

**UNITED STATES AIR FORCE
ARMSTRONG LABORATORY**

**TOXICITY OF PERFLUORO
POLYETHERS IN VITRO**

Nicholas DelRaso

OCCUPATIONAL AND ENVIRONMENTAL
HEALTH DIRECTORATE TOXICOLOGY DIVISION
ARMSTRONG LABORATORY
WRIGHT-PATTERSON AFB OH 45433-7400

**Dan Pollard
Merry Walsh
Marcia Ketcha**

MANTECH GEO-CENTERS JOINT VENTURE
P. O. Box 31009
Dayton, OH 45437

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Occupational and Environmental Health
Directorate
Toxicology Division
2856 G Street
Wright-Patterson AFB OH 45433-7400

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TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Armstrong Laboratory

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PREFACE

This report represents research performed by the Biochemical Toxicology Branch, Toxicology Division, Armstrong Laboratory, from June 1993 to September 1993. This document serves as a final report on the in vitro toxicity of the perfluoroalkylethers Fomblin and Galden, and an additive of Fomblin (MLO 89-184) using primary rat hepatocytes. The research was conducted in support of AFOSR Basic Environmental Research Initiative (Work Unit 2312A202).

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INTRODUCTION

Fomblin and Galden are perfluorinated polyether (PFPE) fluids manufactured by Montefluos (Milan, Italy). These fluids possess excellent heat stability, high wetting power and lubricity, and chemical inertness. These properties make PFPE compounds ideally suited for the aerospace, electronic, electrochemical, nuclear and chemical industries as lubricants, damping fluids, sealing fluids, hydraulic fluids, heat transfer fluids and instrument filling fluids. The continual refinement of technique and technology, as well as the need for specialized applications, has stimulated the development of more sophisticated PFPE products. Very little toxicity data exists on these compounds. Previous work conducted in our laboratory has shown perfluorinated alkane acids, such as nonadecafluorodecanoic acid (NDFDA), to be hepatotoxic in the rat (3) and *in vitro* (1). Furthermore, it has also been shown that a specific oligomer of chlorotrifluoroethylene (PolyCTFE), the 8 carbon chain length oligomer acid, is hepatotoxic *in vivo* (5) and *in vitro* (1). In the present study, we determined if the toxicity of PFPE compounds could be tested *in vitro*. Because other perhalogenated hydrocarbons have shown some effects on the liver, primary rat hepatocytes were used to evaluate PFPE toxicity.

MATERIALS AND METHODS

Materials

Fomblin Z, Galden HT-70, and an additive (MLO 89-184) were provided by Mr. Harvey Paige (Material Laboratory, WPAFB, OH). Unless otherwise stated, all other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

Gas Chromatography

PFPE concentrations were analyzed on a 0.53 mm x 23 m PoraPlot Q column using a Varian 3700 gas chromatograph equipped with an 8100 autosampler (Varian Assoc., Sunnyvale, CA) in conjunction with a electron capture detector (ECD). Helium was used as a carrier gas at a flow rate of 1.9 ml/min. The makeup gas consisted of argon/methane at a flow rate of 15 ml/min. The oven temperature was ramped from 120°C to 200°C at 5°C/min. The injection and detector temperatures were 150°C and 300°C, respectively.

Gas Chromatography/Mass Spectrometry

Combined GC/MS data were acquired using a Hewlett-Packard 5890 GC interfaced to a Hewlett-Packard 5970 MSD quadrupole mass spectrometer (Hewlett-Packard, Avondale, PA) equipped with a 70 eV electron impact source. A PoraPlot Q column, 0.32 mm x 27.5 m, was used for the analysis of PFPE compounds.

Rat Liver Perfusion

Fischer 344 rat livers were perfused, and hepatocytes were isolated and enriched as previously described (2) with the following modifications. Perfusion media (pH 7.2) were

supplemented with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Wash-out perfusion medium was supplemented with heparin (2.0 U/ml) and ethylenebis(oxyethylenitrilo)-tetraacetic acid (EGTA; 0.5 mM). Digestion perfusion medium was supplemented prior to use with collagenase at 0.26 U/ml (based on Wunsche U/mg).

Hepatocyte Enrichment and Culture

Primary rat and primate hepatocytes were isolated by low speed isodensity Percoll (Pharmacia, Piscataway, NJ) centrifugation as described by Kreamer et al. (6). Rat hepatocytes were culture in Williams E culture medium (Gibco, Grand Island, NY) supplemented with HEPES (18 mM), sodium bicarbonate (2.2 mg/mL), bovine serum albumin (0.5 mg/mL), 5-aminolevulinic acid (0.1 mg/ml), Sigma insulin/transferrin/sodium selenite solution (10 mg/ml), gentamicin (50 mg/ml) and 5% fetal bovine serum (FBS; Gibco, Grand Island, NY).

