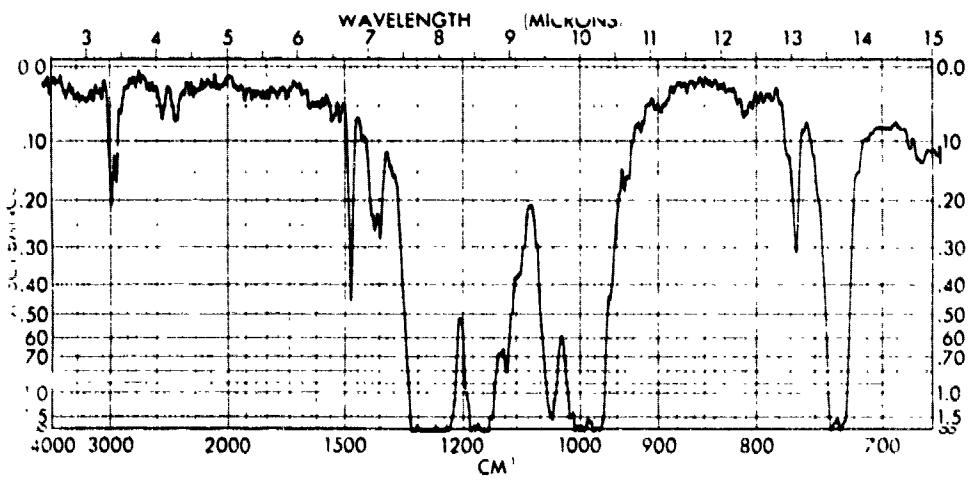
This ether was prepared as in the previous example, (JPL-AB-33) from 25.0 g perfluoro-t-butanol, 10 g KOH and 14.75 g (.0498 mol),of 1,3-diiodopropane. After 22 days the mixture was worked up as before. Yield 12 g, 50% based on perfluoro-t-butanol. The crude product was contaminated with a second component, which was separated by preparative GC and identified as $(CF_3)_3COCH_2CH = CH_2$. JPL-AB-34 is a new compound.

Calculated for: $C_{11}H_6F_{18}O_2$ (512.13): C, 25.80%; H, 1.18%; F, 66.77%; O, 6.25%.

Found: C, 26.06%; H, 1.45%.

Calculated for: $C_7H_5F_9O$ (276.10): C, 30.45%; H, 1.83%; F, 61.93%; O, 5.79%.

Found: C, 30.22%; H, 1.85%.



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
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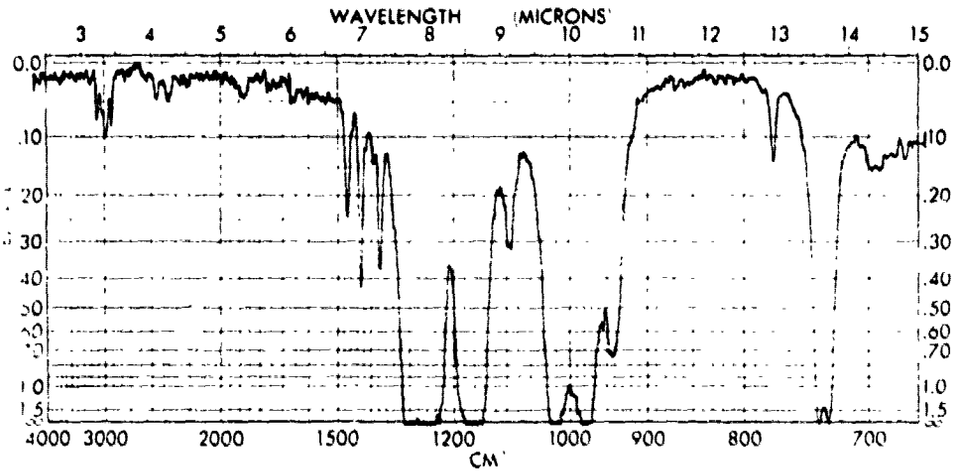
	PURITY	2.	
	PHASE	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
	PURITY	2	
	PHASE	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO
SAMPLE

24. 2,2,5,5-Tetramethylhexane (JPL-AB-35)

$$(CH_3)_3CCH_2COOH \xrightarrow[-2e^-]{NaOMe,MeOh} (CH_3)_3CCH_2CH_2C(CH_3)_3$$

The Kolbe oxidation of 3,3-dimethylbutanoic acid was done as described in the preparation of JPL-AB-32, using 20 g of acid and 125 ml MeOH containing sodium methoxide (MeONa) produced from 0.2 g of Na metal. The methanol solution was diluted with water, the oil which separated was extracted with ether, the ether extract was dried over $MgSO_4$ and evaporated under vacuum. The yield of crude product was 10 g, 50%. The product was purified by short-path distillation at atm. pressure. Boiling point 115°C at 732 mm. JPL-AB-35 is a new compound.

Calculated for: $C_{10}H_{22}$ (142.28): C, 84.41%; H, 15.58%.

Found: C, 84.22%; H, 15.25%.

25. Perfluoro-2-methyl-2-pentylamine (JPL-AB-36)
(by the reduction of 2-(p-nitrophenylazo)-perfluoro-2-methylpentane)

$$CF_3CF_2CF_2(CF_3)_2CN{=}N{-}C_6H_4{-}p{-}NO_2 + Zn(Hg) + HCl \xrightarrow[CH_3COOH]{}$$

$$CF_3CF_2CF_2(CF_3)CNH_2 + p{-}C_6H_4(NH_2)_2$$

Crude azo compound, 5.15 g, (0.109 mol) was dissolved in 200 ml acetic acid at room temperature. To the solution was added 100 ml of 37 percent HCl and 40 g of granulated amalgamated zinc, in portions. Initially, the mixture was kept cold in an ice bath, but as the reaction moderated it was let warm to room temperature. An additional 50 ml of 37 percent HCl was added and the mixture stirred overnight. By the next day all the zinc was consumed, so an additional 20 g of Zn(Hg) and 50 ml of 37 percent HCl added, and stirring continued for 24 hours more at room temperature.

On the third day, excess Zn was still present. A reflux condenser and Dean-Stark trap were attached, and the mixture slowly heated to reflux with an oil bath. Twenty two point eighty four (22.84) g (after drying over $CaCl_2$) of volatile fluorochemicals were collected as a dense lower phase. The ^{19}F nmr spectrum of the product mixture showed about 20 mol-percent of material with peaks consistent with the desired product, as well as the major product, $CF_3CF_2CF_2(CF_3)_2CH$. Neat CF_3SO_3H, 4.7 g, was added to convert the amine present to a salt and permit separation from neutral material; the mixture became warm and separated into two liquid phases. When the volatile neutral material was vacuum distilled (20 torr) into a dry-ice cooled receiver, the residue solidified. About 50 ml of water was then added to the crude residual salt. A lower liquid phase formed, which was steam distilled from the acid. The crude amine so obtained weighed 7.02 g and appeared about 80 percent pure by NMR (yield about 15 percent). JPL-AB-36 is a new compound.

A sample was obtained for analysis and spectra by preparative g.c.

Calculated for $C_6F_{13}NH_2$ (335.06); C, 21.51%; H, 0.60%; F, 73.71%; N, 4.18%.

Found: C, 21.60%; H, 0.64%; N, 4.10%.

26. Perfluoro-2-methyl-2-pentylamine (JPL-AB-36)

(improved preparation from 2-phenylazo-perfluoro-2-methylpentane)

$$CF_3CF_2CF_2(CF_3)_2C\text{-}N{=}N\text{-}C_6H_5 + Zn(Hg) + CF_3COOH \xrightarrow[r.t.]{}$$

$$CF_3CF_2CF_2(CF_3)_2CNH_2 + C_6H_5NH_2$$

Dry powdered potassium fluoride, 50 g (0.86 mol), perfluoro-2-methyl-2-pentane, 100 ml (160 g, 0.53 mol) and dimethylformamide, 500 ml, were combined and stirred magnetically under a dry nitrogen blanket in an ice-bath while benzene diazonium fluoroborate, 96 g (0.50 mol) was added in portions. At the end of the addition, the mixture was stirred for 30 minutes, then filtered by suction into a 2L flask containing about one L of water. The flask and filter cake were rinsed with ether. The combined filtrate and ether washings were made basic by addition of 40 g of NaOH. The ether layer, totaling about one L, was separated, washed several times with 5% NaOH, with 1N H_2SO_4, with saturated NaCl, and dried overnight over $CaCl_2$.

Evaporation of the ether in vacuum, taking care to keep the temperature below 50°C, gave 164.6 g (0.39 mol, 73%) of crude 2-phenylazo-F-2-methylpentane as an orange oil, suitable for reduction to the amine.

Azo compound, 21.2 g, (0.050 mol) was dissolved in 125 g technical CF_3COOH (1.10 moles). To the solution was added, with stirring, 10 g (approximately 0.15 g-atom) of 20 mesh amalgamated zinc, and the mixture was stirred at room temperature. The zinc had been previously amalgamated by washing with dilute HCl and stirring with 10 percent of its weight of $HgBr_2$ in aqueous solution.

The mixture was examined after 15 days by ^{19}F NMR, and at that time the reduction was found to have gone to completion, as evidenced by the disappearance of the original gem-$(CF_3)_2$ peak 12.3 ppm downfield of the TFA peak, and the appearance of a new gem-$(CF_3)_2$ multiplet at 5.5 ppm downfield of TFA. (In a subsequent run on 4 times this scale, the reduction was approximately 30 percent complete after six days at room temperature.)

Thirty ml of 96 percent sulfuric acid was added to the reaction mixture, forming a heavy gray precipitate of $ZnSO_4$. Most of the TFA was distilled from the mixture at atmospheric pressure; 105 g was so recovered. Water was then added cautiously in small portions through the reflux condenser until most of the ppt dissolved. The product amine was collected by direct steam distillation and separation from the co-distilled water. The yield of crude product, showing no impurities by ^{19}F nmr, was 13.2 g, 79 percent. The work-up takes advantage of the very weak basicity of the amine, which is protonated by 96 percent H_2SO_4, but liberated when the acid is diluted with water, so it can then be separated by steam-distillation from zinc, aniline salts, and sulfuric acid.

27. Attempted Preparation of Perfluoro-2-methyl-2-pentylamine (JPL-AB-36)
by reduction of 2-(p-Nitrophenylayo-perfluoro-2-methylpentane

$$P\text{-}O_2N\text{-}C_6H_4\text{-}N{=}N\text{-}C(CF_3)_2CF_2CF_2CF_3 \xrightarrow[(CH_3CO)_2O]{Sn,\ HCl} CF_3CF_2CF_2(CF_3)_2CNH_2$$

50.1 g (0.107 mol) of crude azo compound was dissolved in 227 g acetic anhydride and stirred with 35.6 g mossy tin (0.300 g-atom) in a 3-neck flask equipped with a reflux condenser. 100 ml of 37% hydrochloric acid was added in small portions. A vigorous reaction ensued and all of the tin dissolved. A two-phase reflux was noticed, and after the reaction slowed a Dean-Stark trap was inserted between the pot and the reflux condenser. A dense, mobile lower phase of volatile product was collected by applying heat to the reaction flask. The crude product was separated, dried over $CaCl_2$ and examined by FMR spectroscopy. It was found to consist of essentially pure $CF_3CF_2CF_2(CF_3)_2CH$, recognizable by the distinctive splitting pattern of the geminal CF_3 groups coupled to the lone hydrogen. No signal for geminal CF_3's in the place expected for the amine could be detected. The yield of crude $C_6F_{13}H$ was 23.4 g, 68%.

28. Attempted Preparation of 2-Tert-butoxy-perfluoro-2-methylpentane (JPL-AB-37)

$$CF_3CF_2CF_2(CF_3)_2COH + (CH_3)_2C{=}CH_2 \xrightarrow{TFA} CF_3CF_2CF_2(CF_3)_2COC(CH_3)_3$$

Twenty five point one (25.1) g (0.0747 mol) of perfluoro-2-methyl-2-pentanol was placed in a length of 15 mm Pyrex tubing closed at one end. The tube was cooled to -78 and 4.96 g of isobutene (0.0884) condensed in. The tube was sealed and let warm to room temperature. The mixture was homogeneous. After 24 hours the tube was chilled, opened, and let warm back to room temperature. In the process, most of the isobutene boiled off. A PMR spectrum of the mixture immediately after warming showed no evidence of the desired $-OC(CH_3)_3$ singlet. After 24 hours at room temperature in the narrow-necked tube the net weight of the contents was 25.55 g.

29. Attempted Preparation of Perfluoro-2-methyl-2-pentanol (JPL-AB-38)
by direct O_2 oxidation of Perfluoro-2-methyl-2-pentyl potassium

$$(CF_3)_2C{=}CFCF_2CF_3 + KF + O_2 \xrightarrow{NMP} CF_3CF_2CF_2(CF_3)_2C\text{-}OH$$

Sixty one point four (61.4)g (0.205 mol) perfluoro-2-methyl-2-pentene, 20 g (0.344 mol) anhydrous KF and 100 ml N-methyl-2-pyrrolidinone were combined in a 500 ml pressure bottle and stirred magnetically. Air was displaced from the bottle by repeatedly filling it with O_2 to 25 psig and releasing the pressure, then the bottle refilled with 25 psig of O_2 and stirred at room temperature for 22 hours. No significant pressure drop occurred. The presence of the carbanion was confirmed by adding 25 ml of ethyl bromoacetate (0.226 mol) and stirring long enough for the reaction to go to completion. The mixture was worked up in the usual way and the known $CF_3CF_2CF_2(CF_3)_2CCH_2CO_2C_2H_5$ isolated in 81% yield (67.4 g); no signals consistent with $CF_3CF_2CF_2(CF_3)_2COCH_2CO_2C_2H_5$, the product which would have been expected from the desired alcohol, could be detected in the FMR spectrum of the crude ester.

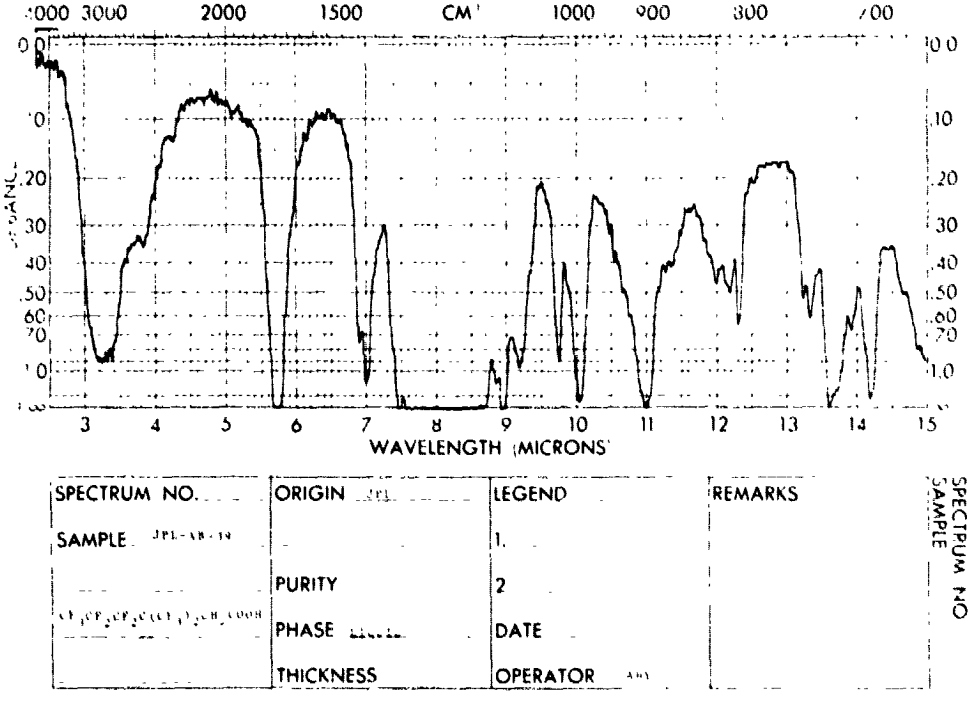
30. 3,3-Bis(trifluoromethyl)-4,4,5,5,6,6,6-Heptafluorohexanoic (JPL-AB-39) acid

$$CF_3CF_2CF_2C(CF_3)_2CH_2COOH \xrightarrow[C_2H_5OH]{85\%\ KOH} CF_3CF_2CF_2C(CF_3)_2CH_2COOC_2H_5$$

The ester, (JPL-AB-24) 68.4 g (0.16 moles) was saponified with a 20% excess of KOH (pellets) in 100 ml 84% ethanol. After stirring at room temperature for 48 hours, the solution was diluted to 500 ml with water and extracted with ethyl ether. The aqueous layer was acidified with HCl to ~ pH 2. The oil which separated was extracted with ether, the extract washed with water, dried over $MgSO_4$, and evaporated on the rotary evaporator. The yield of crude acid was 52.7 g, 83%. An analytical sample was obtained by suspending the acid in hexane, cooling the suspension to -20°C and filtering off the crystalline product. JPL-AB-39 is a new compound.

Calculated For: $C_8H_{13}O_2$ (378.104): C, 25.41%; H, 0.79%.

Found: C, 25.32%; H, 0.97%.



31. 4,4,-Bis(trifluoromethyl)-1,1,1,2,2,3,3,-heptafluoro-6-octene (JPL-AB-40)

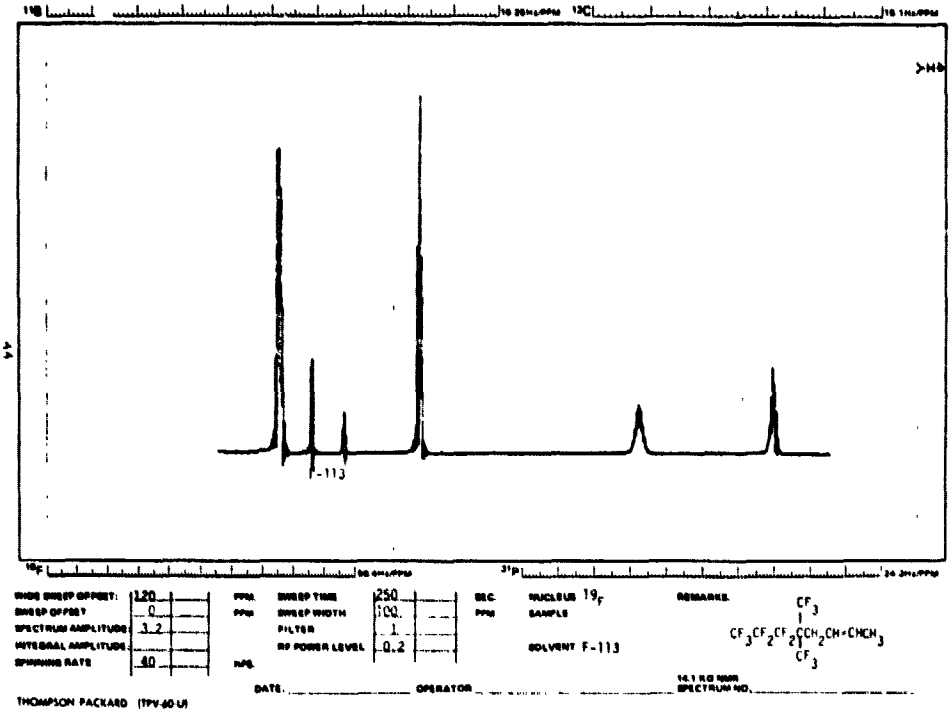
$$(CF_3)_2C{=}CFCF_2CF_3 + CH_3CH{=}CHCH_2Cl \xrightarrow[\text{DMA}]{\text{KF,NaI}} CF_3CF_2CF_2C(CF_3)_2CH_2CH{=}CHCH_3$$

R.T.

A one-liter 3-neck flask was charged with 40 g of dry, ball-milled KF and 500 ml of dry dimethylacetamide. The mixture was stirred magnetically and 134.6 g (0.449 mol) of perfluoro-2-methyl-2-pentene and 54.3 of technical "crotyl chloride" (0.60 mol), consisting of approximately 80% $CH_3CH{=}CHCH_2Cl$ and 20% $CH_3CHClCH{=}CH_2$, added. The flask was stoppered and let stir at room temperature. After 17 hours stirring was stopped and the lower phase examined by FMR; only starting perfluoroolefin was present. Sodium iodide (5 g, 0.033 mol) was added and stirring was resumed. The lower phase was examined again 24 hours later; this time the reaction was essentially complete, the FMR spectrum of the starting perfluoroolefin having been replaced by the characteristic multiplets of the perfluoro-tert-hexyl group. After three more hours of stirring 75 ml of morpholine was added to destroy unreacted crotyl chloride and any remaining perfluoroolefin, and the mixture stirred overnight at room temperature. After 18 hours, the reaction mixture was poured into ca. 1.5 liter of water; the lower fluorochemical phase was separated and washed once with a solution 3 M in HCl and 0.5 M in H_3BO_3. The total crude product weighed 130.4 g, 77.7%, and was substantially pure as judged by FMR and PMR spectroscopy. Before hydrogenation, the crude product was dried over $CaCl_2$, distilled at aspirator pressure, let stand overnight over solid KOH and distilled again. JPL-AB-40 is a new compound.

Calculation for $C_{10}H_7F_{13}$ (374.14): C, 32.10%; H, 1.89%; F, 66.01%.

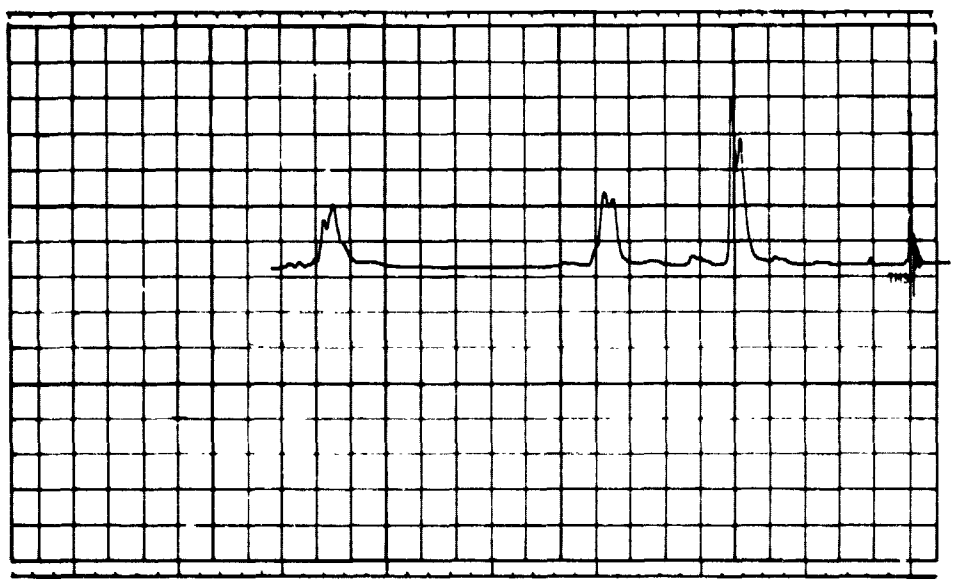
Found: C, 32.22%; H, 2.01%.



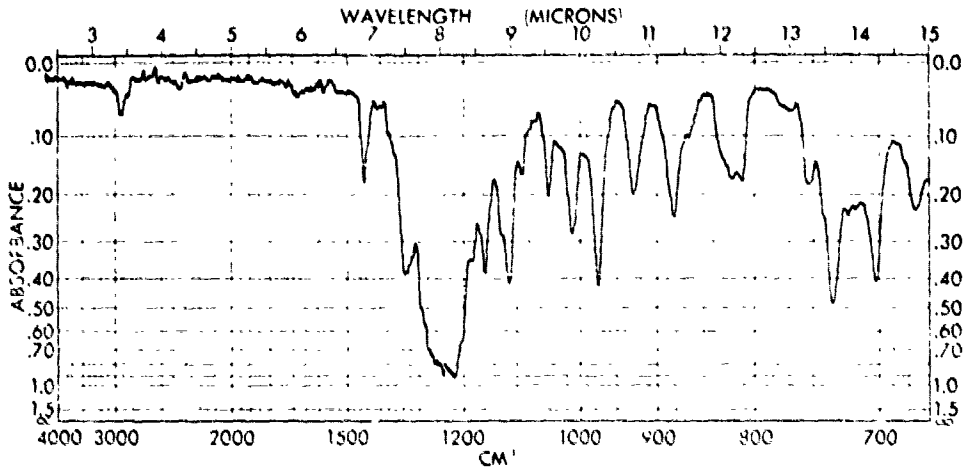
WIDE SWEEP OFFSET:	120	PPM	SWEEP TIME	250	SEC	NUCLEUS ^{19}F	REMARKS
SWEEP OFFSET	0	PPM	SWEEP WIDTH	100.	PPM	SAMPLE	$CF_3CF_2CF_2\underset{\underset{CF_3}{\mid}}{C}CH_2CH{=}CHCH_3$
SPECTRUM AMPLITUDE	3.2		FILTER	1			
INTEGRAL AMPLITUDE			RF POWER LEVEL	0.2		SOLVENT F-113	
SPINNING RATE	40	NPS					

DATE _____ OPERATOR _____ 14.1 KG NMR SPECTRUM NO. _____

THOMPSON PACKARD (TPV-60 U)



0	X		^1H	$CF_3CF_2CF_2\underset{\underset{CF_3}{\mid}}{C}CH_2CH{=}CHCH_3$
3.2		X	In TMS	
40	X	0.05	F-113	



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)CCH_2CH{=}CHCH_3$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

32. 4,4-Bis(trifluoromethyl)-1,1,1,2,2,3,3-heptafluoro-6-methyl-6-heptene (JPL-AB-41)

$$(CF_3)_2C{=}CFCF_2CF_3 + ClCH_2C(CH_3){=}CH_2 \xrightarrow[\text{DMA}]{\text{KF, NaI}} CF_3CF_2CF_2(CF_3)_2CCH_2C(CH_3){=}CH_2$$

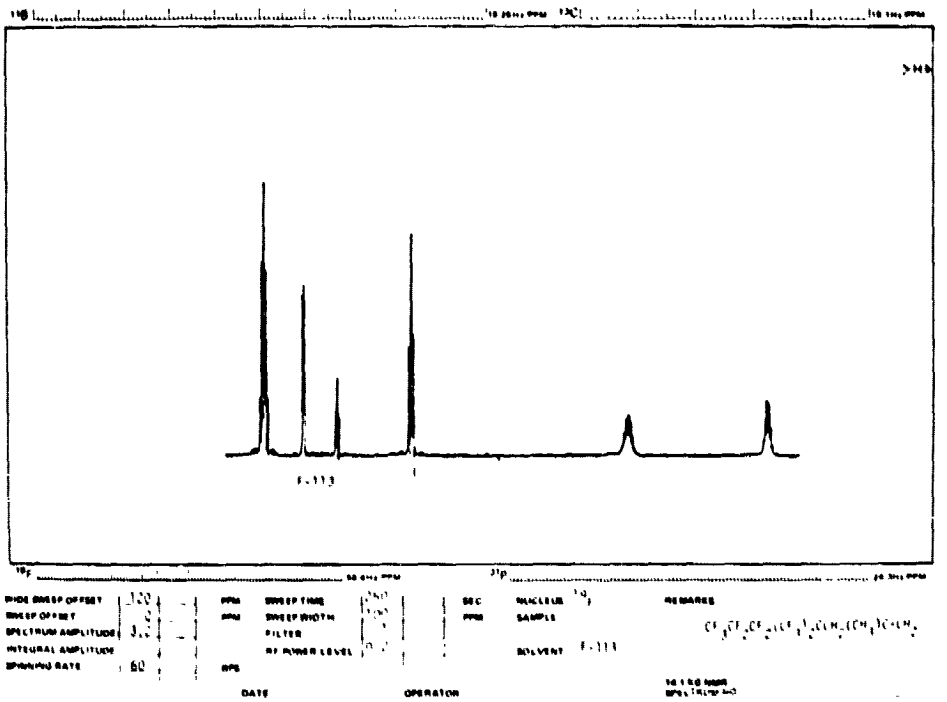
R.T.

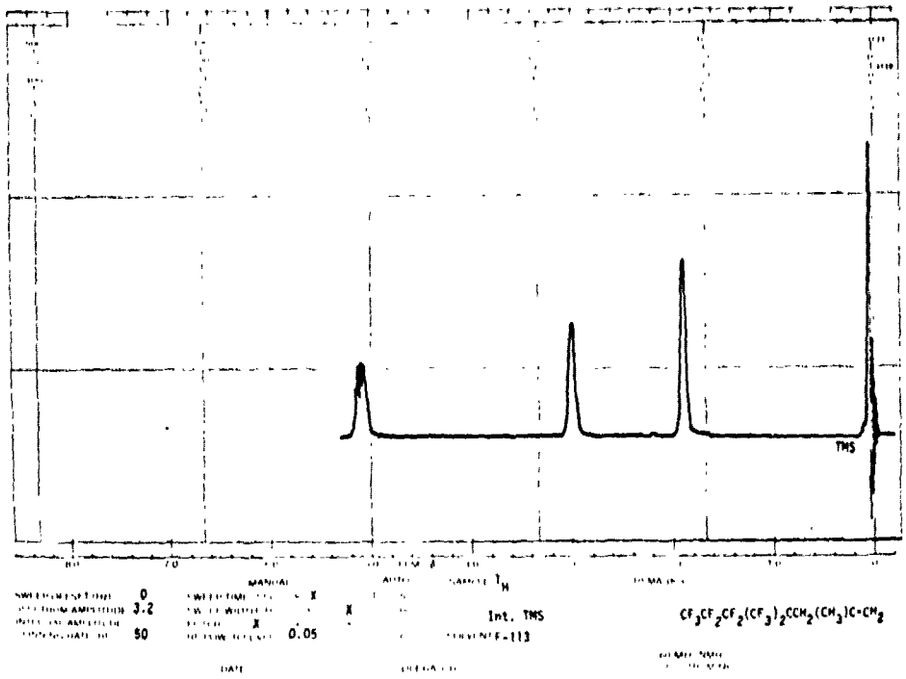
Dry, finely ground potassium fluoride (40 g), dimethylacetamide, 500 ml, perfluoro-2-methyl-2-pentene, 125.6 g (0.419 mol), and 3-chloro-2-methyl-1-propene, 55.6 g (0.613 mol) were combined and stirred at room temperature. No evidence of product formation was found after 17 hours, so 5 g of NaI was added and stirring continued. After 24 hours more the reaction was judged 29% complete. After a total of 350 hours, 50 ml of morpholine was added, and 24 hours later the reaction was worked up as in the preceding example. The yield of crude distilled product, judged substantially pure by FMR and PMR, was 128.7 g, 82.1%. JPL-AB-41 is a new compound.

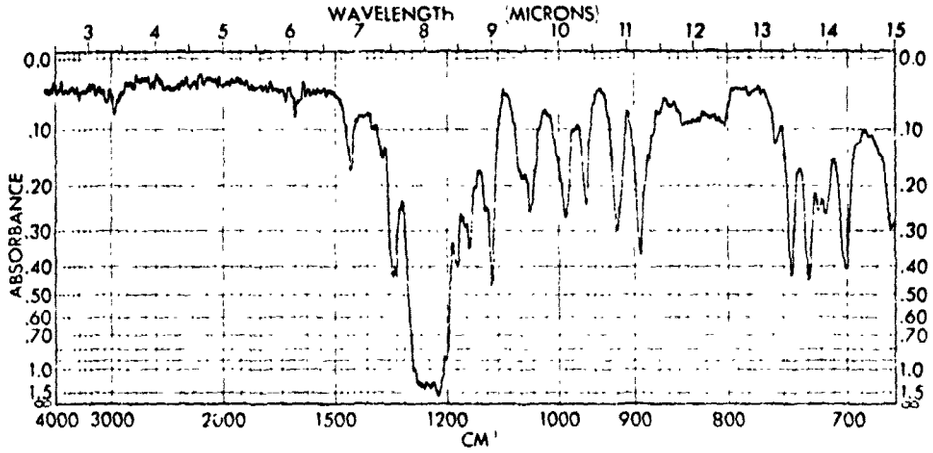
A sample was purified for analysis by preparative gas chromatography.

Calculated for $C_{10}H_7F_{13}$ (374.14): C, 32.10%; H, 1.89%; F, 66.10%.

Found: C, 31.94; H, 2.00.







SPECTRUM NO.	ORIGIN JPL	LEGEND	REMARKS
SAMPLE		1.	
$CF_3CF_2CF_2(CF_3)_2CCH_2(CH_3)C=CH_2$	PURITY	2.	
	PHASE Liquid	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE

33. 2-(p-nitrophenylazo)-perfluoro-2-methylpentane (JPL-AB-42)

$$(CF_3)_2C{=}CFCF_2CF_3 \quad + \quad KF \quad + \quad p\text{-}NO_2\text{-}C_6H_4\text{-}N_2^+BF_4^- \xrightarrow{DMA}$$

$$CF_3CF_2CF_2(CF_3)_2C\text{-}N{=}N\text{-}C_6H_4\text{-}p\text{-}NO_2$$

A mixture of 118 g perfluoro-2-methyl-2-pentene (0.392 mol), 45 g dry, powdered potassium fluoride (0.78 mol) and 500 ml of dry dimethylacetamide was cooled to -16°C and stirred under a blanket of nitrogen while 93 g (0.393 mol) of p-nitrobenzenediazonium fluoroborate was added in small portions. Stirring became difficult as the mixture became porridge-like, and the temperature was let rise to 0° by the end of the addition. After stirring about 0.5 hour more, the mixture was filtered by suction, and the flask and filter-cake rinsed well with ether. Water, ca. one L, was added to the filtrate, and the product was extracted into ca. one L of ether, including the washings above. The ether extract was washed with 5% potassium hydroxide solution several times, once with dilute sulfuric acid, once with saturated brine, dried over $CaCl_2$, and taken to dryness in vacuum. The crude azo compound was a red orange oil weighing 151 g, essentially pure by 19F nmr, 82% yield.

The product (4.9 g) was purified by column chromatography. The column (1 x 30 cm) was packed with Silica Gel 60; 70-230 mesh, EM Reagents-Catalog Nr 7734.

The product was eluted with petroleum ether and twenty 10 ml fractions were collected. Fractions 7 through 12 were combined and evaporated to dryness to yield 1.26 g of oil. A sample was further purified for analysis by crystallizing the oil from cold (-30°C) petroleum ether. JPL-AB-42 is a new compound.

Calculated for: $C_{12}H_4F_{13}N_3O_2$ (469.17): C, 30.72%; H, 0.86%; N, 8.96%.

Found: C, 30.44%; H, 0.92%; N, 9.66%, 10.03%.

SECTION IV

PHYSICAL CHARACTERIZATION

In this section the various methods of physical characterizations used during the second year of the effort are described. The previously reported numerical methods for the calculation of physical properties of fluorocarbon materials (Reference 20) were upgraded. In the following subsections, results using these upgraded methods and the contributions of technical material by the University of Southern California, Rockwell International Science Center, and the University of Utah Research Institute are described. Supporting data and information are presented here and in the pertinent appendixes.

A. ESTIMATION OF HEAT OF VAPORIZATION FROM BOILING POINT DATA (HILDEBRAND'S RULE)

Reevaluation of Hildebrand's Rule for the estimation of heats of vaporization of fluorocarbons from boiling point data: from the boiling point (T_B at 760 mm Hg), the heat of vaporization (ΔH_v) may be estimated by means of the semiempirical Hildebrand's rule (Eq. 1 and 2).

$$\Delta H_{v298\,K} = -2950 + 23.7\ T_b + 0.020\ T_b 2 \qquad (1)$$

or in terms of the boiling point at 760 mm Hg:

$$T_b = \frac{-23.7 + [651.7 + 0.08\ (2950 + \Delta H_v)]^{1/2}}{0.04} \qquad (2)$$

It was pointed out to us by Prof. R. L. Scott (UCLA) that Hildebrand and he did not include fluorocarbon data in the original rule (Eq. 1 and 2) because of a lack of reliable data (1950). The data in the literature since 1950 is extensive enough to allow us to compute a new relationship for fluorocarbons which is:

$$\Delta H_v = 0.0724\ T_b^{\ 2} - 17.17\ T_b + 5309 \qquad (3)$$

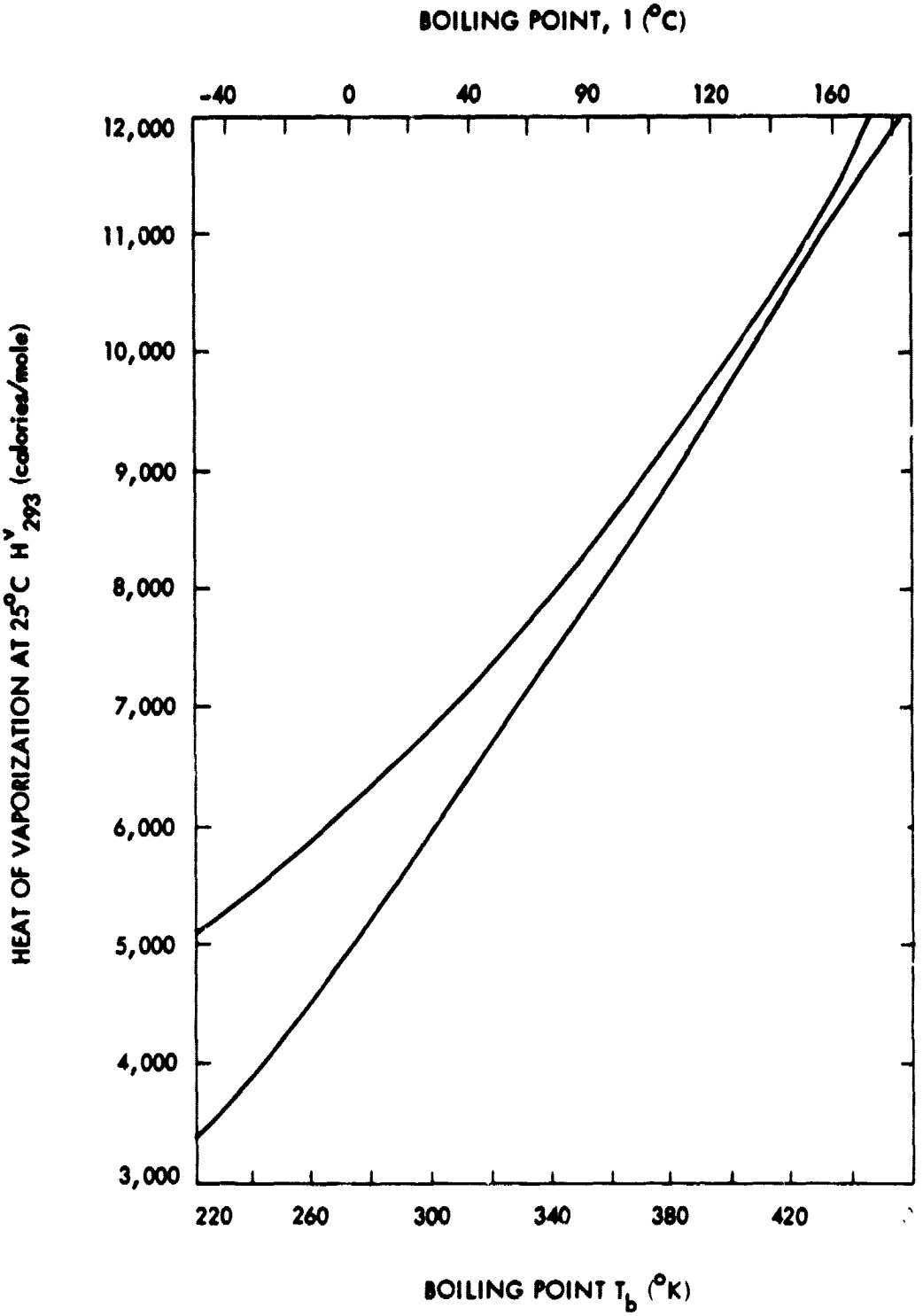


Figure 4-1. Plots of Hildebrand rule (curve B) and that of Perfluorocarbon Materials (curve A).

