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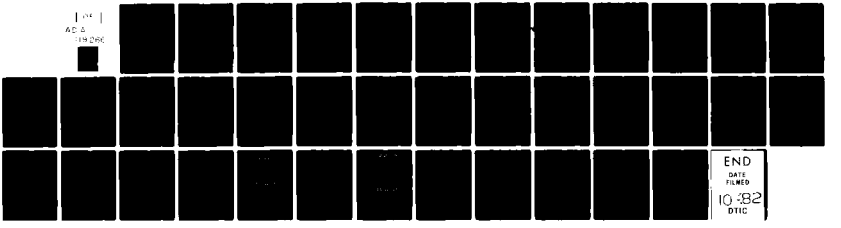

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INTERIM SCIENTIFIC REPORT

ON

MODELING OF INHALATION ADMINISTRATION OF VAPORS WITH CAPACITY LIMITED CLEARANCE

AFOSR Grant No. 81-0210

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June 30, 1981 to June 30, 1982.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Factors, affecting dependence of metabolic clearance of vapors on exposure concentration were investigated. Mathematical solution of multi-compartmental simulation model with one nonlinear elimination pathway was found and programmed for small computer and used for prediction of uptake, distribution and elimination of vapors inhaled under a variety of exposure conditions.

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TABLE OF CONTENTS

SUMMARY

The main achievements between June 30, 1981 and June 30, 1982 are:

1. A program for mathematical solution of a multi-compartmental model for simulation of uptake, distribution, and elimination of vapors having a capacity limited elimination pathway was prepared for the Apple II Plus computer and tested by simulating a variety of trichloroethylene exposures. The model accommodates up to 7 compartments, with optional linear elimination in each compartment and an additional non-linear elimination in one compartment.

2. Using rabbit tracheas we showed that vapors of water soluble substances only (not gases or other vapors) are retained on the walls of respiratory airways during inspiration and are desorped during expiration. Because of this, pulmonary uptake measured from the difference in concentrations of inhaled and exhaled air does not represent systemic uptake, and concentrations measured in end exhaled air do not represent alveolar concentrations, thus explaining why experimental data differ from data predicted for water soluble vapors.

3. We developed a method for determining intrinsic organ clearance in small animals, based on the assumption that during steady state, concentrations in non-excretory organs are equilibrated with arterial blood and the concentration in regional venous blood is equilibrated with the concentration in the appropriate excretory organ. Intrinsic clearance is then calculated from the mass balance across the organ.

4. We showed in rats exposed to non-effective concentrations of halothane that halothane metabolism is a capacity limited process and that anaerobic metabolic pathway is enhanced when oxidative pathway reaches saturation. Halothane oxidation is also inhibited in simultaneous exposure to isoflurane or methylene chloride. Nitrous oxide has no effect on halothane metabolism.

INTRODUCTION

The overall objective of the project is to design economical and informative testing of subacute and chronic toxicity of new volatile substances. The specific objectives are: 1) to prepare a mathematical model for simulation of uptake, distribution, and elimination of vapors having capacity-limited clearance 2) to obtain experimental data supporting the model. 3) to study the factors affecting nonlinearity of clearance (concentration dependence, interference of inhalation of other vapors). The project was planned for two years.

This report describes research conducted in our laboratory during the first year of the project (June 30, 1981 and June 30, 1982). During the first year our efforts concentrated on two items: 1) the preparation of mathematical solutions of a multi-compartmental simulation model describing uptake, distribution and elimination of inhaled vapors having one capacity-limited elimination pathway. 2) the finishing of the manuscript of the monograph entitled "Modeling of Uptake, Distribution, and Elimination of Vapors" to be published in two volumes by CRC press in January of 1983. The principal investigator is the editor and major contributor. One copy of the manuscript of each chapter authored by the principal investigator and of the chapter authored by Dr. J. Vlach were submitted to AFOSR in January 1982, when funds for the second year were requested. These chapters include data generated by AFOSR supported projects, acknowledgement of which is made in the preface

of this book.

This interim scientific report is divided into two parts: Computer simulation, and animal studies.

COMPUTER SIMULATION:

The basic program for Apple II computer for solving the non-linear multi-compartment model was developed in collaboration with Dr. Vlach. This specific model accommodates up to seven compartments with optional linear elimination in each compartment and an additional non-linear elimination in one compartment. Non-linear elimination is considered a saturable process described by an equation similar to the Michaelis-Menten equation for enzymatic reactions (refered to as capacity-limited elimination). The print-out of the computer program is on the next pages.

USER'S MANUAL

DATA STATEMENTS: The information on compartments is given by data statements 2-36.

Statement 2: Indicates number of compartments.

Statements 4-10: are reserved for the description of the compartments. Each statement gives information on one compartment. The order of compartments is arbitrary, with two exceptions: 1) the first statement (statement 4) must relate to the compartment with capacity-limited elimination. 2) the last statement must relate to lung compartment.
Three items of information are given in each statement.