Collagen Coated Mesh

Sterile nylon mesh (20 micron pore size; Small Parts, Inc., Miami, FL) were placed into sterile 100 mm tissue culture plates. Using a micropipetter with sterile tip, 100 ml aliquots of a collagen type I solution (4 mg/ml in 0.02N acetic acid; Upstate Biotechnology, Inc., Lake Placid, NY) were added to the nylon mesh. The collagen was then evenly distributed over the mesh using a sterile "L"-shaped glass rod. The plates containing the collagen coated mesh were then placed into a vapor jar saturated with ammonium hydroxide for 5 min. After 5 min, the plates were removed from the vapor jar to a laminar flow hood and 5.0 ml of sterile water was added to all plates. After 1 h, the water was removed. This procedure was repeated twice. Hank's balanced salt solution (HBSS) containing 5% fetal bovine serum (FBS) is then added to the plates and refrigerated at 4°C for 24 h.

Micro Roller Bottle Culture

The recently developed micro roller bottle (patent pending) is a modified pyrex glass 20 ml gas chromatography (GC) sample vial with two open ends. The micro roller bottle is made air tight by the insertion of Mininert^R valves (Pierce, Rockford, IL) into the open ends of the bottle. Before hepatocytes can be exposed to volatile compounds in this micro roller bottle, they must be attached to the collagen gel coated nylon mesh strip. The HBSS medium containing FCS covering the mesh strips is removed prior to the addition of the hepatocytes. Primary hepatocytes were first concentrated to a cell density of $5 \times 10^5/0.1$ ml in Williams E culture medium supplemented with dexamethasone (0.1 mM). From this concentrated cell suspension, 2×10^6 hepatocytes (0.4 ml) were evenly distributed over the mesh. The plates were then placed in a CO₂ incubator at 37°C for 3 to 4 h to allow for cell attachment. Using forceps, a corner of the mesh in the culture plate was grasped and the mesh was rolled into a narrow tube. The narrow tube of mesh was then inserted into the roller bottle and the mesh unrolled. The forcep was then released by gently rolling the roller bottle so that the mesh unrolled itself. Before placing the other Mininert^R valve into the roller bottles, 1.0 ml of Williams E culture medium was added bringing the total volume of culture medium in the roller bottles to 2.0 ml. The micro roller bottles were injected with volatile test compounds using a 60 cc syringe with side port needle.

Volatile Exposure

Volatile PFPE compounds for hepatocyte exposure were prepared in standard bags at saturation levels. Using 60 cc syringes with side port needles, 60 cc of volatile test compound was removed from each bag and inserted through the Mininert^R valves of the micro roller bottles, respectively. Before flushing the micro roller bottle chamber with the volatile test compound, a second side port needle is inserted through the Mininert^R valve at the opposite end of the roller bottle to prevent excessive pressure build up during injection. After flushing the micro roller bottle chamber with test agent (3 bottles/dose), the two side port needles were removed and both Mininert^R valves were closed. Control roller bottles were flushed with an incubator atmosphere of 95% air/5% CO₂. An atmosphere sample was taken from each of the micro roller bottles, except controls, and analyzed by GC to determine the concentration of test agent. A second sample was taken at the termination of exposure (24 h) to ensure that the test agent was present at a concentration of at least 95% of that measured at dosing. The micro roller bottles were incubated on a modified roller apparatus at 37°C and rotated at 3 rpm for 24 h. At the termination of exposure, medium samples were collected for cellular lactate dehydrogenase (LDH) enzyme leakage determinations.

LDH Assay

Medium lactate dehydrogenase (LDH) enzyme levels were determined using an automated clinical analyzer (ACA Model V, DuPont, Huffman Estates, IL) as previously described (DelRaso et al., 1989).

Statistics

Data were compared by an analysis of variance (ANOVA) using SYSTAT software statistics package (SYSTAT, Inc, Evanston, IL). Means found to be significant by ANOVA were compared with the Tukey post hoc test with Type I error level held at $p < 0.05$.

RESULTS

Solubility of PFPE Compounds

Experiments were conducted to determine if a suitable solvent vehicle could be used to deliver PFPE compounds to cells in aqueous culture. Standard *in vitro* low toxicity solvents, such as dimethylsulfoxide and acetone, used to solubilize nonpolar test agents, were found to be inadequate for the solubilization of PFPE compounds. These compounds were soluble in similarly related compounds such as freon and trifluoroacetone. However, addition of the solubilized PFPE compounds to aqueous medium resulted in the PFPE compounds coming out of solution. Because of its large molecular weight (~10,000), high boiling point (>270°C) and extreme insolubility in aqueous medium, Fomblin and its additive could not be detected by GC methods and was not tested further. Galden's lower molecular weight (<500), lower boiling point (70°C) and partial solubility in water (14 ppm) did allow for GC analysis of tissue culture medium and headspace in the micro roller bottle *in vitro* test system.