In terms of the boiling point:

$$T_b = \frac{+17.17 + [294.9 - 0.2896\ (5309 - \Delta H_v)]^{1/2}}{0.1448} \qquad (4)$$

Equation (3) is a least-squares computer curve fit based on 19 fluorocarbons whose ΔH_v^{298} and boiling point values were found in the literature or calculated by us from vapor pressure-temperature data. The 19 compounds include straight chain fluorocarbons, one substituted C_4 ring, one C_5 ring, two substituted C_6 rings, one decalin, one substituted decalin, and two perfluoro tertiary amines.

Figure 4-1 is a plot of both Equations (1 and 3). As can be noted, fluorocarbons have a higher heat of vaporization than hydrocarbons on a boiling point basis. This is especially true in the lower ΔH_v^{298} range (5,000 - 9,000 cal) after which the fluorocarbons parallel the hydrocarbons fairly closely.

B. ESTIMATION OF PROPERTIES BY GROUP ADDITIVITY METHOD

Estimation of solubility parameters and molar volumes by the group additivity method: our previous group contributions for the energy of vaporization (Δe_i) and molar volume (Δv_i) have been re-examined using the new form of Hildebrand's rule for fluorocarbons (Eq. 3).

In formulating a group additivity system, it is desirable to have energy of vaporization (ΔE_v^{298}) and density data on a large variety of homologous compounds from which the individual group contributions can be calculated. Research uncovered 19 fluorocarbons whose ΔH_v^{298} or vapor-pressure/temperature data appeared good. But, operating on the assumption that Equation 3 is a good first approximation to estimating ΔH_v^{298} for a large number of compounds since a great amount of boiling point data is available for fluorocarbons in the literature.

Thus, by taking the boiling points for perfluorobutane and perfluoropentane one can calculate their individual ΔH_v^{298} which can be used to calculate ΔE_v^{298} ($\Delta E_v^{298} = \Delta H_v^{298} - RT$). The difference between the two ΔE_v^{298} values is the group contribution of a $-CF_2-$ to the energy of vaporization. By using the boiling points for the straight chain fluorocarbons from C3-C13 one can get 10 values for the Δe_i of $-CF_2-$ which can then be averaged.

$CF_3CF_2CF_2CF_3$	$CF_3CF_2CF_2CF_2CF_3$
b.p. = 271.9°K	b.p. = 302.5°K
ΔH_v^{298} = 5991 cal/mole	ΔH_v^{298} = 6738 cal/mole
ΔE_v^{298} = 5399 cal/mole	ΔE_v^{298} = 6146 cal/mole

$$6146 - 5399 = 747 \text{ cal/mole for } -CF_2-$$

Also, from the density data, one can calculate the molar volume, V cm^3/mole, ($V = \frac{MW}{D}$) and analogously get the group contribution of a $-CF_2-$ to the molar volume.

$CF_3CF_2CF_2CF_3$	$CF_3CF_2CF_2CF_2CF_3$
D^{25} = 1.527 g/cm^3	D^{25} = 1.604
MW = 238.03	MW = 288.04
V = 155.9 cm^3/mole	V = 179.6 cm^3/mole

$$179.6 - 155.9 = 23.7 \text{ cm}^3\text{/mole for } -CF_2-$$

Table 4-1 contains a list of the fluorocarbon group additivity values calculated by us and the hydrocarbon values published in a paper by R. F. Fedors (Reference 7). (Polymer Enginering and Science, 14, 147-153, 1974.). Table 4-2 gives a summary of the physical properties of these materials.

C-2

Table 4-1. Group Contributions to the Energy of Vaporization and Molar Volume at 25°C

Group	ΔE_v^{298} cal/mol	V cm^3/mol
CF_3-	1933	54.8
-CF_2-	783	23.1
-CF-	-396	-15.0
-CFH-	422	18.6
-C- (Perfluoro)	-1515	-38.3
-O- (Ether)	8	19.0
-N- (3° Amine)	-914	16.3
Ring: 5 atoms	2023	37.7
Ring: 6 atoms	2272	39.9
$(CF_3)_3C$-	4284	126.1
$CF_3CF_2CF_2C(CF_3)_2$-	5850	172.3
CH_3-	1125	33.5
-CH_2-	1180	16.1
-CH	820	-1.0
-$\overset{\vert}{\underset{\vert}{C}}$-	350	-19.2

C. ESTIMATION BY USE OF SATO EQUATION

Estimation of vapor pressures by use of the Sato Equation (Hydrocarbons and fluorocarbons): materials that have high vapor pressures tend to have adverse physiological effects when used in artifical blood substitute formulations. Therefore, it is desirable to have some method of predicting the vapor pressure before synthesis is attempted.

K. Sato has pointed out that the molar entropy of vaporization (ΔS_v) of normal (i.e., non-polar) liquids is related to their vapor pressures P (in mm. Hg) at given temperatures as follows:

$$\Delta S_v = \frac{\Delta Hv}{T} = \alpha R\left(\frac{P}{T}\right)^{\beta-1} \qquad (5)$$

This relationship was derived from Hildebrand's general rule that compounds have equal ΔS_v's at equal vapor densities. To make it more useful Eq. 5 was recalculated in terms of P (pressure in mm Hg):

$$P = T\left(\frac{\Delta Hv}{\alpha RT}\right)^{\frac{1}{\beta-1}} \qquad (6)$$

K. Sato, however, calculated the constants α and β based on the vapor pressure/temperature data for ten hydrocarbons. We essentially duplicated Sato's calculation with ten fluorocarbons and determined new α and β values which match the literature values for the vapor pressures of fluorocarbons much more closely than Sato's original values. Sato's constants for hydrocarbons and our values for fluorocarbons are:

Hydrocarbon	$\alpha = 11.8822$	$\beta = 0.8810$ (Sato's values)
Fluorocarbon	$\alpha = 12.2497$	$\beta = 0.8846$

Table 4-2 presents the calculated vapor pressures of some fluorocarbon and hybrid-fluorocarbon materials and, when available from the literature, their measured vapor pressures. The accuracy is good enough to make decisions on the feasibility of use as an artificial blood substitute.

Table 4-3 gives a summary of the physical properties of these materials.

D. EMULSION STABILITY CRITERIA (ROCKWELL INTERNATIONAL SCIENCE CENTER)

For the stabilization of fluorocarbon emulsions for artifical blood substitute applications, a number of nonionic emulsifiers were investigated. Stabilization characteristics were described largely in terms of empirical observations. Consequently, the optimization of new fluorocarbon-emulsifier systems requires extensive experimentation. The purpose of this research was to attempt to provide a basis for the development of quantifiable physical/chemical criteria for the stability of artifical blood emulsions and thus aid in the design and optimization of new systems (see Appendix B).

This study focused on the role of interfacial phenomena between the fluorocarbon and the aqueous phase. Inasmuch as stabilization with nonionic macromolecules is controlled primarily by dispersion and polar intermolecular forces, rather than by the electrostatic effects which control ionic surfactants, our investigation was limited to the fluorocarbon-surfactant water system in the absence of electrolytes. Although effects of electrolytes should be investigated as an extension of this study our overall conclusions should not be affected. The fluorocarbons studied were perfluorotributylamine and perfluorodecalin; the surfactant was Pluronic F68 (Wyandotte Chemical Corp.), a commonly used surfactant for artifical blood formulations.

Fluronic F68 is an A-B-A triblock copolymer with A=polyethylene oxide (PEO), and B=polypropylene oxide (PPO). In this type of surfactant, B is the segment which is anchored to the fluorocarbon; A is the stabilizing segment which is highly soluble in water, i.e., the dispersion medium. Results of quantitative measurements of interfacial tension and surface energetics analysis are detailed in the subsequent section, along with the description of a model for micelle formation. This discussion provides an interpretation for the observed decrease in interfacial tension between the fluorocarbon and the aqueous phase and for the oncotic pressure effect that has been observed for the Pluronic emulsifier. The discussion in the remainder of this section is based entirely on data from the literature, and hence is only qualitatively correct. The stabilizing effect depends on the solution thermodynamics of PEO. It has been established that the thermodynamic theta-temperature (i.e., temperature of incipient precipitation for infinite mol wt polymer) for the

stabilizing segment is nearly identical to the critical flocculation temperature for the emulsion. Addition of electrolyte tends to raise the theta-temperature. This suggests that in the absence of electrolyte the emulsion should be stable in the range between room temperature and body temperature, but that once the emulsion is adjusted to physiologic conditions it may become unstable at ambient temperature.

Particle size should be minimized in order to allow passage through capillaries in the circulatory system and to enhance gas exchange. It was observed, however, by Geyer that if the particle size gets below about 300-500 Å, viscosity increases dramatically. The viscosity of an emulsion depends on the volume fraction of the dispersed phase and on inter-particle interactions.

If one assumes that the Pluronic PPO segment is strongly absorbed on the fluorocarbon particle, and assuming as a lower limit to the thickness of the hydrated PEO layer its theta-dimension, one arrives at a thickness of about 50 Å for PEO mol wt 3,500. It is common practice to have the initial emulsion (prior to adjusting for physiologic conditions) contain about 20-24 wt% fluorocarbon, which translates into about 12 vol%. However, if one considers a 300 Å particle size, the PEO 50 Å layer would increase the volume fraction to nearly 30%, whereas for large particles the volume fraction increase would be negligible. A large increase in viscosity for comparable particle size-volume-fraction ranges was reported for latex suspensions. To derive a quantitative viscosity-particle size relationship, the determination of a scaling parameter to account for interparticle interactions would be required.

Table 4-2. Estimated Vapor Pressure for some Fluorocarbons at 25°C

COMPOUND	[1]EST. ΔH_v^{298}	[2]EST. BOILING POINT @760mm	LIT. BOILING POINT @760mm	[3]EST. VAPOR PRESSURE @25°C	LIT. VAPOR PRESSURE @/T°C
$CF_3CF_2CF_2CF_2CF_3$	6807	32	29	519	646/25
$CF_3CF_2CF_2CF_2CF_2CF_3$	7590	59	57	202	220/25
	8316	81	76	92	106/25
	9070	102	102	43	35/25
	10608	141	142	11.1	6.6/25
	11362	158	160	6.1	2.1/25
$CH_3CHF[OCF_2CF(CF_3)]_2F$	8782	94	104	57	56/37.5
$(CF_3)_2CFO(CF_2)_5CF_3$	9918	124	121	19.9	39/37.5
$[(CF_3)_2CFOCF_2CF_2]_2$	10608	142	135	10.5	10/37.5
$CF_3CHF[OCF_2CF(CF_3)]_3F$	11110	152	152	7.4	10/37.5
$CF_3CHF[OCF_2CF(CF_3)]_4F$	13438	201	194	1.4	1.9/27.5
$[(CF_3)_2CFOCF_2)_4]_2$	13812	208	199	1.1	3/37.5
$CF_3CHF[OCF_2CF(CF_3)]_5F$	15766	244	224	0.4	0.4/37.5
$(CF_3CF_2CF_2CF_2)_3N$	12524	183	174	2.6	2.5/37.5

1. Calculated from the values in Table I.
2. Calculated by equation 4.
3. Calculated by equation 6 using the values for fluorocarbons.

E. TECHNICAL CONTRIBUTIONS

Technical contributions were made by the University of Southern California. These were consolidated and made available to specialists in the field through current literature (see Appendices C,D,E,F, and G).

Table 4-3. Summary of Physical Properties

COMPOUNDS	JPL AB NUMBER	OXYGEN SOL FOUND $cc O_2/100 cc C$ @25°C	OXYGEN SOL CALC'D $cc O_2/100 cc C$ @25°C (V=46 ml)	CALC'D VAPOR PRESSURE @ 25°C mm Hg	BOILING POINT 730 mm Hg FOUND	CALC'D BOILING POINT 760 mm	DENSITY FOUND @ 25°C
$CF_3CF_2CF_2C(CF_3)_2CH_2CH_2CH_3$	15	46.7	41.0	19.8	121	124	1.522
$CF_3CF_2CF_2(CF_3)_2CH_2CH_2CH_2CH_3$	16	46.6	39.9	7.5	137.5	152	1.466
$CF_3CF_2CF_2C(CF_3)_2CH_2CH(CH_3)_2$	17	43.6	40.4	10.4	132	143	1.453
$CF_3CF_2CF_2C(CF_3)_2OH$	18	47.9	39.7	60.3	93	--	1.770
$CF_3CF_2CF_2C(CF_3)_2OCH_3$	19	48.1	42.6	86.9	97	83	1.617
$CF_3CF_2CF_2C(CF_3)_2OC_2H_5$	20	49.9	41.3	27.7	109	114	1.571
$CF_3CF_2CF_2C(CF_3)_2OCH_2CH_2CH_3$	21	45.8	40.0	10.1	125	143	1.507
$CF_3CF_2CF_2C(CF_3)_2CH_2OCH_2CH_3$	22	44.3	40.0	10.1	133	143	1.530
$CF_2CF_2CF_2C(CF_3)_2NO$	23	decomp.	43.0	122.7	73	--	1.702

1. ΔE_v^{298} calculated from the values in Table 4-1.

SECTION V

BIOLOGICAL EVALUATION

A. BIOLOGICAL SCREENING

Under contract with the Jet Propulsion Laboratory for "Synthesis and Biological Screening of New and Improved Fluorocarbon Compounds for Use as Artifical Blood Substitutes", the Utah Biomedical Test Laboratory (UBTL) assumed responsibility for the biologic screening of new fluorochemical (FC) compounds that were synthesized by the Jet Propulsion Laboratory. The main goal of the study was to test the premise that from the synthesis of novel fluorocarbons, candidates for safe hemoglobin substitutes could be found, and specifically to test each compound for gross toxicity and tissue retention in mice. Results of the second year's study are included in the UBTL Report (Appendix H).

B. TISSUE CULTURE EVALUATION

Tissue culture studies on 10 compounds were carried out by Professor Geyer of Harvard University. The desirability of obtaining a baseline for results from the overall program was agreed upon at the contractor's meeting held at Air Products, Allentown, PA, May 1977. The results of Dr. Geyer's tests are given given in Appendix I.

C. COMPUTER ANALYSIS

A new computer program was submitted (for use with an HP-97 computer) under the category name "Physical Sciences Chemistry". Given the chemical structure of a fluorochemical, the program calculates certain physical characteristics at 25°C (see Appendix I).

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APPENDIX A

MOUSE TOXICITY TEST PROTOCOL

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MOUSE TOXICITY TEST PROTOCOL

After the compounds are prepared for intra-peritoneal injection, "Absorption Verification Studies" are performed as follows:

Three mice for each compound are injected intra-peritoneal with 20% v/v emulsion in a dose of 100 ml/kg of body weight. They are observed continuously for the first half hour, then at least every half hour until the end of the second hour for 2 hours and finally every 2 hours through the 24th hour. A standard protocol for observation of intoxicated animals is employed, and findings are recorded on a check-off sheet. The mice are sacrificed after 24 hours (unless they died earlier) and their peritoneal cavities washed for recovery of the compound. This helps to establish whether lack of mortality, when it occurs, is due to lack of toxicity or lack of absorption.

If the mice die during this test, and if the controls do not, it is assumed that the test compound was absorbed. However, the three test mice do undergo the peritoneal wash.

After the peritoneal wash, one of the three mice is placed immediately in formaldehyde for later histopathological study and the other two undergo whole body homogenization so possible metabolites can be extracted. The three control mice are injected with the Pl-F68 solution (5.76%).

If the mice do not die during the Absorption Verification Studies, a new group of three mice is injected intra-peritoneally with the same dose (100 ml/kg) of body weight and observed for 2 weeks for death and/or toxic effects. Doses of 10 ml/kg and 1 ml/kg are also administered. This is the Mouse Toxicity Test. If a test compound passes this test, it is recommended for re-synthesis in larger quantity, emulsification and *in vitro* blood testing before going to the more complete exchange transfusion (blood replacement) rat tests. If death or toxicity occur during the Mouse Toxicity Test then other doses will be considered, if additional toxicity data are deemed desirable.

APPENDIX B

A SURFACE ENERGETICS ANALYSIS OF ARTIFICIAL BLOOD SUBSTITUTES

APPENDIX B

August 1978 SC5126.4FR

A SURFACE ENERGETICS ANALYSIS OF ARTIFICIAL BLOOD SUBSTITUTES

Final Report

For Period 08/26/77 through 07/15/78

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A SURFACE ENERGETICS ANALYSIS OF ARTIFICIAL BLOOD SUBSTITUTES

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ABSTRACT

The surface and interfacial tensions for fluorocarbon aqueous emulsions used in artificial blood substitutes are evaluated over a wide range of surfactant concentrations. The surface tension of the nonionic surfactant in water is directly correlated to the interfacial tension between aqueous and fluorocarbon phase by a surface energetics model of isolated dispersion and polar contributions to interfacial work of adhesion and interfacial tension. This study shows that the polymeric surfactant which is an A-B-A triblock copolymer where A = polyethylene oxide and B = polypropylene oxide displays prominent surface and interfacial tension transitions at $c = 1.10^{-7}$ and $c = 0.10$ weight fraction detergent in water. The $c = 10^{-7}$ transition is shown to produce a prominent change in the extensibility of the fluorocarbon to water interface and formation of a strong interfacial film which enhances emulsion stabilization.

INTRODUCTION

The first use of fluorochemical liquids to carry dissolved oxygen and carbon dioxide in a liquid-liquid interface oxygenator was reported in 1965 by Howlett, Dundos and Sabiston[1]. Clark and Gollan in 1966 showed that mice could survive while fully immersed in oxygenated liquid fluorocarbon[2]. Clark and Gollan also showed in late 1966 that an isolated rat heart perfused with a fluorocarbon emulsion would continue to function[3]. Geyer and co-workers in 1968 used perfluorotributyl amine (FC 43 of 3M Co.) in water emulsion as a blood substitute to maintain live rats in pure oxygen atmosphere[4]. Geyer and co-workers have continued to develop better fluorocarbon emulsions so that by 1974 a total blood replacement of rats had been demonstrated[5,6]. These rats survive in pure oxygen atmosphere while rebuilding their material blood cells and serum proteins and continue to live normally afterward.

The stabilization of fluorocarbon emulsions in blood or blood substitute media remains as an important problem which has not been fully defined and made subject of systematic study. The difficulty of other workers to reproduce Clark's and Geyer's clinical survival rates appears traceable to variability in the preparation and stabilization of the fluorocarbon emulsion[7]. As defined by Geyer, the term "artificial blood substitute" should be reserved for synthetic preparations that substitute for both red cells and plasma proteins[8]. Symbols and nomenclature for the principal physical or mathematical terms used in this discussion are summarized in Table 1.

Current Concepts for Emulsion Stabilization

A general discussion of emulsion stabilization by Schnolka enumerates the following factors which enhance emulsion stabilization in artificial blood substitutes[8]:

1. Reduction of interfacial tension.
2. Formation of a strong interfacial film.
3. Relative partial solubility of the surfactants in the two phases.
4. Surfactant chemistry relative to the two phases.
5. Formation of an electric double layer.

The high molecular weight Pluronic® (Wyandotte Chemical Corp.) surfactants were discovered by Geyer to be highly effective fluorochemical emulsifiers for artificial blood[5,6]. The optimum emulsion has a maximum particle size of less than one-half micron diameter to permit easy passage through the smallest capillaries[8]. The most widely used surfactant is Pluronic® F68 which is an A-B-A triblock copolymer with A = polyethylene oxide (PEO) (segment molecular weight M_A = 3500 gm/mol) and B = polypropylene oxide (PPO) (M_B = 1750 gm/mole) with the following structural formula[9]:

$$HO(CH_2\text{-}CH_2\text{-}O)_a\text{-}(\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_b(CH_2\text{-}CH_2\text{-}O)_aH \qquad (1)$$

where the number of segment repeat units are $a \simeq 75$ and $b \simeq 30$.

The discussion of Schnolka[9] indicates that the HLB index (HLB = Hydrophile Lipophile Index) is used as a basis for selecting emulsifiers and defining stability of fluorochemical emulsions. Inspection of the HLB index as detailed by Griffith[10] shows that this system is empirically based and

highly subjective in application. The HLB system states that surfactants with low HLB values will tend to form water in oil (W/O emulsions). Conversely, a surfactant with high HLB value should form oil in water (O/W) emulsions. For nonionic surfactant, such as Pluronic®F68, the HLB number appears to merely indicate the weight fraction of the hydrophilic portion of the molecule. Evidently, the current theory of emulsions and HLB criteria for emulsification require some refinement for application in artificial blood substitutes.

A coherent explanation of the well documented biocompatibility and emulsification efficiency of the Pluronic®F68 block copolymer surfactant presents a major test of the theory of emulsions. Kaelble and Moacanin[11] have recently applied a surface energetics analysis which isolates competing hydrophobic (dispersion-d) and hydrophilic (polar-p) mechanisms of bonding to redefine bioadhesion and thromboresistance for both biological and cardiovascular implant surfaces. Nyilas and co-workers[12], have also applied an analysis using separated dispersion γ^d and polar γ^p components of implant surface tension to analyze for cardiovascular compatibility. This recent approach to the present adsorption theory analysis of bioadhesion applies the new concept of defining the interfacial tension between phases i and j by the following general relation for interfacial tension[11,13]:

$$\gamma_{ij} = (\alpha_i - \alpha_j)^2 + (\beta_i - \beta_j)^2 + \Delta_{ij} \qquad (2)$$

where $\alpha = \sqrt{\gamma}^{\,d}$ and $\beta = \sqrt{\gamma}^{\,p}$ denote the respective square roots of the dispersion-d and polar-p parts of surface energy and Δ_{ij} describes

interactions defined by ionic-covalant bonding theory. A related detailed equation for interdiffusion bonding presents the following relation for interfacial heat of mixing ΔH_{ij} as follows[13]:

$$\Delta H_{ij} = V_{ij}\,\phi_i\phi_j\left[(\delta_i^d-\delta_j^d)^2 + (\delta_i^p-\delta_j^p)^2 + (\delta_i^h-\delta_j^h)^2 + \Delta_{ij}\right] \qquad (3)$$

where V_{ij} is the mixing volume and ϕ_i, ϕ_j the volume fractions of the mixed components from the adjacent i and j interfacial phases. The solubility parameters δ^d, δ^p, and δ^h for phases i and j define the respective dispersion (d), polar (p), and hydrogen bonding (h) mechanisms of mixing and Δ_{ij} denotes an excess term describing ionic-covalent bonding. The formation of stable micro emulsions (of particle size $\mu \leq 0.5$ micron) is predicted to require the minimization of both γ_{ij} and ΔH_{ij}. As mentioned above, Eq. (2) has been successfully applied in earlier studies to determine surface energy requirements for thromboresistance[11,12]. The solubility parameters described by Eq. (3) have been extensively applied by Hansen[14] in empirical definitions of polymer solubility.

Water is by far the most important single substance encountered in bioadhesion and biocompatibility. In Eq. (2) the surface tension of water (at 20 C) is described by $\alpha, \beta = 4.67, 7.14$ $(\text{dyn/cm})^{\frac{1}{2}}$ in bioadhesion analysis[11,12]. In Eq. (3) the solubility parameters for water of δ^d, δ^p, $\delta^h = 6.0, 15.3, 16.7$ $(\text{cal/cc})^{\frac{1}{2}}$ are assigned by Hansen[14]. Thus both the surface energy γ_d and the bulk cohesive energy density δ^2 for water are dominated by polar-p or hydrogen-h bonding contributions. The primary objective in this study of emulsion stabilization in artificial blood

is to delineate the role of the Pluronic® F68 surfactant in stabilizing the aqueous emulsion of perfluorotributyl amine and perfluorodecalin.

EXPERIMENTAL

Control materials for this study were kindly provided by D. D. Lawson and T. Terranova, JPL, and are described in Table 2. In this study the usual electrolytes and buffers described by Geyer[8] in fully formulated artificial blood substitutes are excluded in order to focus the analysis on the interaction between water, Pluronic® F68 surfactant, and fluorochemical components.

A water solution of Pluronic F68 was coated on a glass plate and dried to form a smooth adherent film. This solid film of Pluronic® F68 was characterized by contact angle measurements with both interdiffusing liquids such as water and absorption liquids such as 1-bromonapthalene and h-hexadecane. A standard set of test liquids with measured surface tension reported in Table 2 were employed. The following equations for absorption interactions are applied in data analysis[14]:

$$\gamma_L = \gamma_L^d + \gamma_L^p = \alpha_L^2 + \beta_L^2 \quad (4)$$

$$\gamma_S = \gamma_S^d + \gamma_S^p = \alpha_S^2 + \beta_S^2 \quad (5)$$

$$W_a = \gamma_L (1 + \cos\theta) \quad (6)$$

$$W_a = 2(\alpha_L \alpha_S + \beta_L \beta_S) \quad (7)$$

$$\frac{W_a}{2\alpha_L} = \alpha_S + \beta_S(\beta_L/\alpha_L) \quad (8)$$

where the nomenclature of Table 1 details the meaning of terms in Eqs. (4-8). The above relations form the basis for designing experiments for surface energy analysis which isolate the discrete mechanisms of polar and dispersion bonding at interfaces. The test liquids in Table 3 display a wide range of polar character with β_L/α_L = 1.53 for water to β_L/α_L = 0 for nonpolar hexadecane. Inspection of (8) shows that using measured values of W_a for liquids of known α_L and β_L permits isolation of the solid-vapor surface properties α_s and β_s as the respective intercept and slope of a plot of $W_a/2\alpha_L$ vs. β_L/α_L.

For interdiffusing test liquids such as water on dry Pluronic®F68 coatings a time dependent contact angle θ_t is displayed which reaches a minimum steady-state value θ as shown in Fig. 1 as the interdiffusion process approaches an equilibrium at the advancing contact angle boundary. As shown in Table 3 and Fig. 2 a group of interdiffusing liquids show nonpolar solid surface properties α_s = 4.8 and β_s = 0 for Pluronic®F68 coating. Polar liquids which interdiffuse into the coating impact their own polar surface properties to the swollen coating. As shown in lower Table 3 and the upper curve of Fig. 2 the extensive interdiffusion of water into the Pluronic® F68 coating shifts the solid surface properties α_s = 4.8 $(dyn/cm)^{1/2}$ and β_s = 6.97 $(dyn/cm)^{1/2}$ to become essentially equivalent to bulk water. The transformation of surface properties shown for water interdiffusion correlates with the molecular micellular structures shown in Fig. 3 where the dry Pluronic®F68 film would expose the nonpolar and hydrophobic PPO center segments to the air phase as shown in the left view. Upon water swelling the hydrophobic center segments form small spherical domains where the highly

swollen PEO end segments are now exposed to air. Recent surface energy analysis of protein analogs by Kaelble and Moacanin[11] shows that values of α_s and β_s are sensitive indicators of prior exposure to polar or nonpolar liquids which modify the protein chain conformation and surface chemistry. Sherman, Smith, and Johannessen[15], postulate somewhat similar micellular transitions for block copolymer fluorochemical textile finishes which contain hydrophilic PEO center segments under exposure to water swelling.

The surface energy and surface morphology transitions shown respectively in Fig. 2 and Fig. 3 for Pluronic®F68 should be expected to play an important role in the mechanism by which this nonionic surfactant forms stable fluorochemical emulsions. For combined surface and interfacial tension studies of artificial blood components the special Du Nuoy ring experiment shown in Fig. 4 was developed and applied. As shown in Fig. 4 the upper liquid phase is triple distilled water plus solvated Pluronic®F68 (phase 1) while the lower liquid phase is fluorocarbon. Water or water plus Pluronic®F68 forms a zero contact angle to the flame cleaned platinum-iridium ring in both air and fluorocarbon. The ring is raised at constant rates $\dot{y} = dy/dt = \pm 0.05, \pm 0.1$, and ± 0.5 cm/min to measure phase 1 surface tension γ_{1V} and lowered through the 12 interface to measure interfacial tension γ_{12}. Phase 1 is then removed to measure phase 2 surface tension γ_{2V} by raising the ring through the phase 2 surface. Counterweight (CW) as shown in Fig. 2 is added to ring to offset the bouyancy effect of the more dense fluorocarbon phase 2 on the measurement of γ_{12} interfacial tension.

These interfacial tension measurements implemented the programmed mechanical control and automated weighing system of an Instron TT-M tensile testing instrument. Tests were conducted at ambient temperature 22 $\pm$ 0.5 ^{o}C and a ring translation rate $\dot{y} = dy/dt = \pm$ 0.10 cm/min with alternative rates of $\dot{y} = \pm$ 0.05 and $\pm$ 0.5 cm/min reserved to check for rate dependence of surface or interfacial tension values. Measurements of γ_{1V}, γ_{12}, and γ_{2V} were conducted for a wide range of solution concentrations defined as c = weight fraction Pluronic®F68 in distilled water. A series of typical recorded curves of Du Nuoy ring force versus deflection through the phase 12 interface at different concentration c levels of Pluronic®F68 in distilled water are shown in Fig. 5. The conversion of these measured force versus deflection data to calculated values of surface or interfacial tension γ_{ij} utilizes the standard correction factors of Haskins and Jordon as extended by Zuidema and Waters in the following relations[16,17]:

$$\gamma_{ij} = \frac{M_g F}{4\pi R} = PF \tag{8}$$

$$F = a + \left[\frac{4bP}{(\pi R)^2 (D-d)} + \frac{cr}{R} + d \right]^{\frac{1}{2}} \tag{9}$$

It should be noted that Eq. (9) and Eq. (10) are ordinarily applied in the lifting of an interfacial or surface film against gravity where the algebraic signs of the vector quantities F and g are considered positive. In the measurement of γ_{12} described in the present study wherein the ring is lowered through the 12 interface the algebraic signs of both F and g are

negative and the meaning of Eq. (9) is unchanged. In Eq. (9) the general definition of densities D and d in Table 1 define the respective receding and advancing phases relative to direction of ring motion which correctly reverses the sign of the density difference (D-d) to correlate with the algebraic sign of P. These definitions define both the lifting and lowering of the Du Nouy ring for respective surface and interfacial tension measurement.

Multiple autographically recorded measurements of the ring force versus ring deflection were recorded at each concentration level of surfactant. The curves of Fig. 5 show a regular rise in m to a maximum value -m = M where , by definition, both m and y become negative for interfacial tension measurement which involves ring lowering. Curves as shown in Fig. 5 are extremely reproducible and provide reproducible values of M well within $\pm$ 0.5% variation which represents the accuracy of Instron weighing system calibration and recording. The maximum force values M_{1V} and M_{12} are converted by Eq. (9) and Eq. (10) to the calculated surface tension γ_{1V} and interface tensions γ_{12} values reported in the data summaries in Tables 4-6.

RESULTS AND DISCUSSION

The data of Tables 4-6 report the γ_{1V}, γ_{12}, and γ_{2V} values measured at constant displacement rate $\dot{y} = \pm$ 0.10 cm/min. Higher and slower rates $\dot{y} = \pm$ 0.05 and $\pm$ 0.50 cm/min did not alter M over the range of surfactant concentrations studied from values reported at $\dot{y} = \pm$ 0.10. The curves of Fig. 5 reveal important information in addition to the maximum force M. At

low surfactant concentration $c \leq 1.0 \cdot 10^{-7}$ the ring force deflection curves display a characteristic maximum followed by well defined interface failure with a rapid drop of deflection force to zero. At higher surfactant concentrations as shown by $c = 3 \cdot 10^{-6}$ a definite interface yielding with nearly constant m precedes interface failure. At still higher surfactant concentration $c = 1.0 \cdot 10^{-4}$ the curves of Fig. 5 show a characteristic maximum deflection force followed by extended deflection at essentially constant force without interface fracture. The transition in fracture properties of the 12 interface with increasing surfactant concentration appears associated with formation of strong interface films.

The curves of Figs. 6-8 more clearly delineate the effects of surfactant concentration by plots of log c versus γ_{1V}, γ_{12}, and γ_{2V}. The sigmoidal transition shown in the $_{12}$ curves of Figs. 6-8 at Pluronic®F68 concentrations log c = -7 to -5 are clearly associated with the transition in the extensibility and fracture properties of the fluorocarbon to aqueous phase interface. At still higher Pluronic®F68 concentrations of c = 0.1 to 1.0 curves of Figs. 6-8 show that the magnitude of γ_{12} becomes less than the fluorocarbon surface tension γ_{2V} and tends to decrease toward $\gamma_{12} \approx 0$ as Pluronic®F68 concentration increases to c = 1.0.

All surface tension measurements on the three fluorocarbons shown in Fig. 6-8 showed no change in γ_{2V} due to prior contact with either water or aqueous Pluronic®F68 solutions listed in Tables 4-6. This result is shown by the flat dashed curves of γ_{2V} in Figs. 6-8 and reflects the well known incompatibility of inert fluorocarbons with either hydrocarbon or aqueous phases.

The upper curves of Figs. 6-8 show that the aqueous phase surface tension γ_{1V} closely parallels the curves for γ_{12} interfacial tension over substantial portions of the wide range of surfactant concentration studied. The end point of these upper γ_{1V} curves is shown in Figs. 6-8 to coincide at c = 1.0 with the dispersion surface tension values γ_s = 23.8 ± 0.8 dyn/cm determined for solid films of pure Pluronic® F68 in the data analysis of Table 3. The extrapolated end point of $\gamma_{12} \simeq 0$ at c = 1.0 is developed through analysis of the relation of γ_{12} to γ_{1V} in terms of the dispersion and polar definition adsorption bonding as expressed in Eq. (2). A simple model for the aqueous phase surface tension first assumes that $\gamma_{1V}^d \simeq 21.8 \pm 1.0$ dyn/cm is essentially independent of of Pluronic F68 concentration so that:

$$\alpha_1^2 = 21.8 \tag{10}$$

$$\beta_1^2 = \gamma_{1V} - 21.8 \tag{11}$$

A second assumption for the flucrocarbon phase 2 would assume a negligible polar contribution to surface tension so that $\gamma_{2V}^P \simeq 0$ and the surface tension γ_{2V} is described by the following relation:

$$\alpha_2^2 = \gamma_{2V}^P \tag{12}$$

$$\beta_2^2 = 0 \tag{13}$$

Introducing the above relations in Eqs. (10-13) into Eq. (2) provides with rearrangement the following special relations:

$$(\alpha_1-\alpha_2)^2 = \left[4.67-(\gamma_{2V})^{\frac{1}{2}}\right]^2 \qquad (14)$$

$$(\beta_1-\beta_2)^2 = \gamma_{1V} - 21.8 \qquad (15)$$

Rearranging Eq. (2) to solve for the excess turn $_{12}$ and substituting Eq. (14) and Eq. (15) provides the following relation:

$$\Delta_{12} = 21.8 + \gamma_{12}-\gamma_{1V} - \left[4.67-(\gamma_{2V})^{\frac{1}{2}}\right]^2 \qquad (16)$$

Calculated values of Δ_{12} obtained by substitution of ex; ental values of γ_{12}, γ_{1V}, and γ_{2V} into Eq. (16) are tabulated in the right colums of Tables 4-6 for each concentration c of Pluronic®F68 in wa[illegible] The lower curves of Figs. 6-8 plot these Δ_{12} values versus c for the three artificial blood systems studied. These curves show that Δ_{12} is generally low with respect to measured values of surface and interfacial tension for all three systems. In concentration regions where γ_{1V} and γ_{12} display maximum concentration dependence, at c $\approx 10^{-7}$ and 10^{-1} one notes in Figs. 4-6 that Δ_{12} maximizes for all three systems.

The low amplitude of Δ_{12} in relation to γ_{12}, γ_{1V}, and γ_{2V} reconfirms the validity of the dispersion and polar force model in the surface energetics analysis of artificial blood. The maximum in Δ_{12} at low Pluronic®F68 concentration c $\approx 10^{-7}$ evidently correlates with the change in interface deformation response shown in Fig. 5. The second maximum in Δ_{12} at high Pluronic®F68 concentration c $\approx 10^{-1}$ evidently relates to the morphology transition shown in Fig. 3.

A general study of the interdiffusion bonding and bulk solution properties of Pluronic F68 in water appears as a logical extension to the present study. The low concentration $c = 10^{-7}$ inflection in γ_{1V} and γ_{12} functions of Figs. 4-6, for example, are strongly suggestive of incipient colloidal structure formation between the hydrophobic PPO segments and oriented absorption of these Pluronic®F68 center segments at the 1V surface and 12 interface as described in the experiment of Fig. 4. In studies of lyophilic sole formation by nonionic surfactants of the following structure[18]:

$$C_m H_{2m+1} O(CH_2 CH_2 O)_n H \qquad (17)$$

it is shown that polymolecular hydrate shells are formed around colloidal particles by oriented surfactant adsorption. The above alkyl ether of polyethylene oxide is a simpler structure analog to Pluronic®F68, see Eq. (1), and suggests a similar result obtains in the fluorochemical emulsions in artificial blood. Based on a hydrated polymolecular shell model for emulsion stabilization the importance of the interdiffusion interaction of Pluronic®F68 with the electrolyte and buffer, constituents of artificial plasma formulations remains a subject of much more detailed study. According to Geyer[8] typical electrolytes include NaCl, KCl, $MgCl_2$ or $MgSO_4$, NaH_2PO_4 and $CaCl_2$. Buffers such as bicarbonate, phosphate, lactate, glucose and glycerol have been incorporated in some formulations.

The adsorption-interdiffusion theory which is briefly described in Eq. (2) and Eq. (3) appears as only one form of a broad range of theoretical

models for short and long range interfacial forces. As pointed out in a recent review by Israelachvili and Ninhan[19] many theories are presently competing as tools for interpretation of bioadhesion phenomena and current theory of long range forces is presently in a very dynamic state of development.

This study has focused on the role of interfacial phenomena between the fluorocarbon and the aqueous phase. In as much as stabilization with nonionic macromolecules is controlled primarily by dispersion and polar intermolecular forces, rather than by electrostatic effects which control ionic surfactants, our investigation was limited to the fluorocarbon-surfactant water system in absence of electrolyte. Although effects of electrolyte should be investigated as an extension of this study our overall conclusions should not be effected. This discussion provides an interpretation for the observed decrease in interfacial tension between the fluorocarbon and the aqueous phase and for the oncotic pressure effect that has been observed for the Pluronic emulsifier. It would appear from this study that important contributions to the Pluronic F68 emulsion stabilizing effect depends on the solution thermodynamics of PEO end segments. It has been established that the thermodynamic theta-temperature (i.e., temperature of incipient precipitation for infinite mol. wt. polymer) for the stabilizing segment is nearly identical to the critical flocculation temperature for the emulsion.[21] Addition of electrolyte tends to raise the theta-temperature for PEO from about 25^{o}C in absence of electrolyte to about 40^{o}C in 0.4 M electrolyte; salts of divalent metals raise the theta-temperature more than nonvalent metals.[22] This

suggests that in absence of electrolyte the emulsion should be stable in the range between room temperature and body temperature, but that once the emulsion is adjusted to physiologic conditions it may become unstable at ambient temperature.

Particle size should be minimized in order to allow passage through capillaries in the circulatory system and to enhance gas exchange. It was observed, however, by Geyer that if particle size gets below about 300-500Å, that viscosity increases dramatically.[23] Viscosity of an emulsion depends on volume fraction of the dispersed phase and on inter-particle interactions. If one assumes that the Pluronic PPO segment is strongly absorbed on the fluorocarbon particle, then assuming as a lower limit to the thickness of the hydrated PEO layer its theta-dimension, one arrives at a thickness of about 50Å for PEO mol. wt. 3,500. It is common practice to have the initial emulsion (prior to adjusting for physiologic conditions) contain about 20-24 wt% fluorocarbon, which translates into about 12 vol%. However, if one considers a 300Å particle size, the PEO 50Å layer would increase the volume fraction to nearly 30%, whereas for large particles the volume fraction increase would be negligible. Large increases in viscosity for comparable particle size-volume-fraction ranges was reported for latex suspensions.[23] To derive a quantitative viscosity-particle size relationship the determination of a scaling parameter to account for interparticle interactions would be required.

SUMMARY AND CONCLUSIONS

This study shows that surface and interfacial tensions in artificial blood systems (which exclude electrolyte and buffer constituents) are amenable to surface energy analysis in terms of separated dispersion-d and polar-p interactions as defined by Eq. (2). This result suggests that application of separated dispersion-d, polar-p, and hydrogen-h bonding interactions as expressed by Eq. (3) may be applicable in first approximation analysis of the interdiffusion aspects of colloidal stability in artificial blood systems. The Du Nouy ring experiment identifies several important attributes of Pluronic®F68 effects on fluorochemical emulsion stability in water which are:

1. At concentrations of Pluronic®F68 of $c \geq 1 \cdot 10^{-5}$ weight fraction the water-fluorochemical interface deforms without rupture. This formation of a highly deformable interfacial film is accompanied by a reduction in interfacial tension which further stabilizes the fluorocarbon emulsion.
2. Commercial artificial blood compositions[8] which employ a weight fraction c = 0.02 to 0.04 of Pluronic®F68 in water display a aqueous phase surface tension $\gamma_{1V}^{d} \simeq 22$ dyn/cm and $\gamma_{1V}^{p} \simeq 20$ dyn/cm which closely matches the solid surface properties of adsorbed protein films deposited from blood on implant surfaces[11] and thus may explain the biocompatibility of these emulsions.