Program Non-Linear

Program can have non-linearity defined by Michaelis-Menten equation in the first compartment.

```
1  REM  NUMBER OF COMPARTMENTS
2  DATA  7
3  REM  G,GX,C
4  DATA  15.2,0,65
5  DATA  11.4,0,4.5
6  DATA  6.6,0,31.5
7  DATA  9.5,0,58
8  DATA  24,0,653
9  DATA  4.2,0,6120
10  DATA  6,0,11.1
15  REM  INITIAL CONDITIONS,(TIS.CONC./PART.COEFF.)
16  DATA  0,0
17  DATA  0,0
18  DATA  0,0
19  DATA  0
25  REM  UMAX,KM
26  DATA  325,8
28  REM  STEP IN MINUTES
29  DATA  .2
35  REM  REQUIRED PRECISION
36  DATA  0.01
200  REM  ITERATIONS TERMINATED
205  DIM U(7),UN(7),UOLD(7),F(7),A(7,7)
210  DIM G(7),GX(7),C(7),DX(7)
215  DIM CU(2),CO(2),XI(3)
220  INPUT " WANT PRINTER ? (Y/N)";R$
225  IF R$ = "Y" THEN  PRINT "PR#1"
230  PRINT General information on simulation study
235  INPUT " DATE: ";A$
240  INPUT "OBJECTIVE:  ";B$
245  INPUT " EXPOSED SUBJECT: ";C$
250  INPUT "COMPOUND: ";S$
255  PRINT : PRINT
260  READ N  Read in and print-out the parameters
265  PRINT "NUMBER OF COMPARTMENTS=",N
270  PRINT "G(I)          GX(I)          C(I)"
275  FOR I = 1 TO N
280  READ G(I),GX(I),C(I)
285  PRINT G(I),GX(I),C(I)
290  NEXT I
295  REM  INITIAL CONDITIONS
300  PRINT "INITIAL CONDITIONS"
305  FOR I = 1 TO N STEP 2
310  IF I = N THEN  READ UOLD(I): GOTO 320
315  READ UOLD(I),UOLD(I + 1)
320  IF I = N THEN  PRINT UOLD(I): GOTO 335
325  PRINT UOLD(I),UOLD(I + 1)
330  NEXT I
335  READ UMAX,KM
340  PRINT "UMAX="UMAX,"KM="KM
345  READ H
350  PRINT "STEP SIZE IN MIN.",H
355  READ EPS
360  PRINT "PRECISION =      ",EPS
```

```
365  REM  INITIALIZE VOLTAGES U(I)
370  FOR I = 1 TO N
375 U(I) = UOLD(I)
380  NEXT I
385  REM  INITIALIZE INTEGRALS
390  FOR I = 1 TO 3
395 XI(I) = 0.
400  NEXT I
405  REM  SET JACOBIAN WITHOUT NONLINEARITY
410 NM1 = N - 1
415  FOR I = 1 TO N
420  FOR J = 1 TO N
425 A(I,J) = 0
430  NEXT J
435  NEXT I
440  FOR I = 1 TO NM1
445 A(I,I) = G(I) + GX(I) + C(I) / H
450 A(I,N) =  - G(I)
455 A(N,I) =  - G(I)
460 A(N,N) = A(N,N) + G(I)
465  NEXT I
470 A(N,N) = A(N,N) + G(N) + C(N) / H
475  REM  SET INITIALTIME AND STEP COUNTER
480 T = 0.
485 IST = 0
490 DOLD = 0.
495 HH = H / 2.
500  REM  CO=OLD CURRENTS FOR INTEGRALS
505 CO(1) = 0.
510 CO(2) = 0.
515  REM  TIME STEPPING STARTS HERE
520  PRINT "PR#0"
525  INPUT "WANT PRINTER ? (Y/N)                ";R$
530  IF R$ = "Y" THEN  PRINT "PR#1"
535  PRINT
540  INPUT "SIMULATION DURATION IN MIN =      ";TE
545  INPUT "EXPOSURE CONCENTRATION      =      ";E
550  INPUT " PRINT AFTER STEP                   ";NPR
555 T = T + H
560 IST = IST + 1
565  REM  PREDICT UN(I)
570  FOR I = 1 TO N
575 UN(I) = 2. * U(I) - UOLD(I)
580  NEXT I
585  REM  NEWTON ITERATION STARTS HERE
590 IT = 0
595 IT = IT + 1
```

```
800  REM  RHS VECTOR WITHOUT NONLINEARITY
805 F(N) = 0.
810  FOR I = 1 TO NM1
815 U1 = (UN(I) - UN(N)) * G(I)
820 F(I) =  - U1 - GX(I) * UN(I) - C(I) * (UN(I) - U(I)) / H
825 F(N) = F(N) + U1
830  NEXT I
835 F(N) = F(N) - G(N) * (UN(N) - E) - C(N) * (UN(N) - U(N)) / H
840  REM  ADD NONLIN. CONTRIBUTION
845 U1 = XM + U(1)
850 CX = UMAX * U(1) / U1
855 F(1) = F(1) - CX
860  REM  MODIFY THE ENTRY OF THE JACOBIAN
865 U1 = U1 * U1
870 DN = UMAX * XM / U1
875 A(1,1) = A(1,1) - DOLD + DN
880 DOLD = DN
885  REM  SOLVE THE NEWTON EQUATION
890  GOSUB 945
895  REM  OBTAIN NEW VOLTAGES
900  FOR I = 1 TO N
905 UN(I) = UN(I) + DX(I)
910  NEXT I
915  REM  CHECK CONVERGENCE
920 ER = 0.
925  FOR I = 1 TO N
930 R =  ABS (DX(I))
935  IF R > ER THEN ER = R
940  NEXT I
945  IF IT = 20 THEN  PRINT "REDUCE STEPSIZE OR INCREASE PRECISION"
950  IF IT = 20 THEN  STOP
955  IF ER < EPS THEN  GOTO 980
960  GOTO 595
965  REM  ITERATIONS TERMINATED
970  REM  TRANSFER THE RESULTS
975  REM  CALCULATE CURRENTS AND INTEGRALS
980  FOR I = 1 TO N
985 UOLD(I) = U(I)
990 U(I) = UN(I)
995  NEXT I
800 CU(1) = (E - U(N)) * G(N)
805 CU(2) = CX
810 U1 = (CO(1) + CU(1)) * HH
815  IF U1 > 0 THEN XI(1) = XI(1) + U1
820  IF U1 < 0 THEN XI(2) = XI(2) - U1
825 U1 = (CO(2) + CU(2)) * HH
830 XI(3) = XI(3) + U1
835 CO(1) = CU(1)
840 CO(2) = CU(2)
```

```
845  REM  ITERATIONS TERMINATED,TRANSFER,RESULTS
850  IF IST < NPR THEN  GOTO 930
855  PRINT
860 IST = 0
865 T% = T + .1
870  PRINT "TIME=",T%
875  PRINT "CONCENTRATION VALUES"
880  FOR I = 1 TO N STEP 2
885  IF I = N THEN  PRINT U(N)
890  IF I < N THEN  PRINT U(I),U(I + 1)
895  NEXT I
900  PRINT "RATES": PRINT "PULM.UPTAKE   ELIMINATION"
905  PRINT CU(1),CU(2)
910  PRINT "TOTAL AMOUNT": PRINT "PULM.UPTAKE   WASH-OUT"
915  PRINT XI(1),XI(2)
920  PRINT "TOTAL ELIMINATED (NONLIN)"
925  PRINT XI(3)
930  IF T >  = TE THEN  GOTO 520
935  REM  GO BACK FOR A NEW STEP
940  GOTO 555
945  REM  LU DECOMP. AND SUBSTITUT. Substitute to solve Newton equation
950  FOR K = 1 TO NM1                                    (call in 690)
955 KP1 = K + 1
960  FOR J = KP1 TO N
965 A(K,J) = A(K,J) / A(K,K)
970  FOR I = KP1 TO N
975 A(I,J) = A(I,J) - A(I,K) * A(K,J)
980  NEXT I
985  NEXT J
990  NEXT K
995 DX(1) = F(1) / A(1,1)
1000  FOR I = 2 TO N
1005 IM1 = I - 1
1010 SUM = 0.
1015  FOR J = 1 TO IM1
1020 SUM = SUM + A(I,J) * DX(J)
1025  NEXT J
1030 DX(I) = (F(I) - SUM) / A(I,I)
1035  NEXT I
1040  FOR K = 1 TO NM1
1045 I = N - K
1050 IP1 = I + 1
1055 SUM = DX(I)
1060  FOR J = IP1 TO N
1065 SUM = SUM - DX(J) * A(I,J)
1070  NEXT J
1075 DX(I) = SUM
1080  NEXT K
1085  RETURN
1090  END
```

The first number, G, indicates the FLOW of the vapor to the compartment. The value of G equals perfusion rate, F, of the compartment multiplied by blood-air partition coefficient, λbl/air. The only exception is the last compartment, for which G equals alveolar ventilation, $\dot{V}_{alv}$.

The second number, G_x, indicates INTRINSIC CLEARANCE of the compartment. The second number is zero in the first compartment (statement 4) if the clearance is capacity limited. The second number also equals zero in any statement describing a compartment with no elimination pathway.

The third number, C, indicates the CAPACITY of the compartment to retain the vapor. The value equals volume of compartment V, multiplied by an appropriate tissue-air partition coefficient. The exception is the value for the last compartment, which is determined by equation (1):

$$C_{lung} = FRC + 1/3\ V_{tid} + V_{lung}\lambda_{lung/air} + \dot{Q}\lambda_{bl/air} \quad (1)$$

Where FRC = functional residual capacity, V_{tid} = tidal volume, V_{lung} = volume of lung parenchymal tissue, $\dot{Q}$ = cardiac output and λ's are appropriate partition coefficients at 37C.

Statements 16-19: are reserved for the definition of starting conditions; that is, for concentration values in each compartment at the beginning of simulation. They must be listed in the same order as compartments are listed in statements 4-10. Each statement accommodates for starting conditions of two compartments. To start the first exposure, the values of

starting conditions are equal to zero or to the normal level of vapor in the compartments. For desaturation, or for an additional exposure, the values of starting conditions equal to concentrations of the vapors in the appropriate compartments divided by appropriate tissue-air partition coefficients (refered to as concentration values). The concentrations, which represent starting points of the simulation, can be obtained experimentally (for example, concentrations measured in tissues of animals sacrificed at the end of exposure define the starting conditions for simulation of desaturation), or can be obtained by simulation of previous exposures as concentration values obtained at the end of the immediately preceding exposure (see output data).

