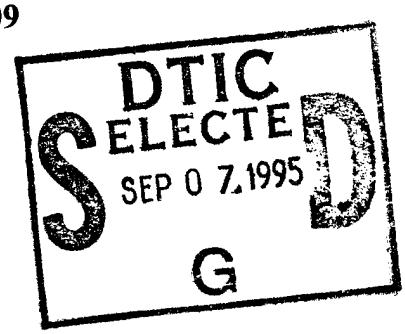
AL/OE-TR-1994-0149

ARMSTRONG LABORATORY

METABOLISM OF 2,2-DICHLORO-1,1,1 TRIFLUOROETHANE (HCFC-123) BY HUMAN HEPATIC MICROSOMES

C. S. Godin
M. M. Ketcha
J. M. Drerup
A. Vinegar

MANTECH ENVIRONMENTAL TECHNOLOGY INC.
P. O. BOX 31009
DAYTON, OH 45437-0009



November 1994

FINAL REPORT FOR THE PERIOD AUGUST 1992 THROUGH APRIL 1993

Approved for public release; distribution is unlimited

AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-7022

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0149

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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 COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1994	Final -August 1992 - April 1993

4. TITLE AND SUBTITLE
Metabolism of 2,2-Dichloro-1,1,1-Trifluoroethane (HCFC-123) by Human Hepatic Microsomes

5. FUNDING NUMBERS
Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630200
WU 63020002

6. AUTHOR(S)
C.S. Godin, M.M. Ketcha, J.M. Drerup, and A. Vinegar

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING;MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1994-0149

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

As part of its safety evaluation, the *in vitro* metabolism of 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), a replacement candidate for Halon 1211, by human hepatic microsomes, was assessed. Microsomal incubations containing HCFC-123 ranging from 6 to 75% (v/v) in the headspace produced increasing amounts of trifluoroacetic acid (TFA); the kinetics suggested substrate-saturation although substrate inhibition was apparent above a concentration of 36%. The rate of TFA formation with respect to pH, time, and protein concentration permitted linear rates of formation to be determined. Rates of TFA formation from incubations conducted at physiological pH, and containing concentrations of HCFC-123 in solution representing estimated concentrations of the chemical in human liver at steady-state, were 67% of those obtained under optimal conditions.

14. SUBJECT TERMS
Halocarbon
Human Metabolism
1,1-dichloro-2,2,2-trifluoroethane
HCFC-123
in vitro

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

This document serves as a final technical report describing the results of the *in vitro* metabolism, by human hepatic microsomes, of 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), a replacement candidate for Halon 1211. This work was conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. The research described herein began in August 1992 and was completed in April 1993. It was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F08). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Occupational and Environmental Health Directorate, Armstrong Laboratory, Toxicology Division.

The authors thank Dr. Michael Olson, General Motors Research Laboratory, Warren, MI, for helpful suggestions and advice. The authors also thank Drs. K. Allen and C. Tyson, Stanford Research Institute, Menlo Park, CA, for assistance in obtaining the human tissues used in this research.

TABLE OF CONTENTS

ABBREVIATIONS

CFC	Chlorofluorocarbon
HCFC-123	2,2-Dichloro-1,1,1-trifluoroethane
PBPK	Physiologically based pharmacokinetic
TFA	Trifluoroacetic acid

SECTION 1

INTRODUCTION

Increasing atmospheric levels of chlorofluorocarbons (CFCs) has resulted in stratospheric ozone depletion (1). As a result, the Montreal Protocol of 1987 called for a phaseout of CFCs by the year 2000. One of these CFCs, Halon 1211, is commonly used as a fire extinguishant. One candidate considered as a replacement for Halon 1211 is 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), an analog of the anesthetic halothane. The metabolism of halothane has been described both *in vivo* and *in vitro* (2,3) but the metabolism of HCFC-123 has not been as extensively studied, and no metabolism studies in humans have been reported. A single *in vivo* metabolism study, in which rats were exposed to 1% HCFC-123 for 2 h, has shown that HCFC-123 is oxidatively metabolized to trifluoroacetic acid (TFA). In that study levels of trifluoroacetylated liver proteins were nearly identical to those detected after an exposure to 1% halothane for 2 h (4). These liver protein adducts are formed from a trifluoroacetyl halide intermediate arising from the oxidative metabolism of the substrate and are believed to be involved in the development of halothane-induced hepatitis in humans (5,6). Because the structure of HCFC-123 is similar to that of halothane, and because the potential for environmental and occupational exposure to HCFC-123 exists, the rates of HCFC-123 metabolism by human hepatic microsomes were assessed as part of the safety assessment of this chemical.