3. At normal Pluronic®F68 concentrations of c ≃ 0.02 to 0.04 the interfacial tension γ_{12} for the fluorocarbon in water slightly exceeds the fluorocarbon surface tension and displays a highly extensible interfacial film which appears to enhance emulsion stabilization.

ACKNOWLEDGEMENTS

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Table 1: Nomenclature of Principal Symbols

Symbol	Meaning
γ_{LV}	liquid-vapor surface tension
γ_{SV}	solid-vapor surface tension
α_L, β_L	square roots of the respective (London) dispersion γ_{LV}^d and (Keesom) polar parts of γ_{LV}
α_S, β_S	square roots of the respective (London dispersion γ_{SV}^d and (Keesom) polar parts of γ_{SV}
W_a	nominal work of adhesion
	steady-state advancing liquid-solid contact angle
γ_{ij}	surface or interface tension
M	maximum ring force (gm)
g	980.665 dyn/gm
R	ring radius = 0.95413 cm
F	ring correction factor
P	$Mg/4\pi R$
D, d	densities of the respective receding and advancing phases
r	ring wire radius = 0.01767 cm
a, b, c, d	constant: a = 0.7250, b = $9.075\ 10^{-4}$, c = -1.679, d = $4.534\ 10^{-2}$

Table 2: Study Materials for Fluorochemical Emulsions in Artificial Blood

Material and Designation	Treatment	Density 25 C (gm/cc)
Perfluorotributyl amine tech. grade (3M Co. FC43)	None	1.900
Perfluorotributyl amine (JPL* 92077 control)	Distilled	1.900
Perfluorodecalin PP-5 (JPL* 91977 control)	Distilled	1.946
Pluronic®F68 (UBTL* 977 control)	Solid Flake	1.060
Water	Triple Distilled	0.997

*JPL = Jet Propulsion Laboratory, Pasadena, Ca.
*UBTL = Utah Biological Testing Laboratory, Salt Lake City, Utah

Table 3: Contact Angle Measurement and Surface Energy

Analysis of Plusonic® F68 Cast Film (Test Liquid Properties from Ref. 14)

No.	Liquid	γ_{LV} (dyn/cm)	θ (deg)	W_a (dyn/cm)	$2\alpha_L$ (dyn/cm)	β_L/α_L	$W_a/2\alpha_L$ $(dyn/cm)^{1/2}$
1.	Water	72.8	10	144.5	9.34	1.53	15.47
2.	Glycerol	64.0	90	64.0	11.66	0.94	5.49
3.	Formamide	58.3	15	114.6	11.37	0.90	10.08
4.	Ethylene glycol	48.3	32	89.3	10.83	0.81	8.25
5.	1-bromonapthalene	44.6	65	63.5	13.36	0.00	4.75
6.	Glycol PG-E200	43.5	41	76.3	10.62	0.74	7.18
7.	Tricresylphosphate	40.9	41	71.8	12.52	.21	5.73
8.	Glycol PG-15-200	36.6	76	45.5	10.20	.64	4.46
9.	Glycol PG-1200	31.3	56	48.8	9.90	.53	4.93
10.	n-hexadecane	27.6	32	51.0	10.51	.00	4.85

Solid surface energy (based on liquids No. 2,5,8,,9,10)

$\alpha_s = 4.90 \pm 0.38$, $\beta_s = 0.00$ $(dyn/cm)^{1/2}$

$\gamma_s^d = 24.9$, $\gamma_s^p = 0$ $\quad \gamma_s = \gamma^d + \gamma^p = 24.9$ (dyn/cm)

Solid surface energy with water interdiffusion (based on liquids No. 1,5,10)

$\alpha_s = 4.80 \pm 0.05$, $\beta_s = 6.97$ $(dyn/cm)^{1/2}$

$\gamma_s^d = 23.0$, $\gamma_s^p = 46.6$, $\quad \gamma = \gamma^d + \gamma^p = 71.6$ (dyn/cm)

Table 4: Surface and Interfacial Tensions of Phase 1 = Pluronic® F68 in Distilled Water and Phase 2 = FC43 (3M Co.) Tech-Grade Perfluorotributylamine

c	γ_{1v} (dyn/cm)	γ_{12} (dyn/cm)	γ_{2v} (dyn/cm)	Δ_{12} (calc) (dyn/cm)
0	72.4	53.2	17.5	2.4
$3 \cdot 10^{-9}$	70.1	51.1	↑	2.6
$1 \cdot 10^{-8}$	69.4	50.8	↑	3.0
$3 \cdot 10^{-8}$	69.3	51.7	↑	4.0
$1 \cdot 10^{-7}$	62.9	50.5	↑	9.2
$3 \cdot 10^{-7}$	58.2	47.5	↑	10.9
$1 \cdot 10^{-6}$	56.6	37.6	↑	2.6
$3 \cdot 10^{-6}$	54.4	34.5	↑	1.7
$1 \cdot 10^{-5}$	52.1	32.2	(Constant)	1.7
$3 \cdot 10^{-5}$	50.5	30.1	↓	1.2
$1 \cdot 10^{-4}$	48.9	29.8	↓	2.5
$3 \cdot 10^{-4}$	48.6	27.6	↓	0.6
$1 \cdot 10^{-3}$	46.5	26.4	↓	1.5
$3 \cdot 10^{-3}$	45.9	26.0	↓	1.7
$1 \cdot 10^{-2}$	43.7	24.3	↓	3.2
$3 \cdot 10^{-2}$	42.3	23.0	↓	2.3
$1 \cdot 10^{-1}$	35.6	21.0	17.5	7.0

Table 5: Surface and Interfacial Tensions of
Phase 1 = Pluronic (R) F68 in Distilled Water and
Phase 2 = Perfluorotributyl amine (JPL* 92077)

c	γ_{1V} (dyn/cm)	γ_{12} (dyn/cm)	γ_{2V} (dyn/cm)	Δ_{12} (calc) (dyn/cm)
0	72.6	55.3	17.6	4.3
$3 \cdot 10^{-9}$	72.6	53.8		2.8
$1 \cdot 10^{-8}$	70.1	53.9		5.4
$3 \cdot 10^{-8}$	67.3	53.9		8.2
$1 \cdot 10^{-7}$	61.7	53.1		13.0
$3 \cdot 10^{-7}$	59.8	46.4		8.2
$1 \cdot 10^{-6}$	57.4	37.8		2.0
$3 \cdot 10^{-6}$	54.5	34.2		1.3
$1 \cdot 10^{-5}$	49.1	32.8	(Constant)	5.3
$3 \cdot 10^{-5}$	51.7	31.3		1.2
$1 \cdot 10^{-4}$	50.4	30.1		1.3
$3 \cdot 10^{-4}$	48.8	29.8		2.6
$1 \cdot 10^{-3}$	47.2	28.1		2.5
$3 \cdot 10^{-3}$	46.7	26.6		1.5
$1 \cdot 10^{-2}$	44.5	25.2		2.3
$3 \cdot 10^{-2}$	43.4	23.9		2.1
$1 \cdot 10^{-1}$	37.4	21.0		5.2
$2.5 \cdot 10^{-1}$	33.1	16.7	17.6	5.2

Table 6: Surface and Interfacial Tensions of
Phase 1 = Pluronic® F68 in Distilled Water and
Phase 2 = Perfluorodecalin (JPL* 91977)

c	γ_{1V} (dyn/cm)	γ_{12} (dyn/cm)	γ_{2V} (dyn/cm)	Δ_{12} (calc) (dyn/cm)
0	72.6	55.6	19.2	4.7
$3 \cdot 10^{-9}$	71.5	55.2		5.4
$1 \cdot 10^{-8}$	70.5	54.8		6.0
$3 \cdot 10^{-8}$	68.4	54.8		8.1
$1 \cdot 10^{-7}$	62.4	53.9		13.2
$3 \cdot 10^{-7}$	60.8	51.0		11.9
$1 \cdot 10^{-6}$	55.3	35.6		2.0
$3 \cdot 10^{-6}$	55.4	33.4		-0.3
$1 \cdot 10^{-5}$	54.8	32.7	(Constant)	-0.4
$3 \cdot 10^{-5}$	52.9	31.9		0.7
$1 \cdot 10^{-4}$	51.7	29.0		-1.0
$3 \cdot 10^{-4}$	50.9	27.4		-1.8
$1 \cdot 10^{-3}$	48.5	27.1		0.3
$3 \cdot 10^{-3}$	47.8	25.3		-0.8
$1 \cdot 10^{-2}$	46.0	23.9		-0.4
$3 \cdot 10^{-2}$	43.8	22.4		0.3
$1 \cdot 10^{-1}$	41.4	18.8		-0.9
$2.5 \cdot 10^{-1}$	33.1	14.2	19.2	2.8

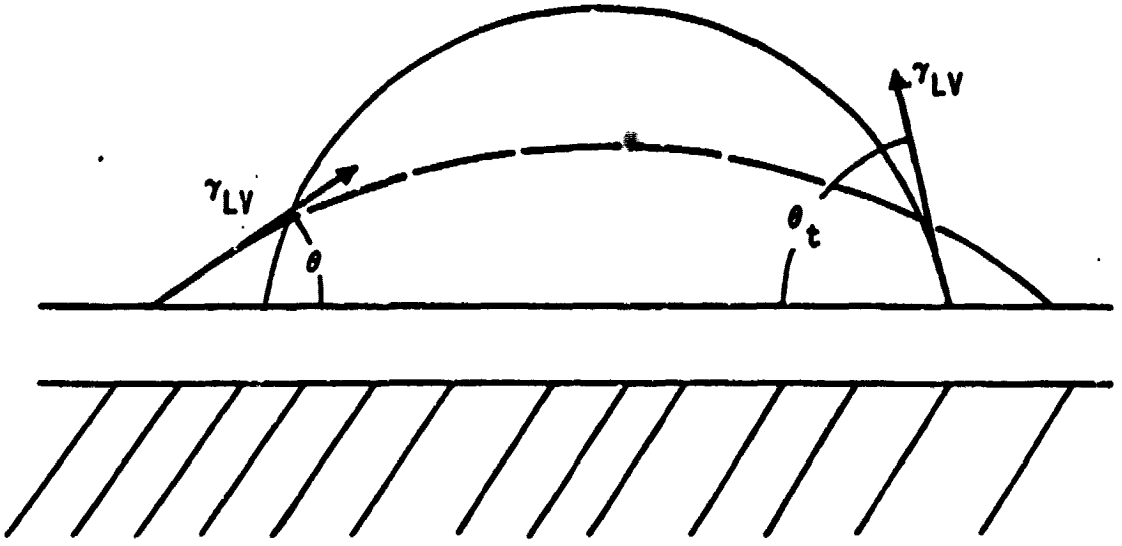

FIG. 1 OBSERVATION OF TIME DEPENDENT CONTACT ANGLE θ_t STABILIZED AT MINIMUM VALUE θ BY INTER-DIFFUSION. FOR PURE ADSORPTION WITHOUT INTER-DIFFUSION $\theta_t \simeq \theta$

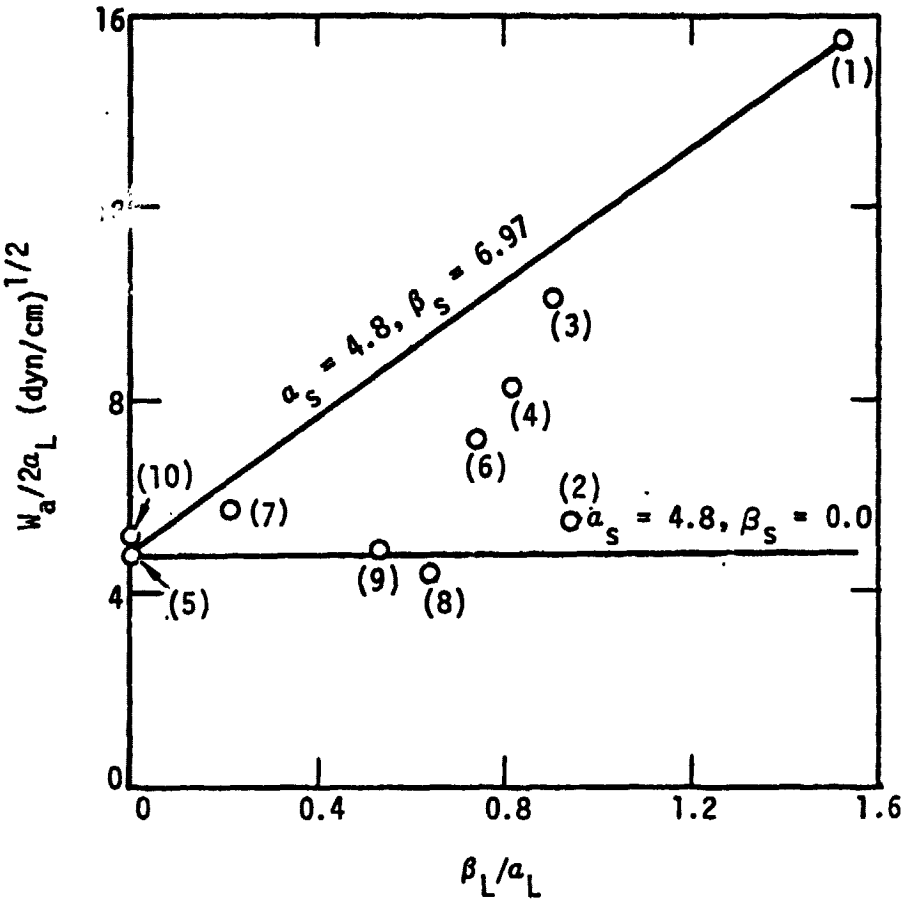

FIG. 2 SURFACE ENERGY ANALYSIS OF PLURONIC® F68 FOR ADSORPTION LIQUIDS (LOWER CURVE) AND FOR ADSORPTION PLUS WATER INTERDIFFUSION (UPPER CURVE).

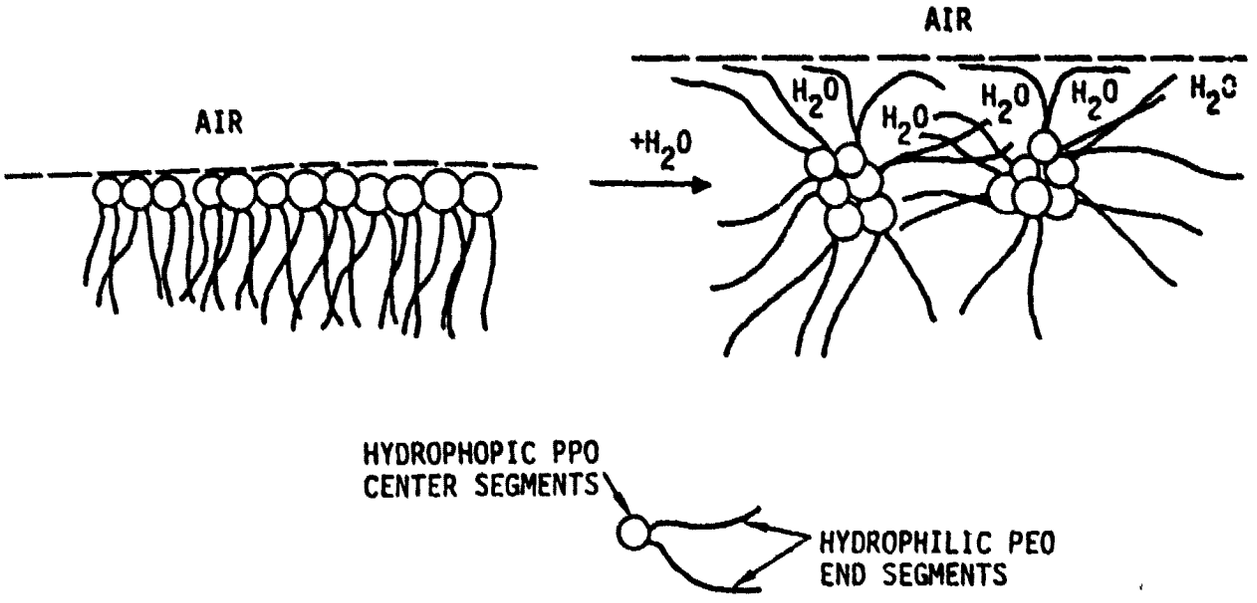

FIG. 3 POSTULATED MICELLE STRUCTURES FOR PLURONIC® F68 IN THE DRY STATE (LEFT VIEW) AND HYDRATED BY WATER INTERDIFFUSION (RIGHT VIEW).

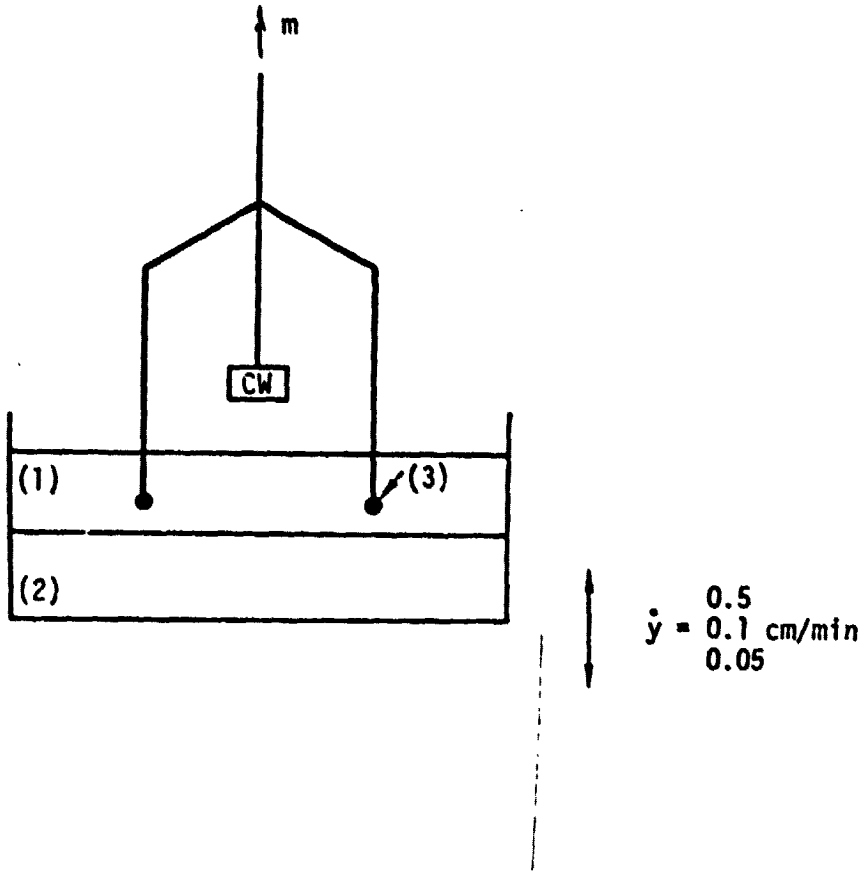

FIG. 4 SCHEMATIC OF SURFACE/INTERFACIAL TENSION MEASUREMENT OF ARTIFICIAL BLOOD SUBSTITUTES. (1) = AQUEOUS PHASE + SURFACTANT, (2) = FLUOROCARBON, (3) = DuNOUY RING.

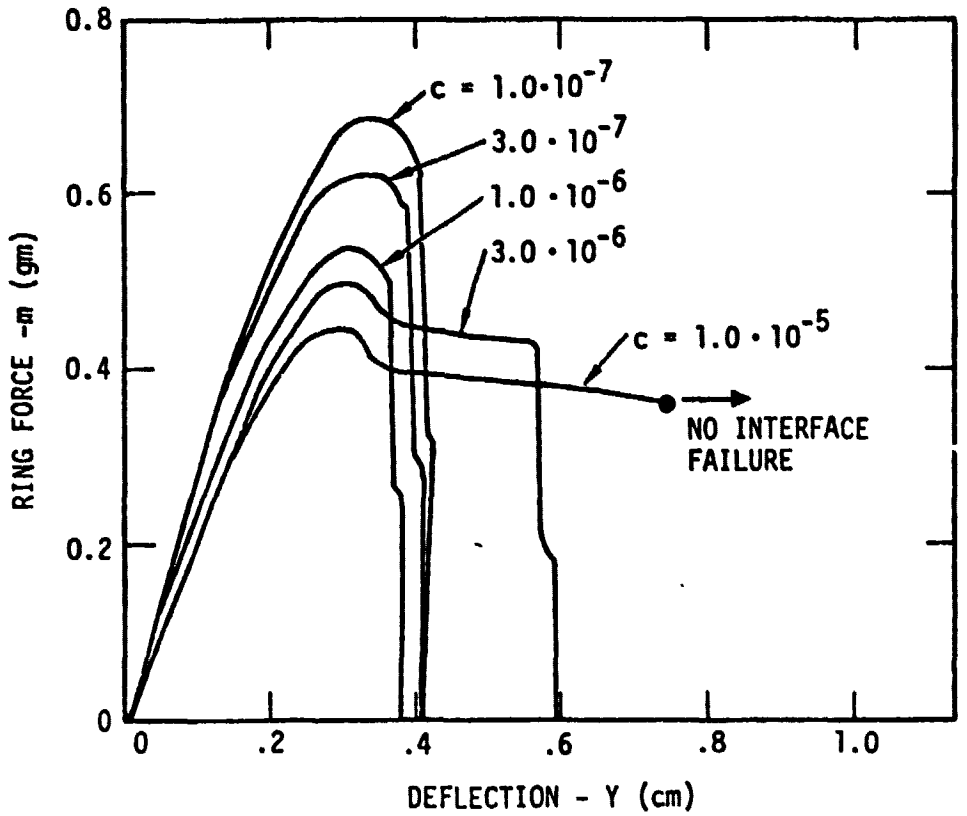

FIG. 5 MEASURED CURVE OF DuNUOY RING FORCE m_{12} VERSUS RING DEFLECTION AT THE PHASE 12 INTERFACE. (Temp = 22°C, DISPLACEMENT VELOCITY $\dot{y}$ = -0.10 cm/min, PHASE 1 = PLURONIC® F68 SURFACTANT IN DISTILLED WATER AND PHASE 2 = FC43 FLUOROCARBON).

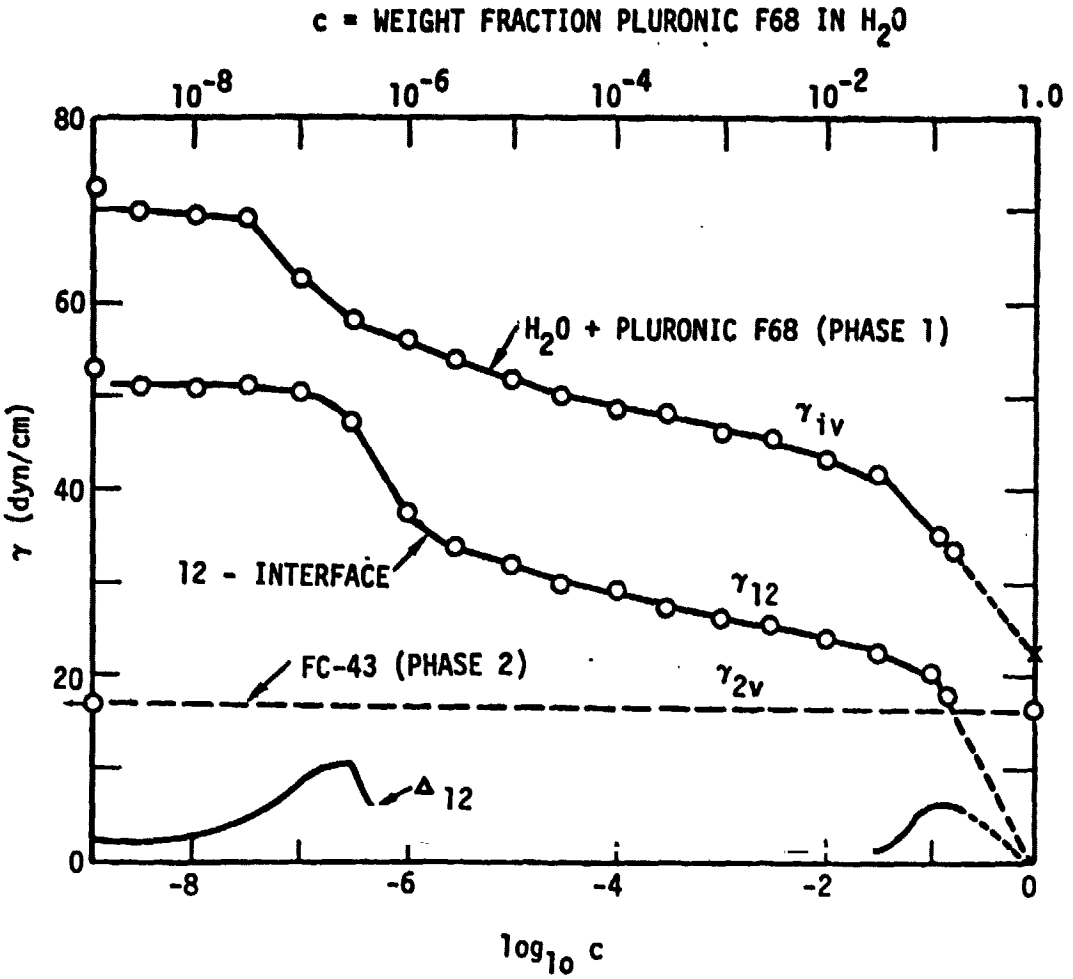

FIG. 6 SURFACE AND INTERFACIAL TENSIONS FOR PHASE 1 = H_2O + PLURONIC F68 AND PHASE 2 = FC43 (3M Co. Tech. Grade perfluorotributylamine)

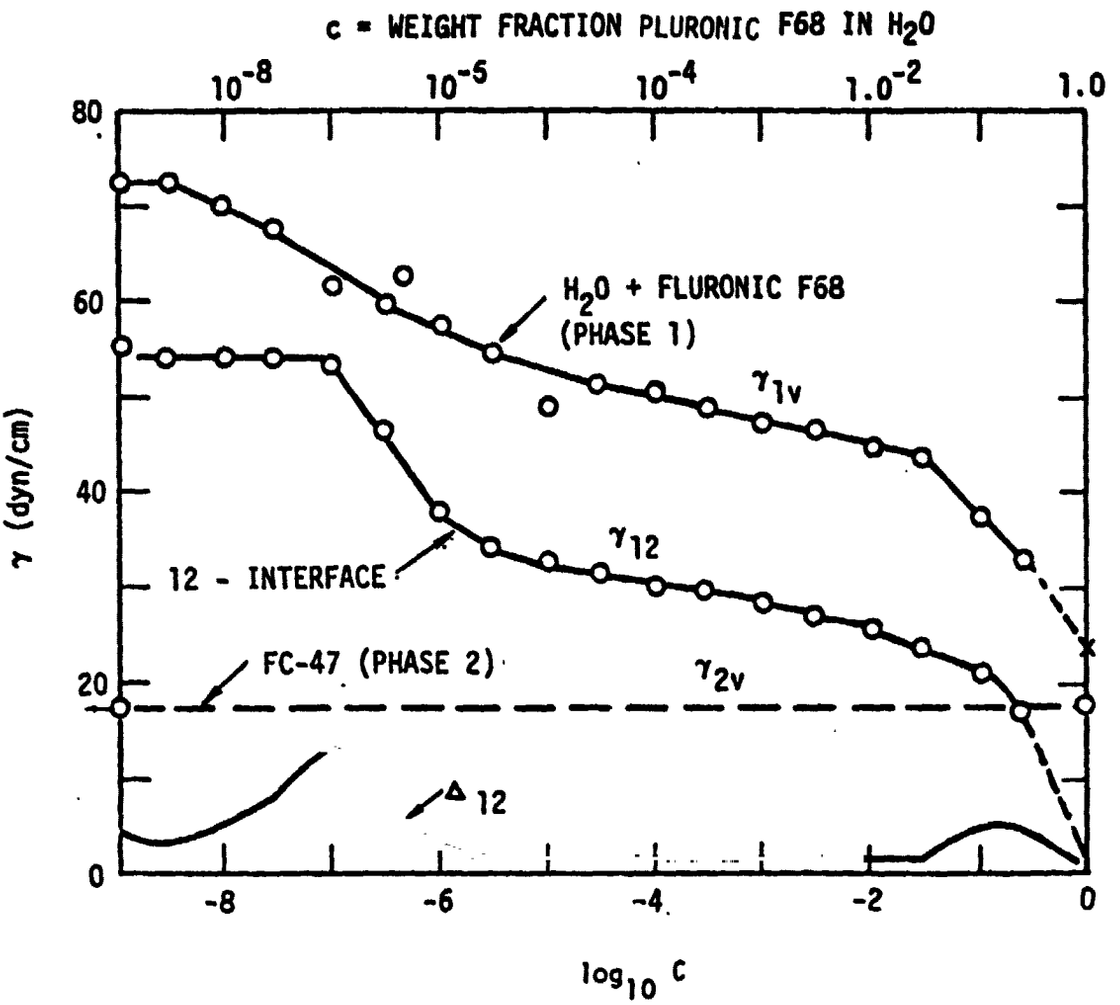

FIG. 7 SURFACE AND INTERFACIAL TENSIONS FOR PHASE 1 = H_2O + FLURONIC F68 AND PHASE 2 = FC47 (redistilled perfluorotributylamine, JPL* 92077).

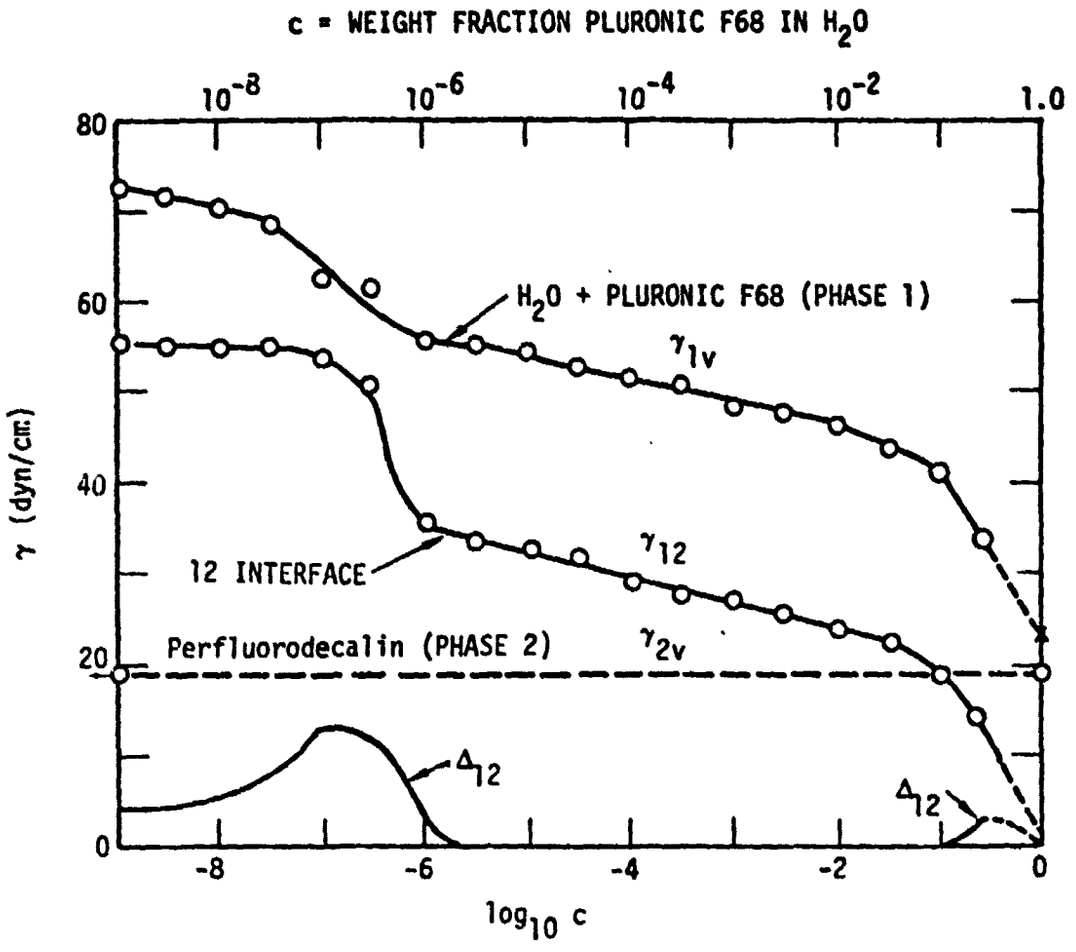

FIG. 8 SURFACE AND INTERFACIAL TENSIONS FOR PHASE 1 = H_2O + PLURONIC F68 AND PHASE 2 = perfluorodecalin (redistilled, JPL * 91977).

APPENDIX C

PERFLUORO-3, 3-DIMETHYL-2(E)-PENTENE BY FLUORIDE ION CATALYZED ADDITION OF OCTAFLUOROISOBUTENE TO HEXAFLUOROPROPENE

APPENDIX C

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PRELIMINARY NOTE

Perfluoro-3,3-dimethyl-2(E)-pentene by Fluoride Ion Catalyzed Addition of Octafluoroisobutene to Hexafluoropropene

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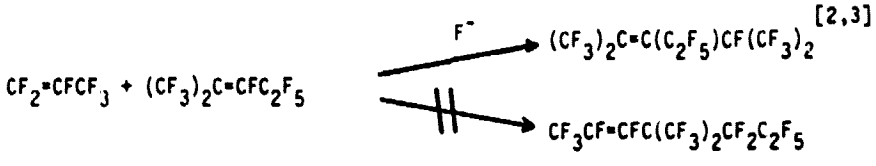
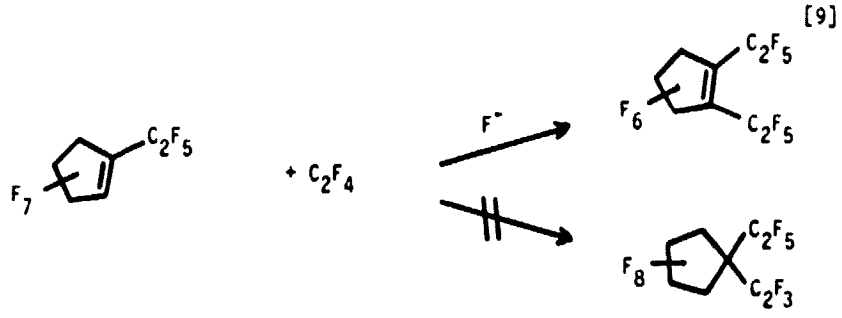
The fluoride ion catalyzed [1] oligomerization of perfluoroolefins has been employed extensively to prepare higher homologs [2,3,5-10]. When unlike partners react, the reaction might take either of two courses, depending on which olefin accepts fluoride ion to become the nucleophile. In all reported cases where the structure of the product allows the mechanism to be inferred, the less substituted (by carbon) olefin becomes the nucleophile, albeit at the more substituted center.
e.g.



[2,3]

and



[9]

The unobserved reactions might have been expected because the tertiary anions should be the more abundant intermediates owing to their greater stability, but considerations of steric hinderance or product stability evidently rule instead. We wish to report a counter-example.

When a mixture of hexafluoropropene (HFP) and octafluoroisobutene (OFIB) [toxic] obtained by pyrolysis of octafluorocyclobutane [11] was passed into a flask equipped with a dry ice-cooled cold finger and containing a stirred suspension of ball-milled, anhydrous potassium fluoride in dimethylformamide, a clear lower layer accumulated. Upon water washing and distillation, the major component of this layer boiled at 71-72°, lacked ir absorption in the double bond region, and had a mass spectrum consistent only with composition C_7F_{14} [12]. These data plus the ^{19}F spectrum establish the structure of the product as 1, rather than 2 which Young and Bennett report for the adduct of the same two olefins over CsF at 200° [5,10].

$E\text{-}(CF_3)_3CCF{=}CFCF_3$ (1)

$(CF_3)_2CFCF{=}C(CF_3)_2$ (2)

The ^{19}F spectrum of 1 consists of a doublet of doublets (J = 14.2 and 16.2 Hz) at -63.3 ppm (upfield) from internal $CFCl_3$, a broadened doublet (J = 16 Hz) at -68.6 ppm, and a multiplet at -150 ppm, in the ratio 9:3:2 [13]. The four and five bond couplings are somewhat larger than those reported by Schumann and Cavagna for $E\text{-}(CF_3)_2CFCF{=}CFCF_3$ [14], presumably owing to greater interaction between non-bonding orbitals in the more crowded tert-butyl compound.

We have found 1 to be a ubiquitous by-product in the F^- promoted chemistry of HFP-contaminated OFIB; the addition of OFIB to other fluoroolefins is under study.

We would like to thank Dr. K. L. Servis for measuring the 94 MHz spectrum of 1 and Mr. R. Haack for the mass spectrum. This paper presents the results of one phase of research conducted at the Jet Propulsion Laboratory, California Institute of Technology, for the National Institutes of Health, by agreement with the National Aeronautics and Space Administration.

1 Amines are also used as catalysts [2,3], but in such cases the effective catalyst is probably a quaternary ammonium fluoride formed in a preliminary reaction between the amine and olefin [4].

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APPENDIX D

METHODS FOR THE ESTIMATION OF VAPOR PRESSURES AND OXYGEN SOLUBILITIES OF FLUOROCHEMICALS FOR POSSIBLE APPLICATION IN ARTIFICIAL BLOOD FORMULATIONS

APPENDIX D

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METHODS FOR THE ESTIMATION OF VAPOR PRESSURES AND OXYGEN SOLUBILITIES OF FLUOROCHEMICALS FOR POSSIBLE APPLICATION IN ARTIFICIAL BLOOD FORMULATIONS.

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SUMMARY

A group additivity system has been generated which makes it possible to estimate, from the structural formulas alone, the energy of vaporization and the molar volume at 25°C of many fluorocarbons and their derivatives. From these two parameters and appropriate thermodynamic relations it is then possible to predict the vapor pressure and the solubility of oxygen in a fluorochemical liquid with sufficient accuracy to estimate its potential for application in fluorochemical emulsion artificial blood.

INTRODUCTION

Fluorochemical emulsion artificial blood has received much research attention in recent years, owing to its perceived potential use in human therapy, where it might help alleviate the shortage of safe donar blood[1]. The fluorochemical phase in these emulsions transports oxygen and carbon dioxide, and must also have a vapor pressure within certain limits. If the vapor pressure is too high at 37°C, gas emboli can form in the circulatory system; whereas too low a vapor pressure contributes to prolonged retention of the fluorochemical in the body[2]. Oxygen solubility is a point of special concern: although fluorochemicals dissolve much more oxygen than ordinary liquids, the oxygen capacity of all emulsions so far prepared is well below that of normal mammalian bloods at the oxygen tension of ambient air, and improved oxygen solubility is a goal of current research.

As one part of a program principally concerned with the synthesis and testing of new fluorochemicals, we have sought to estimate from chemical structure alone, gas solubilities and vapor pressures of fluorochemicals as an aid to optimize selection of compounds for synthesis.

EXPERIMENTAL

Details of the synthesis of the new fluorocarbon-hydrocarbon hybrid compounds described later in this paper will be reported in subsequent publications. All of the new compounds had elemental analyses and mass, ir and nmr (^{19}F and ^{1}H) spectra consistant with the assigned structures. Oxygen solubilities were measured by gas-solid chromatography using the method described by the 3M group[3]. The bulk of the computations were done on a Hewlett-Packard HP 67 programmable calculator and the programs for calculating vapor pressure and oxygen solubility are on file in the Hewlett-Packard Users' Program Library[4].

DISCUSSION AND RESULTS

For more than 50 years, Hildebrand and co-workers have studied the nature of the solubility of gases in liquids and the process of vaporization[5,6]. In the following sections it will be shown how the thermodynamic relations governing these phenomena in conjunction with a group additivity system make it possible to estimate the vapor pressure and oxygen solubility of liquid fluorochemicals from their chemical structure. Therefore it is possible to ascertain before synthesis whether or not a compound will have physical properties appropriate for an artificial blood constituent.