Galden Analysis In Vitro

A standard curve was generated for Galden using a range of concentrations from 0.1 to 3.0 mg/L (Fig 1). Unknown concentrations of Galden in culture medium and headspace of *in vitro* culture apparatus were derived from the standard curve. The concentration of Galden in saturated culture medium (1.0 g/100 ml) at 37°C was found to be relatively constant over 3 days (Table 1). The amount of Galden solubilized in the culture medium represented less than 1% of the amount added to the medium. Analysis of the culture medium by GC indicated the presence of three peaks with retention times of 3.5, 6.1 and 9.0 min (Fig 2). Analysis of these samples by GC/MS resulted in the identification of 4 peaks (Fig 3). These 4 peaks were found to possess mass spectra that closely resembled the mass spectra of 2-(perfluoropropoxy)perfluoro-propionyl fluoride and perfluoroalkylether nitrile (Fig 4). It was determined that the 6.1 min retention time peak indicated by GC was actually composed of two peaks.

When Galden saturated culture medium was added to the *in vitro* micro roller bottle exposure chamber no peaks were found when analyzed by GC. The two peaks of Galden were detected in the headspace of these exposure chambers. Addition of Galden vapor (1 ml/L in 5% CO₂/air) to these exposure chambers resulted in medium equilibration of the 6.1 min retention time peak at 30 ±4 mg/ml (Table 2). However, the more volatile component of Galden remained in the headspace of the exposure chamber. The 9.0 min retention time peak was not used in determining Galden concentrations in medium because it was associated with some of the background peaks.

Primary Hepatocyte Galden Exposure

Because of the design of the micro roller bottle exposure chamber, cells are exposed to the vapor and solubilized components of volatile chemicals. Primary hepatocytes exposed to highest achievable concentration of Galden, whether in the head space only or in both head space and medium, did not exhibit any cytotoxicity as indicated by LDH enzyme leakage after 24 h of exposure (Fig 5).

DISCUSSION

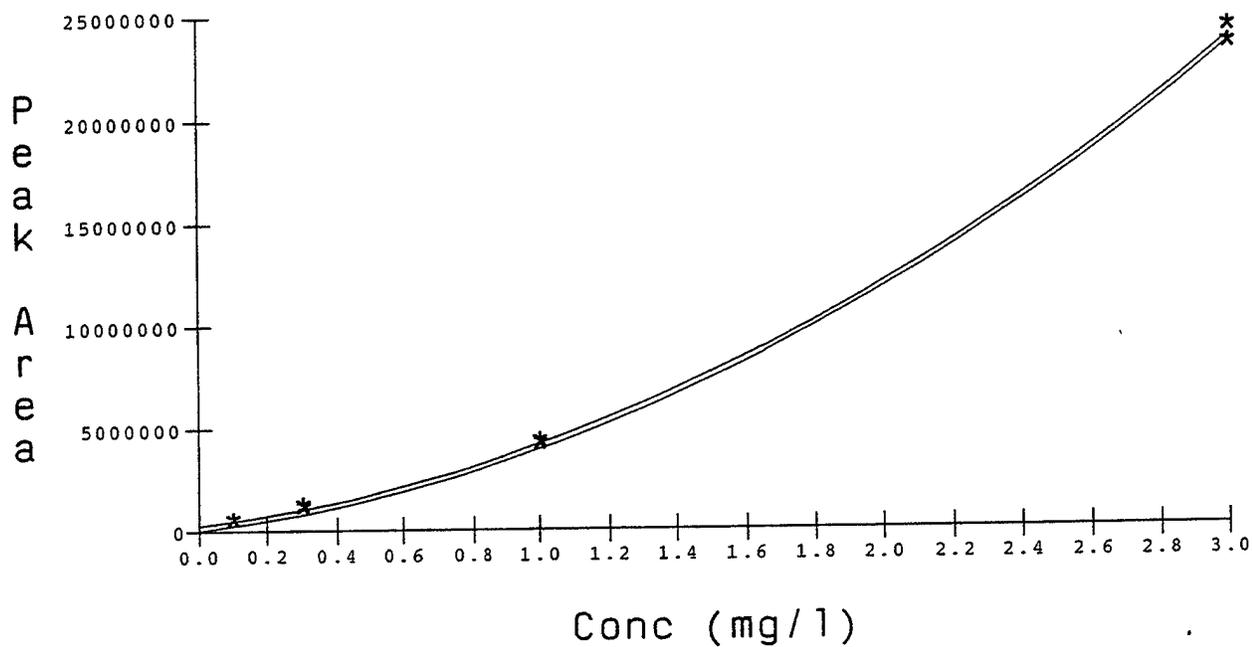
Fomblin is a mixture of linear polymers of PFPEs. The presence of fluorine and oxygen in these polymers contribute to the physical properties of these compounds. Because of the high electronegativity of the fluorine atoms and the bonding of the oxygen atoms in ether linkages, PFPEs are extremely insoluble in aqueous environments due to greatly reduced hydrogen bonding. Furthermore, the long chain length of PFPEs also contributes to their hydrophobicity. In addition, large molecular weight hydrophobic additives (~2000) can be included in the formulation of these compounds. Therefore, the toxic effects of Fomblin (or its additive) *in vitro* could not be assessed. However, Fomblin's high insolubility and chemical inertness would indicate that this compound would exhibit very low toxicity. This is supported by a recent acute and two-week inhalation study in the rat with a similar PFPE produced by Du Pont (Krytox) that produced only mild effects at