Statement 26: describes the nonlinear elimination in the compartment described in statement 4. The first number, V_{max}, indicates the maximum possible clearance in the compartment (assuming no flow restriction). The second number in statement 26, K_m, indicates the vapor concentration in regional (compartmental) venous blood, at which clearance equals one half of V_{max}.

Statements 29 and 36: define the accuracy of the calculation. In general, the smaller the numbers, the greater the accuracy and the longer the calculation time. Inaccuracy apparent mainly in output data for the first minutes of simulation is reduced with time. If the chosen numbers in statements 29 and 36 are inadequate, the display on the

screen will demand that the value for the STEP (statement 29) is reduced or PRECISION (statement 36) is increased. It is advisable to start wit. two short simulations: in the first simulation, the STEP = 0.5 (statement 29) and PRECISION (statement 36) hundred times smaller than the simulated exposure concentration (statement 545). In the second simulation, STEP is reduced by half. If the output data in both simulations is the same, the values used in the first simulation were correct. If there is a large difference between the two outputs, then STEP must be reduced.

IMPUT STATEMENTS: The imput statements 235-250 describe the experiments (Date, objective, exposed subject, compound). Statement 540 gives a choice of exposure duration, statement 545 gives a choice of exposure concentrations, and statements 220,525, and 550 control the printer.

Statements 220 and 525: give option to print the data. If the first call "WANT PRINTER?" (statement 220) is answered "YES" (press "Y"), then the information given in statements 1-36 will be displayed and printed (see example on page 12). If the answer is "NO" (press "N"), then the data will be only displayed. Statement 525 activates the printer in a similar way as statement 220, giving option to print the output data. If the answer is "Y", then all data which appear on the screen are printed.

Statement 550: this statement controls when the data are displayed and printed. The calculated data are available for display or printing for such short simulation period as defined by "STEP" (Statement 29). However, less frequent display of data is

EXAMPLE OF PRINT-OUT

```
RUN
 WANT PRINTER ? (Y/N)Y
```

IMPUT STATEMENTS 220-250
GENERAL INFORMATION

```
 DATE: 15/6/82
OBJECTIVE:  EFFECT OF EXPOSURE CONCENTRATION
 EXPOSED SUBJECT: NORMAL MAN
COMPOUND: TRICHLOROETHYLENE
```

DATA STATEMENTS 1-36

NUMBER OF COMPARTMENTS= 7

Q(I)	QX(I)	C(I)	$F\lambda_{bl/air}$ Cl $V\lambda_{tis/air}$
15.2	0	65	liver
11.4	0	4.5	kidney
6.6	0	31.5	brain
3.5	0	58	VRG
24	0	653	MG
4.2	0	6120	FG
8	0	11.1	lung,

INITIAL CONDITIONS		$C_{tis}/\lambda_{tis/air}$	
0	0	liver	kidney
0	0	brain	VRG
0	0	MG	FG
0		lung	

```
VMAX=325        KM=8
STEP SIZE IN MIN.          .2
PRECISION =                .01
```

IMPUT STATEMENTS 525-550

```
SIMULATION DURATION IN MIN =     5
EXPOSURE CONCENTRATION     =     1
 PRINT AFTER STEP                5
```

OUTPUT STATEMENTS

TIME= 1 .		Simulation duration (min.)	
CONCENTRATION VALUES	Tissue conc./λ	tis/bl=regional ven. bl. conc./λbl/ai	
.0130937345	.0738612829	liver	kidney
.0143733474	.0114999477	brain	VRG
2.75733535E-03	5.24911008E-05	MG	FG
.0916795369		lung	

```
RATES
PULM.UPTAKE    ELIMINATION
5.44992278       .430110174
TOTAL AMOUNT
PULM.UPTAKE    WASH-OUT
5.00402034       0
TOTAL ELIMINATED (NONLIN)
.163034918

TIME=            2
CONCENTRATION VALUES
.021673971       .0978115313
.0302875977      .0246889568
6.25442913E-03   1.21047425E-04
.103216489
RATES
PULM.UPTAKE    ELIMINATION
5.78070106       .825356731
TOTAL AMOUNT
PULM.UPTAKE    WASH-OUT
10.4144478       0
TOTAL ELIMINATED (NONLIN)
.811267532
```

usually prefered. (STEP is usually a fraction of a minute. When "PRINTER AFTER STEP" is displayed, a one integer number must be pressed. This number determines, how many steps before the data will be displayed or printed. For example, the display of calculated data is requested after each simulated minute. If the STEP (statement 29) equals 0.5,the answer is 2 (= 1/0.5). If the data print is requested only after each simulated hour, then the answer is 120 (60/0.5). If the STEP (statement 29) equals 0.2, then the answers are 5 and 300 respectively.

Statement 540: displays "SIMULATION DURATION", asking for information on duration of exposure or desaturation which should be simulated (in minutes). If repetitive exposures are simulated, then the simulation time is counted from the beginning of the first exposure.

Statement 545: displays "EXPOSURE CONCENTRATION", asking for information on exposure concentration which must be expressed in weight/volume units. If exposure was interupted, answer "0" to initiate the calculation of desaturation data.

<u>OUTPUT STATEMENTS</u>: (870-925): print the following information for each simulation period selected in statement 550 (see example of print-out on pg. 12):

a. first the actual simulation time "TIME" is displayed. The time in minutes represents the time interval which collapsed from the beginning of the first simulated exposure.

b. CONCENTRATION VALUES for compartments are next. The

vapor concentrations in compartments are not given directly. To obtain the vapor concentrations in tissues, the displayed concentration values must be multiplied by the appropriate tissue-air partition coefficients. To obtain vapor concentrations in regional venous blood, the concentration values must be multiplied by blood-air partition coefficients.

The concentration values are displayed (printed) in the same order as the compartmental parameters were given in statements 4-10. That is, the concentration value for compartment with capacity limited clearance is displayed first (first line to the left). Next is printed the concentration value for the compartment defined by statement 5 (first line to the right). In the second line are concentration values for the compartments defined by statements 6 and 7, and so on; the last printed concentration value equals the concentration in the alveolar air. If this value is multiplied by blood-air partition coefficient, then the concentration in arterial blood is obtained. If the last printed concentration value is multiplied by lung-air partition coefficient, then vapor concentrations in lung parenchymal tissue is obtained.

The concentration values can be also used to calculate rates by which the vapor is transfered from the lung to the compartment or vice versa. To calculate this transportation rate, the concentration value for the appropriate compartment is substracted from the last displayed concentration value and the difference is multiplied by appropriate G-value (given

in the corresponding statement 4-10). If the value is positive, it indicates tissue uptake rate, if the value is negative, it indicates tissue wash-out rate.

c. pulmonary UPTAKE RATE (left) and ELIMINATION RATE in the first compartment (right) are given next. Pulmonary uptake rate is printed because of its importance. Otherwise, it can be easily calculated by subtracting the last concentration value from exposure concentration (given in statement 545; notice that it can be zero for desaturation) and by multiplying the difference by alveolar ventilation. If the PULMONARY UPTAKE RATE has positive signs, it represents pulmonary uptake. If it has a negative sign, it represents EXHALATION RATE or desaturation rate.

All elimination rates can be easily calculated by multiplying the concentration values of the compartment by the G_x characterizing the compartment. The elimination rate for capacity limited clearance is displayed.