A. Estimation of the Energy of Vaporization and Molar Volume by the Group Additivity Method

A number of group additivity methods already exist in the literature which assume that molecular properties can be partitioned among the individual functional groups and structural components of a molecule. Thus,

simply by summing the group parameters, it is possible to estimate molecular properties from chemical structure alone. An extensive group additivity system for estimating both the energy of vaporization (ΔE_v^{298}) and molar volume (V) of organic liquids has been developed by Fedors[7]. Based upon examination of a large amount of data on simple liquids, he assumed that:

$$\Delta E_v = \sum_i \Delta e_i \tag{1}$$

$$V = \sum_i \Delta v_i \tag{2}$$

where the Δe_i and the Δv_i are the atomic or group contributions to the energy of vaporization and molar volume at 25°C. The deviations between the experimentally measured values and those estimated by this method for a number of liquids were found to be less than 10 percent. The group contributions given by Fedors, although extensive, include only the parameters CF_3- and -CF_2- for fluorochemicals.

Our search of the literature uncovered adequate ΔH_v^{298} or vapor pressure/temperature data for only 19 perfluorochemicals, but extensive boiling point and density data was found. However, the following empirical relationship proposed by Hildebrand and Scott[8] relates the heat of vaporization of nonassociated liquids at 25°C to the boiling point (T_b°K) at one atmosphere:

$$\Delta H_v^{298} = 0.020 T_b^2 + 23.7\ T_b - 2950 \tag{3}$$

When equation 3 was tested on the 19 perfluorochemicals for which ΔH_v^{298} was known, the calculated and experimental values were often in disagreement by several hundred calories. Subsequently, it was brought to our attention[9] that no fluorochemical data were included in calculating the constants in equation 3. Using the data collected by us, the following relation for perfluorochemicals ensues:

$$\Delta H_v^{298} = 0.0724\ T_b^2 - 17.17\ T_b + 5309 \tag{4}$$

or in terms of the boiling point:

$$T_b{}^{\circ}K = \frac{17.17 + [294.9\text{-}0.2[illegible](5309\text{-}\Delta H_v{}^{298})]^{1/2}}{0.[illegible]} \qquad (5)$$

Equation 4 is based on least-squares computer curve fit for the 19 liquid perfluorochemicals whose $\Delta H_v{}^{298}$ and boiling point values were found in the literature or calculated by us from vapor pressure/temperature data. The perfluorinated compounds include eleven straight and branched alkanes, one dimethyl cyclobutane ring, one cyclopentane, two cyclohexanes, decalin, a methyl decalin and two tertiary amines. Figure 1 is a plot of equations 3

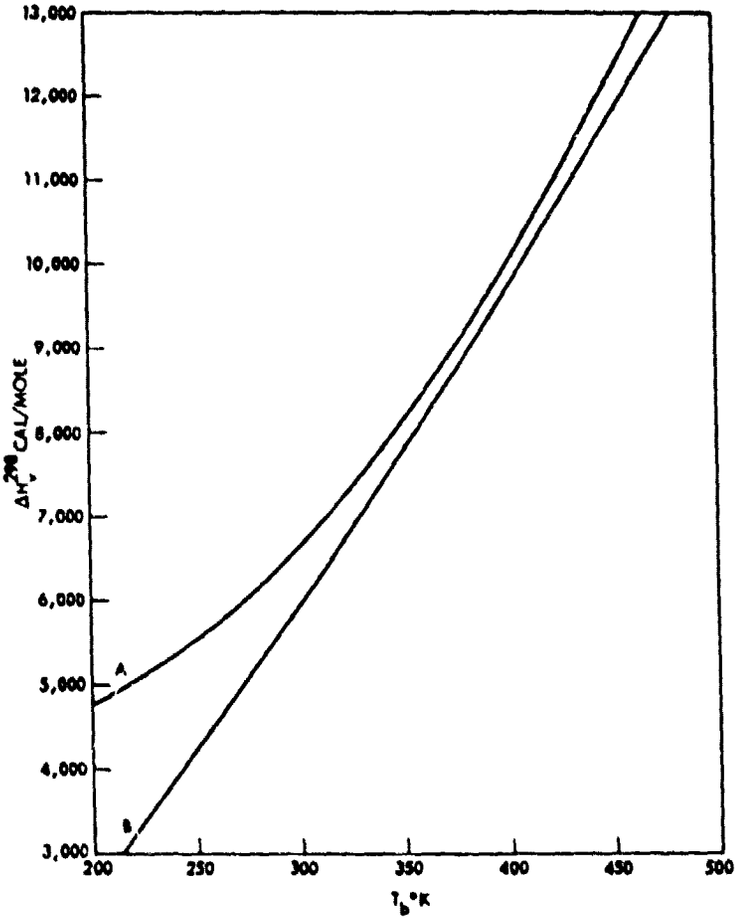

Figure 1. Relationship between $\Delta H_v{}^{298}$ and T_b for: (A) fluorochemicals and (B) nonfluorinated liquids.

and 4. As can be seen, perfluorochemicals have higher heats of vaporization than hydrocarbons of the same boiling point. This is especially true of those with boiling points below 300°K, after which perfluorochemicals parallel non-fluorinated liquids fairly closely.

With the assumption that equation 4 is a good first approximation to estimating ΔH_v^{298} for perfluorochemicals, we calculated ΔH_v^{298} for a large number of such compounds from their literature boiling points. For example, by taking the boiling points for perfluorobutane and perfluoropentane, one can calculate their individual ΔH_v^{298} which can be used to calculate ΔE_v^{298} from:

$$\Delta E_v^{298} = \Delta H_v^{298} - RT \qquad (6)$$

The difference between the two ΔE_v^{298} values is an estimate of the group contribution of a $-CF_2-$ to the energy of vaporization. By using the boiling points for straight chain fluorocarbons from C_3-C_{13}, 10 values for Δe_i of a $-CF_2-$ were calculated, which were then averaged. Also, from the density data the molar volumes were calculated from:

$$V = \frac{MW}{D} \qquad MW = \text{molecular weight; } D = \text{density} \qquad (7)$$

and the Δv_i for $-CF_2-$ calculated in a similar way. Table I contains a list of fluorochemical group additivity values calculated in this work, as well as some hydrocarbon values published by Fedors[7].

By summing the individual group parameters, obtained by inspection of the structural formula of a compound, the energy of vaporization and molar volume can be estimated before it is synthesized. Also, by calculating ΔH_v^{298} from equation 6 and using this value in equation 5, a reasonable estimate of the boiling point may be made. Similarly, the density of the compound can be estimated from the molar volume by equation 7.

The predicted and reported boiling points for a number of perfluorochemicals are presented in Table II.

TABLE I

Group Contributions to the Energy of Vaporization and Molar Volume at 25°C

Group	Δe_i^{298} cal/mole	Δv_i cm^3/mole
CF_3-	1933	54.8
-CF_2-	783	23.1
-CFH-	422	18.6
-CF-	-396	-15.0
-C- (Perfluoro)	-1515	-38.3
-N- (Perfluoro 3° Amine)	-914	-16.3
-O- (Perfluoro Ether)	8	19.0
Ring: 5 atoms	2023	37.7
Ring: 6 atoms	2272	39.9
CH_3-	1125	33.5
-CH_2-	1180	16.1
-CH-	820	-1.0
-HC=	1030	13.5
-C-	350	-19.2
-O-	800	3.8

B. Estimation of Vapor Pressure

Material that have vapor pressures over about 40 torr at 37°C tend to have adverse physiological effects when used as the gas carrying phase of artificial blood substitute formulations. Therefore it is desirable to have some method of predicting the vapor pressure before synthesis is attempted.

TABLE II

Calculated Boiling Point and Vapor Pressure for Some Fluorochemicals

Compound	(1) CALCD. ΔH_v^{298}	(2) CALCD. B.P. °C 760 torr	(4) LIT. B.P. °C 760 torr	(3) CALCD. V.P. @25°C	(4) LIT. V.P. @/T°C
$CF_3CF_2CF_2CF_2CF_3$	6807	32	29	519	646/25
$CF_3CF_2CF_2CF_2CF_2CF_3$	7590	59	57	202	220/25
	8316	81	76	92	106/25
	10608	141	142	11.1	6.6/25
	11362	158	160	6.1	2.1/25
$CF_3CHF[OCF_2CF(CF_3)]_2F$	8782	94	104	57	56/37.5
$(CF_3)_2CFO(CF_2)_5CF_3$	9918	124	121	19.9	39/37.5
$[(CF_3)_2CFOCF_2CF_2]_2$	10680	142	135	10.5	13/37.5
$CF_3CHF[OCF_2CF(CF_3)]_3F$	11110	152	152	7.4	10/37.5
$CF_3CHF[OCF_2CF(CF_3)]_4F$	13438	201	194	1.4	1.9/37.5
$[(CF_3)_2CFO(CF_2)_4]_2$	13812	208	199	1.1	3/37.5
$CF_3CHF[OCF_2CF(CF_3)]_5F$	15766	244	224	0.4	0.4/37.5
$(CF_3CF_2CF_2CF_2)_3N$	12524	183	174	2.6	2.5/37.5

1. from the values in Table I.
2. from equation 5.
3. from equation 9 using the α and β values for perfluorochemicals.
4. from Ref.[16].

From Hildebrand's general rule that compounds have equal entropies of vaporization at equal molar volumes of their vapor[10], the entropy of vaporization for non-polar liquids can be predicted from a relation developed by K. Sato[11].

$$\Delta S_v = \frac{\Delta H_v}{T} = \alpha R\left(\frac{P^*}{T}\right)^{\beta-1} \qquad (8)$$

In terms of the vapor pressure (P in torr), equation 8 becomes:

$$P = T\left(\frac{\Delta H_v}{\alpha R T}\right)^{\frac{1}{\beta-1}} \qquad (9)$$

where T is the temperature in ^{o}K at which the vapor pressure is desired and R is the gas constant in cal $mole^{-1}$ $^{o}K^{-1}$. Thus, equation 9 provides a useful way of estimating the vapor pressure of a non-polar liquid from its heat of vaporization when the constants α and β are known. Sato originally calculated α and β from vapor pressure/temperature data for 10 non-polar liquids containing hydrogen.

New values of α and β for perfluorochemicals have been calculated by the same procedure in this work. BY plotting dP/dT vs. P/T from vapor pressure/temperature data, a family of overlapping curves was generated. The deviations of the vapor from ideality were corrected for by fitting the data for each substance to an equation of the form:

$$\frac{dP}{dT} = \alpha\left(\frac{P}{T}\right)^{\beta} \qquad (10)$$

and then taking the average of the α and β values. Using these new constants, the vapor pressure of perfluorochemicals calculated from equation 9 matches their literature vapor pressure much more closely than when Sato's original α and β values are used. Sato's constants and our values for perfluorochemicals are:

Non-polar liquids:	α = 11.8822	β = 0.8810
Perfluorochemicals:	α = 12.2497	β = 0.8846

Thus, by estimating ΔE_v^{298} of a perfluorochemical from the group additivity values in Table I, calculating its ΔH_v^{298} with equations 6, and

using equation 9 with the α and β constants for perfluorochemicals the vapor pressure of the compound can be estimated before it is synthesized. Alternatively, if the boiling point of the compound is known, its ΔH_v^{298} can be estimated from equation 4 and its vapor pressure predicted from equation 9. Table II also contains vapor pressures of some perfluorochemicals calculated from equation 9 and corresponding experimentally measured values. It is believed that the accuracy of vapor pressures predicted by this method is sufficiently reliable to make decisions on the utility of new perfluorochemicals as artificial blood constituents before they are synthesized.

C. Estimation of Oxygen Solubility.

In selecting gas-carrying materials for artificial blood formulations it is desirable to have a method of making estimates of oxygen and, to a lesser degree, of carbon dioxide and nitrogen solubility in candidate materials. It is possible, using regular solution theory as developed by Hildebrand and others, to make useful predictions of the solubility of a number of gases in a wide range of solvents.

There are two equations based on regular solution theory that are frequently used to calculate gas solubilities[12]. The first is:

$$-\log x_2 = -\log x_2^i + \frac{0.4343\bar{V}_2(\delta_1-\delta_2)^2}{RT} \qquad (11)$$

where the subscript 1 refers to the solvent and 2 refers to the solute. R is the gas constant (cal $mole^{-1}$ $^oK^{-1}$), T(^oK) is the temperature at which the gas solubility is to be estimated, $\bar{V}_2$ is the partial molar volume of the gas in the solvent, δ is the solubility parameter defined as:

$$\delta = \left(\frac{H_v-RT}{V}\right)^{1/2} = \left(\frac{E_v}{V}\right)^{1/2} \qquad (12)$$

and x_2^i is the "ideal" gas solubility calculated from:

$$\log x_2^i = \frac{\Delta H_v}{4.574}\left(\frac{1}{T}-\frac{1}{T_b}\right) \qquad (13)$$

where ΔH_v is the heat of vaporization of the gas at the boiling point, T_b, and T is the temperature at which the gas solubility is to be determined. Equation 11 gives the best results when gas and solvent molecules are similar in size. For solutions where the molecules differ greatly in size a correction of the Flory-Huggins type based upon the ratio of molar volumes is introduced which alters equation 11 to [13]:

$$-\log x_2 = -\log x_2^i + \frac{0.4343\,\bar{V}_2\,(\delta_1-\delta_2)^2}{RT} + \log\left(\frac{\bar{V}_2}{V_1}\right) + 0.4343\left(1 - \frac{\bar{V}_2}{V_1}\right) \qquad (14)$$

In terms of natural logs, equation 14 is:

$$\ln x_2 = \ln x_2^i - \frac{\bar{V}_2\,(\delta_1-\delta_2)^2}{RT} - \left[\ln\left(\frac{\bar{V}_2}{V_1}\right) + \left(1 - \frac{\bar{V}_2}{V_1}\right)\right] \qquad (15)$$

The application of equations 14 and 15 has been described by Gjaldbaek in a number of publications[14]. The pertinent constants for equation 15 when calculating oxygen solubility are:

$$x_2^i = 17.638 \times 10^{-4};\ \bar{V}_2 = 46\ \text{ml./mole};\ \text{and}\ \delta_2 = 5.70\ (\text{cal./cm}^3)$$

The value of $\bar{V}_2$ = 46 ml/mole for oxygen is the measured value obtained by Horiuti[15] in benzene and is the value normally used. However, Horiuti's measurements of the partial molar volume of oxygen in other solvents have shown that this quantity depends on the solvent and varies from 56 ml/mole in diethyl ether to 31 ml/mole in water, while in pure liquid oxygen at the boiling point it is 28 ml/mole.

When $\bar{V}_2$ was changed so that values of oxygen solubility calculated from equation 15 agree for 24 liquid fluorocarbons reported by Clark[16], it was found that $\bar{V}_2$ varies over the range of 30-50 ml/mole. It can be seen from Figure 2 that a reasonable correlation exists between calculated values of $\bar{V}_2$ for these compounds and logarithms of their entropies of vaporization at 25°C, if the cyclic structures are treated separately.

The equations of the least squares fit for the data and their correlation coefficients are:

Open chain compounds:

$$\bar{V}_2 = -19.85 + 15.90 \ln\Delta S_v^{298} \quad , \quad r^2 = 0.8999 \qquad (16)$$

Cyclic Compounds:

$$\bar{V}_2 = -100.15 + 39.90 \ln\Delta S_v^{298} \quad r^2 = 0.8910 \qquad (17)$$

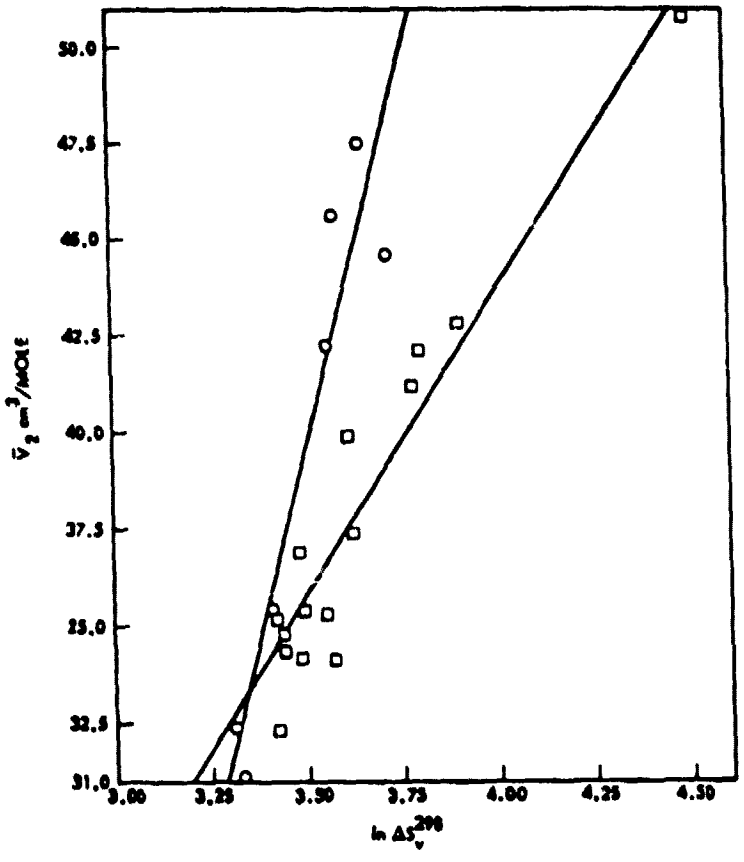

Figure 2. Relationship between $\ln\Delta S_v^{298}$ and $\bar{V}_2$ for Cyclic (O) and Open Chain (□) Fluorochemicals.

By using equation 16 or 17 in conjunction with equation 15, it is possible to predict the oxygen solubility of 22 compounds to ±4 ml. and 14 of these to within ±2 ml. of their experimentally determined values. Since equations 16 and 17 are empirical and we have not measured the partial molar volume of oxygen in these solvents, no physical interpretation of this relationship is attempted. Some compounds with their calculated oxygen solubility and corresponding experimentally measured values are presented in Table III.

TABLE III

Calculated Oxygen Solubility of Fluorochemicals

Compound	[1] b.p. °C	[2] D_4^{25}	[3] ΔH_v^{298}	[4] V	[5] δ	[6] ΔS_v^{298}	[7] $\bar{V}_2$ calc.	[8] O_2 solub. calcd/lit.
	76.3	1.788	8150	195.7	6.21	27.34	31.85	58.1/57.2
$CF_3CHF[OCF_2CF(CF_3)]_2F$	104.4	1.656	9147	272.9	5.60	30.68	34.59	52.4/55.7
$CF_3(CF_2)_7Br$	140.5	1.890	10,595	264.0	6.16	35.54	36.92	49.1/52.7
$(CF_3)_2CF(CF_2)_4Cl$	108	1.77	9283	228.2	6.17	31.14	35.82	52.7/52.7
$(CF_3)_2CFO(CF_2)_5CF_3$	121	1.721	9789	292.9	5.60	32.83	35.66	50.6/52.5
	102	1.783	9057	233.3	6.02	30.38	36.06	51.3/52.2
$(CF_3)_2CF(CF_2)_4Br$	120	1.977	9749	227.1	6.35	32.70	35.60	51.1/51.4
$CF_3CHF[OCF_2CF(CF_3)]_3F$	152	1.738	11,096	355.6	5.43	37.22	37.66	47.0/47.3
$(CF_3)_2CF(CF_2)_6Cl$	151	1.83	11,051	275.4	6.16	37.07	37.59	48.0/45.6
$[(CF_3)_2CFO(CF_2)_4-]_2$	199	1.820	13,342	423.1	5.49	44.75	40.59	43.2/41.8
	160	1.972	11,455	239.6	6.47	38.42	45.43	39.9/38.4
$CF_3CHF[OCF_2CF(CF_3)]_9F$	399	1.848	26,477	873.4	5.44	88.80	51.48	32.9/33.3

1,2: Literature values from [16].
3: From lit. b.p. and eq. 4
4: From lit D_4^{25} and eq. 7.
5: From ΔH_v^{298} and eq. 12.
6: From ΔH_v^{298}
7: From ΔS_v^{298} and eqs. 16 or 17
8: From eq. 15 and 18.

Therefore, to estimate the solubility of oxygen in a fluorochemical from its structure alone, ΔE_v^{298} and V are estimated from Table I. δ_1 is calculated from equation 12, ΔH_v^{298} is calculated from equation 6, and ΔS_v^{298} (= $\Delta H_v^{298}/T$) then determined. From ΔS_v^{298} and either equation 16 or

17, $\bar{V}_2$ is estimated. Then, using the estimated δ_1, $\bar{V}_2$, V_1 and the constants x_2^1 and δ_2 in equation 15, the mole fraction of oxygen, x_2, is obtained. This can be converted to cm^3 of O_2/100 ml. of liquid at 25°C by:

$$\text{cm.}^3\ O_2/100\ \text{ml. of liquid} = \frac{100 \cdot x_2 \cdot 24465}{V_1} \qquad (18)$$

TABLE IV

Sample Calculations of Physical Properties from Chemical Structure

	From Table I	ΔE_v^{298} cal/mole	V^{298} cm^3/mole
	7 x CF_2 =	5481	161.7
	3 x CF =	-1188	-45.0
	1 x CF_3 =	1933	54.8
	2 x 6 atom ring =	4544	79.8
		10,770	251.3

1. ΔE_v^{298} = 10,770 cal/mole
2. V_1^{298} = 253.1 cm^3/mole
3. δ_1 = 6.55 from eq. 12
4. $\Delta H_v^{298} = \Delta E_v^{298} + RT$ = 10,770 + 592 = 11,362 cal/mole
5. T_b°K = 431 or 158°C from eq. 5
6. D_4^{25} = 2.04 from eq. 7
7. P torr = 6.1 from eq. 9 using perfluorochemical α, β values.
8. $\Delta S_v^{298} = \frac{\Delta H_v^{298}}{298.15}$ = 38.11 cal/mole-°K
9. $\bar{V}_2$ = 45.11 cm^3/mole from eq. 17
10. Mole fraction of dissolved O_2 @ 25°C: $\lambda_2 = 4.111 \times 10^{-3}$ from eq. 15.

    $cm^3 O_2/100\ cm^3$ of liquid = 40.0 from eq. 18.

Table IV presents a sample calculation of all the physical properties discussed above for perfluoremethyldecalin. Table V shows the calculated and measured physical properties of some fluorocarbon-hydrocarbon hybrid compounds prepared in our own research efforts. Because these compounds have high hydrogen content, the agreement between calculated and measured physical properties is not quite as good as those for totally fluorinated materials.

TABLE V

Properties of Fluorocarbon-Hydrocarbon Hybrids

	$^1\Delta H_v$298	^1V	$^2\rho^{25}_4$ calc/found	^3T_b °C calc/found	$^4cm^3O_2/100\ cm^3$ calc/found
$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2CH_3$	9927	238.0	1.52/1.52	126/121	46.5/46.7
$CF_3CF_2CF_2(CF_3)_2CCH_2CH_2CH_2CH_3$	11,107	254.1	1.48/1.47	152/138	41.3/46.6
$CF_3CF_2CF_2(CF_3)_2CCH_2CH(CH_3)_2$	10,692	254.4	1.48/1.48	143/132	43.1/43.6
$CF_3CF_2CF_2(CF_3)_2COCH_3$	8367	209.6	1.67/1.62	83/97	56.3/48.1
$CF_3CF_2CF_2(CF_3)_2COCH_2CH_3$	9547	225.7	1.61/1.57	116/100	48.4/49.9
$CF_3CF_2CF_2(CF_3)_2COCH_2CH_2CH_3$	10,727	241.8	1.56/1.51	144/12-	42.6/45.8
$(CF_3)_3CCH_2CH_2C(CF_3)_3$	11,520	284.4	1.64/solid	161/solid	40.2/-
$(CF_3)_3CCH_2CH_2CH_2C(CF_3)_3$	12,700	300.5	1.60/solid	186/solid	36.7/35.8
$(CF_3)_3CCH_2CH=CHCH_2C(CF_3)_3$	13,580	311.4	1.58/solid	206/solid	34.3/-
$(CF_3)_3CCH_2CH_2CH_2CH_2CH_2C(CF_3)_3$	15,060	332.7	1.53/solid	231/solid	31.1/-
$CF_3CF_2CF_2(CF_3)_2CCOH$	8726	182.3	-/1.77	-/93	-/44.3
$CF_3CF_2CF_2(CF_3)_2CCH=O$	8040	206.1	-/1.70	-/73	-/decomp.

1: From Table I.
2: From eq. 7.
3: From eq. 9.
4: From eq. 16, 17 and 18 (The hybrids are similar to the cyclic fluorochemicals in their O_2 solubility behavior).

In summary, the design of fluorochemical phases for use in artificial blood should have a vapor pressure of less than 40 mm Hg at 37.5°C and an oxygen solubility as high as possible. In this paper we report methods for predicting vapor pressure and oxygen solubility for perfluorochemicals based on chemical structure alone; thereby making it possible to guide synthetic strategy in the design of artificial blood substitutes.

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APPENDIX E

F-2-METHYL-2-PENTANOL

APPENDIX E

F-2-Methyl-2-Pentanol, An Easily-Prepared Perfluorinated Tertiary Alcohol, Kirby V. Scherer, Jr.*, Department of Chemistry, University of Southern California, Los Angeles, California, 90007, and Tobia F. Terranova and D.D. Lawson, Energy and Materials Research Section, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91103

Abstract: F-Propene was converted to F-2-methylpentene (1) by reaction at 10-15°C and one atm. with KF and 18-crown-6 in CH_3CN, followed by refluxing with the same catalysts in $CH_3CON(CH_3)_2$. Reaction of 1 with KF and either NOCl or N_2O_4 in $CH_3CON(CH_3)_2$ at -5° gave $CF_3CF_2CF_2(CF_3)_2CNO$, which was oxidized to $CF_3CF_2CF_2(CF_3)_2CONO$ with O_2 or excess N_2O_4 at r.t.; hydrolysis of the nitrite gave $CF_3CF_2CF_2(CF_3)_2COH$ (2), purified by distillation from conc. H_2SO_4; d^{24} 1.770, bp_{728} 93°C, in 57-65% yield from 1. Reaction of 2 with KOH and n-C_4H_9I in DMSO gave 84 $CF_3CF_2CF_2(CF_3)_2CO$-n-C_4H_9, bp_{732} 138°C, d^{21} 1.473.

F-2-Methyl-2-Pentanol, An Easily-Prepared Perfluorinated Tertiary Alcohol[1]

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We report here an efficient synthesis of the title compound (1) which can be carried out in ordinary laboratory glassware from a relatively inexpensive starting material and common reagents. Fluorinated alcohols, particularly 2,2,2-trifluoroethanol and 2-H-F-2-propanol (hexafluoroisopropanol), have attracted attention as specialty solvents, owing to their low nucleophilicity,[2] powerful H-bond donor ability[3] and optical transparency. Perfluorinated tertiary alcohols as a class should possess those qualities as well as exceptional chemical unreactivity.

F-tert-butanol (2), the only commercially available perfluorinated alcohol, has received little attention as a solvent because of its high price and modest liquid range (-15 to +45°C); it has been prepared by disproportionation of F-acetone catalyzed by CsF-H_2O,[4] by halogen exchange from $(CF_3)_2CCl_3COH$,[5] and by several routes from the dangerously toxic F-isobutene.[6] Other

perfluorinated tertiary alcohols have been prepared by reaction of perfluorinated ketones with perfluoroorganometallic reagents,[7] and by SbF_5-catalyzed rearrangement of perfluorinated oxiranes.[8]

(Scheme 1)

Our synthesis of 1 is modeled after Knunyants and Dyatkin's original route to 2,[6a,c] but we have simplified the procedure and reagents. In addition, we report convenient and reproducible conditions for the preparation of F-2-methyl-2-pentene (3), the stable dimer of F-propene, in kilogram quantities wit[illegible]e use of pressure equipment. Alcohol 1 is thus available [illegible] to four steps and over 50% overall yield from F-propene, a fluoropolymer intermediate[9] currently priced at about $6/lb in large quantities.

The anionic dimerization of F-propene to give 3 was described first in the patent literature,[10] and most recently by Dmowski, Flowers and Haszeldine;[11] we find that reaction of F-propene with KF and 18-crown-6 in acetonitrile at atmospheric pressure gives largely the unstable dimer, F-3-methyl-2-pentene (4), and that 4 must be refluxed (ca. 50°C) for several days with the same catalyst in dimethylacetamide (DMA) to effect isomerization to 3. The difference between our conditions and Dmowski, et al's are probably due to the much smaller ratio of catalyst to olefins in our work. We were unable to reproduce the preparation of 3 with the special amine catalyst of von Halasz, et al.[12]

Addition of the elements of nitrosyl fluoride to 3 is conveniently effected by passing either N_2O_4 or NOCl into a cold,

well-stirred suspension of powdered KF and 3 in DMA. If an excess of N_2O_4 is used, the blue nitrosoalkane 5 is slowly oxidized *in situ* to the nitrite (6); with NOCl, 5 is isolated and without purification oxidized to 6 with a slow stream of O_2 at room temperature. The two-step procedure gave a purer product and seems safer, since solutions of N_2O_4 in organic solvents have been reported to explode.[13] Neither 5 nor 6 has been isolated in analytically pure form and their structures are assigned by analogy.[14] The oxidation of 5 by N_2O_4 or O_2 probably occurs through a radical-chain mechanism, and takes place under milder conditions than those reported for the oxidation of $(CF_3)_3CNO$[6c,f]; models suggest that relief of steric repulsions as the $CF_3CF_2CF_3(CF_3)_2C\cdot$ radical becomes planer may account for its more ready formation versus $(CF_3)_3C\cdot$.

Several alkyl ethers of 1 were prepared for evaluation as the oxygen-carrying phase of fluorochemical emulsion artificial blood[15]; a typical procedure for the preparation of one of these is described in the experimental section.

Experimental Section

Potassium fluoride was dried at 180° in a vacuum oven, ground in a ball mill, and manipulated thereafter in a glove box; other chemicals and reagents were used as received. Fluorine NMR spectra were measured on pre-calibrated chart paper using a Varian T-60 spectrometer; the triplet of $CFCl_2CF_2Cl$ was used as an internal reference, and 72.0 ppm was added to the observed chemical shifts to obtain ϕ^*.

F-2-Methyl-2-propene (3). A 3L 3-neck flask was surrounded by a cooling bath and equipped with magnetic stirring, an immersion thermometer, a large cold-finger charged with Dry Ice-isopropanol on the center neck and a $CaCl_2$-filled exit trap. The flask was charged with 30 g KF, 32 g 18-crown-6/acetonitrile complex and 524 mL of spectrograde CH_3CN. F-propene was introduced through a side neck directly from a cylinder at a rate such that a thin stream of condensate fell from the condenser tip into the stirred KF suspension. The pot temperature rose initially to 50°, but for the rest of the reaction was kept between 10 and 15°C by periodic additions of Dry Ice to the external isopropanol bath. Refluxing of F-propene stopped almost immediately if the introduction was interrupted, and the mildly exothermic dimerization resumed when the gas flow was re-started. After about 3 h the working capacity of the flask was reached, and the mixture was allowed to stir overnight and come to room temperature. Stirring was then stopped and the clear lower layer siphoned out. The yield of crude dimers was 2430 g; analysis by NMR indicated ca. 27% 3 and 73% 4; trimers

were not detected. In another run, three successive batches of mixed dimers totalling 8263 g were obtained using the same sample of catalyst, without noticeable loss of activity.

Isomerization of 4 to 3: The 2430 g of dimer mixture was combined in another 3L r.b. flask with 20 g 18-crown-6/acetonitrile complex, 30 g KF, and 250 mL DMA. The mixture was refluxed under an efficient condenser for 13 days, when NMR analysis showed isomerization to 3 to be > 95% complete. Direct distillation from the pot gave 2111 g of 3, b.p. 51-60°C, suitable for the preparation of 1.

F-2-methyl-2-pentanol (1). A mixture of 500 mL DMA, 50 g dry KF (.86 mol) and 171.3 g 3 (.571 mol) in a one L three neck flask was stirred at 4 - 7°C (external ice-salt bath) while 109 g N_2O_4 (1.18 moles) was slowly distilled in in a slight flow of O_2. A -28°C (boiling CF_2Cl_2) cold finger was used to minimize escape of NO_2. The addition was slightly exothermic and was interrupted whenever the pot temperature reached +7°. Immediately after the addition the reaction mixture was deep blue; NMR examination of the lower fluorochemical phase showed complete consumption of starting olefin and a single predominate product, presumably $CF_3CF_2CF_2(CF_3)_2CNO$ (5, gem-CF_3 quintet at ϕ* 63.7). The reaction mixture was let warm slowly to room temperature with stirring. After 18 h the blue color had disappeared and the lower phase was light yellow, consistent with $CF_3CF_2CF_2(CF_3)_2CONO$ (6, gem-CF_3 quintet at ϕ* 68.5). After ca. 48 h additional, most of the fluorochemical had dissolved in the DMA and much white solid was

present. Water, ca. 400 ml, and 6.2 g H_3BO_3 (0.1 mol) were added; the lower phase re-formed and most of the solid dissolved. Sulfamic acid was added in small portions to destroy the nitrous acid present and the reaction mixture was distilled at atmospheric pressure until the condensate was clear. The distillate consisted of a lower fluorochemical phase and an upper aqueous phase, both almost colorless. Potassium hydroxide, 37.4 g (.66 mol), was added to the distillate, whereupon most of the lower phase dissolved in the aqueous layer. A small amount of neutral material was distilled under aspirator pressure into a -78° trap, then the clear aqueous KOH solution was acidified with H_2SO_4. The lower phase which formed was separated, mixed with 25 mL of 96% H_2SO_4 and distilled at aspirator pressure into a Dry Ice-cooled receiver. Yield 108.9 g of 1 as a dense, water-white liquid, 56.7%, bp_{728} 93°C, d^{24} 1.770 g cm^{-3}. NMR: ϕ* 68.9 (6F,t of t), 76.8 (3F, t), 109.9 (2F, m), 118.9 (2F, m).

Anal. Calcd for $C_6F_{13}OH$: C, 21.45; H, 0.30; O, 4.76; F, 73.49. Found: C, 21.19; H, 0.49.

The neutral fraction weighed 17.5 g (approximately 9% yield) and consisted of a mixture of two still-unidentified $CF_3CF_2CF_2(CF_3)_2CX$ products. The gem-$(CF_3)_2$ t of t of the major (~60%) component appears at ϕ* 63.9; the gem-$(CF_3)_2$ t of t of the minor component at ϕ* 67.6.

Alternate procedure: Dried KF, 58 g (1.0 mol), 550 mL DMA and 201.3 g (0.671 mol) 3 were combined and stirred magnetically in a one L three neck flask equipped with a cold finger condenser,

thermometer well, and drying tube. The mixture was cooled to -20° in a Dry Ice-isopropanol bath, and nitrosyl chloride was passed in, keeping the temperature below -5°, until a green color persisted (the reaction product forms a clear blue lower layer, and the presence of excess yellow-brown NOCl causes the mixture to appear green). An NMR spectrum of the blue lower layer showed complete consumption of starting material. The reaction mixture was poured into a 2 L separatory funnel containing 30 g of boric acid in ca. one L of ice water. The blue lower layer was drawn off and without further purification, placed in a 250 mL three neck flask equipped with a cold finger condenser and oxidized with a slow stream of oxygen. The blue liquid turned green, then yellow within 30 minutes; the oxidation is exothermic. The crude yellow nitrite ester was poured into 800 mL of water and stirred until gas evolution ($NO + NO_2$) ceased, then made strongly basic by addition of 60 g KOH pellets. Insoluble material (40.8 g) was removed by direct steam distillation; distillation was stopped when the distillate ran clear of insoluble material. The residual aqueous solution was cooled and acidified with sulfuric acid. The lower layer which formed was separated, mixed with 75 mL of 96% H_2SO_4, and the mixture distilled at aspirator pressure into a dry ice-cooled receiver. Yield 145.5 g (64.5%) of colorless 1, pure by NMR.

2-n-Butoxy-F-2-methylpentane. Dimethylsulfoxide, 125 g; alcohol 1, 25.0 g (0.0744 mol); KOH pellets, 9.9 g (0.115 mol);

and 1-iodobutane, 18.4 g (0.100 mol) were combined and stirred at room temperature for three days (when 1 was mixed with DMSO, heat was evolved; on a larger scale, provision for cooling should be made.) At the end of the reaction period the mixture was poured into ca. 300 mL of water and the crude lower layer separated and dried over solid KOH. Distillation of the crude product at aspirator pressure into a Dry Ice-cooled receiver gave 24.8 g of colorless liquid, 84% yield. The analytical sample was obtained by preparative gas chromatography. Constants: bp $_{732}$ 138°C, d^{21} 1.473 g cm^{-3}.

Anal. Calcd. for $C_{10}H_9OF_{13}$: C, 30.63; H, 2.31; O, 4.08; F, 62.98. Found: C, 30.47; H, 2.25.

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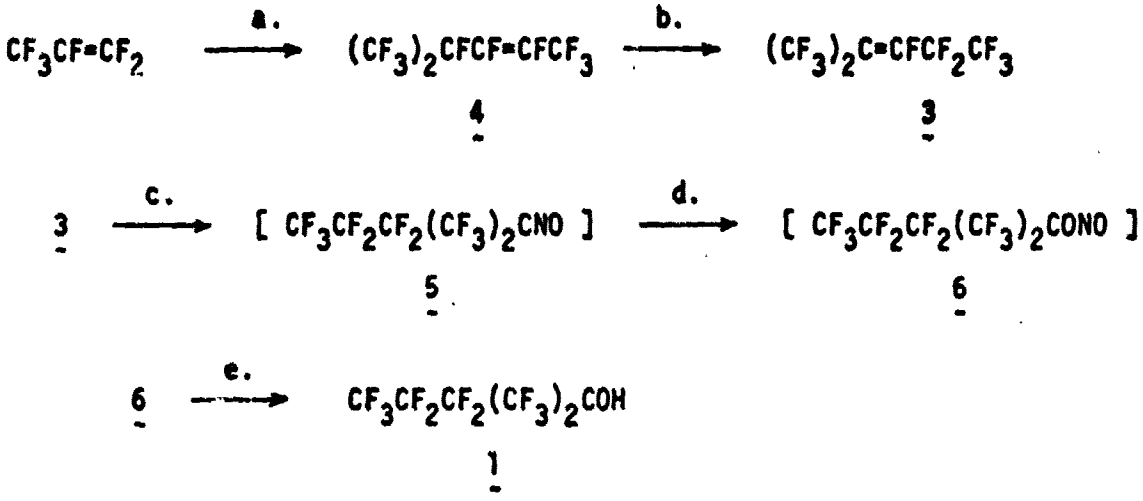
SCHEME I



a. KF, 18-crown-6, acetonitrile, 10 - 15° C; b. KF, 18-crown-6, dimethylacetamide, reflux; c. KF, NOCl or N_2O_4, dimethylacetamide, ca. 0° C; d. O_2 or N_2O_4 at room temperature; e. H_2O.

APPENDIX F

HYBRID FLUOROCHEMICALS AS CANDIDATES FOR THE OXYGEN CARRYING PHASE IN ARTIFICIAL BLOOD: SYNTHESIS

APPENDIX F

207. HYBRID FLUOROCHEMICALS AS CANDIDATES FOR THE OXYGEN CARRYING PHASE IN ARTIFICIAL BLOOD: SYNTHESIS. K. V. Scherer, Jr., Dept. of Chemistry, Univ. of Southern Calif., Los Angeles, CA 90007; T. F. Terranova, D. D. Lawson and E.N. Washington, Energy and Materials Research, Section 346, Jet Propulsion Laboratory, Pasadena, CA 91103.

Alkylation of perfluorinated tertiary carbanions, formed by equilibrium addition of potassium fluoride to readily available perfluoroolefins in a polar aprotic solvent, leads in good yield to "hybrid" fluorochemicals with physical properties appropriate for application in artificial blood. The tert-butyl carbanion derived from octafluoroisobutene reacts with n-alkyl iodides or bromides, whereas the tert-hexyl carbanion derived from perfluoro-2-methyl-2-pentene requires the more reactive allylic iodides or bromides for good yields in acceptable time. E.g.:

$$(CF_3)_2C{=}CFCF_2CF_3 + KF + CH_2{=}CHCH_2Br \xrightarrow{DMA} CF_3CF_2CF_2C(CF_3)_2CH_2CH{=}CH_2$$

Gas-phase hydrogenation gives the saturated compounds without loss of fluoride.