high exposure levels (4). This study also demonstrated that unlike some other fluorinated oils (PolyCTFE), no observable effects were found in the liver with respect to liver weight, liver enzyme, peroxisomal β -oxidation or histopathologic examination (4). While extreme measures may be possible to solubilize Fomblin for *in vitro* studies they are not realistic with respect to actual exposure scenarios and should not be attempted. Another factor to consider in evaluating PFPE toxicity is the toxicity of its breakdown products. Based on limited animal data (4), PFPEs appear to be only mildly toxic. However, their thermal breakdown products (hydrogen fluoride and carbonyl fluoride) exhibit a high degree of toxicity.

Galdens are lower molecular weight distillation fractions of PFPEs. This was demonstrated by GC/MS analysis of Galden that indicated that the 4 peaks identified had similar mass spectra. These compounds do exhibit some degree of solubility in water. Therefore, the toxicity of these compounds can be assessed by *in vitro* methods. Based on its similar structure and physical characteristic to Krytox, it is likely that Galden would also exhibit a low degree of toxicity. In the present study, primary rat hepatocytes exposed to the highest achievable level of Galden in cell culture medium did not produce any cytotoxicity as determined by LDH enzyme leakage. It can be concluded from this study that *in vitro* methods can not be used to conduct toxicity determinations of large molecular weight PFPEs. It must also be noted that only one cell system was used in this study (primary hepatocytes) and that it is possible that other cell systems may be affected by exposure to PFPEs. Possible other target cells can be identified by conducting preliminary *in vivo* studies. It is possible that PFPEs may be taken up and stored in the fat producing delayed effects. This again stresses the need for some *in vivo* studies. Preliminary *in vivo* work with Krytox indicates that PFPEs appear to have low toxicity. However, future *in vitro* studies may be considered if preliminary studies, utilizing small numbers of animals, identify potential target organs for PFPEs.

REFERENCES

1. **DelRaso, N.J., Walsh, M.J., Fazekas, T., and Schmidt, W.J.** The use of isolated rat and primate hepatocytes in hepatotoxicity screens for halogenated fatty acids. *In Vitro* 27:157; 1991.
2. **DelRaso, N.J., Mattie, D.R., and Godin, C.S.** *In vitro* toxicity of solubilized 2,3,4-trimethylpentane I. Cytotoxicity and metabolism of TMP using primary hepatocytes. *In Vitro* 25:1031-1038; 1989.
3. **George, M.E. and Andersen, M.E.** Toxic effects of nonadecafluoro-n-decanoic acid in rats. *Toxicol. Appl. Pharmacol.* 85:169-180; 1986.
4. **Kelly, D.P., Ross, P.E., and Cook, J.C.** Acute and two-week inhalation toxicity studies with Krytox fluorinated oil. *The Toxicologist* 13:396; 1993.
5. **Kinkead, E.R., Bungler, S.K., Wolfe, R.E., Flemming, C.D., Whitmire, R.E., and Wall, H.G.** Repeated-dose gavage studies on polychlorotrifluoroethylene acids. *Toxicol. Indust. Health* 7:295-307; 1991.
6. **Kreamer, B.L., Staecker, J.L., Sawada, N., Sattler, G.L., Hsia, M.T.S., and Pitot, H.C.** Use of a low-speed iso-density percoll centrifugation method to increase the viability of isolated hepatocyte preparations. *In Vitro* 22:201-211; 1986.



$$1942802.050629 * X^{**2} + 1981659.705781 * X + 155450$$

Fig. 1 Golden HT-70 standard curve. Peak area reflects total area of 3 peaks with retention times of 3.5, 6.1 and 9.0 min. Data points reflect average peak areas on 2 different days.