Finally, the output gives numerical integrals of pulmonary uptake rates and of the elimination rate in the first compartment (capacity limited clearance). The integrals represent total amounts retained and eliminated. The integral for pulmonary uptake is split into two values: 1) if uptake rates are positive, then the integral represents total pulmonary uptake (on the left). 2) if the uptake rate is negative, then the integral represents the pulmonary wash-out (on the right). This means that during the saturation period, the

integral on the left increases with time and the integral on the right remains constant. On the other hand, during desaturation period, the integral on the right increases and the integral on the left remains constant.

The last value is the integral of capacity-limited elimination. This integral, which represents total amount eliminated, always rises. If pulmonary wash-out integral and elimination integral are subtracted from the pulmonary uptake integral, the body burden is obtained.

COMMENTS

1. The consistency of units. It is essential that all parameters be expressed in the same units. The concentrations must be expressed in weight/volume units. For example, if time, volumes, and flows are given in minutes, liters and liters per minute respectively, and exposure concentrations are given in mg/l, then in output statements concentration values are in mg/l, rates are in mg/min. and total amounts are in mg. For small animals, min., ml., ml/min. and µg/ml can be prefered. For small exposure concentrations, µg/l or ng/ml can be prefered. K_m - values must be in the same units as concentrations; V_{max} - value must be in the same units as rates, and intrinsic clearances must be in the same units as flows.

2. Computation time on Apple II Plus is rather long. It takes about 10 hours of computation to simulate a 24 hour exposure.

3. Modeling of repetitive exposures or exposures with varying exposure concentrations can be done with the program if the exposure is

presented as a number of consecutive exposures to constant exposure concentrations (When exposure is interrupted, the exposure concentration equals zero.)

This is achieved using the following steps:

a. The data statements are prepared.

b. When "SIMULATION DURATION" is displayed, the duration of the first exposure period is pressed.

c. When "EXPOSURE CONCENTRATION" is displayed, the exposure concentration for the first exposure period is pressed.

d. The program runs, and the output is displayed and/or printed in intervals pre-scheduled by statement STEP.

e. The program returns to statements calling for information on the second exposure period. SIMULATION DURATION now equals the sum of durations of the first and second exposure, and the EXPOSURE CONCENTRATION refers to the second exposure period.

f.. Repeat steps b-e for each exposure period, making simulation duration equal to the sum of exposure durations.

In the computer print-out, the time periods and integrals are added, so that simulation time (the first line in each output) gives the time from the beginning of the first exposure (= sum of exposure durations), and the integrals represent total amounts retained, exhaled or eliminated during the whole simulation time; that is, from the beginning of the first exposure until the time indicated in the first line of each print-out. The amounts retained, exhaled or eliminated during each exposure period, are calculated as the difference

between the corresponding integrals at the end of the two consecutive exposure periods.

4. Scheduling of printer. Rational scheduling of printer makes the data easier to survey and economizes on computer time and printer paper. The output data is needed usually more frequently, when the values are rising or declining rapidly, while less frequent output data is needed if values change slowly. To arrange for such a rational scheduling, the exposure is simulated in more than one run. After each run, the calculation is returning to statement 540, inquiring about "SIMULATION DURATION", "EXPOSURE CONCENTRATION" and "PRINT AFTER STEP". For example, it is desirable to obtain print-outs after each minute during the first ten minutes of exposure; after each ten minutes between 10 and 60 minutes of exposure, and after each 30 minutes between one hour to eight hours. Then, the exposure is interrupted and print-outs are needed each minute during additional five minutes of desaturation. Such exposure is simulated in four runs. Assuming that exposure concentration equals 7 and step equals 0.2, the imput statements for each run are as follows:

Run 1: Exposure conc. 7 Simulation duration 10 Print after step 5
Run 2: Exposure conc. 7 Simulation duration 60 Print after step 50
Run 3: Exposure conc. 7 Simulation duration 480 Print after step 300
Run 4: Exposure conc. 0 Simulation duration 485 Print after step 5

5. Simulation of changes in physiological parameters. If any parameter described in input data (statements 1-36), changes during exposure, then simulation must be interrupted and the program returned to the beginning (press "RESET"). Changes in parameters must be made

in appropriate data statements. To secure continuity of simulation, the final concentration values in first simulation must be used as initial conditions for the second simulation (indicated in statements 16-19). An example of such simulation is exposure during which the pulmonary ventilation and cardiac output rise temporarily because of strain excercise.

6. Preparation of data. On page 20 is a format of the protocol we are using for preparation of data for simulation. The protocol sheet is self-explanatory. For demonstration, a protocol sheet for simulation on page 12 is shown on page 21.

We have by now available a series of simulations of different trichloroethylene exposures. These simulations, compared with experimental data, confirm the validity of the model.

TABLE 1

NON-LINEAR PROGRAMS

Objective: _____

Vapor: _____ Study No.: _____

Exposed subject: _____ Date: _____

Statements	COMPARTMENT	Flow ℓ/ min.				Capacity ℓ			
		F	λbl/air	G	G_x ℓ/min.	V	λtis/air	C	Starting Concentration Values
*4									
5									
6									
7									
8									
9									
10								☆	

*V_{max} (mg/min.)		Step	
K_m (mg/ℓ)		Precision	

Exposure schedule:

RUN	1	2	3	4	5	6	7	8
Exposure duration (min.)								
Simulation duration (min.)								
Exposure concentration (mg/ℓ)								
Printer: after m-minutes								
after step = $\frac{m}{step}$								

☆ $C_{lung} = FRC + 1/3\ V_{tid} + V_{lung}\lambda_{lung/air} + Q\lambda_{bl/air}$

TABLE 2

NON-LINEAR PROGRAMS

Objective: Effect of Exposure Concentration.

Vapor: Trichloroethylene Study No.: see page 12

Exposed subject: Normal man Date: June 15, 1982

Statements	COMPARTMENT	Flow ℓ/ min. F	λbl/air	G	G_x ℓ/min.	Capacity ℓ V	λtis/air	C	Starting Concentration Values
*4	Liver	1.6	9.5	15.2	0	2.6	25	65	0
5	Kidney	1.2	9.5	11.4	0	0.3	15	4.5	0
6	Brain	0.7	9.5	6.6	0	1.5	21	31.5	0
7	VRG	1.0	9.5	9.5	0	3.9	20	58	0
8	MG	2.55	9.5	24	0	34.4	19	653	0
9	FG	0.45	9.5	4.2	0	10.2	600	6120	0
10	Lung	6		6	0		14	☆ 11.1	0

*V_{max} (mg/min.)	325	Step	0.2
K_m (mg/ℓ)	8	Precision	0.01

Exposure schedule:

RUN	1	2	3	4	5	6	7	8
Exposure duration (min.)	5							
Simulation duration (min.)	5							
Exposure concentration (mg/ℓ)	1							
Printer: after m-minutes	1							
after step = $\frac{m}{step}$	5							

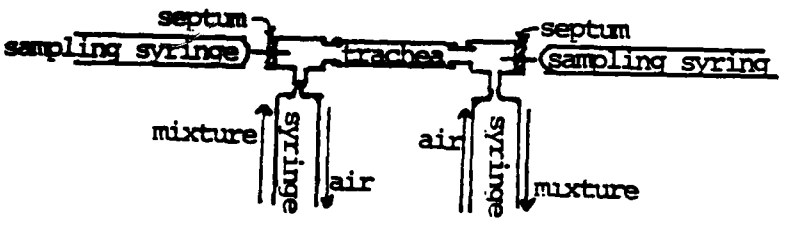
☆ $C_{lung} = FRC + 1/3\ V_{tid} + V_{lung}\lambda_{lung/air} + Q\lambda_{bl/air}$

ANIMAL STUDIES

1. Retention of vapors in respiratory airways. The simulation models developed in our laboratory reliably predicted the bioavailability of vapors and gases of organic solvents, but failed to predict the bioavailability of vapors and gases of such substances as acetone and alcohols. For such substances as acetones and alcohols, it was apparent from comparison of predicted and experimental data, that transfer of vapor from ambiental air in arterial blood was less than predicted. We suggest that discrepancy between predicted and experimental data is caused by adsorption and desorption of the substances in the walls of respiratory airways. This study was undertaken to evaluate this adsorption and desorption.