SECTION 2

EXPERIMENTAL

HCFC-123 (CAS No. 306-83-6) was supplied by Allied Signal Inc. (Morristown, NJ) and was found to contain about 5% 1,2-dichloro-1,1,2-trifluoroethane as an impurity. All other reagents were obtained from Sigma Chemical Co. except as specified. The human liver specimens used in this study were obtained from Stanford Research Institute, Menlo Park, CA. Liver specimens from organ donors were stored at -135 °C, shipped frozen, and stored at -70 °C until used for the preparation of microsomes. Prior to the original acquisition of the livers, the organs had been perfused in preparation for possible organ transplant. The livers were negative for the presence of hepatitis A and B viruses as well as human immunodeficiency virus.

Livers were thawed at room temperature and homogenized in 4 vol of ice-cold 0.154 M KCl/0.05 M Tris-HCl (pH 7.4). The homogenate was centrifuged at 4 °C at 500 and 10,000 xg for 10 min each. The supernatant fraction obtained after the final spin was centrifuged at 104,000 xg for 60 min at 4 °C and the microsomal pellet was washed with and resuspended in the 0.154 M KCl/0.05 M Tris-HCl(pH 7.4) prior to storage at -80 °C. Protein concentrations were determined by the BCA method (Pierce Chemical Co., Rockford, IL). The cytochrome P450 content of sodium dithionite-reduced microsomes in 0.1M Tris-HCl (pH 7.6) was determined by the method of Omura and Sato (7).

Initial experiments were conducted to establish conditions leading to linear reaction rates of TFA formation with respect to time and protein concentration. Experiments also were conducted to determine the optimal pH and saturating substrate concentration. Microsomal incubations were conducted in 25 ml Erlenmeyer flasks sealed with Teflon-lined silicon septa. The headspace atmosphere was prepared by mixing compressed air and nitrogen with gas-flow controllers (Dwyer Instruments, Michigan City, IN) to yield an oxygen concentration of 5%. The headspace of each flask was purged with this gas mixture, which was chosen because the hepatic vein of humans contains approximately 4 to 5% oxygen (8). Microsomes were thawed, diluted with 0.1 M Tris buffer (pH 7.0) to give the appropriate protein concentration, and bubbled for 2 min with the same gas mixture used to purge the flasks. A 2-ml volume of the diluted microsomal suspension was added to each flask with a gas-tight syringe, HCFC-123 was added to the headspace of each flask with a Hamilton syringe, and the flasks were preincubated at 4 °C with vigorous shaking for 15 min. The reactions were initiated by the injection of 25 μl of ß-NADPH solution through the septum to yield a final concentration of 1 mM,

and terminated after the appropriate incubation period by rapidly heating the flasks to 60 °C.

For physiological metabolism studies, incubations were conducted as described above. Incubations contained 2 mg/ml of human hepatic microsomal protein in 0.1 M Tris buffer adjusted to a pH of 7.37. HCFC-123 (7.3 μl) was added to the flasks that were incubated for 7 min following the addition of ß-NADPH. This amount of HCFC-123 resulted in a 1.13 mM concentration in solution as determined from determining the partitioning of HCFC-123 into the reaction mixture. This concentration (1.13 mM) was derived from physiologically based pharmacokinetic (PBPK) estimates of the steady-state concentration of HCFC-123 in human liver following an exposure to 1% (v/v) of HCFC-123.

The supernatants of all incubations were analyzed for TFA by derivitization to form volatile methyl esters using the method of Maiorino et al. (9). The chromatographic separation of the TFA-methyl ester was conducted according to the method described by Brashear et al. (10).

SECTION 3

RESULTS

As shown in Figure 1A, the optimal pH value was approximately 7.0 for the oxidative metabolism of HCFC-123 to TFA. Therefore, 0.1 M Tris buffer at this pH was used for all subsequent optimization experiments. The rate of TFA formation with respect to time is presented in Figure 1B. The rate was approximately linear for the first 7 min, and became distinctly nonlinear between 7 and 60 min. A time of 7 min was chosen for determination of optimal rates of TFA formation. The effect of increasing amount of microsomal protein is presented in Figure 1C. The reaction is apparently nonlinear over the entire range of concentrations but a concentration of 2 mg/ml was chosen for determination of optimal rates of TFA formation.