Oxidation of the same carbanions, e.g. with dinitrogen tetroxide, gives the tert-nitrite which may be hydrolyzed to the tertiary perfluoro alcohol. Reaction of the alcohols with potassium hydroxide and an alkyl iodide in dimethyl sulfoxide leads to the corresponding ethers in excellent yield. All of these reactions are performed at room temperature or below in ordinary laboratory glassware, and afford good yields of isomerically pure products.

(From Abstracts of Papers, American Chemical Society, 175th ACS National Meeting, Organic, 207, 1978.)

APPENDIX G

HYBRID FLUOROCHEMICALS AS CANDIDATES FOR THE OXYGEN CARRYING PHASE IN ARTIFICIAL BLOOD: PHYSICAL AND PRELIMINARY TOXICOLOGICAL TEST RESULTS

APPENDIX G

63. HYBRID FLUOROCHEMICALS AS CANDIDATES FOR THE OXYGEN CARRYING PHASE IN ARTIFICIAL BLOOD: PHYSICAL PROPERTIES AND PRELIMINARY TOXICOLOGICAL TEST RESULTS. K. V. Scherer, Jr., Dept. of Chemistry, Univ. of Southern California, Los Angeles, CA 90007; D. D. Lawson, J. Moacanin and T. F. Terranova, Energy and Materials Research, Section 346, Jet Propulsion Laboratory, Pasadena, CA 91103.

A series of partially fluorinated alkanes and ethers of the general formula $(C_nF_{2n+1})(CF_3)_2C$-R and $(C_nF_{2n+1})(CF_3)_2C$-OR, R = saturated alkyl, has been prepared as candidates for the oxygen carrying phase in fluorochemical emulsion artificial blood. A semi-empirical treatment based on group contributions to molar volume and intermolecular attraction, and on regular solution theory (Hildebrandt), has been developed which predicts O_2 solubility and vapor pressure from chemical structures alone. These are the physical properties most significant for predicting physiological utility in this application. A comparison of the predicted and experimental vapor pressures and O_2 solubilities for the new compounds and for compounds in the literature will be presented. Several of the new fluorochemicals dissolve more oxygen than perfluorodecalin or perfluorotributyl amine, while having vapor pressures within acceptable limits. Animal tests are in progress, but cell-culture tests suggest that the compounds below possess very low toxicity bordering on biological inertness. Examples of compounds prepared to date, with measured O_2 solubility in ml/100 ml and [extrapolated] v. p. in torr, both at 25 °C, include: n-$C_3F_7(CF_3)_2C$-n-C_3H_7, 46.7 ml, 14.3 torr; n-$C_3F_7(CF_3)_2C$-n-C_4H_9, 46.6 ml, 6.8 torr; n-$C_3F_7(CF_3)_2C$-O-C_2H_5, 49.9 ml, 24.4 torr; n-$C_3F_7(CF_3)_2C$-O-C_3H_7, 45.8 ml, 11.1 torr.

(From Abstracts of Papers, American Chemical Society, 175th ACS National Meeting, Medicinal, 63, 1978.)

APPENDIX H

FINAL REPORT
UNIVERSITY OF UTAH RESEARCH INSTITUTE

APPENDIX H

Contract No. 954595

FINAL REPORT - YEAR II

Artificial Blood Substitute
July 15, 1977 to July 14, 1978

TR 222-022

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TABLE OF CONTENTS

SECTION I: INTRODUCTION

Under contract with Jet Propulsion Laboratory for "Synthesis and Biological Screening of New and Improved Fluorocarbon Compounds for Use as Artificial Blood Substitutes," Utah Biomedical Test Laboratory (UBTL) assumed responsibility for biologic screening of new fluorochemical compounds (FCC) synthesized by Jet Propulsion Laboratory (JPL). The main goal of the study was to test the feasibility that from the synthesis of novel fluorocarbons, candidates for safe hemoglobin substitutes could be found.

Summary of First Year's Study (1976-1977)

In the original submission of our proposal to the National Blood Institute in July, 1975, JPL/UBTL detailed a two-year plan for the synthesis and biological screening of new and improved fluorochemical compounds (FCC) for use as artificial blood substitutes. The Institute requested that the proposal be modified and resubmitted to comply with a one-year feasibility study with the possibility that the project may continue for a second year. The one-year study was granted with the main goal of the study directed toward testing the feasibility of the hypothesis that from the synthesis of novel fluorocarbons, candidates for safe hemoglobin substitutes could be found. As one of the objectives of this study, it was proposed that approximately 20 newly synthesized compounds would be quickly tested by UBTL for gross toxicity and tissue retention in mice as an initial crude screen.

In the original plan, selected compounds passing the initial mouse screen would be resynthesized as necessary and formulated into emulsions for blood replacement in rats to allow for more refined toxicity, tissue retention, and efficacy testing. It was anticipated that approximately four new compounds, formulated into artificial blood, would be tested in

rats at the 50% blood-replacement level.

However, after having completed 6 months of work on the first year's contract, the National Blood Institute requested that we submit a proposal to them for continuation of studies into the second year and as part of the site visit for this renewal, recommendations were made by the site team altering the originally planned studies. In compliance with the site team recommendations, UBTL concentrated their efforts on mouse toxicity screen testing, cancelling plans to do infusion studies on rats for this year and the next.

The candidate compounds synthesized by JPL which met basic physicochemical requirements as stated in the RFP were subjected to basic gross toxicity and tissue retention testing in mice at UBTL. For these tests UBTL usually received approximately 5-10 grams of each compound only. Emulsions of test FCCs were prepared at UBTL as needed.

Screening methods and protocols were developed for dosing and testing mice for toxicity with the new FCCs. Because the intraperitoneal route for administration of the FCC was proposed, absorption studies were developed to validate absorption of FCCs from the peritoneal cavity. Using FC-47 as a reference standard, absorption kinetics for this FCC indicated a half time of less than two hours. Gas chromatography was used for assay of the FCCs in fluids and tissues.

The screening methods and protocols developed for the candidate compounds include an Absorption Verification Study and a more prolonged Mouse Toxicity Screen Test.

Because of the relatively small amount of test compound received, the protocol developed for screening in mice had to be adapted to each individual compound and could rarely be followed in its entirety. Flexibility in the application of the protocol was essential so that the tests selected would yield the most information for a particular compound.

A total of 13 newly synthesized FCCs were screened with the above methods and 20 commercially available surfactants were screened *in vitro*

for blood compatibility. Of the 13 FCCs tested, one FCC, JPL-AB-6, appeared to be the most promising candidate for further study. A second FCC, JPL-AB-13 was considered as the next most promising. Two others were considered for further screening, and nine were rejected, classified as too toxic for further testing.

For protocol and testing details, see Appendix I and II. For the complete report, the reader is referred to UBTL Final Report, "Artificial Blood Substitute," (TR 222-013) or JPL Publication 77-80, "Synthesis and Biological Screening of Novel Hybrid Fluorocarbon Hydrocarbon Compounds for Use as Artificial Blood Substitutes," (Annual Report, July 1976 to July 1977).

Summary of Second Year's Study (1977-1978)

Under terms of the second year's contract, UBTL was given the charge of biologically screening approximately 20 new FCCs to be done in mice only. Studies were to be in greater depth using previously developed protocols, modified to meet current test plans. No rat or dog studies were to be included.

It was directed that emphasis should be placed on FCCs that proved relatively non-toxic and that investigations should continue with at least two of the promising compounds from the previous study.

These conditions were met insofar as possible with the biological testing dependent on JPL for the supply of FCCs to test.

In this second year of study, a total of 14 newly synthesized FCCs were screened with biological testing. Of these 14 FCCs tested, two were non-toxic and can be considered as candidates for further testing; these were compounds JPL-AB-14 and JPL-AB-32.

In addition to the biological screening described above, other studies were done as follows:

— JPL-AB-6, a promising candidate from the first year's study, was studied in greater detail and preparations were made for more detailed phar-

macokinetic studies.

— Preliminary studies were done on the "shelf-life" of JPL-AB-6.

— LD_{50} techniques were investigated using FC-47 at various dose levels.

— Sonication experiments were done to investigate the effects of pH, CO_2, N_2O, N_2 and O_2 on fluoride ion generations and concentrations.

SECTION II: MATERIALS AND METHODS

Screening of New FCCs

The 14 FCCs were investigated with the biological screening methods adapted from those used in the first year's study.

These methods are detailed in Appendices I and II and consist mainly of intraperitoneal absorption studies, observation for toxicity and death, organ distribution studies with gas chromatography, and histopathological studies on tissues.

Absorption of the FCC from the intraperitoneal injections was verified by peritoneal wash techniques for recovery of any residuals as detected by gas chromatography. If the mice did not die within 24 hours and absorbed most of the injected sample, a new group were similarly injected and observed for a two-week period. Organ distribution of the FCC with gas chromatography was determined, and histopathological studies were done in the mice surviving the two-week screening study.

In-depth Study of JPL-AB-6

The most promising FCC from the first year's study, JPL-AB-6, was studied in greater detail this year but was more limited than anticipated due to resynthesis problems at JPL. Protocols as indicated above were used to study 8 mice, 2 for Absorption and 5 for Mouse Toxicity Screen studies.

Shelf-life Studies. As part of the program plan for the second year study, UBTL looked at the stability of both the neat and emulsified JPL-AB-6. The emulsion was aged for about 2 weeks and the neat for 4 weeks at 37° C, intermittently exposed to room air. Then the neat was emulsified for injection, after aging in that form, and injected i.p. into 3 mice. The aged emulsion was injected i.p. into one mouse. Followup studies were done to compare results with those found in mice injected with the original un-aged FCC and are reported in the next section "Results".

One factor to consider with this series of JPL-AB-6 studies compared to last year's is that JPL stated that this batch of the FCC was more carefully purified before it was sent to UBTL.

LD_{50} Studies

We investigated the LD_{50} of an FC-47 emulsion at various dose levels. With a 50% emulsion, 3 mice were injected i.p. for each of the following dose levels: 50, 100, 200 and 500 gm/kg.

Sonication Experiments

In sonication experiments, 20% emulsions of FC-47 at pH 7.2, each under one atmosphere of CO_2, N_2O, N_2 or O_2 respectively, were first purged by bubbling the specific gas through the solution for 5 minutes then sonicated continuously up to 90 minutes to assess generation of fluoride ion. Fluoride ion was determined at 30 minutes intervals by a fluoride ion-specific electrode. The calibrating standard solutions were adjusted to match the ionic composition of the emulsions. In a related experiment, 20% FC-47 emulsions were similarly sonicated at pH's of 4.0, 7.0, and 10.0, under one atmosphere of O_2. Sonication was performed in a 25 ml rosette cell immersed in a water-ice bath.

The sonicating device used here and also for the other FCCs screened has a lead zirconate and titanate probe (Heatsystems Ultrasonics Inc., Plain View, New York, Model No. 350).

Data Management

Again, this year, because of the small amounts of new FCCs (5-10 gm and occasionally up to 16 gm), only a few mice per compound could be studied as a rule. With such small numbers of animals per compound, statistical analyses are not feasible, so judgments on each compound were necessarily made on observations of essentially individual mice under different test circumstances for a given compound.

Interpretation of observations such as animal behavior, time of death after injection, results of microscopic pathology, and some FCC analyses, are reported in the next section "Results".

SECTION III: RESULTS

New FCCs Screened

The results of biological screening of the 14 FCCs for this year are described below. Table 1 shows the JPL-AB number, the structure, the general animal reaction and the results in brief. The table includes the FCCs we screened during the first year (JPL-AB-3 to -13), but excludes JPL-AB-1 and -2 which were "start-up" FCCs.

Two FCCs, JPL-AB-14 and -32 were the only two that appeared to be promising during this year's screen. The others were rejected for further study.

In most cases, these FCCs were injected intraperitoneally as a 20% emulsion and some with the neat form of the FCC. Control mice were also injected at the same time. The majority of the FCCs were very low in fluoride ion; that is, less th 7 ppm, our lowest standard. In those FCCs that were high in fluoride ion, the ion was extracted with water until low enough. It was then emulsified prior to injection.

1. JPL-AB-14. Two mice were injected i.p. for the 24-hour Absorption Verification Study. The mice lived and were sacrificed at the end of 24 hours. The spleen was very large, approximately two times normal size, and was whitish in color. The peritoneal recovery study indicated 7% remaining after 24 hours. Organ tissues were assayed for FCC levels in one mouse and the other underwent histopathological studies.

Because of the encouraging results of the Absorption Verification Study, three more mice were injected for the two-week Mouse Toxicity Screening. These mice were sacrificed at the end of the two-week period and FCC assays were done on peritoneal washes and tissues. All three had peritoneal washes performed, but only two were assayed for tissue levels, and the third was reserved for histopathological studies.

The FCC assay levels are shown in Table 2 and indicate that the FCC was readily absorbed from the peritoneum, (93% in 24 hours and 99.5% in 14 days), that high concentrations are observed in the

Table I

Toxicity of Intraperitoneal Injection of Fluorocarbon-Hydrocarbon Hybrid Compounds

No.	Structure	Reaction	Result
3.	$(CF_3)_3C\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Ataxia & Violent Shaking	Death 10-40 min
4.	$(CF_3)_3C\text{-}CH_2\text{-}C(=O)\text{-}O\text{-}CH_2\text{-}CH_3$	Ataxia	Death <15 min
5.	$(CF_3)_3C\text{-}NH_2$	Violent	Death <10 min
6.	$(CF_3)_3C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}C(CF_3)_3$	None	Non toxic
7.	$(CF_3)_3COH$	Total Relaxation	Instantaneous Death
8.	$(CF_3)_3C\text{-}C(Cl)(F)\text{-}C(Cl)(F)\text{-}CF_3$	Ataxia	Death 24-48 Hrs.
	1/7 Dose Neat Fluid	None	Non toxic
9.	$(CF_3)_3C\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}C(CF_3)_3$ (1 mouse - 0.69 gm)	Decreased Activity & Tone	Impaired but lived.
10.	$(CF_3)_3C\text{-}CH_2\text{-}CH_2\text{-}CHI$ (F^- Generated with Sonication)	Ataxia, Gasping, Convulsions	Death 1-3 Hrs.
11.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}CH_2\text{-}CH = CH_2$ (F^- Generated with Sonication)	Tetany	Death 1-2.5 Hrs.

C-3

FCC'S STUDIED TO DATE (continued)

No.	Structure	Reaction	Results
12.	$(CF_3)_3C\text{-}CH_2\text{-}COONa$ (H_2O Soluble, High F^- Generation)	Ataxia	Death 5 min
13.	$(CF_3)_3C\text{-}(CH_2)_5\text{-}C(CF_3)_3$	None	Non toxic
14.	$(CF_3)_3C\text{-}(CH_2)_2\text{-}C(CF_3)$	None	Non toxic
15.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Ataxia - Prostrate	Death 3-24 Hrs.
16.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Sick - Prostrate	Death 24-48 Hrs.
18.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}OH$	Relaxation	Instantaneous Death
20.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}O\ CH_2\text{-}CH_3$	Ataxia	Death 4-12 Hrs.
21.	$CF_3\text{-}CF_2\text{-}CF_2\text{-}C(CF_3)_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH_3$	Ataxia, Gasping	Death 8-24 Hrs.

FCC'S STUDIED TO DATE (continued)

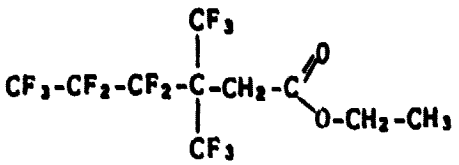
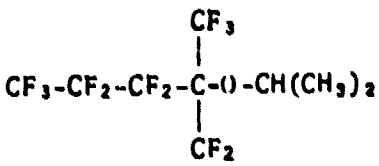
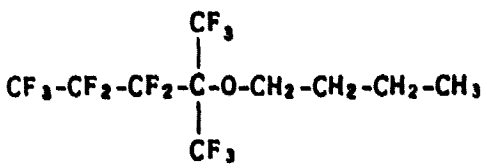
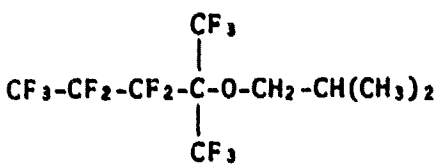
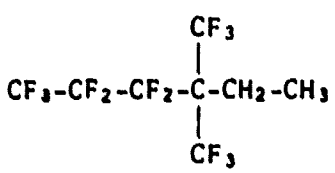
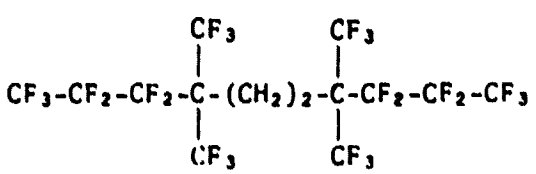
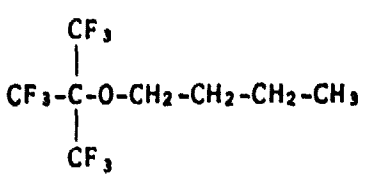
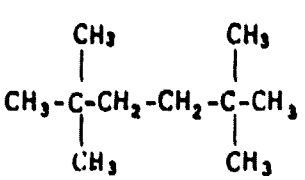
No.	Structure	Reaction	Results
24.		Agitated & Violent	Death 1-2 Hrs.
27.		Prostrate-convulsions	Death 3-6 Days
28.		Ataxia-Prostrate	Death 18-24 Hrs.
29.		Ataxia-Prostrate	Death within 5-6 hrs. or recover & survive
31.		Ataxia-Prostrate	Death 3-5 Days
32.		None	Non toxic
33.		Choking-Prostrate	Death 5-7 Hrs.
35.		Prostrate	Death 4-5 Hrs.

TABLE 2. FLUOROCARBON TISSUE DISTRIBUTION FOLLOWING INTRAPERITONEAL INJECTION

SAMPLE	FC No. 6				FC No. 14				FC No. 32			
	24 Hr.		14 Days		24 Hr.		14 Days		24 Hr.		14 Days	
	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected	mg/g Tissue	% of Total Injected
Intraperi-toneal Wash	-	4.3	-	9.4	-	7.0	-	6.5	-	3.0	-	2.6
Liver	-	-	27.5	7.5	112	21.0	73.7	36.3	38.2	7.0	18.8	4.9
Spleen	-	-	87.9	9.2	166	4.6	87.9	12.6	159	2.8	82.4	5.4
Brain	-	-	0.05	0	3.1	0.2	.5	0.02	6.0	0.4	0.6	0.1
Blood *	-	-	2.2	0.7	0	0	0	0	28.1	9.8	0	0
Kidney	-	-	-	-	26.7	1.4	2.7	.4	25.4	2.2	2.6	0.4
Remainder of Body	-	-	1.7	4.7	0.1	14.7	.02	0.1	0.4	1.2	0.2	0.4
TOTAL				22.5		48.9		49.9		26.4		13.8

* Blood volume of 10% of total body weight was assumed.

liver and spleen at 24 hours, that these concentrations in the liver and spleen drop by about one-half in 14 days, and that the FCC is about 50% eliminated at the end of 24 hours. We must emphasize, however, that Table 2 data was obtained from individual animals in some cases and an average of 2 or 3 animals at most. This accounts for some discrepancies in specific comparisons, yet the general trends are similar for all three FCCs.

Pathology studies showed gross enlargement of the liver and especially the spleen at 24 hours and at 14 days. The spleen exhibited a gray-white cast. Histopathology studies on the 24-hour mouse showed "lipid-laden" macrophages in the spleen and other minor changes, but these changes are not as marked as seen in other test animals. Tissues from other organs showed only minimal changes. In the two-week mouse, lipid-laden macrophages are seen in the liver and spleen with fairly marked tissue reaction (swollen cells) throughout all other tissues examined.

Status: This FCC appears to be similar to JPL-AB-6, a promising compound studied in the first year. It is recommended for further study and more complete evaluation.

2. JPL-AB-15. Two of the three mice injected with the emulsion died within 5 hours, not violently but huddled and depressed. One was alive at 24 hours but appeared very sick and prostrate. This animal was sacrificed for possible peritoneal wash to assess absorption by G.C. methods, but this procedure was considered redundant when findings on the neat were observed.

A few days later three mice were injected with the neat FCC, and all died within a five-day period. One assumption for the delayed death with the neat was that the neat was probably absorbed more slowly than the emulsion, allowing this group of mice to live longer.

Status: JPL-AB-15 is considered toxic and is rejected from the study at this time.

3. JPL-AB-16. Several groups of mice were studied with i.p. injections of the 20% emulsion and the neat. A total of 10 mice received the 20% emulsions and were very sick, dying within 1 to 3 days except for two which were sacrificed at 24 and 48 hours respectively. A total of 7 mice received the neat form and lived up to 3 weeks, some being sacrificed at various times for residual FCC and/or pathology studies.

Pathology studies were done on 3 mice and showed varied changes. The mouse sacrificed at 48 hours, with the 20% emulsion injection, was prostrate at the time. Micropathology studies showed alterations mainly in the spleen with marked cell degeneration and histiocytic proliferation. The kidneys, liver and brain showed lesser involvement. The mouse sacrificed at 48 hours, with the neat injection, was well at the time. Micropathology studies showed minimal changes, generally with only moderate congestion of the spleen. The third mouse was well and was sacrificed 3 weeks after the neat injection. Studies showed some liver cord swelling and degeneration, the lungs were congested chronically and the kidney tubules and glomeruli showed some degeneration.

Several FCC assay studies were planned to clarify the differences noted clinically and pathologically between the neat and the emulsion injected mice but these were not completed due to contract contraints toward the end of the study.

Status: This FCC was considered to be generally toxic and rejected for further study at this time. Reassessment should not be completely ruled out.

1. JPL-AB-18. Three mice were injected with 10 microliters of this FCC. All died within 5 minutes.

Status: Rejected; highly toxic.

5. JPL-AB-20 and -21. The two groups of three mice each injected with JPL-AB-20 and -21 all died quietly within 24 hours, huddled together. Also all the mice injected with the neat form of these two FCCs died within 2 days.

Status: Rejected because of toxicity.

6. JPL-AB-24. The two mice injected with this FCC were dead within one to two hours, dying an extremely violent death and very agitated.

Status: Rejected for further study.

7. JPL-AB-27. Three mice survived the AVS study (24 hours) but were sick. The two-week studies were begun but the three mice injected were dead 3-4 days later.

Status: Rejected for further study.

8. JPL-AB-28. Three mice were injected with this FCC and died within 18 to 24 hours. Three mice injected with the neat died in less than 24 hours.

Status: Rejected for further study.

9. JPL-AB-29. Appears to be unstable during sonication, generating fluoride ion. Two groups of three mice each were injected i.p. (30 gm/kg) and all died but one. Incomplete absorption is possible in the mouse that lived since sonication was done for 15 seconds only to minimize fluoride ion generation. Not enough left for neat studies.

Status: Rejected for further study.

10. JPL-AB-31. Of three mice injected, all were dead by the sixth day. One mouse was injected i.p. with the neat FCC and lived 2 weeks, and at the time of sacrifice the liver was enlarged and the spleen was very small. No FCC left for additional studies.

Status: Rejected for further study.

11. JPL-AB-32. Two mice survived the 24-hour AVS study. One mouse was sacrificed for histopathological studies and one for FCC tissue assay. Two additional mice survived the two-week MTS study and were sacrificed for histopathological and tissue assay studies. The histopathological studies are pending.

Table 2 shows the FCC levels for this FCC. The data generally parallels that seen with JPL-AB-14. The FCC was readily absorbed and there were rather high concentrations in the spleen (liver moderate)

at 24 hours dropping to about half in these organs in 14 days. About 74% of the total FCC injected was eliminated in 24 hours and 86% in 14 days. Of note is the relatively high concentrations in the blood at 24 hours, with a drop to zero at 14 days.

This data, like that for JPL-AB-6 and -14, was obtained from single animals in some cases and the statistical validity is thus impaired. However, the trends are similar for all three FCC's. Furthermore, we did not have enough JPL-AB-32 to adequately perform extraction efficiency studies, but, because the extraction efficiencies of JPL-AB-6, -13, and -14 were essentially 85%, and because of similarities in molecular structure, we assumed an 85% extraction efficiency for JPL-AB-32.

Status: This FCC is similar to JPL-AB-6 and -14 and classified as a promising compound.

12. JPL-AB-33. Two mice died within 6 hours with a 20% emulsion injection and one mouse within 24 hours with the neat injection.

Status: Rejected for further study.

13. JPL-AB-35. Three mice were injected with this compound which is not a fluorochemical. All died within 5 hours.

Status: Rejected for further study.

In-depth Study of JPL-AB-6

JPL-AB-6, the most promising FCC from the first year's study, was resynthesized by JPL for further study in the second year.

UBTL received 15.3 grams of JPL-AB-6 from JPL on November 2, 1977. A summary of the mice dosed with this FCC is shown in Table 3:

Table 3. Summary of Mice Dosed with JPL-AB-6

Injection Form	Dose	Time Alive Post Injection	Follow-up Studies	No. of Mice
Emulsion	30 g/kg	Sacrified - 24 hrs.	Perit. Wash	3
	30 g/kg	Sacrificed - 2 wks.	Perit. Wash, Tissue Levels Histopath	5
		(1 of the 5 mice died at 17 hrs.)		
"Aged" Emulsion	30 g/kg	Alive - 1 month	Perit. Wash, Tissue Levels Histopath	1
"Aged" Neat Emulsified for injection	30 g/kg	Alive	Perit Wash, Tissue Levels Histopath	3
		(All controls alive and well.)		

Three mice were injected i.p. with the emulsion for the 24 hours' Absorption Verification Study. These lived but were sacrified at 24 hours for FCC assay of peritoneal wash. Table 2 indicates that approximately 95% of the fluorocarbon is absorbed in 24 hours. This fast rate of absorption insures that the mouse is exposed to the full dose physiologically.

Five were injected i.p. with the emulsion for the two-week Mouse Toxicity Screen (MTS). One mouse died on the second day, quiet and sick. The other four remained alive and well along with the controls and were sacrificed at two weeks according to the two-week Mouse Toxicity Screening Protocol. Two mice were preserved for a histopathology examination after their peritoneal wash. Recovery of JPL-AB-6 from the peritoneal washes and selected organ tissues of the remaining mice are tabulated in Table 2. These data reveal findings similar to those of JPL-AB-14 and -32. The highest levels are in the liver and spleen. Furthermore, they indicate that essentially all of the fluorocarbon has been absorbed and that approximately 80% of it has been eliminated from the body during the 14 days post injection. This fluorocarbon compound appears to be similar to the FC-47 in its time distribution with the spleen and liver scavanging most of the emulsion and that these data are very encouraging because

they indicate a reasonable rate of excretion of the compound. Its duration is long enough to be effective as a blood replacement, yet it appears to be excreted from the body at a reasonable time.

1. Shelf-life Studies. During the screening studies done in the first year of the contract, it was noted that JPL-AB-6 appeared to become more toxic with time. This was based on the observation that all mice died which were injected several days after the initial testing of JPL-AB-6.

Table 3 shows that the four mice were still alive following the i.p. injection of both aged emulsion and aged neat. The neat was emulsified for injection after aging in that form.

One factor to consider with this series of JPL-AB-6 studies compared to last year's is that JPL indicated that the FCC was more carefully purified before testing.

2. Pathology Studies. Detailed reports were received from UBTL's pathology consultant, Dr. John Carlquist, on the mice studied with AB-6. The following summarizes his report:

Two mice were injected with a 20% emulsion of the JPL-AB-6 and were sacrificed at the end of two weeks. Marked degenerative changes were noted, especially in the spleen, with histiocytic proliferation in the spleen. This was interpreted to indicate a marked phagocytosis. There was a rather marked degree of edema of the lungs, marked degeneration of the liver cord cells, and the kidneys showed some epithelial degeneration. It was reported that the two mice were essentially the same from a standpoint of pathological features with one showing more degenerative changes in the liver and kidney than the other mouse. Dr. Carlquist emphasized that the striking feature of the examination was the marked histiocytic proliferation within the spleen which apparently represents deposition of the chemical in the spleen.

One mouse was injected with "aged" JPL-AB-6 to study the effect

of shelf life. This was a 20% emulsion. At the end of four weeks, the mouse was sacrificed; mild changes were noted in the epithelium of the liver and kidneys. Again, the major alteration was a marked histiocytic proliferation in the spleen.

One of the five mice injected with JPL-AB-6 (20% emulsion) died at the end of 18 hours, while the other four lived and were sacrificed at the end of the two-week period. Marked changes were noted in all tissues with marked cell degeneration as the most striking feature of the splenic section. It should be added that gross and microscopic inspection revealed blood in the small intestines with sections showing frank necrosis.

3. Summary of JPL-AB-6. A summary for this FCC was prepared for NIH.BI by copy of their memorandum dated April 14, 1978. This memo and the summary are found in Appendix III. Tissue culture, animal studies, oxygen solubility, vapor pressure and so on, are presented.

LD_{50} Studies

We investigated LD_{50} techniques using FC-47 emulsion at various dose levels. With a 50% emulsion, 3 mice were injected i.p. for each of the following dose levels: 50, 100, 200 and 500 gm/kg. At 50 mg/kg, all mice lived. The mice given 100 and 200 gm/kg all died within 2 days, and at the 500 gm/kg level, 2 died and 1 lived, suggesting the LD_{50} was between 50 to 100 gm/kg. Additional mice studies confirmed this range but the end-point was not sharp.

It is possible that the deaths could have been due to volume overload of the peritoneal cavity.

Sonication Studies

In sonication experiments, using 20% emulsions of FC-47, each under one atmosphere of CO_2, N_2O, N_2 and O_2 respectively, a significant increase of fluoride ion with time (up to 90 minutes) was seen with the O_2 and N_2 atmospheres. See Figure 1. Very little increase occurred with N_2O and CO_2 atmospheres. Using an atmosphere of O_2, the pH did not affect the amount of fluoride ion generated at pH of 4.0, 7.0 and 10.0.

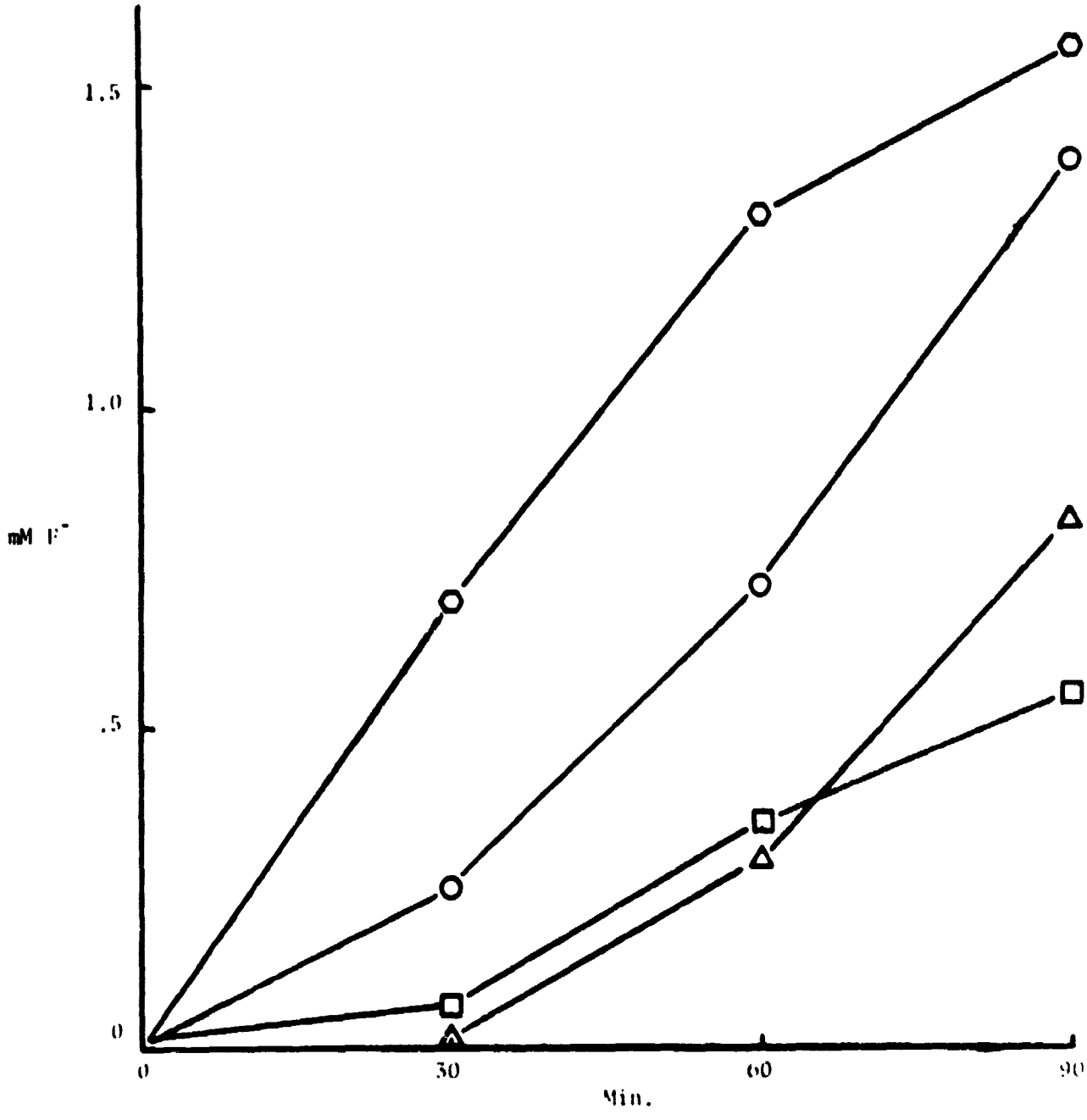


Figure 1. Fluoride ion produced upon sonication of 15 ml of a 20% (v/v) emulsion of FC-47 in a 25 ml rosette cell maintained in a water-ice bath. The emulsion was first purged by bubbling the gas through it for 15 minutes then maintained under one atmosphere of the gas during sonication ○, O_2; ○, N_2; △, N_2O; □, CO_2.

SECTION IV: DISCUSSION

Introduction

Most of the fluorocarbons synthesized by JPL and subsequently tested by UBTL differ chemically from the "perfluorocarbons" which have dominated hemoglobin substitute research. The test compounds are largely fluorocarbon-hydrocarbon hybrids. Such hybrids not only have a greater potential to become metabolically altered, and hence possibly generate toxic metabolites, but they probably also have different distributions, hence different biological activities, than perfluorocarbons. Consequently, the Mouse Toxicity Screen was designed specifically to test for toxic effects that derive from the inherent properties of the test FCC rather than its biophysical properties, its emulsion or adverse effects of rapid volume expansion (see "Route of Administration" below).

Owing to the small amounts, usually 5-10 grams, of the Test FCCs made available to us from initial synthesis, we were obligated to use them sparingly but at the same time to clearly identify those FCCs that were significantly toxic or nontoxic. Retesting was often not possible. Thus, the candidate compounds synthesized by JPL which met basic physicochemical requirements as stated in the RFP were subjected to basic gross toxicity and tissue retention testing in mice at UBTL.

Also, because of the relatively small amount of test compound received, the protocol developed for screening in mice had to be adapted to each individual compound and could rarely be followed in its entirety. Flexibility in the application of the protocol was essential so that the tests selected would yield the most information for a particular compound.

Materials and Methods

The biological screening methods consisted mainly of intraperitoneal (i.p.) injections with related i.p. absorption studies, observations for

toxicity and death, organ distribution studies using extraction and gas chromatography for assay of the FCC and histopathological studies on tissues. Absorption of the FCC from i.p. injection was verified by peritoneal wash techniques and G.C. analysis for recovery of any residuals. Twenty-four hour studies and 2 week studies on mice formed the basic protocols.

1. Route of Administration. The intraperitoneal route was chosen for three reasons: 1) The intravenous route is unsuitable for the purpose. Unexpected coalescence of particles of FCC in the emulsion may give rise to droplets large enough to effect FCC-embolism and hemolysis and/or intravascular clotting may accrue due to the sudden contact between emulsion and blood, or the oncotic properties of Pluronic F-68 may not be manifested in the expected way in the presence of certain FCCs. In such instances, "toxicity" should not be attributed to the test FCC but rather to a failure in appropriate make-up of the emulsion. Furthermore, adverse effects can follow the very rapid expansion of the intravascular volume. Consequently, it is desirable to employ a route other than the intravenous route. 2) The specification for emulsions to be administered intraperitoneally are much less demanding than for emulsions to be given intravenously, hence a saving in time and materials can be made in preparing emulsions for the intraperitoneal route. 3) Absorption time by the intraperitoneal route is usually adequately rapid, even when rather unstable suspensions of quite insoluble drugs are used.

a. Validity of the Intraperitoneal Route. In establishing the protocol to be used for screening the new FCCs, it was critical to prove the hypothesis that the intraperitoneal (i.p.) route of dosing mice with FCCs was valid. This hypothesis was tested using FC-47 because of its widespread use in experimental animals and a relatively large literature reserve documenting its use. The test results supported the hypothesis and that FC-47 could be used as our control FCC. Details are described in Appendix I, but to summarize: experiments

done in these laboratories with a 20% v/v emulsion of FC-47 in Pluronic F-68 iso-tonic saline solution have shown that the absorption half-time is less than 2 hours with a dose that is approximately equal to that contained in a total blood replacement (approximately 100 ml per kg); by 24 hours nearly all of this huge dose is absorbed.

Early in the study there was some discussion raised by Dr. Scherer of JPL regarding the use of perfluorodecalin as the "standard" reference compound rather than the FC-47 we specified in the proposal. Because perfluorodecalin is not retained in the animal preparation and evaporates through lung/skin, it was proposed that this might serve as better reference standard for our compounds. A change to this standard posed several problems such as availability from England at that time, difficulties in emulsification, purity and presence of unidentified compounds, and eventual approval by NHLBI if we recommend this change. Later discussion with the UBTL research group led to the decision to stay with FC-47 as the reference standard.

b. Absorption Verification Studies. Although the absorption studies with FC-47 indicated the suitability of the intraperitoneal route for the Mouse Toxicity Screen, it was possible that there may be FCCs that were not absorbed well by this route. In order to attribute correctly non-mortality or non-morbidity to lack of toxicity, it was necessary to establish that the FCC was, infact, absorbed. Consequently, prior to testing for toxicity, each new FCC was tested in mice for the ability of an emulsion of it to be absorbed from the peritoneal cavity. A dose of 100 ml/kg of 20% emulsion (v/v) made with 5.76% Pluronic F-68 in phosphate buffer, pH 7.4 was given, and these animals were observed for toxic effects according to the description given under Item 4 in Appendix II. After 24 hours, the peritoneal cavity was opened and the residual FCC content removed. If the animals died before 24 hours and the controls did not, absorption was assumed to have occurred and peritoneal wash done only if especially indicated.

Peritoneal washes in mice injected with JPL-AB-6 and -8 in which test mice lived longer than 24 hours showed a recovery of 27% and 3%

respectively, indicating absorption was highly probable. Later studies with JPL-AB-6, -14 and -32 showed a recovery of 7.0% at the most (see Table 2).

To rule out any possibility that toxicity and death in these mice could be due to the emulsification process and the production of unexpected toxins, one or more mice were usually injected with the neat FCC (unemulsified) if enough was left over.

2. General Comments. It should be emphasized that not all studies in the protocol could be completed on a given FCC because of the limited amount of the newly synthesized FCC we received. For example, if a new FCC proved overtly toxic killing the test mice in a matter of minutes to hours, then it was assumed to be toxic and not investigated further. Also, as we gained experience in screening, it was deemed cost effective to omit such things as peritoneal washes, histopathology and residual FCC tissue studies in FCCs that were overtly toxic.

The main purpose for such flexibility in the application of the protocol was to obtain maximum information on the first batch of every new FCC to conserve on resynthesis effort, time and cost. Our task was to show with reasonable certainty that an FCC was either toxic enough to be rejected or safe enough to qualify for resynthesis and further detailed studies in animals.