Method:

New Zealand rabbits (weighing about 2.5 kg) were used for the study. The rabbits were sacrified, the tracheas were immediately removed, and the ends of the tracheas were attached to swagelock T-pieces, as shown in the scheme.



Tracheas (usually about 4cm. long with inner surface area 6 cm^2) were wrapped in a soft, wet cloth and kept at 37C. Sampling syringes were

50 µl Hamilton gas-tight syringes. Gas and vapor concentrations used in the studies were 5 µM/l, 25 µM/l, and 125 µM/l. The studied gases and vapors are listed in table 3. From the side of the preparation where the larynx side of the trachea was connected with the T-piece, 20 ml. of gas mixture (equal to tidal volume of a rabbit) were pushed rapidly through the trachea from a glass syringe. Immediately after, the gas samples (25 µl) were collected simultaneously on both ends of the preparation and analyzed by gas chromatography. The retention in trachea was calculated from the difference of concentrations in inflow and outflow ends. The retentions in table 3 are expressed as a fraction of the injected concentration.

$$R = \frac{C_{in} - C_{out}}{C_{in}}$$

The trachea was washed with five strokes of clean air from the other side of the preparation, and the gas mixture was injected again. The procedure was repeated ten times. The mean value was used for evaluation of retention in each trachea. The means in Table 3 were calculated from retentions obtained with n-number of tracheas. For compounds which had large retention in trachea, desorption was observed. Immediately after 20 ml of gas mixture was passed through the trachea, 20 ml of clean air was passed through the trachea from the opposite direction, and gas samples were collected simultaneously on both ends of the preparation. Concentrations of the studied gas were measured and desorption was evaluated from the difference of concentrations across the trachea.

$$D = \frac{C'_{out} - C'_{in}}{C_{in}}$$

Where C'_{in} and C'_{out} relate to inflow and outflow concentrations after injecting clean air.

For substances with small retention, D was smaller than 0.001. However, a significant fraction of original concentration of ethanol (D = 0.2) and acetone (D = 0.1) remained on trachea walls washed with a tidal volume of clean air. Traces of ethanol and acetone were found in the fifth wash of the tracheas with a tidal volume of clean air. This study is finished.

Conclusions

The retention of vapors on the surface of the rabbit trachea is shown in table 3. The absolute value of the measured retention is questionable, since only retention in the trachea was measured. In vivo, the buccal absorption and adsorption in other parts of respiratory airways would increase the retention. However, since retentions shown in Table 3 were determined under the same experimental conditions, the values indicate the relative retention among the substances.

Our model is based on the assumption that no vapor or gas is retained in respiratory airways. Under this assumption, the concentration in end-exhaled air equals the concentration in alveolar air, and pulmonary uptake, measured as the difference of vapor concentration in inhaled and exhaled air multiplied by minute ventilation, represents systemic uptake. However, if adsorption and desorption in respiratory airways take place, then concentrations in end-exhaled air collected during exposure is larger than alveolar concentration, and pulmonary uptake does not necessarily represent systemic uptake. These studies indicate that our existing model is not suitable for simulation of uptake, distribution, and elimination of water-soluble compounds, and

TABLE 3

Retention of Vapors in Rabbit Trachea *In Vitro*.

Substance	Percentage of Inflow Concentration Retained			
	n	mean	SD	SE
2,2,2-trifluoro-1-chloroethane	6	-0.1	3.3	0.9
1,1-difluoro-2-chloroethylene	5	0.0	3.1	1.0
Methylene Chloride	5	2.5	9.5	3.0
Halothane (CF_3 - CHClBr)	5	6.7	1.5	0.5
Freon-12 (dichlorodifluoromethane)	5	6.9	1.0	0.3
Trichloroethylene	5	8.3	5.0	1.6
Toluene	10	10.6	5.5	1.2
Ethylene Oxide	4	13.9	4.1	1.4
Styrene	6	17.6	6.0	1.7
Ethyl Acetate	9	17.7	9.9	2.4
Acetone	9	21.9	7.5	1.8
n-Amyl alcohol	5	41.1	4.1	1.3
Acetyl Acetone	6	45.8	4.9	1.4
1-Butanol	7	48.2	11.4	3.0
1-Propanol	5	54.0	4.6	1.5
Ethanol	11	58.2	11.4	2.4
1,4-Dioxane	3	59.5	4.1	1.7
Methanol	5	68.6	2.3	0.7

Retention was calculated from the difference between inflow and outflow concentrations:

$$R = 100 \frac{C_{in} - C_{out}}{C_{in}} \%$$

n equals the number of experiments performed.

requires further modification.

2. A new method for determination of intrinsic clearance in vivo was developed. Calculation of clearance is based on the concentration difference of vapor in arterial and regional venous blood. The concentrations are derived from vapor concentrations in brain (or muscle) and in the excretory organ at steady state by dividing the tissue concentrations by the appropriate tissue/blood partition coefficients. This method is simpler than the previously suggested determination from arterial blood, and is now used for the determination of intrinsic clearance required by our simulation model.

3. The thermodynamic theory for estimation of partition coefficients was tested. Stern and Shiah (Molecular Pharmacology, 19, 56, 1981) published a new correlation between partition coefficients and critical values of substances. Since determination of partition coefficients is cumbersome, we attempted to use thermodynamic theory for prediction of blood/air and tissue/air partition coefficients. The attempt was unsuccessful, because the differences between predicted and measured values were too large (up to 100%).

4. Dependence of halothane metabolites formation in rats on exposure concentration and on presence of other vapors.
Procedure: Sprague-Dawley female rats (about 200 g) were exposed for three hours to halothane or to a mxiture of halothane and some other vapor (methylene chloride, isoflurane or nitrous oxide). At the end of the exposure, rats were sacrified by decapitation, and tissues were excised and analyzed for halothane and its metabolites (trifluoroacetic acid, 1,1-difluoro-2-chloroethylene and 1,1,1-trifluoro-2-chloroethane) and for the presence of other vapors used in the exposure. The exposure

procedure and analytical method were the same as in our previous project (Biomathematical Modeling of Chronic Toxicity, Progress Report to AFOSR September, 1979). The results are presented in Figures 1-6. Each bar represents mean and standard error of the mean for five rats.

a. Dependence of metabolites formation in exposure concentration is shown in Figure 1. The range of exposure concentration varied between 70 p.p.m. - 3,200 p.p.m. (recommended TLV is 50 p.p.m., anesthetic alveolar concentration is 6,500 p.p.m.). In the graph, concentrations in tissues and in air are expressed in μM/kg or μM/l respectively. These units permit comparison of the formation of metabolites on the equi-molar basis. Formation of both volatile metabolites (trifluoro-monochloroethane and difluoro-monochloroethylene are gases) increases sharply with increasing exposure concentration. Concentration of the major metabolite, trifluoroacetic acid, remains unchanged. The nonlinear dependence of metabolites formation on exposure concentration is more apparent if ratios of tissue concentration to exposure concentration are plotted against exposure concentration (Figure 2). If formation of metabolites is a first order process, then the tissue-exposure concentration ratios would be independent of exposure concentration. The graphs show, however, that concentrations of metabolites in tissues increase less than exposure concentration, and halothane concentration in tissues increase more than exposure concentrations. The conclusion is that metabolite formation is a saturable, flow-limited process. At exposure concentration 25mg/l (0.32%), the liver exposure air concentra-