When HCFC-123 was introduced into the headspace of the flasks, the amount of TFA formed (normalized per mg of microsomal protein) increased in response to increasing halocarbon concentration (Figure 2) up to a concentration of 36% (v/v). However, the relationship was nonlinear indicating possible substrate saturation. Above a concentration of 36% in the headspace, the rate of TFA formation was apparently suppressed. Although there is no clear evidence of substrate saturation, a double reciprocal transformation of the first 4 data points on this curve suggested an apparent maximum velocity of 4 nmoles TFA/mg protein/20 min and a half-maximal substrate concentration of 2.9% (v/v) HCFC-123 (Figure 2 inset).

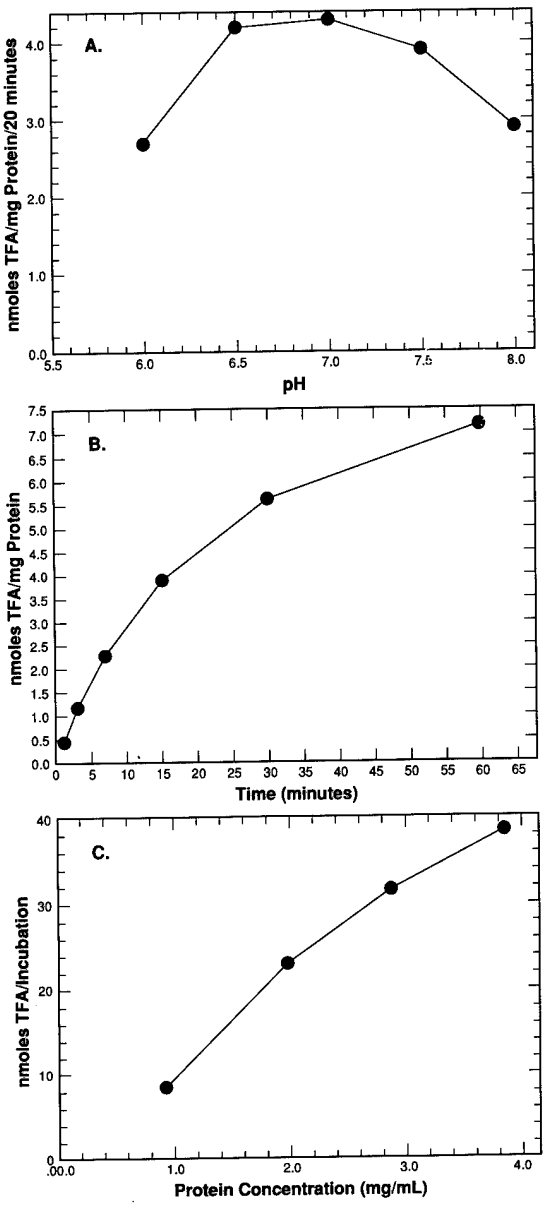


Figure 1. (A) Effect of pH on the rate of TFA formation from HCFC-123 in human hepatic microsomes. Data are from a single experiment. (B) Time course of the formation of TFA from HCFC-123 in human hepatic microsomes. Data are from a single experiment. (C) Effect of human hepatic microsomal protein concentration on the formation of TFA from HCFC-123. Data are from a single experiment.