In addition to the problem of getting the most information with small FCC quantities, there were problems related to preparing the samples for animal injection. Some FCCs did not readily form emulsions, and the solids required heating and sonication while hot. When we checked the [F], we found that some FCCs required considerable "clean up" and followup screening in animals with the clean compound.

Screening of Candidate Compounds

Of the 14 FCCs synthesized by JPL and sent to UBTL for screening this year, 12 were rejected for further study and 2 proved to be promising candidates for further study, i.e., JPL-AB-14 and -32. JPL-AB-6,

one of last year's best candidates, was resynthesized and studied further this year. JPL-AB-13, the other primising FCC from last year's study was not resynthesized by JPL for further study and accordingly was not studied this year..

1. Promising Candidates. Both JPL-AB-14 and -32 were nontoxic according to criteria for our two biological screening methods. They are structurally similar to JPL-AB-6 and -13 which proved nontoxic in last year's study. All four are essentially FCCs with a small amount of hydrocarbon buried in the center of the molecule, sterically hindered from enzyme attack. Last year's JPL-AB-13 will not be reviewed for this report. The sequence of discussing these FCCs will be altered from the previous sections and they will be described according to the time sequence in which we studied them.

a. JPL-AB-6. This FCC was considered to be the most promising compound studied during the first year. Additional JPL-AB-6 was resynthesized for further studies this year. The amount of FCC available was again relatively meager allowing only very few mice to be studied. However, based on last year's experience, these studies could be conducted a bit more uniformly and again showed promising results.

JPL-AB-6 data indicate that virtually all the FCC was absorbed by the mice and that approximately 80% was eliminated from the mice after 14 days. The spleen and liver showed the greatest retention (see Table 2).

Pathology studies seem to parallel the tissue retention of FCC in the liver and spleen. In particular, the spleen showed marked degenerative changes with histiocytic proliferation, interpreted as indicating marked phagocytosis and deposition of the chemical in the spleen. It is interesting to note that our control FCC, FC-47, exhibited similar involvement of the liver and spleen. One of five mice injected for the two week study died at 17 hours and pathology studies suggest a possible mishap with the i.p. injection because of blood and necrosis in the small intestine.

To summarize, this FCC appears to be similar to FC-47 in its tissue distribution and pathology findings which suggest spleen and liver scavenging of most of the emulsion. It is absorbed effectively and is

excreted at a rate that should be desirable for an "interim" circulatory adjuvant.

The pilot "shelf life" studies on this FCC seem to indicate that the compound is relatively stable at least over a 2 to 4 week period.

b. JPL-AB-14 and -32. As with JPL-AB-6, only limited studies were possible due to the small amount of FCCs available for study. The peritoneal absorption studies, tissue distribution, and histopathological studies for these two FCCs indicate the same general trend observed for JPL-AB-6. Essentially all the FCC was absorbed from the peritoneal cavity at the end of 14 days, the liver and spleen showed the highest concentrations with a significant drop in concentration with time (14 days), and at least half or more of the FCC had been eliminated at the end of 14 days. JPL-AB-32 showed a relatively high blood level at 24 hours but speculation on this finding is difficult because this data represents the findings in only one animal.

The pathology findings for JPL-AB-14 are quite similar to those found in JPL-AB-6 studies and similar studies are pending on JPL-AB-32.

To summarize, these three nontoxic compounds, considered as candidates for further testing, were found to have been readily absorbed from the peritoneum; they produced no gross long-term toxicity; they showed moderate histopathological changes, in most tissues, but usually marked changes in the spleen; and they were eliminated from the body at reasonable rates. Only limited pharmacokinetics could be done on these three FCCs due to the small amounts of available compounds. These three FCCs along with JPL-AB-13 deserve further detailed investigation with continuation of pharmacokinetic studies and eventual exchange transfusions in rats.

c. Data Constraints. The data we obtained is presumptive evidence for the general conclusions we have drawn, showing trends that are similar for the three FCCs reported. In many of the studies, only

one mouse could be subjected to a specific analysis, i.e., histopathological or FCC tissue retention studies, because of the necessarily small quantities of FCC sent for testing. However, 2 or 3 mice were used whenever possible. These constraints account for lack of some refinements in the data presented and our inability to satisfactorily resolve some discrepancies. But, we do feel that trends are reasonably well-demonstrated based on the similarity of patterns observed between the three FCCs. We do recognize, however, that the data could not withstand statistical analysis at this time.

2. Rejected Compounds. Compounds JPL-AB-15, -16, -18, -20, -21, -24, -27, -28, -29, -31, -33, and -35 were all considered too toxic for further testing, killing the test animals within minutes, hours, or a few days.

In some cases the animals injected with the neat fared well but died with the emulsion. This was especially true for JPL-AB-16. We have hypothesized that their longevity and well-being is likely due to the slow uptake of the neat, not being as well absorbed as the emulsion form. Peritoneal washes would help clarify this point. Another possibility is that the emulsification process may alter the FCC in some way rendering it toxic. JPL-AB-29 was especially unstable during sonication, always generating abundant fluoride ion.

General Observations: Years I and II

In reviewing the total FCCs studied over the two years, most proved to be toxic, with only four (JPL-AB-6, -13, -14, and -32) out of the 25 in Table I proving non-toxic as assessed by the two biological screening techniques used. Compounds JPL-AB-10, -11, -12, and -29 were unstable as indicated by their release of $[F^-]$ and presumed concommitant toxicity due to $[F^-]$ release. Some FCCs caused very rapid death and are presumed highly toxic to some vital process (JPL-AB-4, -5, -7, -12, and -18); these, in general, have more chemically reactive groups than the slower acting toxic FCCs.

From the above observations in this study, it appears that any fluorocarbon-hydrocarbon hybrid has the potential of toxicity if the hydrocarbon portion of the molecule is so located in that molecule that it is vulnerable for metabolic attack or capable of interacting with other physiologic entities such as membranes. However, if the hydrocarbon is not so exposed and it appears as a perfluorinated compound, then its toxicity is greatly decreased.

In general, the histopathological results suggest that for the promising compounds, tissue changes are very minimal in the mice sacrificed at 24 hours which received the 20% emulsion. Those sacrificed at two weeks or more regularly showed the most marked changes in the liver and spleen:

- Liver: Vacuolated swollen liver cell cords. Degenerative changes.
- Spleen: Marked histiocytic response with macrophages laden with "lipid" material.

There were a variety of additional findings with kidney glomeruli or tubule changes, lung edema, brain cells swollen, etc., but the degree varied from animal to animal. The pathologist interpreted these liver and spleen findings as a "toxic" effect on the liver and a marked phagocytosis by splenic histiocytes of a lipid substance.

For the toxic rejected FCCs, only occasional histopathological studies were justified. In mice with rapid early death (minutes), changes were always minimal, becoming more moderate in mice who lived long enough (hours to days) to develop tissue changes.

SECTION V: CONCLUSIONS

A total of 24 new fluorochemical compounds (FCCs) were synthesized by JPL over the past 2 years for toxicological screening in mice by UBTL. Three other compounds were screened but two were considered as "preliminary" and one, JPL-AB-35, was not a fluorochemical.

Most FCCs proved to be toxic, with only four out of the 24 proving nontoxic as assessed by the two biological screening techniques used. These four FCCs, JPL-AB-6, -13, -14, and -32, are structurally similar with a small amount of hydrocarbon buried in the center of the molecule, sterically hindered from enzyme attack. They were found to have been readily absorbed from the peritoneum; they produced no gross long-term toxicity; they showed moderate histopathological changes in most tissues, but usually marked changes in the spleen; and they were eliminated from the body at reasonable rates. Only limited pharmacokinetics could be done on three of these FCCs due to the small amounts of available compounds but they do deserve further detailed investigation with continuation of detailed pharmacokinetic studies and eventual exchange transfusions in rats. A pilot "shelf life" study on JPL-AB-6 seems to indicate that the compound is relatively stable at least over a 2 to 4 week period.

Of the toxic FCCs, compounds JPL-AB-10, -11, -12, and -29 were unstable as indicated by their release of [F^-] and presumed concommitant toxicity due to [F^-] release. Some FCCs caused very rapid death and are presumed highly toxic to some vital process (JPL-AB-4, -5, -7, -12, and -18); these, in general, have more chemically reactive groups than the slower acting toxic FCCs. In some cases the animals injected with the neat fared well but died with the emulsion. We have hypothesized that their longevity and well-being is likely due to the slow uptake of the neat, not being as well absorbed as the emulsion form. Another possibility is that the emulsification process may alter the FCC in some way rendering it toxic.

From a chemical structure standpoint, it appears that any fluorocarbon-hydrocarbon hybrid has the potential of toxicity if the hydrocarbon portion of the molecule is so located in that molecule that it

is vulnerable for metabolic attack or capable of interacting with other physiologic entities such as membranes. However, if the hydrocarbon is not so exposed and it appears as a perfluorinated compound, then its toxicity is greatly decreased.

Owing to the small amounts of the Test FCCs made available to us from initial synthesis, we were obligated to use them sparingly but at the same time to clearly identify those FCCs that were significantly toxic or nontoxic. Retesting was often not possible.

Also, because of the relatively small amount of test compound received, the protocol developed for screening in mice had to be adapted to each individual compound and could rarely be followed in its entirety. Flexibility in the application of the protocol was essential so that the tests selected would yield the most information for a particular compound.

As a result of these contraints, the data we obtained is considered presumptive evidence for the general conclusions we have drawn, showing trends that are similar for the four promising FCCs. In many of the studies, only one mouse could be subjected to a specific analysis. These constraints account for lack of some refinements in the data presented and our inability to satisfactorily resolve some discrepancies. We do recognize, however, that the data could not withstand statistical analysis at this time.

Recomendations. Additional, more comprehensive in-depth studies should be done on the four promising FCCs. This means that larger amounts of each compound should be resynthesized so that statistically significant numbers of mice could be studied to verify the encouraging trends noted in these studies. Such studies would assure that these FCCs merit later more complex exchange transfusion studies in rats and large animals.

Additional studies should include a more complete look into pharmacokinetics, oxygen solubilities and physical characterization, LD_{50} in some instances, tissue culture, mutagenicity testing, and blood compatibility studies.

APPENDIX I

1. Absorption studies

2. Screening Methods for Candidate Compounds

APPENDIX I

1. Absorption Studies

In preparation for testing the new FCCs to be received from JPL absorption of FCCs from the peritoneal cavity by the i.p. route required validation to support UBTL's basic hypothesis that this would be an effective route of administration. The i.p. route for dosing mice, if it proved effective for systemic FCC absorption, was elected because the investigators felt that this approach would serve as a more reliable test for toxic effects which might be classified as biochemical in nature rather than those effects caused by mechanical or vascular damage which might occur when the emulsion is injected intravascularly. It also would allow for injection of the "neat" compound without emulsification. To implement this validation procedure, UBTL established:

— Reliable gas chromatographic techniques for biological testing of fluorochemical compounds using the commercially available FCCs, Fluosol-43* and FC-47.

*The Green Cross Corporation -1/3, Gamou-cho, Joto-Ku, Osaka, Japan, Available in U.S.A. through Grand Island Biological Company, Pine and Mogul Street, Chagrin Falls, Ohio 44022

— Reliable protocols for effectively dosing mice via the intraperitoneal (i.p.) route with FCCs and evaluating dose absorption.

— Optimum peritoneal recovery techniques and tissue level assays to more objectively evaluate the effectiveness of FCC absorption by the i.p. route, as monitored by FID-GC. The procedures developed for these absorption studies are described below.

a. Gas chromatography. In the development of gas chromatographic analysis and solvent extraction procedures for quantifying the absorption of FC-43 and FC-47 from the peritoneal cavity of approximately 35 mice, preliminary extraction experiments were conducted using the more uniform commercial Fluosol-43. This was used because of some difficulty initially encountered in emulsification of FC-47 with Pluronic-F68** solution.

The extraction of FC-43 from 1 ml of Fluosol-43 using 10 ml of FREON-113/ethanol (5:1) in vitro gave a quite reproducible recovery response as determined by gas chromatography (coefficient of variation 3-4%). The coefficient of variation of replicate standards was 2%. Standard curves were carefully constructed with numerous points, in order to prevent error from non-linearity. Data on extraction recovery is shown in Appendix II as Table 1.

**BASF Wyandotte Corporation, Wyandotte, Michigan 48192

The development of in-house sonication techniques allowed similar studies to be done with FC-47; subsequently, FC-47 has been used exclusively.

b. Absorption Studies in Mice. These studies involved the injection of test fluorochemicals into the mouse peritoneum as a route of administration, so that problems associated with the intravascular route could be avoided. To verify that absorption occurs by the intraperitoneal route and to rule out the possibility that injected FCCs may be retained in the peritoneal cavity, a series of studies in mice were performed by injecting an emulsion of FC-47 with Pl-F68. The FC-47 is the standard to which new FCCs were to be compared.

1) Experiments on Peritoneal Recovery. Initial experiments on recovery of FC-47 from the peritoneum were done in live mice. However, because of ongoing absorption from the peritoneum and consequent variability in residual FCC, it was decided to perform recovery experiments in freshly killed mice. In these experiments, the FC-47 was administered after the mouse had been killed with chloroform. The dead mouse model represents "time zero" from injection, with the mouse sacrificed immediately after injection before any absorption could take place.

Three peritoneal wash techniques were investigated in order to determine the technique that yielded the highest recovery and greatest reproducibility. Recoveries with two dose levels of FC-47 were

determined (30 g/kg and 3 g/kg). Five mice were dosed intraperitoneally (i.p.) with FC-47/Pl-F/68) in each of the 6 studies.

The three wash methods investigated were: 1) Whole body peritoneal wash in which the peritoneum was opened before shaking the whole mouse with the solvent, 2) perfusion of the peritoneum, and 3) rinsing the open peritoneum and viscera. The first method, the "whole body" peritoneal wash, appeared to be the best method since it yielded the highest recoveries (90% for high dose, 97% for low dose), and was the most consistent of the three. Further details on the recovery methods are found in Appendix II. "Dead Mouse Peritoneal Recovery Methods."

The "whole body" peritoneal wash was used to obtain the quantitative data for the "Absorption Kinetics Studies" which follow.

2) Absorption Kinetics Studies. These studies were intended to determine the rate of absorption of FC-47 from the peritoneum. The dose employed was 80 ml/kg of a 20% v/v emulsion, a dose volume that is approximately equal to the total blood volume of the mouse. After i.p. injection, mice were sacrificed at 1 minute, and 2, 8 and 24 hours. These mice were subjected to whole body peritoneal wash and absorption was implied by determining the recovery of FC-47 from the peritoneal wash. Five mice were studied at each time interval. The absorption curve is shown in Figure 1.

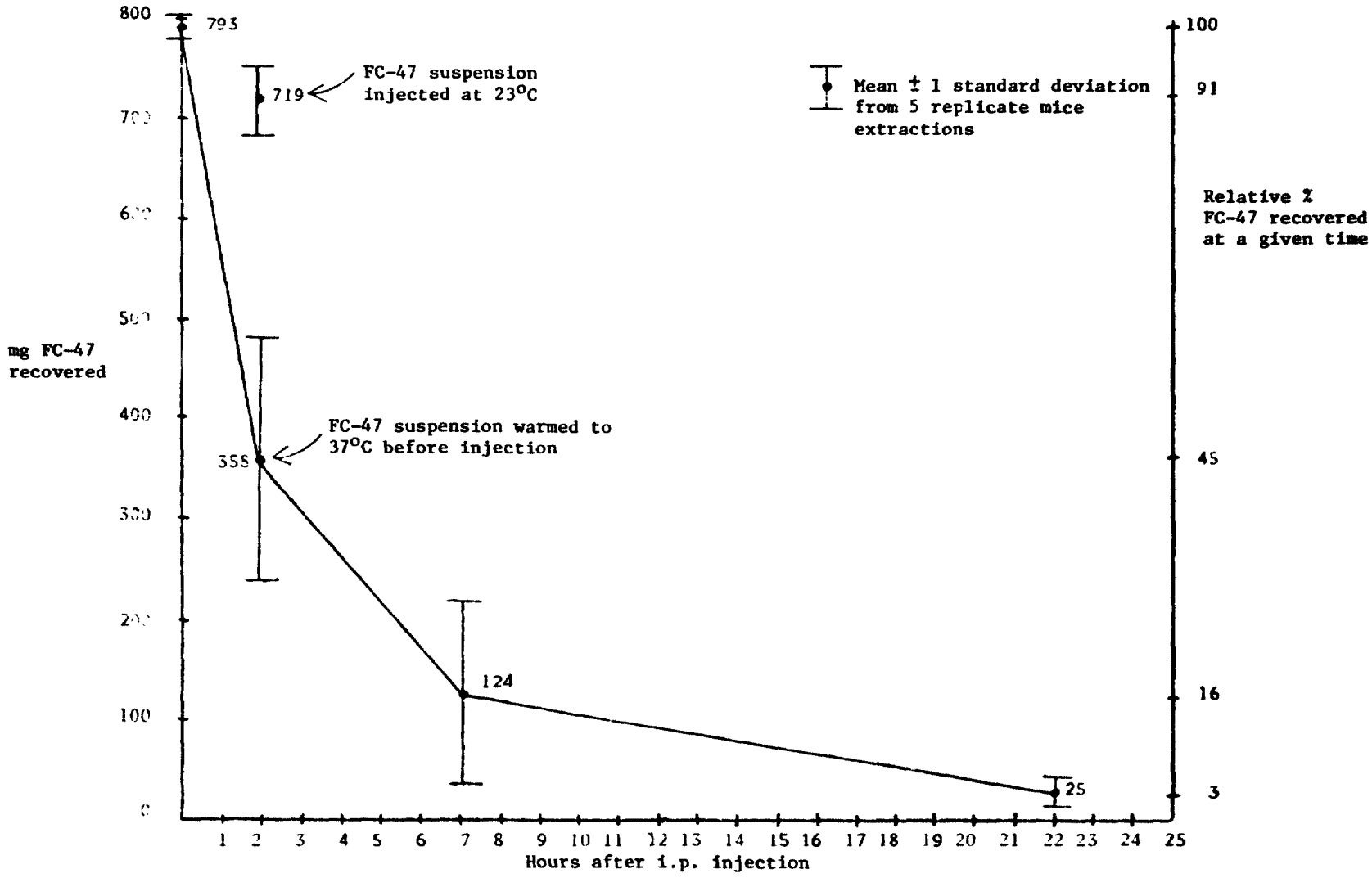


Figure 1. Recovery of FC-47 from Peritoneal Cavity of Live Mice after i.p. Injection

In these studies the FC-47 content of the liver, spleen, and blood of all mice injected were also determined, to correlate their kinetics with the recovery (absorption) curve. A total of ninety-five tissue specimens were assayed for FC-47 by gas chromatographic analysis for this tissue recovery study. This tissue analysis complements the peritoneal assay and a plot of the recovery values is shown in Figure 2 which should be compared to values in Figure 1. The G.C. tissue analysis data is in Appendix II, Table II.

Technical problems with the analysis of the blood samples in the "22 hour" mice rendered this part of the blood assay invalid.

As anticipated, these data verify absorption of FC-47 by the peritoneal injection route in mice over a 22 hour period, indicating that this method of dosing mice is a plausible route for the new FCCs to be studied.

c. Distribution Studies. A pilot study on the distribution of the FCC, using Fluosol-43 and emulsified FC-47 as models, was also completed. The Fluosol-43 was given both i.v. and i.p. and the FC-47 only i.p. in rats. After one week, the brain, heart, liver, spleen, and blood were assayed for FCC by an FID-GC extraction procedure similar to that of Yamanouchi, et al.,1975*. Detectable quantities of FCC were found only in the liver and spleen, the spleen having the higher content. It is of interest that the hepatic and splenic contents of FC-47 given i.p. were higher than those of Fluosol-43 given by either the i.v. or i.p. route. This indicates the bioavailability of the FCC emulsions prepared in this laboratory, and are in agreement with the absorption studies.

* Yamanouchi, K., Suzuki, A., Utsumi, I., and Naito, R., "Determination of Per Fluorochemicals in Organs and Body Fluids by Gas Chromatography," Chem. Pharm. Bull. (Tokyo) 22:2966-71.

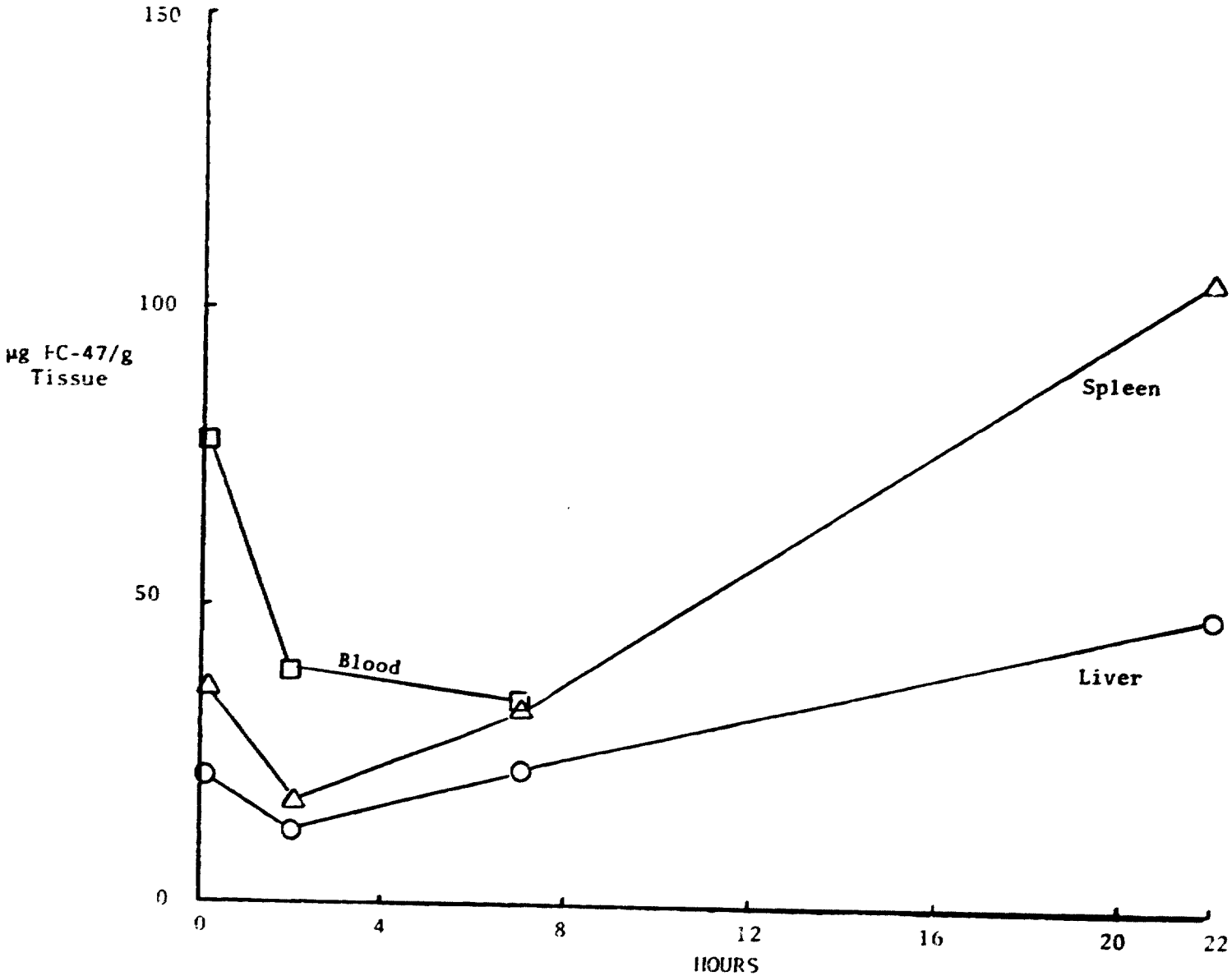
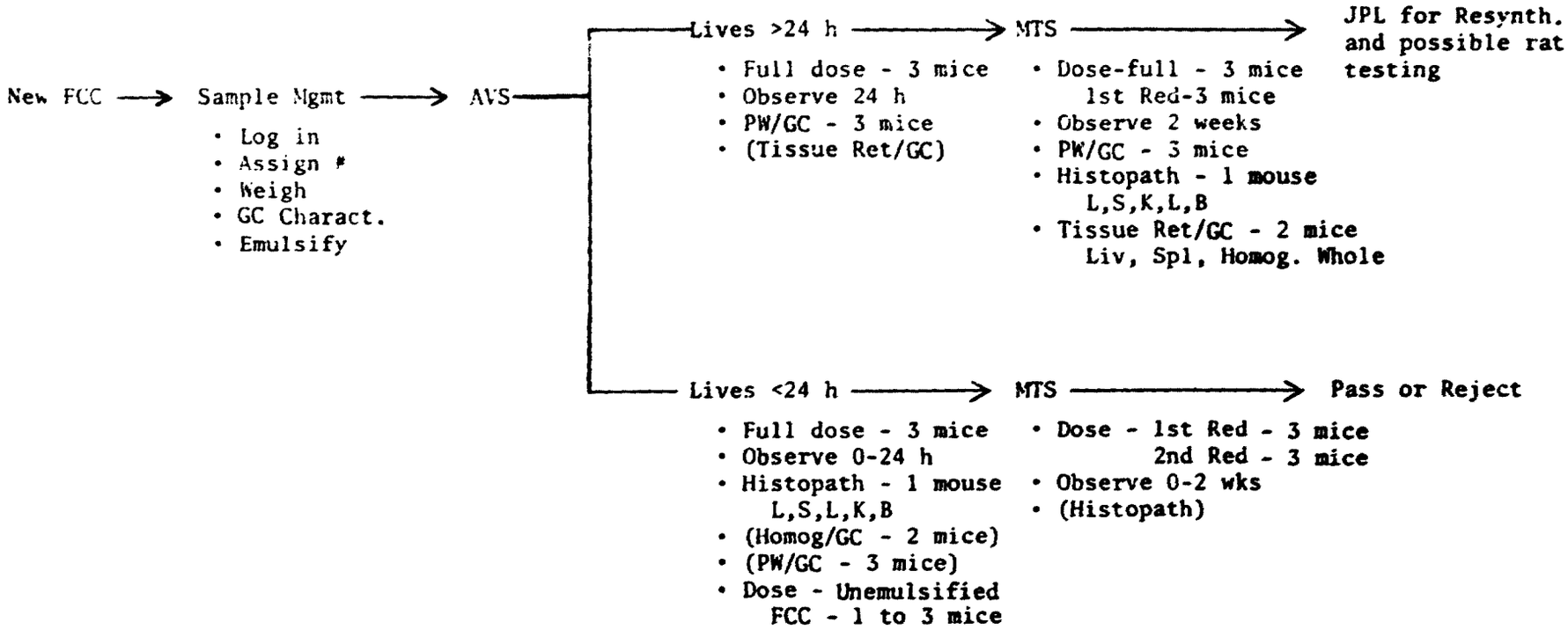


Figure 2. Recovery of FC-47 from Tissues of Live Mice After i.p. Injection



Legend:

AVS - Absorption Verification Study
FCC - Fluorochemical Compound
GC - Gas Chromatography
L,S,K,L,B - Liver, spleen, kidney, lung, brain
MTS - Mouse Toxicity Screen
PW - Peritoneal Wash
Red - Reduction -1st Reduction Dose = 0.1 max. dose
2nd Reduction Dose = 0.01 max. dose
Ret - Retention
(Tissue, PW/GC, Homog) - Studies to be done only in special cases are in (-)

Comment:

1. Above protocol adapted for maximum results when not enough FCC available to do all phases.
2. Unemulsified FCC injected in 1 to 3 mice if death occurs with emulsified FCC to rule out possible toxicity due to emulsification procedures.

Figure 3. Protocol Flow Diagram for New FCCs

2. Screening Methods for Candidate Compounds

With the establishment of reliable G.C. methodology and validation of FC-47 absorption from the i.p. route in mice, we developed protocols supported by these studies for screening the new FCCs to be received from JPL.

a. Sample Management. Upon receipt of JPL samples for screening, the samples are logged in, weighed and refrigerated. Some of the sample was retained for G.C. characterization if the compounds proved non-toxic.

As a rule, all samples are prepared for animal i.p. injection after emulsification with Pl-F68 by means of sonication in a CO_2 atmosphere for 3 to 4 minutes. A 20% (v/v) emulsion was made usually with 1.0 ml of FCC and 4.0 ml of Pl-F68 solution (5.76% wt/vol in isotonic phosphate buffer).

From our preliminary experiments, as well as available literature, it appears that sonication* is the most effective method for the preparation of small volumes of fluorocarbon emulsions. However, sonication procedures may possibly produce toxicologically significant levels of fluoride ion in the emulsions; therefore, we have purchased a fluoride ion selective electrode to monitor fluoride ion levels. If necessary, most of the fluoride can be removed with a simple water

*Model #150 with 13 mm O.D. probe, 150 watt sonic generator, Artex Systems Corporation, 275 Adams Boulevard, Farmingdale, N.Y. 11735.

extraction.

Since the emulsions prepared for screening purposes need not be as finely emulsified as those used in intravenous injections, suspensions of test FCCs have been sonicated at about 100 watts per cm^2 for only 3-5 minutes, a time which generates minimal amounts of fluoride from most FCCs.

b. Screening Tests. After the compounds were prepared for i.p. injection, "Absorption Verification Studies" (AVS) were performed and were followed by "Mouse Toxicity Screen" (MTS) tests. Figure 3, "Protocol Flow Diagram for New FCCs" outlines the procedures a new candidate compound ideally goes through. However, this protocol could not routinely be adhered to in its entirety with every new compound because it was not always possible to produce enough FCC in the initial synthesis to do this. Therefore, decisions had to be made to adapt the protocol for maximal testing results.

1) Absorption verification studies (AVS). These were conducted as follows: Three mice for each compound were injected i.p. with 20% v/v *emulsion in a dose of 100 ml/kg of body weight. They were observed continuously during the first half hour after injection, then at half-hour intervals for one and a half hours, and then at two hour intervals up to 24 hours. (In the MTS, twice daily for 14 days). Not only was mortality noted but also changes in behavior, movement, responsiveness, appearance, etc., were recorded, as well as the characteristics of dying. Over 90 routine observations were made accord-

* Or 30% w/v if the FCC was a solid (assumed density = 1.5)

ing to a check list. A summary of the types of observations is given here in Table I and the check-off sheet relating to these observations is in Appendix II as Table III.

The mice were sacrificed after 24 hours (unless they died earlier) and their peritoneal cavities washed for recovery of the compound. This helped to establish whether lack of mortality, when it occurred, was due to lack of toxicity or lack of absorption.

If the FCC-treated mice died during this 24 hour test, and the controls did not, then it was assumed the test compound was absorbed.

After the peritoneal wash, one of the three mice was placed immediately in formaldehyde for later histopathological study and the other two underwent whole body homogenization so possible metabolites could be extracted. To rule out any possibility that toxicity and death in these mice could be due to the emulsification process and the production of unexpected toxins, one or more mice were usually injected with the unemulsified FCC if enough was left over. Three control mice were injected with the Pl-F68 solution (5.76%).

2) Mouse Toxicity Screen (MTS). In some cases, when death occurred under 24 hours in injected mice, a special Mouse Toxicity Screen test was performed at two dose levels reduced from the initial maximum dose of 100 ml/kg body weight. Three mice would be injected with 10 ml/kg and three with 1 ml/kg to assess the general nature of the toxic response basing the assessment on standard observation

Table I

Types of Observations Made on Mice

Behavior: exploratory, aimless wandering, nuzzling, wall-licking, staring, preening, scratching

Movement: Waltzing, circling, shovel-nose, ataxia, writhing, paralysis, prostration, subconvulsive and convulsive, nystagmus, tail attitude and posture

Respiration: frequency and depth

Responses: foot withdrawal to pinch, touch sensitivity, startle response to hand clap

Reflexes: Pinnal, lash, corneal, consensual light reflex, hind limb placing, grasping, righting

Miscellaneous neurological: rotarod balance, inclined screen, tremors, muscle tone

Miscellaneous: defecation, diarrhea, blood feces, miosis, mydriasis, photophobia, exophthalmos, blinking, conjunctival and scleral injection, corneal chemosis or glazing.

criteria for mouse toxicity testing. Even though the FCC in question may eventually be rejected, results from these tests may possibly influence the chemist's approach to synthesis of future FCCs.

If the mice did not die during the Absorption Verification Studies, a new group of three mice were injected i.p. with the same maximum dose of 100 ml/kg body weight and three with a reduced dose of 10 ml/kg. Further dose reductions were made when indicated if enough sample was available. The mice were observed for 2 weeks for death and/or toxic effects. This is the "upper" Mouse Toxicity Screen test shown in Figure 3. If a test compound passed this test, it was to be recommended for re-synthesis in larger quantity, emulsification and in vitro blood testing before going to the more complete exchange transfusion (blood replacement) rat tests; these advanced studies were not begun this year as originally planned.

It should be emphasized that this protocol was rarely followed through its completion because of the small amounts of compound available for testing. The tests in this protocol were selected that would yield the most information for that particular compound.

APPENDIX II

1. Gas Chromatographic Analysis of Fluosol-43 and FC-47

2. Dead Mouse Peritoneal Recovery Methods

3. Absorption Kinetics: g.c. tissue analysis

4. Standard Protocol for Observation of Toxic Mice

Appendix: Table I. Gas Chromatograph Analysis of the Extract Obtained from Extraction of 1 ml of Fluosol-43 with 10 ml of FREON-113/Ethanol (5:1 v/v)

	Fluosol-43 Extract		G.C. Standard (23.6 µg/µl FC-47)	
Sample #	(mm) Peak Height	(Disc) Peak Area	(mm) Peak Height	(Disc) Peak Area
1	97	785	100	845
2	95	835	105	855
3	96	820	105	840
4	87	765	103	870
5	92	820	103	850
6	92	810	102	820
7	90	810	105	
8	88	760		
9	90	--		
10	92	--		
MEAN	91.50	800.63	103.29	846.67
Standard Deviation	3.31	27.44	1.89	16.63
Coefficient Variation	4%	3%	2%	2%

Estimated extraction efficiency for FC-43 into FREON-113 with ethanol present was 75%.

All gas chromatograph injections were 1 µl.

Dead Mouse Peritoneal Recovery Methods

Initially, three methods of washing the peritoneal cavity after injection of a FC-47 suspension were carried out to determine which if any of the wash methods might be suitable for recovering unabsorbed FC from the peritoneal cavity. Freshly killed mice were used in order to reduce the possibility of transport of injected material out of the peritoneal cavity, thereby increasing possible variation observed among washing procedures samples. Test groups consisting of 5 mice each were injected with a high dose (2.0 ml FC-47 suspension per mouse, I.P.) or a low dose (0.2 ml FC-47 suspension per mouse, I.P.) and then a given wash procedure was used on those mice.

The procedure which appeared to give the best overall precision (C.V. ≤ 0.05) entailed placing the injected mouse in a polyethylene bag (Whirl-Pak) and carefully opening the abdominal cavity with a longitudinal incision and then a lateral incision to facilitate exposure of peritoneal cavity to extraction material. After the above process was completed, 25.0 ml of Ethanol/Freon 113 1:5 was added and the bag was sealed by rolling the top of the bag. After shaking the bag with mouse and solvent by hand for two minutes, the lower organic phase was drained from the bag and placed in another polyehtylene bag with 40 ml of distilled water and shaken for one minute by hand. This second extract was necessary to remove the ethanol from the Freon 113. Finally, the Freon 113 phase was removed and placed in small vials which were frozen at -70°C until analyzed by gas chromatography.

The other two wash procedures were: 1) Perfusion of the peritoneum and 2) open peritoneal rinse. The precision of these methods varied with the dose given and the Coefficient of Variability (C.V.) ranged from 0.02 to 0.94.

Standard controls (no mice) were established in replicates of five in which either 2.0 ml or 0.2 ml of the FC-47 suspension were added to a polyethylene bag and extracted with 25 ml of extraction solvent. The precision of these extractions was C.V. = 0.02-0.04. Using the control samples to represent 100% recovery from the polyethylene bag, the recovery of the high dose of FC-47 suspension was about 90% and the recovery for the low dose was about 97%.

Appendix: Table 11. Absorption Kinetic Studies: g.c. Tissue Analysis
Recovery of FC-47 from Tissues of Mice Injected i.p. with FC-47

Spleen

Time	µg FC-47/g tissue	$\bar{X}$	Standard Deviation
1 min.	50.08		
	39.14		
	13.78		
	41.85	36.21	15.66
2 hr.	17.25		
	16.63		
	27.49		
	14.33		
	5.44	16.23	7.87
7 hr.	36.63		
	27.91		
	29.80		
	50.41		
	19.99	32.95	11.42
21 hr.	2.48		
	57.29	29.89	38.76
21 hr. 30 min.	78.61		
	3.16		
	86.10	55.96	45.88
22 hr.	72.20		
	115.89		
	130.70		
	133.67		
	77.92	106.08	29.17
Combined 21, 21.5, 22 hr		75.80	46.06

Liver

Time	µg FC-47/g tissue	$\bar{X}$	Standard Deviation
1 min	18.05		
	30.87		
	19.05		
	17.48	21.36	6.37
2 hr	36.82		
	4.83		
	5.42		
	10.77		
	2.85	12.14	14.11

Appendix: Table H. (continued)

Liver Tissue (cont.)

Time	µg FC-47/g tissue	$\bar{X}$	Standard Deviation
7 hr	15.03		
	18.50		
	22.47		
	16.59		
	39.17	22.35	9.80
21 hr.	9.11		
	2.89	6.00	4.40
21 hr. 30 min.	29.80		
	1.46		
	35.53	22.26	18.24
22 hr.	36.54		
	53.78		
	66.72		
	41.03		
	58.66	49.35	9.92
Combined 21, 21.5, 22 hrs		32.55	21.65

Blood

Time	µg FC-47/g tissue	$\bar{X}$	Standard Deviation
1 min	86.98		
	54.28		
	66.25		
	104.78	78.07	22.35
2 hr	18.80		
	29.20		
	87.63		
	29.70		
	31.17	39.30	27.46
7 hr.	46.79		
	36.81		
	11.90		
	105.46		
	42.45	48.68	34.50
21 hr.	0.37		
	89.96	45.17	63.35
21 hr. 30 min.	0.51		
	0.00		
	154.85	51.79	89.26

Appendix: Table II. (continued)

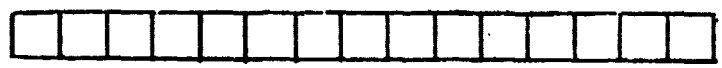
Blood (cont.)

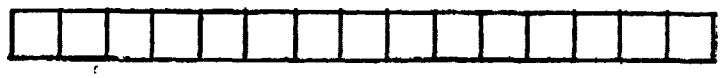
Time	µg FC-47/g tissue	$\bar{X}$	Standard Deviation
22 hr.	140.79		
	97.16		
	246.13	161.36	76.58
Combined 21, 21.5, 22 hr		91.22	88.92

Appendix: Table III. Standard Protocol for Observation of Toxic Mice

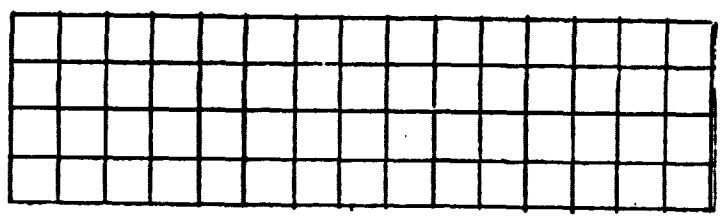
PHYSICAL EXAMINATION
AND OBSERVATION OF MICE IN TOXICITY TESTING

MOUSE NO. _____

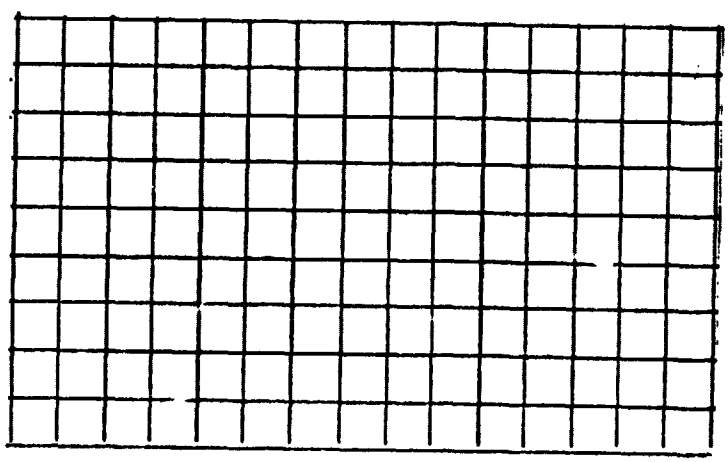
Date . . . . . 