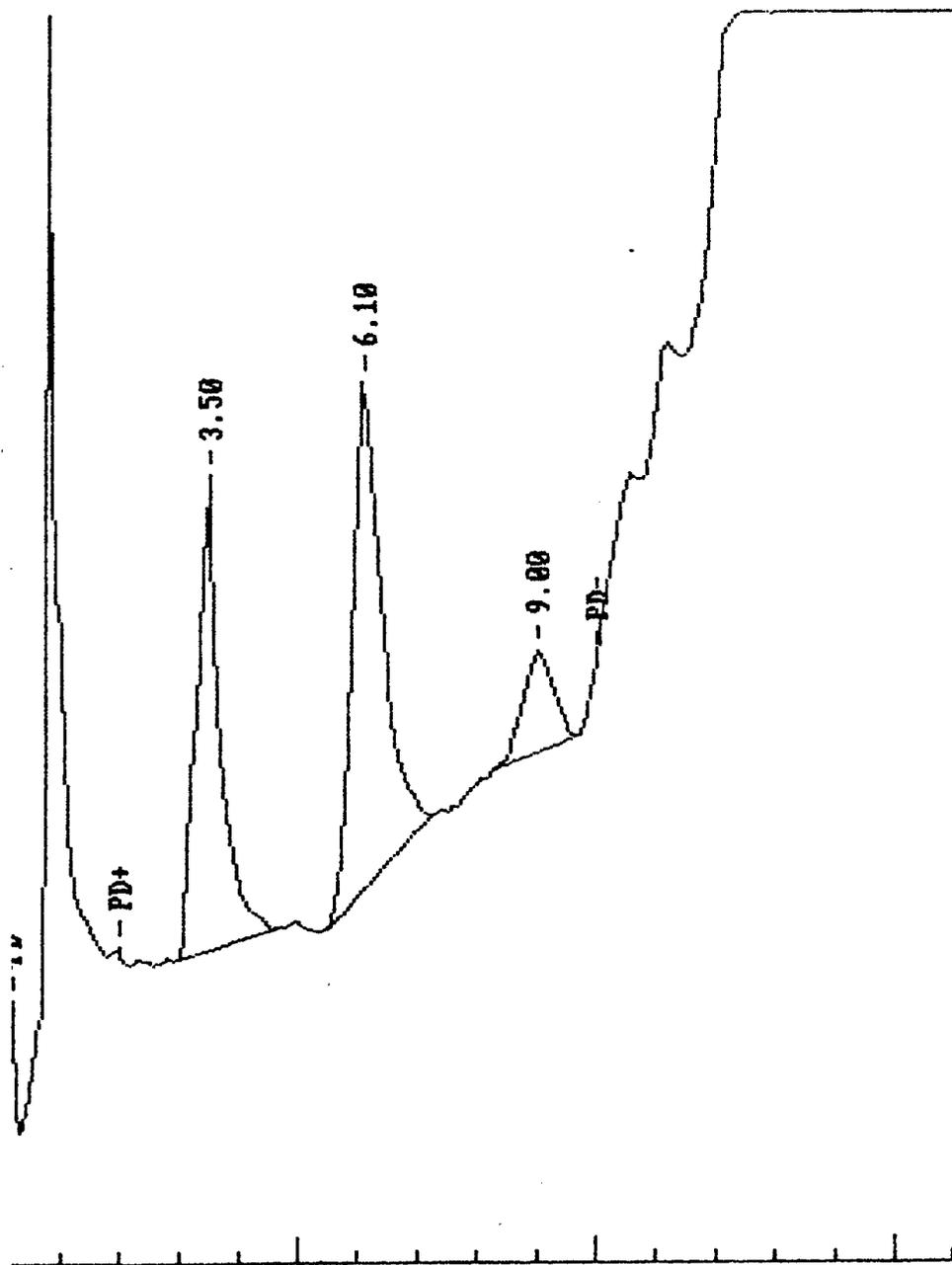


Fig 2. GC tracing of Galden HT-70 at 1.0 mg/L. Values represent retention times in minutes. The tracing was recorded over 16 min using a range of 10 mV.

