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14. ABSTRACT

The overarching goal of this research project was to develop a mathematical dosimetry model for aerosolized JP-8 to better understand human exposure to inhaled JP-8. The JP-8 dosimetry model could then be used to determine human health risks from exposure to JP-8.For the first time, individual hydrocarbon data were collected to characterize the atmospheric exposures and dosimetry for laboratory animals exposed to vaporized or aerosolized JP-8. Both chamber atmosphere and body burden data were collected using analytical methods developed by our team. Our dosimetry work was curtained at end of this grant and remains to be completed. However, preliminary findings suggest that important differences in exposure of rats to individual hydrocarbons occur when JP-8 is aerosolized vs vaporized. This provide some evidence for explaining the apparent discrepancies reported for the toxicology of JP-8 when exposed to vapor vs. aerosol/vapor mixtures.

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FINAL REPORT
DEVELOPMENT OF A PBPK MODEL FOR JP-8

For Walt Kozumbo

Jeff Fisher
University of Georgia

November 15, 2006

EXECUTIVE SUMMARY

PERSONNEL: This grant provided partial or full support for 3 MS students, 1 Ph.D. student and two post doctoral students. The MS theses in pdf format can be found at the University of Georgia web site:
http://dbs.galib.uga.edu/cgi-bin/ultimate.cgi?dbs=getd&userid=galileo&action=search&_cc=1
Type in the name in the space provided and follow directions: Andrew Quin Smith, Ulrich Reiko Perleberg, Katherine Denise Dietzel. The dissertation for Shonetta Delaine Gregg should be available as a pdf by May 2007.
The people involved in the grant were:
Dr. Michael Bartlett, Co-PI (chemist), his Ph.D. student, Shonetta Gregg
Dr. Jeff Fisher, PI and his students, Andrew Smith, Reiko Perleberg, and Kathy Dietzel, Jerry Campbell and Satheesh Anand. All papers are cited in the summary of research below.

RESEARCH: For the first time, individual hydrocarbon data were collected to characterize the atmospheric exposures and dosimetry for laboratory animals exposed to vaporized or aerosolized JP-8. Both chamber atmosphere and body burden data were collected using analytical methods developed by our team. Our dosimetry work was curtained at end of this grant and remains to be completed. However, preliminary findings suggest that important differences in exposure of rats to individual hydrocarbons occur when JP-8 is aerosolized vs vaporized. This provides some evidence for explaining the apparent discrepancies reported for the toxicology of JP-8 when exposed to vapor vs. aerosol/vapor mixtures. The *overarching goal* of this research project was to develop a mathematical dosimetry model for aerosolized JP-8 to better understand human exposure to inhaled JP-8. The JP-8 dosimetry model could then be used to determine human health risks from exposure to JP-8. Dosimetry models for laboratory animals are required because toxicology studies were conducted using laboratory animals. These laboratory animal dosimetry models will be used to extrapolate to humans. The overarching goal of the research was not realized by the end of this grant. However, several important steps were achieved toward this goal.

BACKGROUND

The *overarching goal* of this research project was to develop a mathematical dosimetry model for aerosolized JP-8 to better understand human exposure to inhaled JP-8. The JP-8 dosimetry model could then be used to determine human health risks from exposure to JP-8. JP-8 is the primary fuel used for aircraft in the US military and NATO countries. Many active duty and civilians are exposed the JP-8 while performing their duties. Antidotal health complaints from the employees working with JP-8 over the last decade and laboratory animal toxicology studies conducted in the 1990s raised concerns about the safety of JP-8. To assist in assessing adequate protection of the workforce, this project was devoted to the development of a mathematical model referred to as a physiologically based pharmacokinetic (PBPK) model. PBPK models have gained acceptance in the US and European regulatory communities as a scientifically sound computational tool to estimate health heath risks from exposures to chemicals. JP-8 is a challenging material to work with because JP-8 is a mixture of hundreds of hydrocarbons, significantly complicating the task of PBPK dosimetry model development. In addition, for inhalation, the primary route of exposure to JP-8, both liquid droplets (aerosols) and vapor phases of JP-8 need to be considered. This final report summarizes the accomplishments, to date, towards achieving the *overarching goal* of this project.