Time . . . . . 

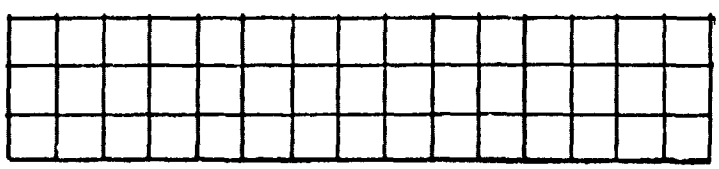
1. Activity
   - Dec exploratory activity
   - Inc exploratory activity
   - Jumping
   - Huddling



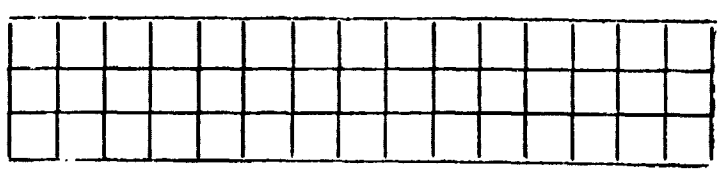
2. Bizarre reaction
   - Circus movements
   - Aimless wandering
   - Backward movements
   - Waltzing
   - Nuzzling
   - Licking Compartment walls
   - Shovel-nose movement
   - Glassy-eyed stare
   - Circling movements



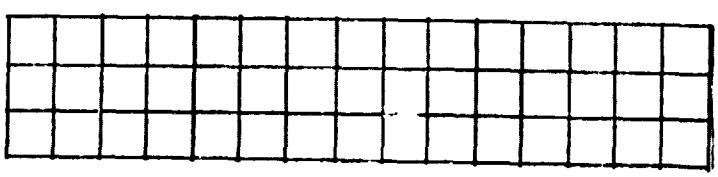
3. Phonation
   - Inc phonation
   - Dec phonation
   - Abnormal phonation



4. Sensitivity to pinch of hind foot
   - Increased
   - Decreased
   - Analgesia

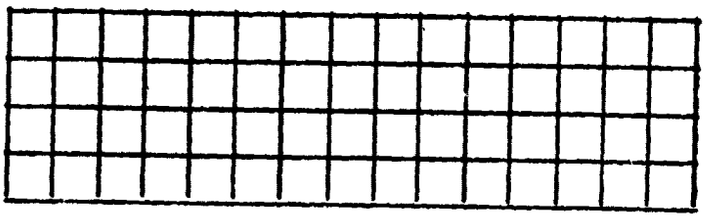


5. Sensitivity to hand clap
   - Increased
   - Decreased
   - No reactivity

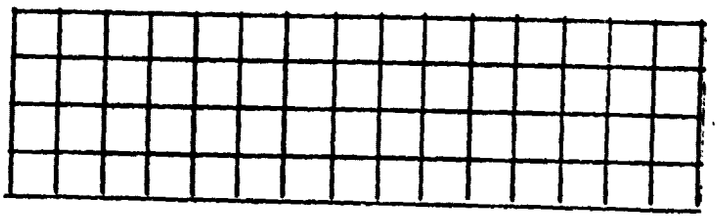


Appendix: Table III. (continued)

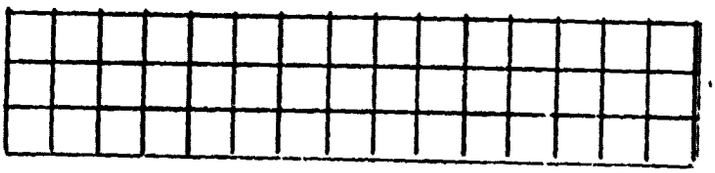
6. Sensitivity to touch
   - Increased
   - Decreased
   - Pinnal reflex depr
   - No pinnal reflex



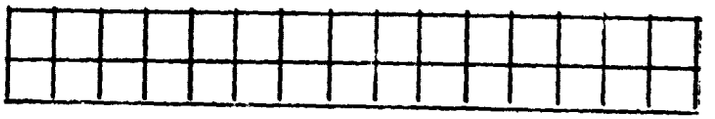
7. Social interaction
   - Inc rearing
   - Inc speed of rearing
   - Dec rearing frequency
   - Dec rearing height



8. Abnormal tail
   - Rigid tail
   - Straub tail
   - Limp tail

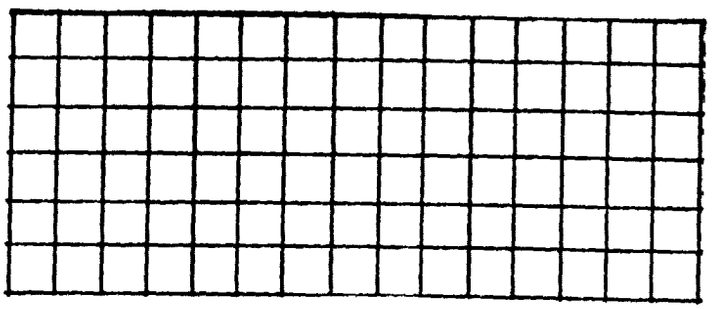


9. Aggressive behavior
   - Increased
   - Decreased

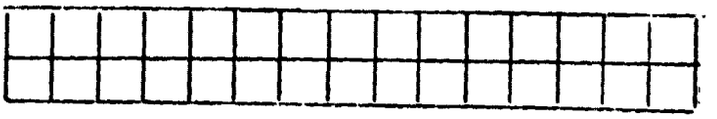


10. Ataxia

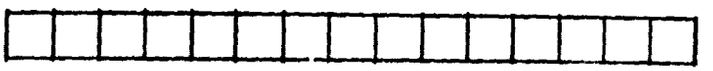
11. Convulsions
    - Tonic convulsions
    - Clonic convulsions
    - Mixed convulsions
    - Sub-convulsions
    - Sub-convulsive movements
    - Audiogenic seizure



12. Muscle tone
    - Inc muscle tone-limbs
    - Dec muscle tone-limbs

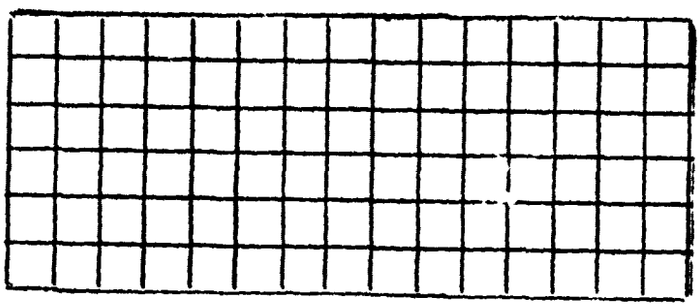


13. Paralysis

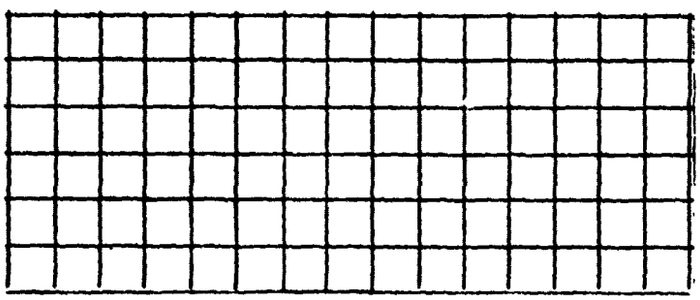


Appendix: Table III. (continued)

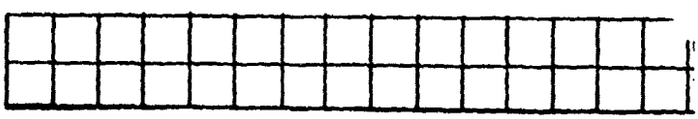
14. Somatic response
    - Inc preening
    - Dec preening
    - Rubbing nose
    - Inc scratching
    - Dec scratching
    - Writhing



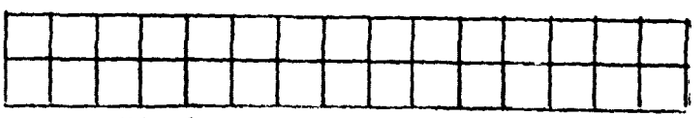
15. Postural reflexes
    - Hind limb placing reflex depr
    - Hind limb placing reflex absent
    - Grasping reflex depr
    - Grasping reflex absent
    - Righting reflex depr
    - Righting reflex absent



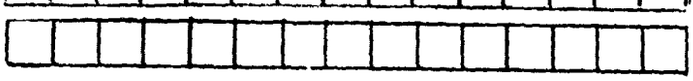
16. Prostration
    - Prostration
    - Loss of consciousness



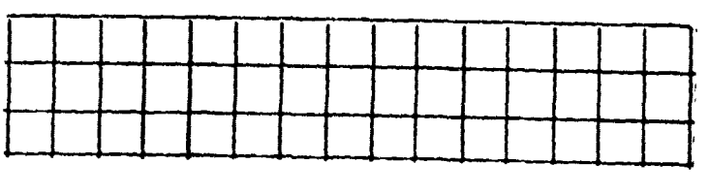
17. Tremors
    - Tremors at rest and in movement
    - Tremors in movement only



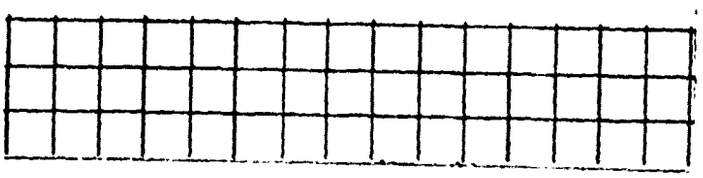
18. Exophthalmos



19. Eye irritation
    - Eye opacity
    - Blinking excessively
    - Iritis



20. Corneal reflex
    - Corneal reflex absent
    - Corneal reflex depr
    - Lash reflex absent



Appendix: Table III. (continued)

21. Lacrimation

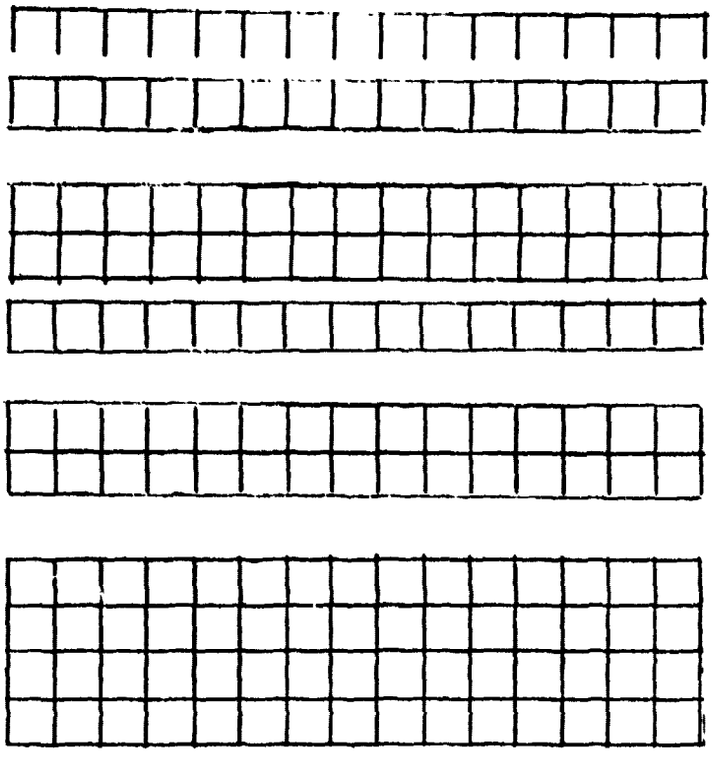
22. Nystagmus

23. Pupillary light reflex
    - Absent
    - Depressed

24. Photophobia

25. Pupil size
    - Mydriasis
    - Miosis

26. Defecation
    - Increased
    - Decreased
    - Diarrhea
    - Blood in stool



APPENDIX III

1. Memorandum from NHLBI, "Choosing the Most Desirable Fluorocarbons"

2. Summary of JPL-AB-6 Studies in Response to Memorandum

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

TO : Participants in Fluorocarbon Project DATE: April 14, 1978

FROM : Chief, Blood Resources and Transplantation Branch, DBDR

SUBJECT: Choosing the Most Desirable Fluorocarbons

On ~~August~~ April 13, 1978, at Atlantic City, New Jersey, a group of representatives from the current fluorocarbon project met to discuss the best candidate compounds for continued production in a new initiative. Several members of the group felt that they were not ready at this time to declare specific compounds. Rather, time was spent in developing a list of criteria by which the best compounds would be judged. These criteria are as follows:

1. Oxygen solubility. This should be in the range of 35 to 50 volumes percent.

2. Toxicity. This should be judged by tissue culture and animal injection of the unmodified material (animal injection would be intraperitoneal). Intravenous replacement studies, in the form of an emulsion, may be done but rigorous data with this type of testing would not be required.

3. Dwell time. No quantitative measures were agreed upon, but it was thought that minimal residual should be present after one year.

4. The compound should ~~not be metabolized~~ result in toxic metabolites

5. Emulsion stability.

   a. Use of a nontoxic stabilizer should be possible.

   b. The material should form a nontoxic emulsion when given intravenously.

   c. The emulsion should be stable on the shelf for one to two months and for a short period of time (? several hours) when mixed with physiological solutions.

6. Vapor pressure. The vapor pressure of the compound should be such that it does not interfere with the respiratory function of the recipient.

7. The compound must be already synthesized and large-scale production should be practical.

It was further agreed that members of the current project would respond as soon as possible with any candidate compounds and would be prepared to respond more definitively at a meeting of the group to be shceduled in late June.

Joseph C. Fratantoni, M.D.

Addressees:

Dr. D. Lawson
Dr. R. Geyer
Dr. R. Moore
Dr. L. Clark
Mrs. L. Clark
Dr. J. Fratantoni
Dr. D. Therriault
Dr. R. Landaburu

CANDIDATE

HYBRID FLUOROCARBON ARTIFICIAL BLOOD COMPOUND

JPL-AB-6

1,3-Di (NONAFLUORO-t-BUTYL) PROPANE

$(CF_3)_3C\text{-}(CH_2)_3\text{-}C(CF_3)_3$

$C_{11}H_6F_{18}$ (MW = 480.13)

Calc.	C	27.52%	Found C	27.64
	H	1.26	H	1.35

MP 32 refractive glass-like prisims from methanol

SUMMARY*

Oxygen Solubility

Calc. 36.7 cm^3 0_2/100 cm^3 at 25°C

Found 35.8 cm^3 0_2/100 cm^3 at 25°C

Toxicity

1. Tissue Culture. Studies by Professor Geyer at Harvard Medical School indicate that JPL-AB-6 was non-toxic in his tissue culture method.

2. Animal Studies. Mice injected intraperitoneally (i.p.) with JPL-AB-6 emulsion for 24 hour absorption verification studies showed no immediate effects that differed from the control mice. These mice survived without incident for the full 24 hours and, according to protocol, were sacrificed for peritoneal recovery studies. Approximately 5% of the fluorocarbon (FC) injected i.p. was recovered from the peritoneum at the end of 24 hours, indicating that 95% was absorbed during this time (see Table 1).

*Data presented in the format suggested by NIH/NHLBI memo April 14, 1978 as per meeting Atlantic City 4-15-78.

Histopathology studies showed essentially normal tissue patterns with only mild congestion.

According to protocol, since the test mice survived the 24 hour test and were sacrificed, another group of mice were injected i.p. for the two-week toxicity screen, at the end of which they were sacrificed for assay of JPL-AB-6. A brief summary of the findings related to this two-week study is described in the next section "Dwell Time".

Dwell Time (Sojourn)

During this two-week study, the mice showed no significant distress as compared to controls. After sacrifice, recovery of JPL-AB-6 from the peritoneal wash and from selected organ tissues of these mice indicated that essentially all of the FC had been absorbed. Furthermore, this data showed that nearly 80% of this FC had been eliminated from the body during the 14 days' post-injection (see Table 2).

Histopathology studies in the two-week animals showed marked histiocytic proliferation in the spleen, probably representing deposition of the FC here. Widespread degenerative changes, especially in the liver, were noted, suggesting the FC had had some toxic effect but one which may possibly be reversible. No striking inflammatory reaction was evident.

The above findings are encouraging because they indicate a reasonable absorption and a reasonable rate of excretion of the FC. Its duration in the body seems to continue long enough to be effective as a blood replacement, yet it appears to be excreted within a desirable time.

Toxic Metabolites

This fourth item regarding identification of toxic metabolites in the seven criteria addressed in the referenced memo above (see title page) has not yet been investigated by UBTL for JPL-AB-6 due to several constraints involving, for example, the availibility of large enough amounts of this compound, contractual limitations (beyond scope) and the like. We do know, however, that there are no Freon-113 extractable metabolites in this compound.

Emulsion Stability

This compound exhibits relative stability as an emulsified system suitable for i.p. injection. Pleuronic F-68 is apparently an adequate stabilizer exhibiting no obvious serious toxic effects. However, in-depth emulsification studies remain to be done and will become important when blood replacement studies are to be considered.

Shelf-Life Studies

During the screening studies done in the first year of the contract, a trend was noted with some compounds that suggested the possibility that the new FC's may become more toxic with time. This was based on the observation that more severe reactions occurred in mice injected days later with a given FC when compared to those initially dosed.

Enough JPL-AB-6 was available after the two-week studies to continue with "Shelf-Life" studies in a limited way. Mice were still alive at about 1 month following the i.p. injection of both "aged" emulsion and "aged" neat. The neat was emulsified for injection after aging in that form.

The emulsion was aged for about 2 weeks and the neat for 4 weeks at 37°C, intermittently exposed to room air.

Vapor Pressure

The measured vapor pressure of JPL-AB-6 is 2.0 mm at 30°C. The calculated vapor pressure is $\sim$ 4.8 mm. Both values are well below the 40 to 50 mm upper limit suggested by Geyer and Clark.

TABLE 1

24-HOUR ABSORPTION OF AB-6

Mouse #	Amount Recovered I.P.	% of Injected
125	29.79 mg	3.9%
126	25.41 mg	3.4%
127	51.45 mg	6.5%

TABLE 2

TISSUE CONTENT OF AB-6 AFTER 14 DAYS

Tissue	Average mg/g tissue	Average mg/ organ	% of Injected
Peritoneum *	---	3.0	0.4
Liver	27.5	60.0	7.5
Brain	0.05	0.02	0
Blood **	2.2	5.4 (estimated)	0.7
Spleen	87.9	76.8	9.2
Remainder of Body (inc. Blood)	1.7	38.9	7
TOTAL		178.7	21.8 **

* Average of 4 mice. The remainder of the tissue averages were done on 2 mice.

** Figures shown for "Blood" are also included in "Remainder of Body" and are not repeated as part of the "Total".

APPENDIX I

TISSUE CULTURE STUDIES WITH JPL COMPOUNDS

APPENDIX I

TISSUE CULTURE STUDIES WITH JPL COMPOUNDS

NO.	CODE	STRUCTURAL FORMULA $CF_3CF_2CF_2C(CF_3)_2-$	VOLUME TESTED (ML)	RESPONSE OF CELLS GROWTH* DAY 1	GROWTH* DAY 2	VIABILITY DAY 1	VIABILITY DAY 2
1	JPL-AB-15	$-CH_2CH_2CH_3$	0.4	90	92	99	99
2	JPL-AB-16	$-CH_2CH_2CH_2CH_3$	0.4	86	75	100	100
3	JPL-AB-17	$-CH_2CH(CH_3)_2$	0.4	63	40	99	99
4	JPL-AB-18	$-OH$	0.001	17	3	99	89
5	JPL-AB-19	$-OCH_3$	0.1	8	1	72	70
6	JPL-AB-20	$-OCH_2CH_3$	0.4	89	89	100	99
7	JPL-AB-21	$-OCH_2CH_2CH_3$	0.4	88	99	99	99
8	JPL-AB-22	$-CH_2OCH_2CH_2CH_3$	0.4	55	40	98	99
9	JPL-AB-23	$-NO$	—	—	—	—	—
10	JPL-AB-25	$(CF_3)_3CH_2CH_2-OCH_2CH_3$	0.002	50	8	8	67

* PERCENT OF CONTROL

(Performed by R. P. Geyer, Dept. of Nutrition, Harvard University School of Public Health)

APPENDIX J

COMPUTER PROGRAM

APPENDIX J

Program Submittal

[x] New Program

[ ] Revision to Program No. [ ]

HP-67 Serial No. [ ] HP-97 Serial No. [1807A0008 7]

Program Title (Underline 1 or 2 Keywords) [97 - ESTIMATION OF PHYSICAL PROPERTIES FROM CHEMICAL STRUCTURE]

Keyword(s) Underlined in Title 1 [PHYSICAL]

2 [PROPERTIES]

No. of Steps [224]

Category No. [0602]

Category Name [PHYSICAL SCIENCES - CHEMISTRY]

Abstract- 75 Word Maximum: Given the chemical structure of a fluorochemical, group additivity constants are summed to calculate the energy of vaporization, molar volume, solubility parameter, boiling point, vapor pressure, and solubility of oxygen in 100 ml. of the neat liquid. The equations are only applicable to liquids at 25 degrees Celsius.

Name Tobia (First) F. (Initial) Terranova (Last)

Address Jet Propulsion Laboratory, 4800 Oak Grove Drive

City Pasadena State Ca. Zip Code 91103

Acceptance Choice: [ ] Four Programs or [x] Two Programs and 10 blank cards.

[0006 6D] [0014 2D] [ ] [ ]

Submittal Checklist: Please use the checklist below to insure submittal of all the proper program documentation.

- [x] Program Submittal
- [x] Program Description I
- [x] Program Description II
- [x] User Instructions
- [x] Program Form(s)
- [x] Magnetic Card(s)

ACKNOWLEDGMENT AND AGREEMENT

To the best of my knowledge, I have the right to contribute this program material without breaching any obligation concerning nondisclosure of proprietary or confidential information of other persons or organizations. I am contributing this program material on a nonconfidential nonobligatory basis to Hewlett-Packard Company ("HP") for inclusion in its program library, and I agree that HP may use, duplicate, modify, publish, and sell the program material, and authorize others to do so without obligation or liability of any kind. HP may publish my name and address, as the contributor, to facilitate user inquiries pertaining to this program material.

Signature Tobia Fred Terranova Date 3/6/79

Program Description I

Program Title Estimation of Physical Properties from Chemical Structure.

Contributor's Name Tobia F. Terranova

Address Jet Propulsion Laboratory, 4800 Oak Grove Drive.

City Pasadena **State** California **Zip Code** 91103

Program Description, Equations, Variables Given the chemical structure of a fluorochemical, group additivity constants* are summed to calculate the energy of vaporization (ΔE_v cal/mole), molar volume (V cm^3/mole), solubility parameter (δ_1 $cal^{1/2}/cm^{3/2}$), boiling point (T_b °C), and solubility of oxygen gas in 100 ml. of the neat liquid at 25°C. The pertinent equations are:

1.) $\Delta E_v = \Sigma e_i$ 2.) $V = \Sigma v_i$ 3.) $\delta_1 = (E_v/V)^{1/2}$ 4.) $P = T\left(\frac{\Delta H_v}{\alpha RT}\right)^{\frac{1}{1-\beta}}$ $\alpha = 12.2497$ $\beta = 0.8846$

5.) $T_b\ °C = \left[\frac{-B+[B^2 - 4A(C-\Delta H_v)]^{1/2}}{2A}\right] - 273.15$ $A = 0.0724$, $B = -17.17$, $C = 5309$

6.) $\ln x_2 = \ln x_2^1 - \frac{\bar{V}_2(\delta_1 - \delta_2)^2}{RT} - \left[\ln\left(\frac{\bar{V}_2}{V_1}\right) + \left(1 - \frac{\bar{V}_2}{V_1}\right)\right]$ $x_2^1 = 17.638 \times 10^{-4}$, $\delta_2 = 5.7$, $RT = 592.424$

7.) $cm^3\ O_2/100$ ml. liq. $(x_2)\frac{(2.4465 \times 10^6)}{V_1}$

8.) $\bar{V}_2 = -19.85 + 15.90 \ln \Delta S_v^{298}$ (open chain compounds)

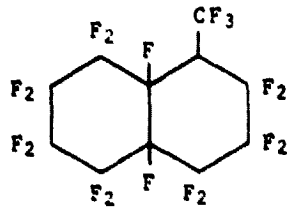
9.) $\bar{V}_2 = -100.15 + 39.90 \ln \Delta S_v^{298}$ (ring compounds)

* see references

Operating Limits and Warnings Always start each new example with GSB 0. This ensures that registers and the stack start with a value of zero.

Program Description II

Sketch(es)



Sample Problem(s) Calculate E_v^{298}, V_1, T_b, P, and O_2 solubility of perfluoromethyldecalin. The group additivity constants for equations 1.) and 2.) are:

GROUP	e_i	v_i
CF_2	783	23.1
CF_3	1933	54.8
CF	-396	-15.0
6 atom ring	2272	39.9

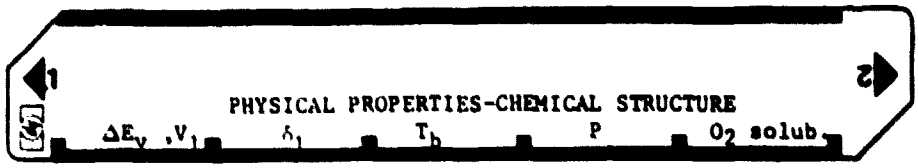
Solution(s)

(Keystrokes)

1933.00	ENT↑	-396.00	ENT↑	39.90	ENT↑		GSBE
54.80	GSBA	3.00	X	2.00	X	1.00	R/S
54.80	***		ENT↑		GSBA	39.88	O_2 ***
1933.00	***	-15.00	ENT↑	251.30	V_1 ***		
		3.00	X	10770.00	ΔE_v***		
783.00	ENT↑		GSBA				
7.00	X	171.50	***		GSBB		
	ENT↑	6226.00	***	6.556	δ_2 ***		
23.10	ENT↑				GSBC		
7.00	X	2272.00	ENT↑	157.95	T_b ***		
	GSBA	2.00	X		GSBD		
216.50	***		ENT	6.13	P ***		
7414.00	***						

Reference(s) D. D. Lawson, J. Moacanin, K. V. Scherer, T. F. Terranova, and J. D. Ingham, J. Fluorine Chem., 12, 221, (1978).

User Instructions



STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS		OUTPUT DATA/UNITS
1	Load sides 1 and 2 of program card				
2	Ensure all registers and stack are zero.		GSB	0	0.00
3	Input e_i value of group.	e_i		Enter	
4	Input v_i value of group.	v_i			
5	Sum e_i into R_0 and v_i into R_9. Continue until all e_i and v_i values representing the structural parameters in the molecule have been summed.			A	e_i
6	Calculate the solubility parameter (δ_1).			B	$\delta_1 cal^{\frac{1}{2}}/cm^{\frac{3}{2}}$
7	Calculate the boiling point (T_b°C)			C	T_b°C
8	Calculate the vapor pressure (P torr)			D	P(torr)
9	Calculate O_2 solubility/100 ml. of liquid.			E	0.00
10	Enter structure code: 0 for open chain compounds or 1 for cyclic compounds.	0 or 1			
11	To get the O_2 solubility of the compound hit R/S.			R/S	cm^3 O_2/100 ml.
12	For next example, repeat steps 2-11.				

Contents of Registers

R_1: Molar Volume (V_1) of compound
R_a: Energy of Vaporization (ΔE_v) of compound.
R_b: Solubility Parameter (δ_1) of compound.
R_c: Boiling Point (T_b) of compound.
R_d: Vapor Pressure (P) of compound.
R_e: O_2 solubility of compound.
R_0: Heat of Vaporization (ΔH_v) of compound.
R_1: Entropy of Vaporization (ΔS_v) of compound.
R_2: Partial Molar Volume of O_2 ($\bar{V}_2$)in compound.
R_3: Not used.
R_4: Mole Fraction (x_2) of O_2 in liquid.
R_5: Not used
R_6: Not used
R_7: Not used
R_8: Working Register
R_9: Working Register

Program Listing I

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLA	21 11	
002	ST÷9	35-55 09	
003	RI	-31	
004	ST÷8	35-55 08	Calculates ΔE_v.
005	RCL9	36 09	
006	PRTX	-14	Calculates V_1.
007	STOI	35 46	
008	RCL8	36 08	
009	PRTX	-14	
010	STOA	35 11	
011	SPC	16-11	
012	RTN	24	
013	*LBLB	21 12	
014	RCLA	36 11	
015	RCLI	36 46	
016	+	-24	Calculates δ_1.
017	√X	54	
018	STOB	35 12	
019	PRTX	-14	
020	RTN	24	
021	*LBLC	21 13	
022	RCLA	36 11	
023	5	05	
024	9	09	
025	2	02	
026	.	-62	
027	4	04	
028	2	02	
029	4	04	
030	+	-55	
031	STO0	35 00	
032	CHS	-22	
033	5	05	Calculates T_b.
034	3	03	
035	0	00	
036	9	09	
037	+	-55	
038	.	-62	
039	2	02	
040	8	08	
041	9	09	
042	6	06	
043	x	-35	
044	CHS	-22	
045	2	02	
046	9	09	
047	4	04	
048	.	-62	
049	8	08	
050	1	01	
051	+	-55	
052	√X	54	
053	1	01	
054	7	07	
055	.	-62	
056	1	01	
057	7	07	
058	+	-55	
059	.	-62	
060	1	01	
061	4	04	
062	4	04	
063	8	08	
064	+	-24	
065	2	02	
066	7	07	
067	3	03	
068	.	-62	
069	1	01	
070	5	05	
071	-	-45	
072	STOC	35 13	
073	PRTX	-14	
074	RTN	24	
075	*LBLD	21 14	
076	RCL0	36 00	
077	1	01	
078	2	02	
079	.	-62	
080	2	02	
081	4	04	
082	9	09	
083	7	07	
084	ENT↑	-21	
085	5	05	
086	9	09	
087	2	02	
088	.	-62	
089	4	04	
090	2	02	
091	4	04	Calculates P.
092	x	-35	
093	+	-24	
094	8	08	
095	.	-62	
096	6	06	
097	6	06	
098	5	05	
099	5	05	
100	CHS	-22	
101	Y^x	31	
102	2	02	
103	9	09	
104	8	08	
105	.	-62	
106	1	01	
107	5	05	
108	*X	-35	
109	STOD	35 14	
110	PRTX	-14	
111	RTN	24	
112	*LBLE	21 15	

REGISTERS

0	1	2	3	4	5	6	7	8	9
S0	S1	S2	S3	S4	S5	S6	S7	S8	S9

A	B	C	D	E	I
ΔE_v	δ_1	T_b °C	P torr	$cm^3 O_2/100$ ml	V_1

Program Listing II

STEP	KEY ENTRY	KEY CODE	COMMENTS
113	RCL0	36 00	
114	2	02	
115	9	09	
116	8	08	
117	.	-62	
118	1	01	
119	5	05	
120	+	-24	
121	STO1	35 01	
122	CLX	-51	
123	STO9	35 09	
124	R/S	51	
125	X≠0?	16-42	
126	GTO1	22 01	
127	RCL1	36 01	
128	LN	32	
129	1	01	
130	5	05	
131	.	-62	
132	9	09	Calculates O_2 solubility.
133	x	-35	
134	1	01	
135	9	09	
136	.	-62	
137	8	08	
138	5	05	
139	CHS	-22	
140	+	-55	
141	STO2	35 02	
142	GTO2	22 02	
143	*LBL2	21 02	
144	RCL2	36 02	
145	RCL1	36 46	
146	+	-24	
147	CHS	-22	
148	1	01	
149	+	-55	
150	ST-9	35-45 09	
151	RCL2	36 02	
152	RCL1	36 46	
153	+	-24	
154	LN	32	
155	ST-9	35-45 09	
156	RCLB	36 12	
157	5	05	
158	.	-62	
159	7	07	
160	$\div$	-45	
161	x^2	53	
162	RCL2	36 02	
163	x	-35	
164	5	05	
165	9	09	
166	2	02	
167	.	-62	
168	4	04	
169	2	02	
170	4	04	
171	+	-24	
172	ST-9	35-45 09	
173	6	06	
174	.	-62	
175	3	03	
176	4	04	
177	0	00	
178	3	03	
179	CHS	-22	
180	ST+9	35-55 09	
181	RCL9	36 09	
182	e^x	33	
183	STO4	35 04	
184	2	02	
185	4	04	
186	4	04	
187	6	06	
188	5	05	
189	0	00	
190	0	00	
191	x	-35	
192	RCL1	36 46	
193	+	-24	
194	STOE	35 15	
195	PRTX	-14	
196	SPC	16-11	
197	SPC	16-11	
198	PREG	16-13	
199	RTN	24	
200	*LBL1	21 01	
201	RCL1	36 01	
202	LN	32	
203	3	03	
204	9	09	
205	.	-62	
206	9	09	
207	x	-35	
208	1	01	
209	0	00	
210	0	00	
211	.	-62	
212	1	01	
213	5	05	
214	CHS	-22	
215	+	-55	
216	STO2	35 02	
217	GTO2	22 02	
218	*LBL0	21 00	Clears registers and stack.
219	CLRG	16-53	
220	CLX	-51	
221	ENT↑	-21	
222	ENT↑	-21	
223	ENT↑	-21	
224	RTN	24	

LABELS					FLAGS	SET STATUS		
A $\Delta E_v, V_1$	B δ_1	C T_b °C	D P torr	E O_2 solub.	0 CLR Reg.	FLAGS	TRIG	DISP
a	b	c	d	e	1	ON OFF 0 ☐ ☒ 1 ☐ ☒	DEG ☒ GRAD ☐ RAD ☐	FIX ☒ SCI ☐ ENG ☐
0	1	2	3	4	2	2 ☐ ☒ 3 ☐ ☒		n
5	6	7	8	9	3			

APPENDIX K

A SURFACE ENERGY ANALYSIS OF BIOADHESION

APPENDIX K

A surface energy analysis of bioadhesion*

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(Received 20 October 1976; revised 14 December 1976)

This report applies recently developed surface energy and fracture mechanics relations to the analysis of bioadhesion and biocompatibility. The dispersion α and polar β components of 190 biological and implant surfaces are analysed. The surface energetics relations between bioadhesion and biocompatibility point out that a strongly adsorbed plasma protein film on the implant surface provides the best blood compatibility and low thrombogenic effects. The surface energy relations provide means of selecting optimum implant surface properties and mapping on surface energy diagrams the three phase interactions which define bioadhesion.

INTRODUCTION

A number of literature reports document the general progress in development of biomaterials and surface treatments designed for application in cardiovascular devices[1-6]. The interfacial interaction of biopolymers with blood remains as one of the central problems in establishing biocompatibility of cardiovascular devices[6]. The exposure of blood to a foreign surface produces a complex set of concurrent and sequential events which appear to correlate with the dispersion (London-d) and polar (Keesom-p) components of surface tension for the implant material. Baier and coworkers[1-7] have reported detailed studies of contact angle measurements of well characterized liquids on biopolymer surfaces. These measurements and surface energetics analysis follow the methodology and definitions of critical surface tension γ_c for wetting of a solid substrate developed by Zisman and coworkers[9].

More recently, Nyilas and coworkers[10], have demonstrated that the wettability data of Baier and coworkers can be utilized in defining the dispersion and polar components of solid surface tension for candidate implant surfaces. This report of Nyilas and coworkers also describes new semi-quantitative relations between blood flow, implant surface energetics, and thrombosis. The analysis utilized by Nyilas and coworkers to isolate the dispersion and polar properties of implant surfaces follows definitions and calculations developed and extensively applied by Kaelble and coworkers[11-13]. The surface energetics analysis of Kaelble and coworkers has recently been extended[14] to define the relations between surface energetics and the Griffith fracture mechanics criteria for spontaneous interface bonding and debonding under consitions of combined liquid immersion and added mechanical stress.

This paper discusses the results of applying the new surface energetics criterion of bonding and debonding for improving the quantitative definition of bioadhesion and to clarify the relationship between bioadhesion and biocompatibility. These new surface energy relations now permit mapping the zones of bonding, termed wettability envelopes on surface energy diagrams of dispersion α *versus* polar β components of surface energy. These surface energy α *versus* β diagrams permit graphic presentation of the surface properties and zones of bonding and debonding for the three phases which constitute the interfacial boundary in the biocompatibility analysis.

* Presented at the First Cleveland Symposium on Macromolecules, Structure and Properties of Biopolymers, Case Western Reserve University, Cleveland, Ohio, USA, October 1976.

SURFACE ENERGY ANALYSIS

The general concept for regular adsorption bonding of interfaces utilized in this discussion is summarized in the following relation for interfacial tension[13]:

$$\gamma_{ij} = (\alpha_i - \alpha_j)^2 + (\beta_i - \beta_j)^2 + \Delta_{ij} \qquad (1)$$

where the parameters are defined in *Table 1* and subscripts denote interactions from phase i and j. Interfaces dominated by Van der Waal's interactions are termed regular interfaces

Table 1 Surface energetics relations

$\gamma_{LV} = \gamma_{LV}^d + \gamma_{LV}^p = \alpha_L^2 + \beta_L^2$	(a)
$\gamma_{SV} = \gamma_{SV}^d + \gamma_{SV}^p = \alpha_S^2 + \beta_S^2$	(b)
$W_a = \gamma_{LV}(1 + \cos\theta) \simeq 2\gamma_{LV}$	(c)
$W_a = 2(\alpha_L\alpha_S + \beta_L\beta_S)$	(d)
$\frac{W_a}{2\alpha_L} = \alpha_S + \beta_S(\omega_L/\alpha_L)$	(e)
$\frac{W_a}{2\alpha_S} = \alpha_L + \beta_L(\beta_S/\alpha_S)$	(f)

where γ_{LV} = liquid–vapour tension, γ_{SV} = solid–vapour surface tension; α_L, β_L = square root of the respective (London) dispersion γ_{LV}^d and (Keesom) polar γ_{LV}^p parts of γ_{LV}, α_S, β_S = square roots of respective dispersion γ_{SV}^d and polar γ_{SV}^p, W_a = nominal work of adhesion, θ = liquid–solid contact angle

Table 2 Fracture mechanics relations

$$\sigma_c = \left(\frac{2E\gamma_G}{\pi c}\right)^{1/2} = \left(\frac{2E}{\pi c}\right)^{1/2} (R^2 - R_0^2)^{1/2} \quad > 0 \qquad \text{(a)}$$

$$\gamma_G = R^2 - R_0^2 \qquad \text{(b)}$$

$$R_0^2 = 0.25\,[(\alpha_1 - \alpha_3)^2 + (\beta_1 - \beta_3)^2] \qquad \text{(c)}$$

$$R^2 = (\alpha_2 - H)^2 + (\beta_1 - K)^2 \qquad \text{(d)}$$

$$H = 0.5\,(\alpha_1 + \alpha_3) \qquad \text{(e)}$$

$$K = 0.5\,(\beta_1 + \beta_3) \qquad \text{(f)}$$

where σ_c = critical crack propagation stress; γ_G = Griffith surface energy for fracture; E = Young's modulus; c = crack length; α_1, β_1 = surface properties of adhesive (ink) phase 1; α_2, α_2 = surface properties of environment phase 2; α_3, β_3 = surface properties of adherend phase 3

and the values of the excess term Δ_{ij} of equation (1) which describes interdiffusion or ionic–covalent interactions can be considered negligible. This is a much more general case than one might expect and permits application of surface energy analysis to a wide range of materials. When $\Delta_{ij} = 0$, equation (1) defines an ideal interface with $\gamma_{ij} = 0$ as the special case where $\alpha_i = \alpha_j$ and $\beta_i = \beta_j$.