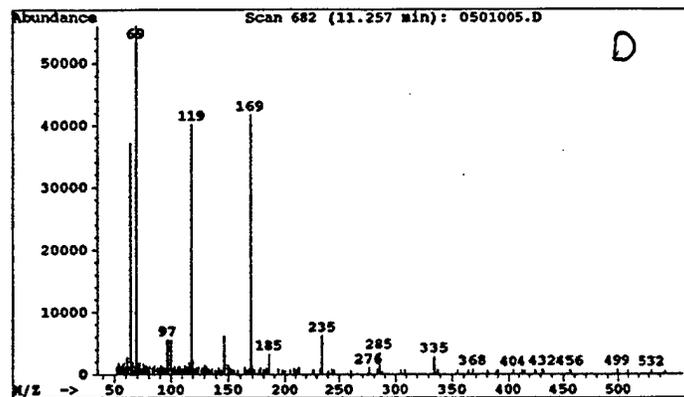
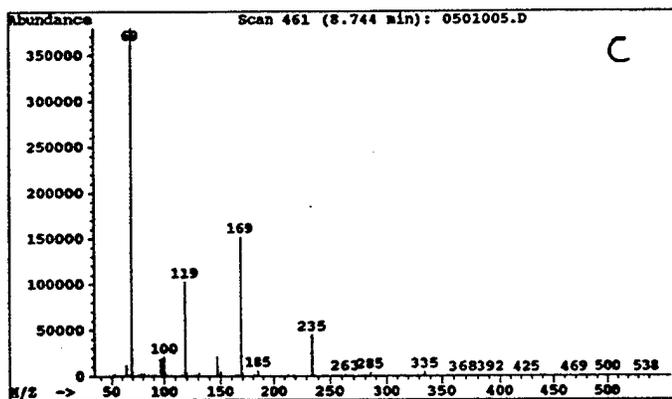
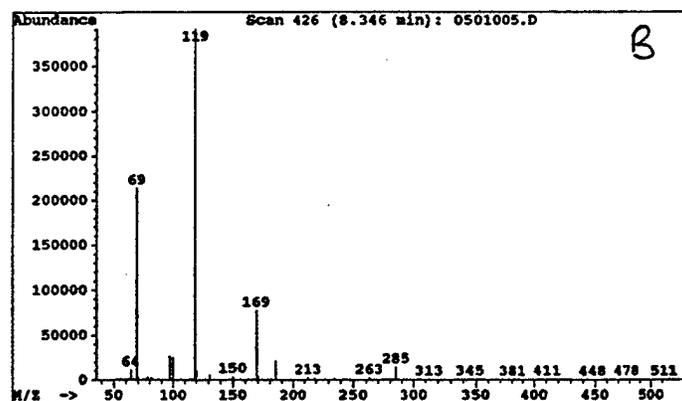
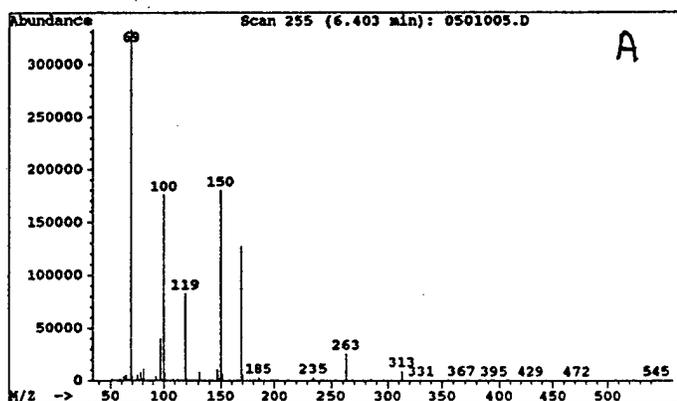
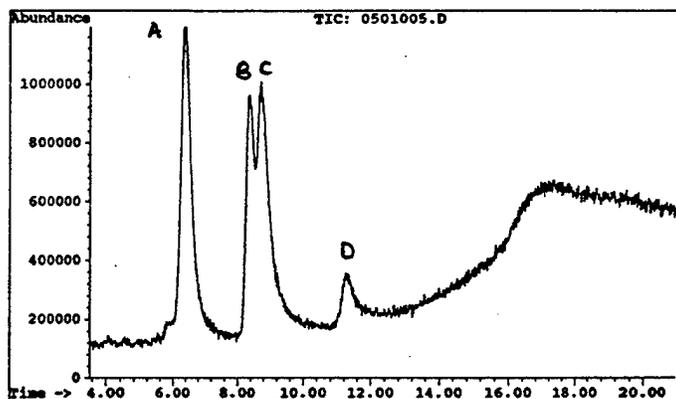


Fig 3. GC/MS tracing and spectra of Galden HT-70 at 1.0 mg/L. Four peaks were identified with similar spectra. The double peak at ~ 8.5 min corresponds to the single GC peak of Fig 2 at 6.1 min.