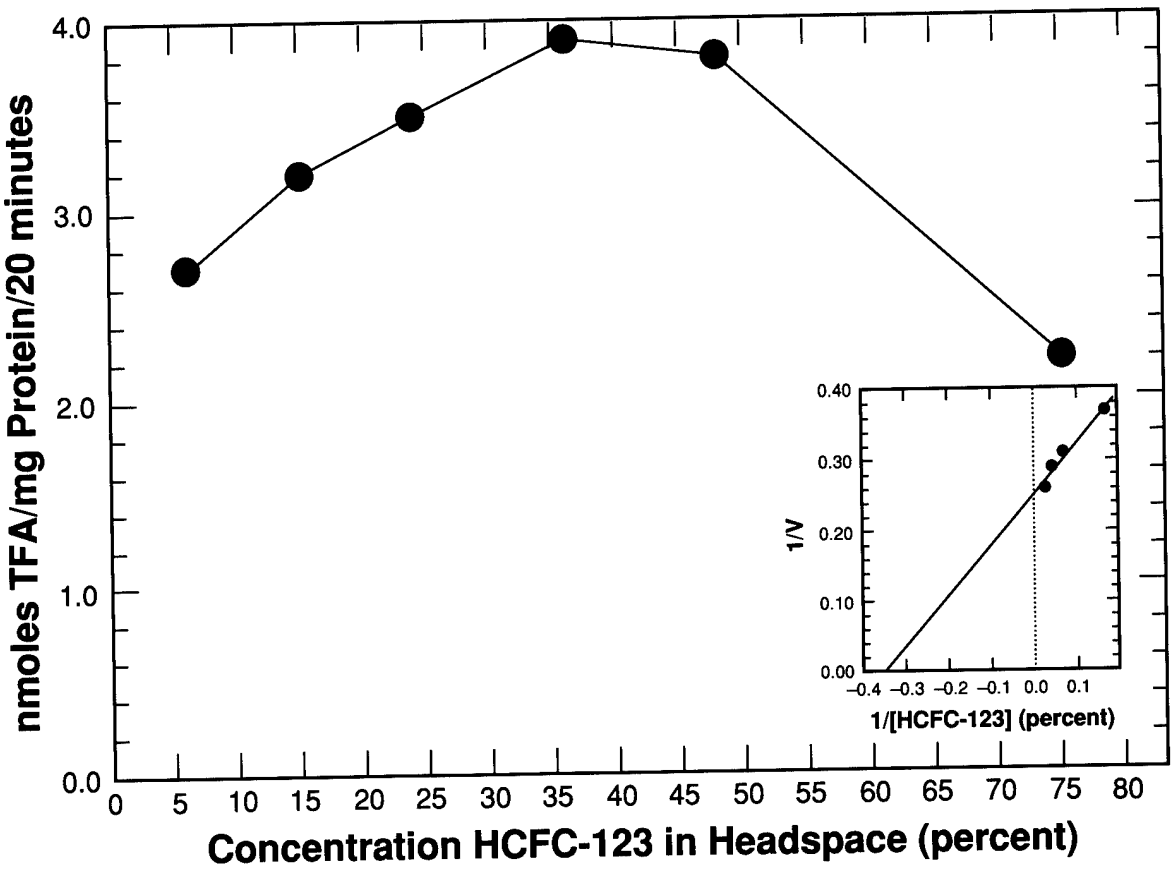


Figure 2. Substrate-dependent kinetics of TFA formation from HCFC-123 by human liver microsomes. Data are from a single experiment. Inset; double reciprocal plot of the first 4 points. (Apparent maximum rates and half-maximal substrate concentrations are provided in the RESULTS section).

The linear rates of TFA formation from HCFC-123 conducted under optimal and physiological conditions are presented in Table 1. Under conditions of

physiological pH, and with substrate concentrations in the liver representing those expected under steady-state conditions, the metabolism of HCFC-123 to TFA was approximately 67% of that observed under optimal conditions.

Table 1 - Comparison of Physiological and Optimal Rates of TFA Formation

Subject	Cytochrome P450 (nmole/mg protein)	Rate of product formation (nmoles/mg protein/min)[a]	
		Optimal	**Physiological**
H-27	1.01	0.41 (0.39,0.43)	0.27 (0.29,0.24)
H-62	0.59	0.33 (0.34,0.32)	0.24 (0.20,0.28)
H-64	0.44	0.24 (0.20,0.27)	0.14 (0.15,0.13)
Mean ± SD	0.68 ± 0.30	0.33 ± 0.09	0.22 ± 0.07

[a] Values represent the average of duplicate incubations conducted on separate days. The individual values for each subject are given in parentheses.

SECTION 4

DISCUSSION

The biotransformation of halothane has been reported in several animal species *in vitro* (11,12,13). Although studies describing the metabolism of halothane by humans *in vivo* have been reported (14,15), there are only limited reports concerning the metabolism of halothane by humans *in vitro* (11). The metabolism of HCFC-123 results in the qualitative production of the same metabolites afforded by the metabolism of halothane (4,10,16), however there have been no reports, to our knowledge, of the metabolism of HCFC-123 by humans either *in vivo* or *in vitro*. Thus, the results of this study provide the first indication that HCFC-123 is metabolized by the human.