The special combination of surface energy and fracture mechanics parameters which enter the modified Griffith relation are defined in *Table 2* and show that the Griffith fracture energy γ_G is defined by the following relation:

$$\gamma_G = -\frac{S_2}{2} = R^2 - R_0^2 \qquad (2)$$

A circular parabola in $\gamma_G, \alpha_2, \beta_2$ Cartesian space is defined by equation (2). The surface energies α_2 and β_2 for the immersion phase 2 which provide the condition $R < R_0$ provides that the spreading coefficient S_2 for phase 2 is positive with $S_2 > 0$. The predicted consequence for $S_2 > 0$ is that phase 2 should spontaneously debond phase 1 from phase 3 in the absence of rheological restraints. When $R < R_0$ the Griffith fracture energy becomes positive and a critical mechanical stress σ_c which depends on γ_G (*see Table 2*) is now required for crack extension.

The relations of *Table 1* and *Table 2* form the basis for designed experiments which isolate the discrete mechanisms of polar and dispersion interactions across interface. The test liquids of *Table 3* display a wide range of polar character in surface tension with $\beta_l/\alpha_l = 1.53$ for water to $\beta_l/\alpha_l = 0$ for linear hydrocarbons. Inspection of equation (e) in *Table 1* shows that using measured values of work of adhesion W_a by contact angle measurements for liquids of known α_l and β_l permits isolation of the solid–vapour surface properties α_s and β_s. The intercept of the plot of $W_a/2\alpha_l$ *versus* β_l/α_l isolates α_s as the intercept and β_s as the slope. An alternative method of computerized determinant solutions of equation (d) (*Table 1*) has been described and extensively applied which solve for averaged values of γ_{sv}^d, γ_{sv}^p and γ_{sv} their respective standard deviations $\pm\,\delta^d$, δ^p, and δ from mean values[11,12]

In this study contact angle data where $\theta > 0$ and $W_a < 2\gamma_{lv}$ published by Baier and coworkers[1-7] is combined with the liquid surface tension data of *Table 3* in the computerized determinant calculations for γ_{sv}^d, γ_{sv}^p, γ_{sv} and $\pm\,\delta^d$, δ^p, and δ. The surface energetics of 190 biological and implant surfaces were analysed and the results summarized as values of $\alpha_s = (\gamma_{sv}^d)^{1/2}$ and $\beta_s = (\gamma_{sv}^p)^{1/2}$ and the percent standard deviation ($\delta \times 100/\gamma_{sv}$) of γ_{sv} in *Table 4*. The surface number sequence of *Table 4* correlates with the appearance of the original experimental data in the referenced literature to facilitate ease of cross reference. The reference articles and reports cover an important six year period of biomaterials development and testing from 1970 through 1975.

Surfaces no. 1 and no. 2 of *Table 4* represent important biological surfaces and the illustrative results of the surface energy analysis are graphed in *Figure 1*. *Figure 1b* shows the wettability data for human fibrinogen thin film as defined by equation (e) of *Table 1*. The solid linear curve of *Figure 1b* graphs the computer calculated average values $\gamma_{sv}^d = 24.6$ and $\gamma_{sv}^p = 13.5$ dyne/cm while the broken curves define the standard deviation $\delta^d = \pm 0.7$ and $\delta^p = \pm 0.9$ dyne/cm. As shown in *Figure 1b* the experimental values of $W_a/2\alpha_l$ form a reasonably linear curve which conforms well with the theory of *Table 1* and the computer solutions.

The data points and linear curves of *Figure 1a* show the larger uncertainty in α_s and β_s values for vein intimal surface no. 2 of *Table 4*. Referring to the discussion of Baier *et al.*[1] it is evident that this surface is soft and deformable, the experiment complicated, additionally possible interdiffusion modifies the contact angle data for water and glycerol. As shown in *Figure 1a*, this analysis permits isolation of a low dispersion and high polar surface energy for the vein intimal surface. Considering the highly hydrated state of the vein intimal surface, it is to be expected that the vein surface properties $\alpha = 4.42$ (dyne/cm)$^{1/2}$ and $\beta = 6.18$ (dyne/cm)$^{1/2}$ are closely similar to the surface tension properties of water where $\alpha = 4.67$ (dyne/cm)$^{1/2}$ and $\beta = 7.14$ (dyne/cm)$^{1/2}$.

Available literature references[14,15] describe the surface tension of blood plasma as equivalent to saline solution with surface tension $\gamma_{lv} = 72$ to 74 dyne/cm, which is essentially equivalent to pure water with $\gamma_{lv} = 72.8$ dyne/cm. Further studies may show that surfactant effects of blood plasma constituents can produce variable values of β_l with nearly constant α_l in which $\beta_l = 7.14$ (dyne/cm)$^{1/2}$ represents a maximum value. This result has been described by Kaelble[16] for aqueous detergent solutions above the critical micelle concentration. For this discussion, the surface properties of pure water with $\alpha_l = 4.67$ and $\beta_l = 7.14$ (dyne/cm)$^{1/2}$ are

Table 3 Surface tension properties of test liquids at 20°C

Test liquid	γ_{lv} (dyne/cm)	α_l	β_l	Reference
Water	72.8	4.67	7.14	10, 13
Glycerol	63.4	6.10	5.12	10
Formamide	58.2	6.28	4.32	10
Dithioglycol	54.0	6.20	3.94	10
Methylene iodide	50.8	6.83	2.06	10
Ethylene glycol	48.3	5.41	4.36	13
1,1,2,2-Tetrabromoethane	47.5	6.40	2.32	10
α-Bromonaphthalene	44.6	6.68	3.59	10
Tricresyl phosphate	40.9	6.26	1.30	13
1-Methylnaphthalene	38.7	5.04	3.65	10
Dicyclohexyl	33.0	5.74	0	10
n-Hexadecane	27.6	5.24	0	13
n-Tetradecane	26.7	5.17	0	10
n-Tridecane	25.9	5.09	0	10
n-Dodecane	25.4	5.04	0	10
n-Decane	23.9	4.89	0	10
n-Nonane	22.8	4.77	0	10
n-Octane	21.8	4.67	0	10
n-Heptane	20.3	4.51	0	10

Table 4 Dispersion (a) and polar (d) surface energy properties of biological and implant materials

Reference	Surface no. 4	Surface	a (dyne/cm)$^{1/2}$	d (dyne/cm)$^{1/2}$	$\frac{1000}{\gamma_{sv}}$
1	1	Human fibrinogen thin film	4.96	3.67	1.17
1	2	Vein intimal surface, dog, air–liquid interface	4.42	6.18	13.00
1	3	Stellite plate–normal polish–distilled water rinse (a)	4.87	1.40	2.94
1	4	Same after vigorous detergent wash (b)	5.01	1.96	2.33
1	5	Stellite cage of Starr–Edwards valve after vigorous detergent wash (c)	5.08	2.90	1.80
1	6	Stellite 21 ring normal polish (d)	5.21	1.62	2.96
1	7	Same after implantation and after vigorous detergent wash (e)	4.99	5.00	5.03
1	8	Stellite 21 ring poorly organic coated (f)	5.08	1.73	2.66
1	9	Same after implantation and adsorption of protein (g)	5.03	4.73	5.74
1	10	Same after vigorous detergent wash (h)	4.83	6.87	11.99
1	11	Stellite 21 ring only partly organic coated (i)	5.75	2.95	2.65
1	12	Same after implantation and adsorption of protein (j)	5.02	4.38	4.36
1	13	Same after vigorous detergent wash (k)	4.84	3.83	13.14
2	14	Thromboresistant TDMAC heparinized silicone – before implantation	5.92	4.09	21.47
2	15	14 (above) after implantation	5.22	6.01	7.43
2	16	Glow discharge treated Stellite 21 before implantation	5.00	5.76	7.27
2	17	16 (above) after implantation	4.83	5.87	9.09
2	18	Diamond polished carbon before implantation	7.38	2.04	10.26
2	19	18 (above) after implantation	4.94	4.79	6.19
2	20	Polyurethane based electret before implantation	5.70	4.89	1.96
2	21	20 (above) after implantation	5.01	5.97	10.20
3	22	Poly(γ-methyl L-glutamate) PMG–α sheet (a)	5.24	4.06	1.40
3	23	PMG–β sheet (b)	5.36	2.80	1.06
3	24	Mixed PMG α/β sheet (c)	5.20	3.89	1.11
3	25	PMG β sheet modified by chloroform (d)	5.15	3.67	2.36
3	26	PMG β sheet after randomizing treatment (e)	5.04	4.47	1.73
3	27	PMG α sheet on water (f)	5.01	4.39	2.16
3	28	Polyglycine (Nylon 2)	5.28	4.71	1.89
3	29	PMG cast from dichloroacetic acid (DCA)	5.45	3.35	1.29
3	30	Poly(benzyl glutamate)PBG cast from DCA	5.51	2.97	2.72
3	31	PMG cast from chloroform	5.31	3.49	1.50
3	32	PMG swelled in formic acid	5.08	2.42	3.95
3	33	PMG cast from formic acid	5.14	4.74	1.69
4	34	Poly(methacrylic ester) of perfluorinated octanol	3.73	1.20	1.99
4	35	Polypyrrolidone (Nylon 4)	5.32	5.03	3.60
4	36	Glass cover slip flame dried from methanol	5.13	5.69	6.20
4	37	Cellophane sheet	5.46	3.96	2.14
4	38	Dimethyl siloxane (Silastic) sheet	4.25	2.11	4.24
4	39	Polytetrafluoroethylene (Teflon) sheet	4.56	1.24	1.91
4	40	Polyethylene sheet	6.26	2.79	10.53
4	41	Nylon sheet	5.31	5.22	3.70
4	42	Poly(vinyl fluoride) (Tedlar) sheet	5.03	2.71	1.97
4	43	Poly(vinyl chloride) sheet	6.62	2.10	5.66
4	44	Poly(ethylene terephthalate) sheet	6.04	3.66	2.22
4	45	Cellulose acetate sheet	5.80	3.92	1.99
4	46	GE Sample 1, unbacked silicone membrane, stock silicone rubber	5.56	2.56	16.14
4	47	GE Sample 1, Side No. 1, reverse of Porous side, 80 to 85% silicone	4.56	1.47	2.97
4	48	GE Sample 2, Side No. 2, porous support side	6.11	2.48	15.22
4	49	GE Sample 2, Side No. 1, polycarbonate copolymer (65% silicone)	5.08	3.32	10.97
4	50	GE Sample 3, Side 2 facing cord	5.56	3.08	16.77
4	51	GE Sample 4, Side 1, facing up, Stock MEM-213 (52–56% silicone)	5.39	2.07	9.01
4	52	GE Sample 4, Side 2, facing cord	4.07	1.75	9.44
4	53	GE Sample 5, Side 1, facing up, polycarbonate copolymer (29% silicone)	5.80	2.34	13.40
4	54	GE Sample 5, Side 2, facing cord	4.79	2.02	5.02
4	56	UC Mylar sheet	6.26	2.36	4.26
4	58	UC Teflon sheet	4.31	1.03	1.99
4	57	UC Sample 9076-53-1H shiny side (mylar cast)	5.80	2.65	7.43
4	58	Same dull side as received (Teflon cast)	5.88	1.86	9.66
4	59	UC Sample 9076-53-1H shiny side, detergent washed	5.84	2.04	5.03
4	60	UC Sample 9076-53-2H, as received, shiny side	5.13	3.35	1.21
4	61	UC Sample 9076-53-2H, as received, dull side	5.37	1.62	4.19
4	62	UC Sample 9076-53-4H, as received, smooth side	5.30	2.96	3.91
4	63	UC Sample 9076-53-4H, as received, dull side	5.78	1.56	6.82
4	64	UC Sample 9076-53-5H, as received, smooth side	5.03	4.20	2.49
4	65	UC Sample 9076-53-5H, as received, dull side	5.26	2.32	2.69
4	66	UC Sample 9076-53-5H shiny side, detergent washed	5.34	3.33	1.91
4	67	UC Sample 9076-53-6H, as received, smooth side	5.01	3.82	2.43
4	68	68 (above), dull side	5.04	1.86	2.73
4	69	UC Sample 9076-53-7H, smooth side	5.30	2.88	3.09
4	70	UC Sample 9076-53-7H, dull side	6.14	1.22	3.88
4	71	UC Sample 9076-53-7H, smooth side, detergent wash	5.83	2.30	1.61
4	72	UC Sample 9076-53-8H, smooth side	5.23	3.04	2.49
4	73	UC Sample 9076-53-8H, dull side	6.38	1.82	10.43
4	74	UC Sample 9076-53-9H, smooth side	5.70	1.85	4.08
4	75	UC Sample 9076-53-9H, dull side	5.72	1.90	7.19

Reference	Surface no. 4	Surface	α $(dyne/cm)^{1/2}$	β $(dyne/cm)^{1/2}$	$\frac{100\beta}{\gamma_{sv}}$
4	76	UC Sample 9076-83-9H, smooth side, detergent wash	5.44	3.07	2.34
4	77	UC Sample 9076-83-10H, smooth side	5.67	2.42	1.32
4	78	UC Sample 9076-83-10H, dull side	5.89	1.61	5.01
4	79	UC Sample 9076-83-11H, smooth side	5.43	2.57	1.17
4	80	UC Sample 9076-83-11H, dull side	5.84	1.25	4.70
4	81	UC Sample 9076-83-11H, smooth side, detergent wash	5.21	3.78	2.48
4	82	UC Sample 9076-83-12H, smooth side	5.74	2.24	2.77
4	83	UC Sample 9076-83-12H, dull side	5.78	1.62	6.44
4	84	UC Sample 9076-83-13H, shiny side	4.96	3.46	2.18
4	85	UC Sample 9076-83-13H, dull side	5.43	1.68	4.88
4	86	UC Sample 9076-83-13H, shiny side, detergent washed	4.89	2.76	1.44
4	87	UC Sample 9076-83-14H, shiny side	4.87	4.04	4.11
4	88	UC Sample 9076-83-14H, dull side	5.25	1.91	4.47
4	89	UC Sample 9076-83-15H, shiny side	5.22	3.44	2.20
4	90	UC Sample 9076-83-15H, dull side	5.87	1.42	3.89
4	91	UC Sample 9076-83-15H, shiny side, detergent washed	4.90	4.11	2.43
4	92	UC Sample 9076-83-16H, shiny side	5.56	2.76	1.77
4	93	UC Sample 9076-83-16H, dull side	5.17	1.49	4.71
4	94	UC Sample 9076-83-17H, shiny side	4.97	3.99	3.61
4	95	UC Sample 9076-83-17H, dull side	5.74	1.74	5.84
4	96	UC Sample 9076-83-17H, smooth side, detergent washed	5.15	3.58	1.86
4	97	UC Sample 9076-83-18H, shiny side	5.04	2.48	3.72
4	98	UC Sample 9076-83-18H, dull side	5.20	1.74	10.34
4	99	UC Sample 9468-18-1, shiny side	5.04	2.04	3.28
4	100	UC Sample 9468-18-1, dull side	5.35	1.27	3.87
4	101	UC Sample 9468-18-1, shiny side, detergent washed	5.46	3.15	7.84
4	102	UC Sample 9468-18-2, shiny side	5.70	2.23	4.35
4	103	UC Sample 9468-18-2, dull side	5.32	2.82	9.38
4	104	UC Sample 9468-18-3, smooth side	5.33	2.32	1.66
4	105	UC Sample 9468-18-3, rough side	5.82	1.74	6.72
4	106	UC Sample 9468-18-3, smooth side, detergent washed	5.31	3.88	5.50
4	107	Parylene AF-4	6.52	1.56	5.22
4	108	Parylene after glow discharge	4.30	4.67	5.82
5	109	UC Sample 9076-83-3H, shiny side	4.98	3.67	2.81
5	110	UC Sample 9076-83-3H, shiny side, detergent washed	5.40	2.86	2.26
5	111	UC Sample 9076-83-3H, dull side	5.30	1.51	2.00
5	112	Nickel ring, glow discharge (GD) treated, after 2 h implantation, patent	4.35	6.25	9.90
5	113	Nickel ring, GD treated, after 2 h implantation, thrombosed	6.02	2.48	1.00
5	114	Magnesium ring, GD treated, after 2 h implantation, patent	4.96	5.13	7.96
5	115	Stellite ring, GD treated, stored in distilled water	5.02	6.02	8.15
5	116	Stellite ring, GD treated, after 2 h implantation, patent	5.01	5.29	7.47
5	117	Stellite ring, GD treated, after 2 week implantation, patent	5.19	5.32	9.35
5	118	Chemically polished copper ring stored 16 h in absolute alcohol	5.36	5.89	8.82
5	119	Copper ring No. 2 thrombosed in 2 h	5.08	5.52	7.42
5	120	Copper ring No. 3 thrombosed in 2 h	4.86	6.09	8.65
5	121	Copper ring No. 4 thrombosed in 2 h	5.63	3.89	2.04
5	122	GDT copper ring No. 5, patent after 2 h	4.79	6.01	9.29
5	123	GDT copper ring No. 2, 2nd acute implant, small junction thrombus	4.96	5.04	3.90
5	124	GDT copper ring No. 4, 2nd acute implant, thrombosed	5.77	3.21	1.77
5	125	GDT aluminium ring No. 2, patent, acute	4.97	3.83	3.16
5	126	GDT aluminium ring No. 3, patent after 2 h	4.70	5.92	5.68
5	127	Aluminum ring No. 4 after chronic implant	5.30	4.06	3.03
5	128	GDT aluminium ring No. 6, patent after 2 weeks	5.06	5.22	8.64
5	129	GDT aluminium ring No. 3, thrombosed in 2 h	5.42	4.83	7.80
5	130	Chemically polished ring No. 4 (aluminum) thrombosed after 2 h	5.67	3.70	1.12
5	131	Chemically polished aluminum ring No. 6 thrombosed at 2 h	5.18	5.67	8.88
5	132	GDT aluminium tube No. 1, patent after 2 h	5.13	3.61	5.80
6	133	Serum Sepcrap monolayer on GE prism	4.82	1.67	2.36
6	134	Siliconized GE plate, ultrasonic cleaned in acetone	4.88	1.46	1.96
6	135	Siliconized GE plate	4.66	1.27	2.19
6	136	Siliconized GE plate rinsed in seawater and fresh water	4.62	1.26	1.86
6	137	Fibrinogen on GE prism (siliconized), distilled water rinse	4.75	1.62	1.07
6	138	Polycarbonate ring, detergent wash, distilled water rinse	5.66	3.41	1.42
6	139	Siliconed abdominal aorta (rabbit) air dried	5.19	3.38	4.36
6	140	Siliconed right iliac artery (rabbit) air dried	5.36	3.26	1.62
6	141	Left iliac artery (rabbit) air dried	5.29	3.51	3.00
6	142	Fluorinated ethyl cellulose film, air side, detergent washed	4.81	2.22	2.07
6	143	Fluorinated ethyl cellulose film, glass side, detergent washed	4.71	2.34	1.72
6	144	Hepan, dehydrated, smooth side, USDA	4.83	5.24	8.07
6	145	Hepan, hydrated, smooth side, USDA	5.37	3.67	7.86
6	146	SRI segmented polyether urethane	5.62	2.72	3.84
6	147	SRI polyether urethane	5.58	2.70	3.26
7	148	PAS coated coverslip, as received	4.93	1.83	3.86
7	149	PAS coated coverslip (from AVCO)	5.03	1.70	4.23
7	150	PAS coated coverslip (from AVCO)	4.85	1.60	3.40

Reference	Surface no. 4	Surface	α (dyne/cm)$^{1/2}$	β (dyne/cm)$^{1/2}$	$\frac{100\beta}{\gamma_{sv}}$
7	151	AVCOTHANE balloon, outside surface, as received	4.44	1.36	3.06
7	152	AVCOTHANE balloon, outside surface, after fatigue test	4.48	1.36	3.16
7	153	Polyglycine cast from dichloroacetic acid, distilled water leach	5.38	5.05	2.59
7	154	Poly(L-alanine) cast from DCA, as prepared	4.67	7.05	15.38
7	155	Poly(L-alanine) film distilled water leached	6.34	2.10	7.16
7	156	Poly(L-alanine) film distilled water leached	5.65	3.74	2.46
7	157	Clean teflon plate	4.46	1.33	61
7	158	Teflon plate after 5 min contact human plasma	4.74	1.98	1.58
7	159	Fluorinated polyacrylate, Type F film	3.55	1.59	2.36
7	160	Fluorinated polyester Type CT film	3.45	0.95	1.34
7	161	Cell adhesive treated polystyrene	4.99	5.28	3.43
7	162	Polished Stellite 21, 30 sec contact 0.2% fibrinogen	5.88	2.92	2.49
7	163	Polished Stellite 21 plate as received	4.88	1.36	3.47
7	164	Grafted Hydrogel continuously hydrated	5.81	3.86	10.97
7	165	Grafted Hydrogel air dried	5.25	3.44	1.64
7	166	Unalloyed pyrolytic carbon, low temperature isotropic (LTI)	6.48	1.97	6.97
7	167	Carbon coated GE	6.75	2.42	6.65
7	168	Carbon coated prism, 2 min contact with 0.2% fibrinogen	5.88	2.64	3.18
7	169	Carbon coated prism, 30 sec contact 0.2% fibrinogen	5.38	3.51	1.61
7	170	Carbon coated steel, 30 sec contact 0.2% fibrinogen	5.19	3.49	1.60
7	171	Biomer segmented polyether urethane autoclaved	5.19	2.20	2.78
7	172	Dow Corning 236 dispersion coated film	4.82	1.50	3.47
7	173	Ethyl cellulose perfluorobutyrate (PFB) coating, as received	4.14	2.62	6.75
7	174	Ethyl cellulose PFB coating, hydrated	4.35	2.13	1.65
7	175	Air dried side PFB coating exposed to fibrinogen solution	5.08	3.07	2.80
7	176	Hydrated PFB coating exposed to fibrinogen solution	5.55	2.85	2.28
7	177	Sample No. 1, Hydrated acrylamide graft (HAG) on SRI urethane	5.74	2.64	0.85
7	178	Sample No. 2, HAG on SRI polyurethane, fibrinogen exposed	5.73	3.87	5.58
7	179	Sample No. 6, HAG on SRI urethane preirradiated with UV	4.32	6.66	8.76
7	180	Sample No. 7, HAG with 0.25% crosslinker on SRI urethane	5.38	3.95	13.29
7	181	Sample No. 9, HAG with 0.25% crosslinker on SRI urethane, fibrinogen exposed	5.33	1.95	0.95
7	182	Sample No. 12, HAG with 0.25 crosslinker on preirradiated SRI urethane	5.28	4.10	10.11
7	183	Plasma nitrogen–acetylene–water coated slip cover	5.23	3.87	2.11
7	184	Poly(vinyl acetate–2% crotonic acid) 60% ionomer hydrated	5.74	2.35	3.52
7	185	Polyelectrolyte hydrogel after air drying	5.29	3.18	1.11
7	186	Ion beam deposited (IBD) carbon film on GE, fibrinogen exposed	5.80	2.46	2.06
7	187	IBD carbon film on GE prism No. 49	6.08	1.97	4.43
7	188	IBD carbon film on GE prism No. 59	6.36	1.58	7.51
7	189	IBD carbon film on GE prism No. 31	6.14	1.77	6.18
7	190	IBD carbon film on GE prism No. 42	6.19	2.12	6.99

taken as the analogue for the surface properties of blood plasma. This assumption sets forth the proposition that protein molecular segments (phase 1) are competing with the water molecules of blood plasma (phase 2) for bonding sites on the implant surface (phase 3).

The surface energy properties of fibrinogen, vein intima, can be graphically displayed on a surface energy diagram of α *versus* β as shown in *Figure 2*. This diagram also locates the surface tension properties of water (≈blood plasma) and graphically displays the close relation between the vein intima surface properties and those of water. Inserting the appropriate values for α and β into equation (1) for $\Delta_{ij} = 0$ one calculates an interfacial tension $\gamma_{ij} = 12.1$ dyne/cm between blood and fibrinogen as compared to a much lower value of $\gamma_{ij} = 1.0$ dyne/cm between the vein intima surface and water. The vein surface thus provides a much closer approach to an ideal interface where $\gamma_{ij} = 0$ than does the fibrinogen film.

BIOADHESION AND BIOCOMPATIBILITY

Substantial evidence[1-8] now shows that the first event that follows the exposure of blood to an implant material is the adsorption of plasma borne protein which covers the implant surface. This adsorbed plasma protein film should modify the surface energy of the implant so as to lower its interfacial tension to blood plasma. From equation (1) the specific set of interfacial tensions between the implant (phase 3), blood plasma (phase 2) and adsorbed plasma protein film (phase 1) are started as follows:

$$\gamma_{12} = (\alpha_1 - \alpha_2)^2 + (\beta_1 - \beta_2)^2 \quad (3)$$

$$\gamma_{13} = (\alpha_1 - \alpha_3)^2 + (\beta_1 - \beta_3)^2 \quad (4)$$

$$\gamma_{23} = (\alpha_2 - \alpha_3)^2 + (\beta_2 - \beta_3)^2 \quad (5)$$

For adsorbed plasma protein (phase 1) to spontaneously displace blood plasma (phase 2) from the implant (phase 3) the spreading coefficient S, for phase 1 must be positive. The phase 1 spreading coefficient is defined as:

$$S_1 = \gamma_{23} - \gamma_{13} - \gamma_{12} \quad (6)$$

As shown in equation (6), protein adsorption is favoured by large values of γ_{23}. As shown in equation (5) a large mismatch in implant surface properties α_3, β_3 and blood plasma $\alpha_2 \approx 4.67, \beta_2 \approx 7.14$ (dyne/cm)$^{1/2}$ is seen to increase γ_{23}

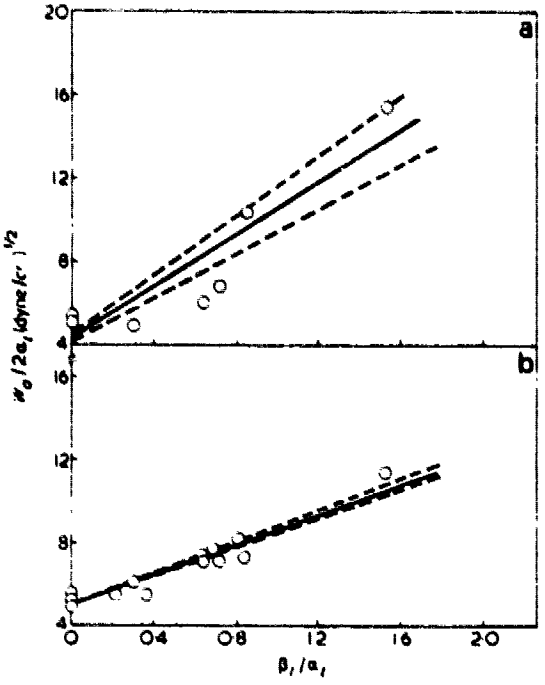

Figure 1 Analytic definition of surface tension properties of (a) vein intimal surface, dog(surface 2 of *Table 4*), $\gamma d = 19.5 \pm 2.6$; $\gamma p = 38.2 \pm 9.5$ dyne cm; $\alpha = 4.42$; $\beta = 6.18$; (b) human fibrinogen thin film (surface 1 of *Table 4*), $\gamma d = 2.46 \pm 0.7$, $\gamma p = 13.5 \pm 0.9$ dyne/cm, $\alpha = 4.96$, $\beta = 3.67$

and enhance protein adsorption.

The complementary proposition in equation (6) is that minimum values of both γ_{13} and γ_{12} also favour protein adsorption. Equation (3) shows that a minimum mismatch in the surface properties $\alpha_1 \simeq \alpha_2$ and $\beta_1 \simeq \beta_2$ reduces γ_{12}. A similar minimization of γ_{13} is obtained by achieving $\alpha_1 \simeq \alpha_3$ and $\beta_1 \simeq \beta_{13}$ as shown in equation (4). The present analysis of protein analogues, see surfaces no. 22–23 in *Table 4*, shows that the polar β part of surface energy is substantially varied by solvent–polymer interactions and resultant polymer chain conformation. It is reasonable to presume that plasma protein will be adsorbed on an implant surface with chain conformations which tend to minimize both γ_{12} and γ_{13} by spontaneous adjustment of α_1 and β_1 values in the protein film. By spontaneously minimizing γ_{12} and γ_{13}, the adsorbed plasma protein film evidently performs an important biological adaptation function.

The physiochemical description of the plasma displacement and protein adsorption on the implant surface is analogous to 'priming' as familiarly described in paint technology. Attachment of platelets with formation of thrombus and subsequent thrombus release to produce embolism appears to be mediated by the protein prime layer. Clinical tests of biocompatibility are briefly described in the Appendix by two in vivo tests in common usage. The vena cava test developed by Gott and coworkers[17,19] is limited to the detection of thrombus. The renal embolus test, developed by Kusserow and coworkers[18,19] detects both thrombus and embolism generated by thrombus release from the implant surface. Recent studies by Baier and coworkers[7] now reveal that implant surfaces with evident high thrombo-resistance in the vena cava test are shown to be thrombogenic in the renal embolus test where inspection of the kidney reveals extensive implant damage. The indications are that the thrombus can form on the implant surface and continuously spall off to be carried and deposited in the kidney. The end result is a relatively thrombus free implant surface that acts as a continuous embolus generator. Simple inspection of the implant surfaces at the site of implantation is therefore not a sufficient test for biocompatibility.

A central issue in a revised definition of biocompatibility can be related to the resistance to detachment of the plasma protein film which 'primes' and biologically modifies the implant surface. The modified Griffith relations of *Table 2* provide a quantitative means for evaluating the Griffith energy γ_G required to detach the adsorbed protein film (phase 1) from the implant (phase 3) in the presence of blood plasma (phase 2). The studies summarized in *Table 4* include surface energy analysis of implant materials before and after implantation. Surfaces no. 14–21 studied by Baier and coworkers[7] furnish data for the calculations of the Griffith surface energy γ_G as summarized in *Table 5*.

The calculations in *Table 5* show that the Griffith surface energy γ_G and related critical mechanical stress σ_c decrease as the polar component β_3 of the implant surface increases. The clinical ratings of biocompatibility listed in the lower portion of *Table 5* show a direct correlation between increased γ_G and improved blood compatibility. The results of *Table 5* can be mapped on surface energy diagrams of α *versus* β as shown in *Figure 3*. *Figure 3b* for polished carbon defines high γ_G with decreasing values of γ_G in clockwise direction to show surface cleaned stellite 21 metal in the lower right view as most highly thrombogenic. The surface energy diagrams of *Figure 3* show the points *H*, *K* defined in *Table 2* as the origin of the R and R_0 vectors which define the Griffith fracture energy:

$$\gamma_G = R^2 - R_0^2 \tag{7}$$

as defined in *Table 2*. The magnitude of R_0 which subtracts

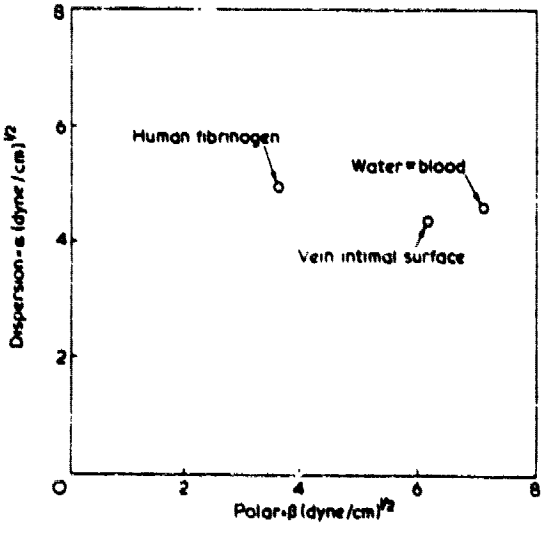

Figure 2 Dispersion (α) and polar (β) surface properties of biological materials

Table 5 Calculated Griffith energy w_c required to detach adsorbed protein layer (1) from implant surface (3) in blood plasma (2)

Implant	Diamond polished carbon	TDMAC* heparinized silicone	Polyurethane electret	GDT* Stellite 21
Surface no (see *Table 4*)	18,19	14 15	20 22	16 17
a_3	7 38	5 92	5 70	5 00
b_3	2 04	4 09	4 69	5 76
a_1	4 94	5 22	5 01	4 63
b_1	4 79	6 01	5 9	5 87
a_2	4 67	4 67	4 67	4 67
b_2	7 14	7 14	7 14	7 14
R^2	6 15	5 57	5 36	4 82
A	3 42	5 01	5 33	5 82
R_0^2	3 38	1 04	0 53	0 04
R_1^2	16 03	5 35	3 75	1 76
$w_c = R^2 - R_0^2$	12 69	4 31	3 70	1 72
Blood compatibility	Excellent[5]	Good[5]	Medium[5]	Poor[5]

* TMDAC = tridodecylammonium chloride GDT = glow discharge treated

from w_c is related to the mismatch in surface properties between implant (phase 3) and adsorbed protein layer (phase 1) Conversely the magnitude of R which adds to w_c is related to the mismatch between H, A which defines an averaged property of the 1 3 interface and the blood plasma phase As shown in *Table 5* in all cases the magnitude of R^2 dominantly controls the magnitude of w_c in the examples graphed in *Figure 5* The design concept for blood compatibility described by equation (7) incorporates a general argument which details the competition between blood plasma (phase 2) and plasma protein (phase 1) for bonding sites on the implant surface A high w_c, indicative of blood compatibility favours both the spontaneous formation and strong retention of the adsorbed protein film

DISCUSSION

The previous two sections have briefly introduced and illustrated methods for analysis of surface energy and bioadhesion The detailed discussion of all relevant aspects of the extensive data compilation in *Table 4* is beyond the scope of this brief report Review of *Table 4* in conjunction with the extensive experimentation and discussion by Baier and coworkers in the original references[1 5] shows general agreement with the results illustrated here wherein a combination of high dispersion (a) combined with low (b) correlates with high blood compatibility The previous section relates this result with the strong adsorption and stable retention of a plasma protein adsorption layer on the implant surface

If the virgin implant surface has a highly polar character which approaches the δ values for water or blood plasma the examples show that the protein layer is weakly adsorbed and held on the implant surface In this latter case, the implant completely covered implant would appear to operate efficiently as a thrombus and embolus generator

This preliminary application of surface energy analysis and the modified Griffith criteria of *Table 1* and *Table 2*, respectively, is quite encouraging Nyilas and coworkers[10] have reported a series of studies of thrombus formation under closely controlled blood flow conditions Within a given category of implant materials a high polar property δ for the implant surface is shown to correlate with low thrombus resistance for a given shear rate of blood flow This result of Nyilas and coworkers[10] is in agreement with the modified Griffith analysis of critical stress σ_c or energy w_c for debonding applied in this report

The adsorption theory outlined in *Table 1* and *Table 2* and applied in this report does not directly treat the effects of either surface roughness or interdiffusion effects related to solvolytic interactions at the interface Kaelble[12] has developed the thermodynamic extensions of adsorption theory of interfaces to treat both roughness and interdiffusion The effects of microroughness at the implant surface is potentially a dominant issue in surface energetics at the microfibric scaffold surface used for anchoring viable fibroplastic and endothelial cells to produce a 'living' blood compatible surface

The adsorption theory is also ill equipped to deal quantitatively with time dependent changes in bioadhesion arising from interdiffusion effects This latter point is graphically evident in the data scatter shown for blood vessel intima in *Figure 1a* The natural linings of blood vessels are hydrogels Synthetic hydrogel coatings consisting of a coherent three dimensional polymer network containing a large proportion of water display promise as implant surfaces[5] A more detailed description of the role of both *incorporated* and *absorbed* water on the bioadhesion of hydrogel coatings and biologically deposited protein films require use of a combined adsorption interdiffusion (AD) theory

The right column of *Table 4* lists the percent standard deviation from the mean ($100\,\delta w_c^{-1}$) for computed average values of w_{ci} Of the 120 surfaces examined, 134 display standard deviations of less than 8 , and 158 surfaces give deviations less than 10 The maximum standard deviation for solid No 14 is 21 4 Large standard deviations are generally related to roughness and interdiffusion effects and readily identified in data displays as shown in *Figure 3* Imprecise values of a and b are, of course, carried forward into the calculations of w_c as presented in *Table 5*

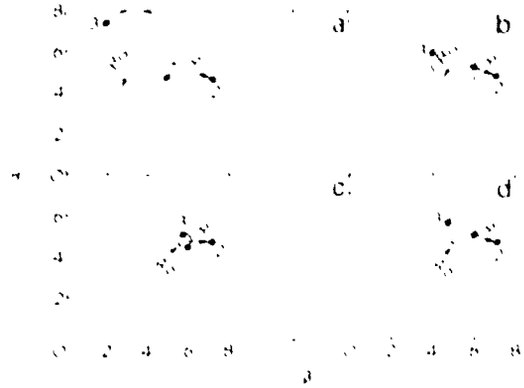

Figure 3 Surface energy analysis of interfacial interactions between plasma protein (phase 1) blood plasma water (phase 2) and implant (phase 3) (a) 1 3 (above) after implantation 2 water * blood plasma 3 diamond polished carbon (b) 1 3 (above) after implantation 2 water blood plasma 3 TDMAC heparinized silicone (c) 1 3 (above) after implantation 2 water * blood plasma 3 GDT Stellite 21 (d) 1 3 (above) after implantation 2 water blood plasma 3 polyurethane electret

CONCLUSIONS

As a result of this study, some conclusions concerning the role of surface energetics in bioadhesion can be made as follows

(A) Surface energy analysis is successfully applied to define the dispersion (α) and polar (β) surface energies of 190 biological and implant surfaces

(B) These dispersion polar surface energies are introduced into fracture mechanics relations for bioadhesion and biocompatibility

(C) These fracture mechanics calculations indicate that blood compatibility of an implant is enhanced by spontaneous absorption and strong retention of a plasma protein film on the implant surface

(D) High dispersion -low polar surface energy for the implant as exemplified by low temperature isotropic (*LTI*) carbon with $\alpha \geq 6.0$ (dyne cm)$^{1/2}$, and $\beta \leq 2.0$ (dyne cm)$^{1/2}$, provide surface energetics favouring stable plasma protein film retention

(E) Low dispersion -high polar surfaces, typified by surface treated Stellite 21 with $\alpha \approx 5.0$ (dyne cm)$^{1/2}$ $\beta \geq 5.0$ (dyne cm)$^{1/2}$, provide surface energetics, appear to favour weak adsorption and retention of the plasma protein such that the implant may continuously generate and spall off emboli into the blood stream

(F) The present analysis can be extended to describe the effects of interface roughness and interdiffusion which represent dominant considerations in microfibre scaffold surfaces, hydrogel coatings, and biological intimal surfaces of the cardiovascular system

The extensive listing of surface energies in *Table 4* is intended to be used in conjunction with the referenced data sources. The main objective in the discussion is to illustrate, by examples, the usefulness of this surface energy analysis (see *Figure 1*) and the application of the analysis of spontaneous bonding and debonding (see *Figures 2* and *3*).

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APPENDIX

Clinical tests for implant biocompatibility

Vena cava test. This test was developed by Gott and coworkers[17,19]. Rings (length = 9 mm, and diameters o.d. = 8 mm and i.d. = 7 mm) with streamlined edges to prevent turbulence are fabricated from the test material or surface coated with the test material. Implantation is made in the inferior vena cava of a 7.7 to 19.4 dg dog. The rings are inserted with a special device to insure noncontact with the atrial wall or contamination with tissue fluid during implantation.

For *acute* studies (2 h) the chest remains open. At the end of the two hour period the vena cava above and below the ring is doubly clamped and the ring is quickly excised. The inside of the ring is examined within a minute. The extent and nature of the gross thrombus is recorded immediately on a standard ring chart.

For *chronic* studies (2 weeks) the same implantation techniques are used. After implantation, the chest is closed and the animal maintained two weeks before sacrifice. The chest cavity is quickly opened, the ring excised and examined for the extent and nature of thrombus and results recorded on the standard ring chart. In addition, there is a gross inspection of the lungs for any pulmonary pathology and pulmonary embolae.

Renal embolus test. This test was developed by Kusserow and coworkers[18,19]. A ring (length = 10 mm, and diameters o.d. = 8.6 mm and i.d. = 7 mm) is implanted into the abdominal aorta of the test animal immediately above the origin renal arteries. A construction is made in the aorta slightly below the origin of the renal arteries so that over 90% of the blood flowing through the ring must pass through the kidneys. After an implantation period of three to five days an autopsy is performed. The extent of surface thrombus of the ring is assessed by direct visual observation. Embolism is evaluated by direct visual and microscopic examination of both kidneys. The kidneys serve as efficient biological accumulators for the embolic phenomena because the emboli produce recognizable infarcts in these organs.