FIGURE 1

CONCENTRATIONS OF HALOTHANE METABOLITES IN LIVER OF RATS EXPOSED FOR 3 HOURS TO DIFFERENT CONCENTRATIONS OF HALOTHANE

n= 5; ±S. E.; studied halothane exposure concentrations in v/v %; 0.007; 0.009; 0.029; 0.067; 0.16; 0.32

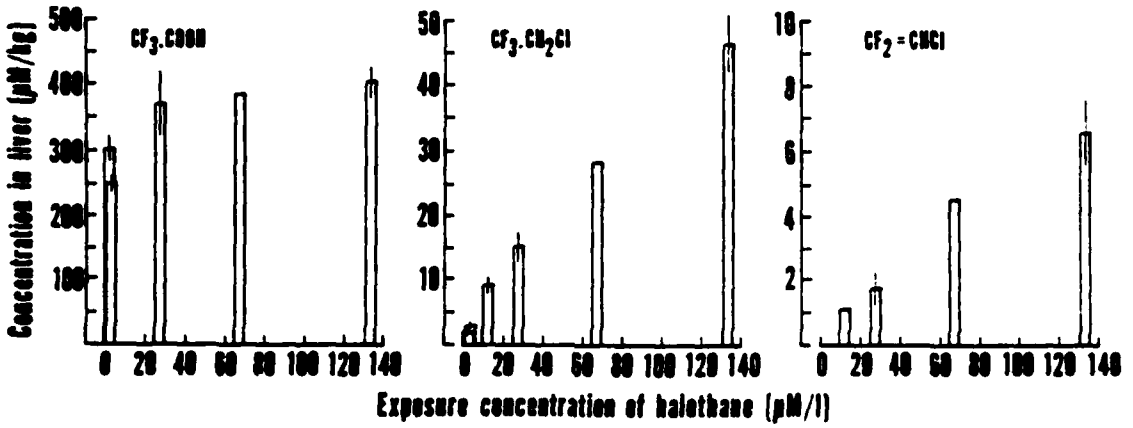
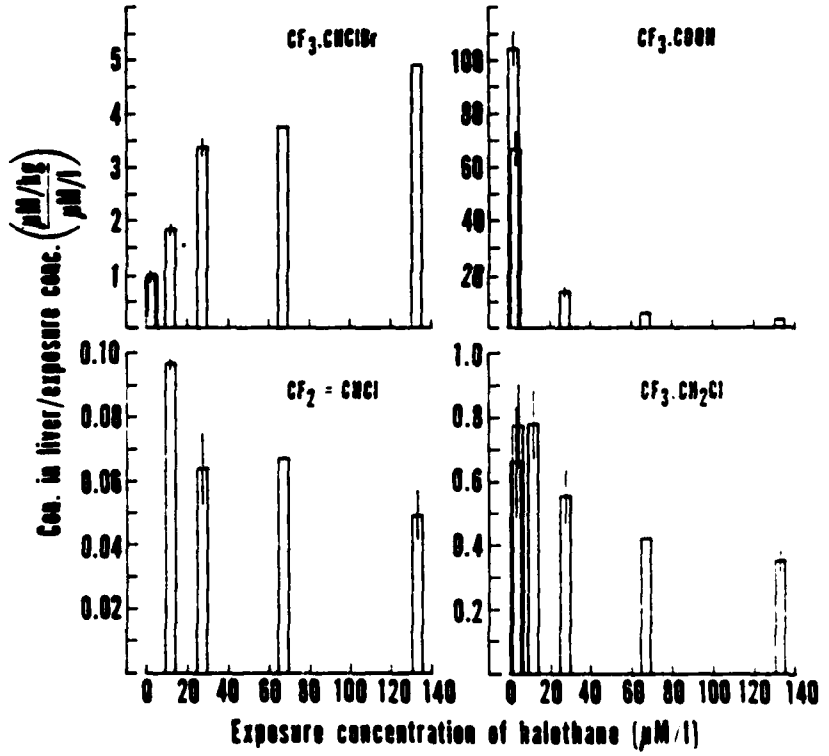


FIGURE 2

DEPENDENCE OF HALOTHANE METABOLITES FORMATION IN LIVER ON EXPOSURE CONCENTRATION OF HALOTHANE

n= 5; ±S. E.; 3-hour exposure of rats to concentrations in v/v %; 0.007; 0.009; 0.029; 0.067; 0.16; 0.32



tion ratio approaches the liver-air partition coefficient ($\lambda_{liver/air}$= 6.2). This indicates that at this concentration metabolic clearance can be considered for flow-limited. It is suggested that the enzymatic system is saturated if exposure concentrations exceed 0.03% (300 p.p.m.), since the concentrations of trifluoroacetic acid were the same in tissues of rats exposed to 0.03% of halothane as in rats exposed to 0.32% of halothane.

After a three-hour exposure, trifluoroacetic acid concentration in liver is always greater than halothane concentration, but concentrations of volatile metabolites are always smaller, than halothane concentration. (Figure 1). Trifluoroacetic acid and halothane were also found in other tissues in a significant amount, but the two volatile metabolites were not detectable in other tissues by our method (sensitivity of the method is 0.5 μM/kg). The explanation is that the volatile metabolites formed in liver are removed from blood circulation by effective pulmonary clearance, thus preventing distribution of the two gaseous metabolites in the body. On the other hand, the trifluoroacetic acid is not removed in lung and is distributed by arterial blood through the whole body. Since renal clearance is inefficient, trifluoroacetic acid accumulates in the body, and after a short exposure, concentrations of trifluoroacetic acid in tissues exceed halothane concentrations.

b. <u>Effect of simultaneous exposure to another vapor on formation of halothane metabolites</u>. Rats were exposed to a mixture of two vapors in which halothane was always present at a concentration 5 mg/l (625 p.p.m.). The mixtures of halothane with three vapors were studied so far. Each vapor was inhaled in four sub-anesthetic concentrations. The data is summarized in figures 3-6. The mean tissue-concen-

FIGURE 3

EFFECT OF NITROUS OXIDE ON HALOTHANE METABOLITES FORMATION
IN RATS EXPOSED FOR 3 HOURS TO MIXTURES OF HALOTHANE AND NITROUS OXIDE
n= 5; $\bar{x}$±S.E.; halothane exposure concentration = 5 mg/l = 625 p.p.m. = 0.062 % = 25.4 μ M/l