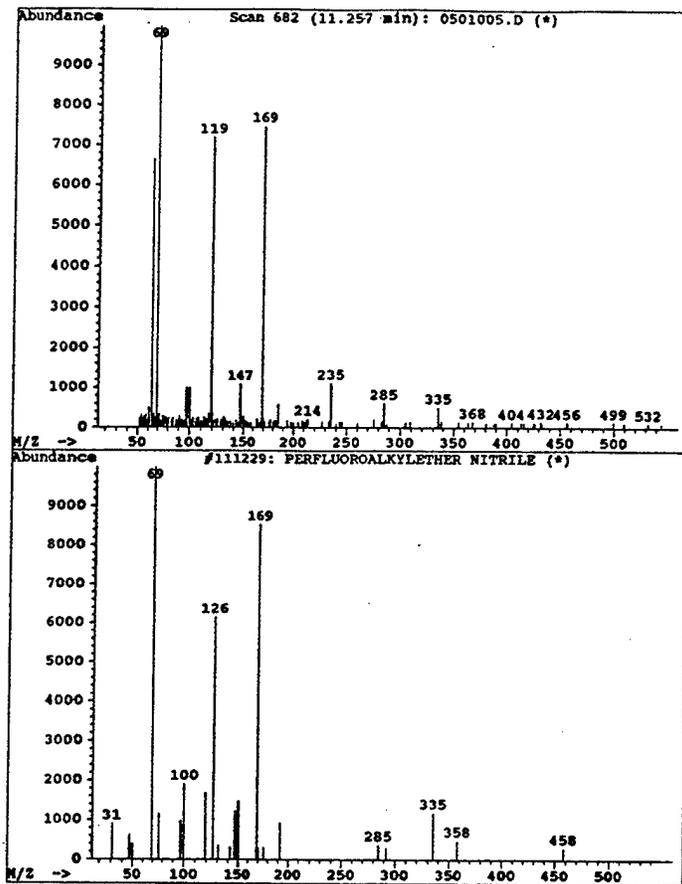
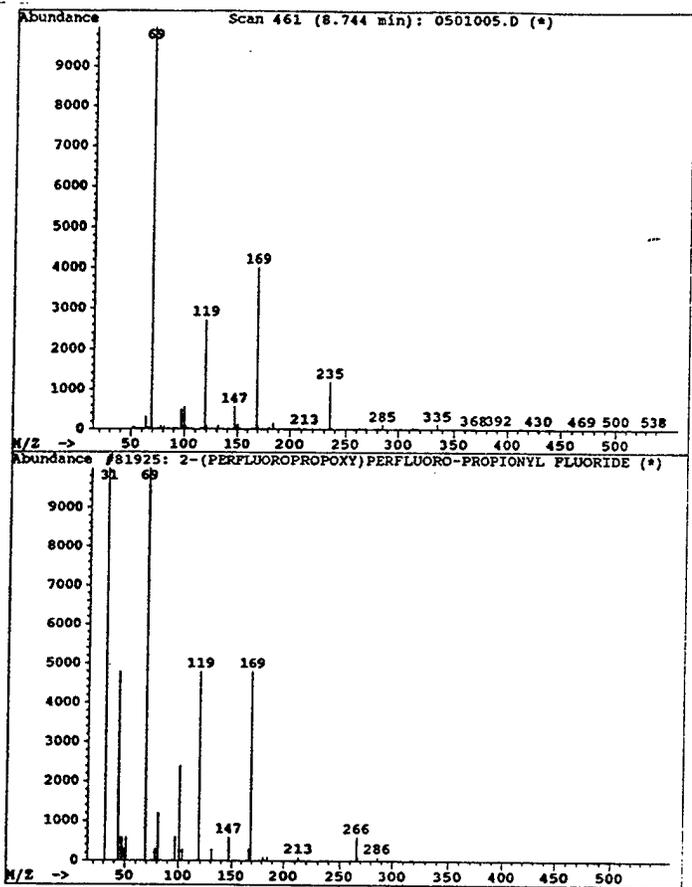


Fig 4. Mass spectra of GC/MS library match to various Galden HT-70 peaks. Top; Galden HT-70 (retention times in parentheses). Bottom; Identification of compound with similar mass spectra.