Gruenke et al. (11) examined the rate of TFA formation from human hepatic microsomes exposed to a concentration of 0.6% (v/v) halothane in the headspace and obtained a rate of 2.05 nmol TFA/mg protein/30 min. In the present study, 5.6 nmol TFA/mg protein/30 min was obtained in incubations containing 36% (v/v) HCFC-123 in the headspace. However, a rate of 1.7 nmol TFA/mg protein/20 min has been obtained in our laboratory from an incubation of human hepatic microsomes containing 0.6% (v/v) HCFC-123 in the headspace indicating that the two substrates are probably metabolized to the same extent *in vitro* (Godin, unpublished observation). Thus the findings reported in the present study are significant because they indicate that humans are capable of producing TFA from HCFC-123 at rates similar to those reported for halothane. The extent to which this reaction occurs in humans *in vivo* is unknown but is important in light of the hepatotoxicity associated with halothane metabolism. Trifluoroacetyl halide intermediates are produced from halothane *in vivo* and form covalent adducts with several hepatic proteins (5,6). Humans develop serum antibodies that have been shown to recognize these adducts (5,6), and their reaction has been linked to the onset of halothane-induced hepatitis. Products from the metabolism of halothane also are thought to be responsible for the hepatotoxicity observed in up to 20% of patients anesthetized with halothane (5,17). The metabolism of HCFC-123 also affords the production of trifluoroacetylated adducts (4), but it must be pointed out that the system used in this study places an enriched cell fraction in direct contact with the chemical in solution. Whereas a PBPK prediction of liver steady-state concentrations of HCFC-123 in humans following an exposure to a 1% (v/v)

atmosphere suggested that the incubations should be conducted at this same concentration it is unlikely that humans would be exposed to a high enough concentration of HCFC-123, and for sufficient periods of time to result in a similar internal concentration. Although the amount of adduct formation resulting from brief accidental exposure may be low, there is currently no information on the amount of adduct required to induce an immune response. In sensitized individuals therefore, hepatitis may develop after subsequent exposure to HCFC-123 or after anesthesia with halothane but this risk cannot be assessed.

SECTION 5

REFERENCES

1. Molina, D.M., and Rowland, F.S. (1974) *Nature* 249, 810-812.

2. Van Dyke, R.A., and Gandolfi, A.J. (1976) *Drug Metab. Dispos.* 4, 40-44.

3. Cousins, M.J., Sharp, J.H., Gourlay, G.K., Adams, J.F., Haynes, W.D., and Whitehead, R. (1979) *Anaesth. Intens. Care* 7, 9-24.

4. Harris, J.W., Pohl, L.R., Martin, J.L., and Anders, M.W. (1991) *Proc. Natl. Acad. Sci. USA* 88, 1407-1410.

5. Pohl, L.R., Kenna, G., Satoh, H., Christ, D.D., and Martin, J.L. (1989) *Drug Metab. Rev.* 20, 203-217.

6. Satoh, H., Martin, J.M., Schulick, A.H., Christ, D.D., and Kenna, J.G. (1989) *Proc. Natl. Acad. Sci. USA* 86, 322-326.

7. Omura, T., and Sato, R. (1964) *J. Biol. Chem.* 239, 2370-2378.

8. Nauck, M., Wolfle, D., and Katz, N. (1981) *Eur. J. Biochem.* 119, 657-661.

9. Maiorino, R.M., Gandolfi, A.J., and Sipes, I.G. (1980) *J. Anal. Toxicol.* 4, 250-254.

10. Brashear, W.T., Ketcha, M.M., Pollard, D.L., Godin, C.S., Leahy, H.F., Lu, P.P, Kinkead, E.R., and Wolfe, R.E. (1992) AL-TR-1992-0078, Armstrong Laboratory, Wright-Patterson Air Force Base, Ohio.

11. Gruenke, L.D., Konoptka, K., Koop D.R., and Waskell, L.A. (1988) *J. Pharmacol. Exp. Ther.* 246, 454-459.

12. Ghantous, H.N., Fernando, J., Gandolfi, A.J., and Brendel, K. (1990) *Drug. Metab. Dispos.* 18, 514-518.

13. Nakao, M., Fujii, K., Kinoshita, H., Yuge, O., and Morio, M. (1991) *Hiroshima J. Med.* Sci. 40, 23-28.

14. Rehder, K., Forbes, J., Alter, H., Hessler, O., and Stier, A. (1967) *Anesthesiology* 28, 711-715.

15. Cohen, E.N., Trudell, J.R., Edmunds, H.N., and Watson, E. (1975) *Anesthesiology* 43, 392-401.

16. Godin, C.S., Drerup, J.M, and Vinegar, A. (1993) *Drug Metab. Dispos.* in press.

17. Wright, R., Eade, O.E., Chisholm, M., Hawksley, M., Lloyd, B., Moles, T.M., Edwards, J.C., and Gardner, J.M. (1975) *Lancet* 1, 817-820.

U. S. Government Printing Office 1995 650-075/00093