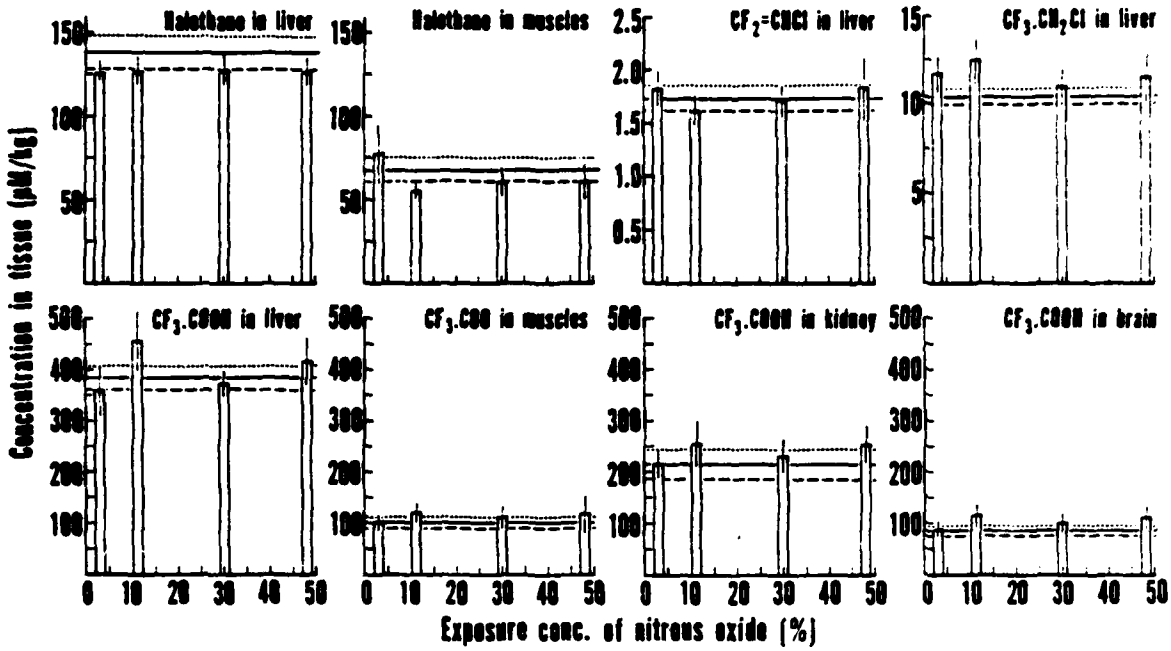
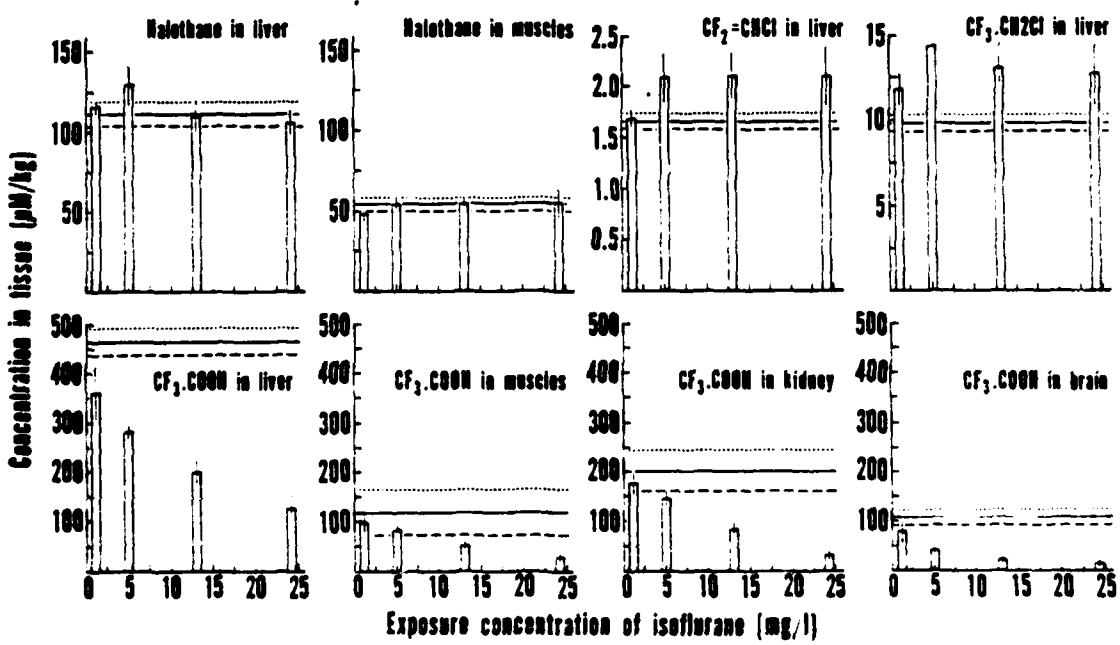


FIGURE 4

EFFECT OF ISOFLURANE ON HALOTHANE METABOLITES FORMATION
IN RATS EXPOSED FOR 3 HOURS TO MIXTURES OF HALOTHANE AND ISOFLURANE
n = 5; $\bar{x}$±S. E.; halothane exposure concentration = 5 mg/l = 625 p.p.m. = 0.062 % = 25.4 μ M/l
studied isoflurane exposure concentration in v/v %: 0.015; 0.066; 0.17; 0.32



tration during each exposure to a mixture of vapors, and standard errors of the means, are shown by bars. The mean concentrations in tissues of control rats exposed only to halothane, and standard errors of the means, are shown by the horizontal lines.

1b.) Nitrous oxide was present in the mixtures in concentrations, 3,10,30 and 50% respectively. Oxygen was added to the mixture so that oxygen concentration in the mixture was 20%. The presence of nitrous oxide had no effect on metabolites formation (Figure 3).

2b.) Isoflurane, a fluorinated anesthetic ($CF_3 \cdot CHCl \cdot O \cdot CHF_2$), is metabolized to a very small extent (less than 1%). The molecular ratios of halothane to isoflurane in the inhaled mixtures were: 1:0.24; 1:1.1; 1:2.7; and 1:5.2. In all exposures, the ratio of halothane concentrations in liver and inhaled air equals four. This indicates that systemic clearance of halothane is flow limited and unaffected by isoflurane. (Liver-gas partition coefficient equals 6). However, isoflurane affects the individual pathways of halothane. Trifluoroacetic acid formation is suppressed in the presence of isoflurane. The suppresion is concentration dependent. The concentrations of volatile metabolites are slightly increased in liver of rats exposed to those mixtures in which isoflurane concentrations are greater than halothane concentrations. Even if the concentrations of volatile metabolites in liver indicate only slight enhancement

of formation of volatile metabolites in liver of rats exposed simultaneously to isoflurane, the enhancement in fact can be large, since the efficient pulmonary clearance prevents cumulation of volatile metabolites in tissues. It is concluded that, quantitatively, isoflurane does not affect the systemic clearance of halothane to any significant extent. However, isoflurane, poorly metabolized by itself, inhibits the aerobic metabolic pathway of halothane, the end product of which is trifluoroacetic acid. Isoflurane enhances the anaerobic pathway which results in the formation of volatile metabolites (CF_3 - CH_2Cl and CF_2 = CHCl). Since CF_3 - CH_2Cl is hepatotoxic (Brown B.R., et al. Env. Health Perspectives, 21, 185, 1977), it is possible that isoflurane abet hepatotoxicity of halothane.

3b.) Methylene chloride also inhibits metabolism of halothane (Figures 5 and 6). Results of the experiment with methylene chloride are pictured in figure 5. The molar ratios of halothane to methylene chloride in exposure mixtures are: 1:1.1; 1:1.9; 1:4.5; 1:6.8 and 1:15.8. Halothane concentrations in liver show that systemic clearance of halothane is either unaffected or slightly enhanced by the presence of methylene chloride. The only exception is exposure to a mixture of 5 mg/l of halothane and 30 mg/l of methylene chloride (1:15.8), during which systemic clearance seems to be reduced. Concentrations of methylene chloride in liver

FIGURE 5

EFFECT OF METHYLENE CHLORIDE ON HALOTHANE METABOLITES FORMATION IN RATS EXPOSED FOR THREE HOURS TO MIXTURE OF HALOTHANE AND METHYLENE CHLORIDE.

n = 5; $\bar{x}$ ± S.E.; Halothane Exposure Concentration = 5 mg/l = 629 p.p.m. = 0.063% = 25.4 mM.
Methylene Chloride Exposure Concentrations: 0.07; 0.12; 0.28; 0.42; 0.70 % (v/v).