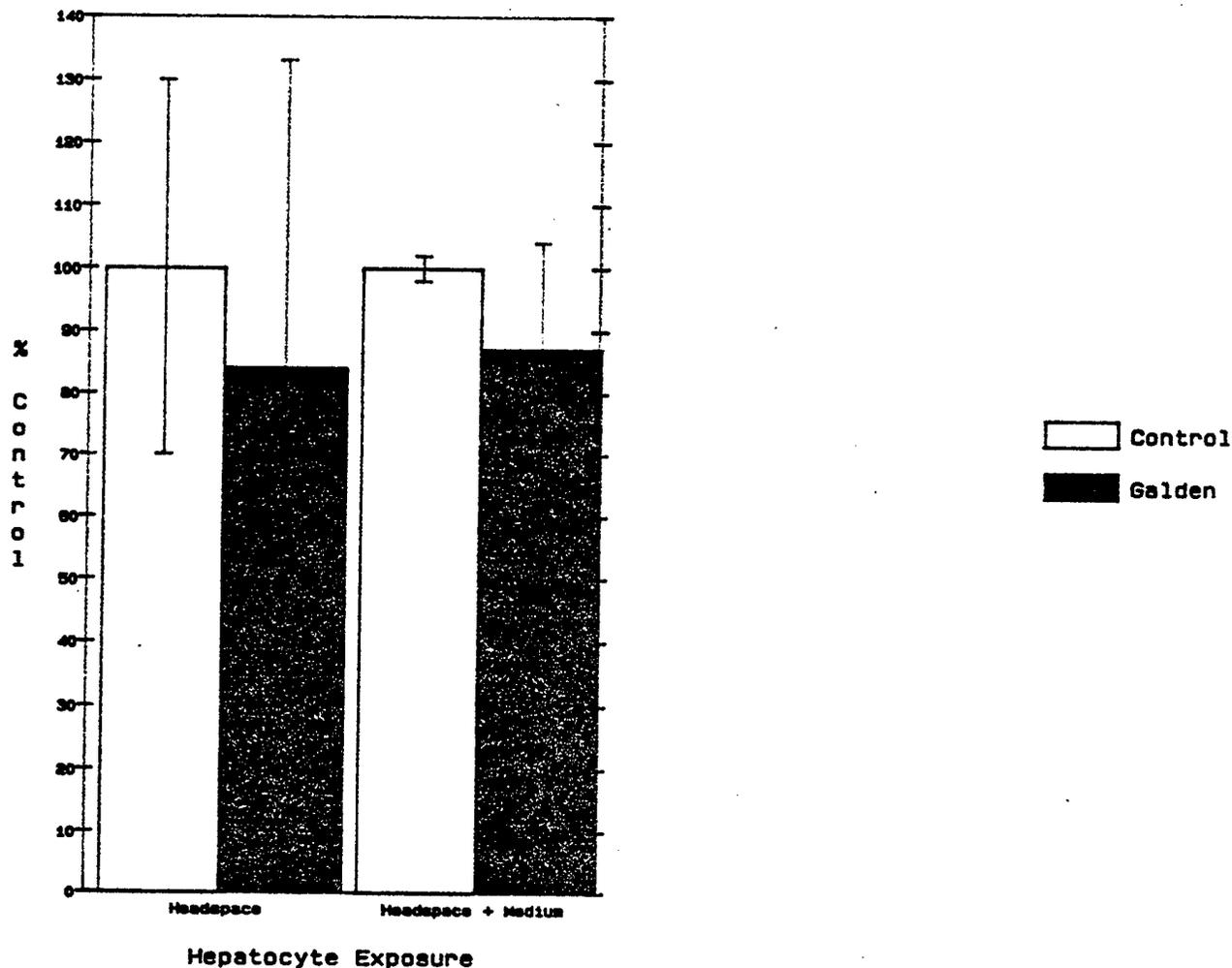


Fig 5. Percent of control LDH leaked by primary rat hepatocytes exposed to Galden vapor in the headspace or in the headspace and medium (30 ± 4 mg/ml). The headspace of exposure chambers were flushed with Galden vapor (1 ml/L 5%CO₂/air). Cultures were incubated for 24 h at 37°C. Control LDH leakage was less than 10% of the total available.

Table 1

Galden HT-70 Concentration in Saturated Tissue Culture Medium Over 72 H

Sample	Medium Conc (mg/ml)	Mean	StDev (mg/ml)	(mg/ml)
24 h	81.0	87.2	±8.6	
	78.7			
	92.6			
	96.4			
48 h	87.3	81.5	±6.0	
	74.9			
	89.0			
	76.5			
	83.6			
	77.4			
72 h	86.6	86.0	±2.1	
	83.3			
	88.7			
	84.6			
	86.7			

Data derived from 4 to 6 samples taken from the same bottle of saturated William's E culture medium. Medium was saturated by adding 1.0 g of Galden to 100 ml of medium. Medium was held at 37°C over the 72 h period.

Table 2

Galden HT-70 Concentration in Culture Medium in In Vitro Micro Rollerbottle Exposure
Chambers at 4 and 24 H

Sample	Medium Conc (mg/ml)	Mean	StDev (mg/ml)	(mg/ml)
4 h	42			
	36			
	31		42	±9
	51			
	50			
24 h	29			
	28			
	34		30	±4
	25			
	29			
	35			

Data derived from a sample taken from each of 5 to 6 different rollerbottles containing saturated William's E culture medium. Headspace of rollerbottles were flushed with Galden vapor (1 ml/L in 5% CO₂/air). Rollerbottles were incubated at 37°C for 24 h with samples taken at 4 and 24 h.