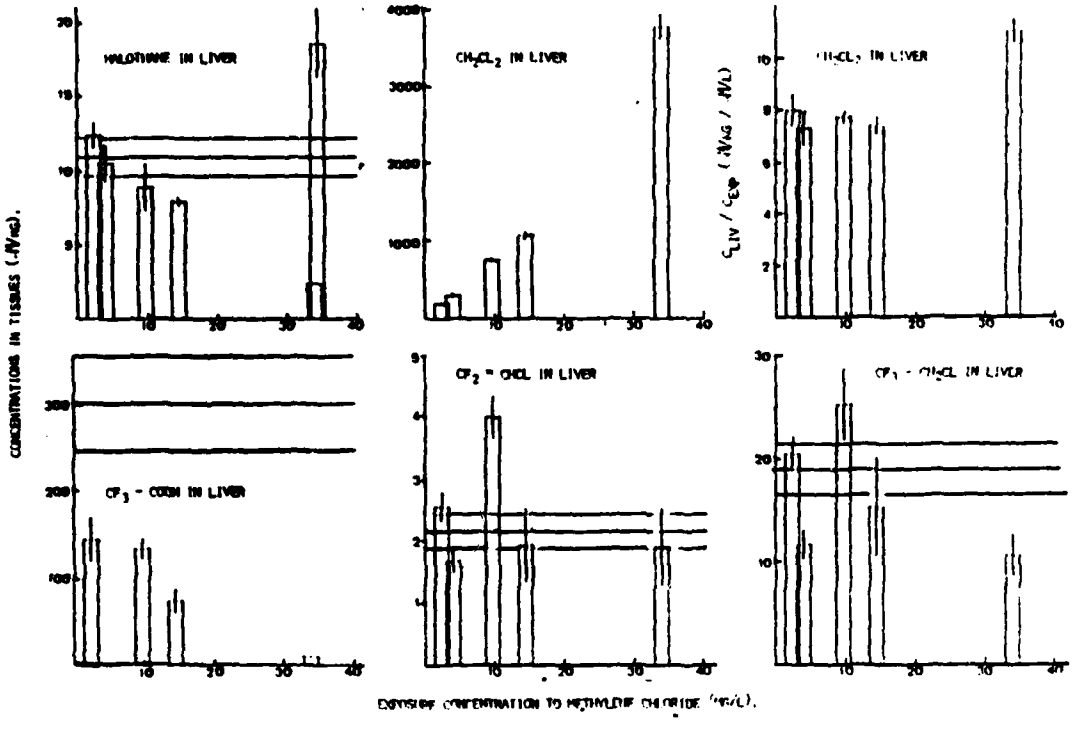
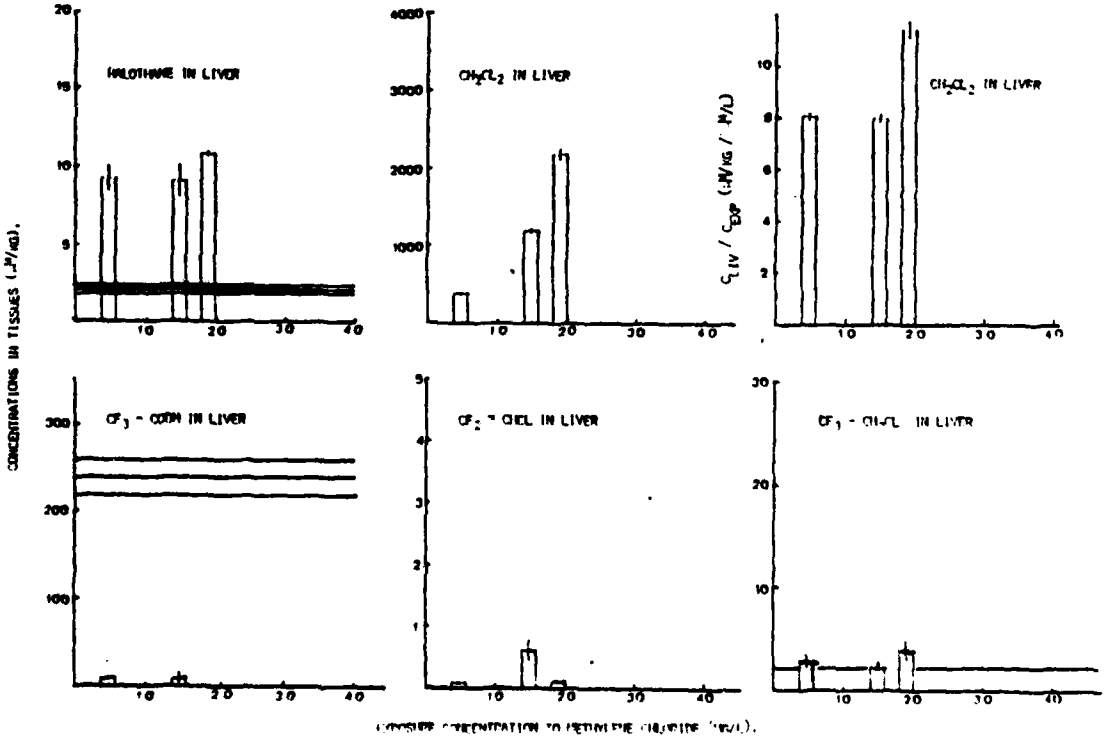


FIGURE 6

EFFECT OF METHYLENE CHLORIDE ON HALOTHANE METABOLITES FORMATION IN RATS EXPOSED FOR THREE HOURS TO MIXTURE OF HALOTHANE AND METHYLENE CHLORIDE.

n = 5; $\bar{x}$ ± S.E.; Halothane Exposure Concentration = 0.5 mg/l = 62.5 p.p.m. = 0.0063% = 2.54 mM.
Methylene Chloride Exposure Concentrations: 0.13; 0.43; 0.55% (v/v).



correlate with exposure concentration. The ratio of methylene chloride in liver and in inhaled air oscillates around the liver-air partition coefficient ($\lambda_{liver/air}$ = 9.1), suggesting that metabolic clearance of methylene chloride in the studied concentration range was not flow limited. The formation of trifluoroacetic acid is largely inhibited in the presence of methylene chloride. The inhibition is concentration dependent. Concentrations of volatile metabolites in liver of rats exposed to mixtures of halothane and methylene chloride vary widely around concentrations measured in liver of rats exposed only to halothane, but the variation is not concentration dependent.

We attempted to perform similar studies using ten times smaller concentrations of halothane (Figure 6). Metabolism of halothane at such exposure concentration (62 p.p.m.) appears to be a flow--limited process of first order. Accordingly, the ratio of halothane concentration in liver to exposure concentration was very small (0.8 compared to liver-air partition coefficients equal to 6). In the presence of methylene chloride, this ratio increased to 4, indicating reduction of systemic clearance of halothane. At the same time, formation of trifluoroacetic acid was reduced, so that concentration of trifluoroacetic acid was below the sensitivity limit of our method. Difluoro-monochloroethylene, which was present in liver of rats exposed

only to 62 p.p.m. of halothane in concentrations smaller than detectable by our method, was measurable in liver of rats exposed to a halothane-methylene chloride mixture. Concentrations of trifluoro-monochloroethane were the same, whether halothane alone or in mixture, was used for exposure.

We conclude that methylene chloride supresses systemic clearance of halothane, profoundly inhibiting the aerobic metabolic pathway (formation of trifluoroacetic acid) but not significantly affecting the anaerobic pathway (formation of volatile metabolites). We speculate that the inhibiting effect is caused by methylene chloride and halothane competing for microsomal mixed function oxidase, or by desactivation of cytochrome P-450 by carbon monoxide released by biodegradation of methylene chloride.

This study is not yet completed. We are initiating similar experiments with mixtures of halothane and trichloroethylene.