EXPOSURE TO HYDROCARBONS

The first technical hurtle of this project was to better understand 'what' the laboratory animals are exposed to when aerosolized JP-8 exposures are conducted, since the fuel consists of hundreds of hydrocarbons. Jet fuel inhalation toxicology studies conducted over the last 3 decades characterized the chamber atmosphere, in which the animals were exposed, simply as the mass of total hydrocarbons (fuel) divided by volume of chamber air (mg of total hydrocarbons/ volume of air, meter^3). With advances in analytical chemistry instrumentation, individual hydrocarbons that comprise significant portions of the aerosol droplets and the vapor phase could be discerned. To this end, we purchased an Agilent 6890N GC with a 5973N Quadruopole MSD, 7683 liquid autosampler, a Tekmar HT7000 headspace sampler, and a Gerstel CIS 4 (cooled injection system) with Gerstel TDS 3 (thermal desorption system). These instruments coupled with the use of a 150 M gas chromatography column allowed for adequate separation of major hydrocarbons found in aerosolized JP-8 atmospheres and their subsequent identification and quantification. The first task was to identify and quantify individual hydrocarbons found in an inhalation chamber with aerosolized JP-8. This was undertaken at the University of Arizona where most of the recent inhalation toxicology studies have occurred. Several important findings resulted from this project:

1) About 85 to 95% of the aerosolized fuel exposure was vapor phase fuel and the remainder aerosol droplets.
2) At least 40% of the aerosol droplets consisted of n-alkanes (C11-C17), with C14 and C15 n-alkanes representing the large percent of identified hydrocarbons.
3) The method of calculation of the JP-8 chamber concentration (mg/m^3) at the University of Arizona was in error, thus many published toxicology studies from this group have under reported the aerosolized JP-8 concentrations up to 6-fold for low concentrations (50-100 mg/m^3) and perhaps 2-fold for high concentrations (1500-2500 mg/m^3).

Publications

Dietzel, K.D., J.L. Campbell, M.G. Bartlett, M.L. Witten, and J.W. Fisher. 2005. Validation of a gas chromatography/mass spectrometry method for the quantification of aerosolized Jet Propellant 8. Journal of Chromatography A, 1083, 11-20.

Shonetta Gregg, Jeffrey W. Fisher and Michael G. Bartlett. 2006. A review of analytical methods for the identification and quantification of hydrocarbons found in jet propellant 8 and related petroleum based fuels. Biomedical Chromatography, 20, 492-507.

PBPK MODEL DEVELOPMENT

The proposed approach for the development of a PBPK model for aerosolized JP-8 was to select specific hydrocarbons that represent different fractions of the hydrocarbon mixture. Taken together, the JP-8 PBPK model will consist of 4-6 hydrocarbon sub-models. The most studied hydrocarbon fraction of JP-8 is the light end of hydrocarbons with high vapor pressure, which consists of aromatics, such as toluene, ethylbenzene, xylenes, and benzene. Several PBPK models have been developed for the aromatics and will be used as sub-model(s) in the PBPK model for JP-8. Other fractions, representing less volatile fractions, such as n-alkanes, have not been well studied. Therefore, laboratory experiments were focused on understanding some of the important properties of semi-volatile n-alkanes which would allow us to develop PBPK models for this fraction of the fuel. These properties include solubility in tissues or partition coefficients and metabolism rates. Key findings from these studies were:

1) The measured tissue:air partition coefficient values for n-alkanes C8 to C12 increased as a function of carbon length for muscle, fat, liver, blood. The measured n-alkane brain solubility values were peculiar and still require further study because of irregular experimental findings.
2) Tissue:air partition coefficients values for n-alkanes C13-C17 were estimated using regression techniques with the data sets collected for C8-C12 and octanol:water partition coefficient values for the entire data set.
3) *In vitro* derived maximum metabolic rates, using rat hepatic microsomes, decreased as a function of carbon length (C9, C10 and C14). The calculated Km value or affinity constant for C9 (nonane) and C10 (decane) were similar.

Alkanes are very lipophilic and their kinetic behavior is complex. We conducted kinetic laboratory experiments with rats by exposing rats to decane vapors and collecting time course data for blood and several tissues. A PBPK model for decane was developed. Key findings are:

1) The kinetics of uptake and clearance of decane were described as diffusion limited for brain, bone marrow, fat, skin, and spleen. The measured partition coefficient values were abandoned in attempts to fit the tissue and blood time course data.
2) New PBPK modeling approaches are needed for describing the kinetics of n-alkanes C9 and greater. One recommendation to consider is: The compartments

are not well-stirred compartments, but consistent of a 'shallow pool' which exchanges readily with blood and a 'deep pool' that does not exchange with the blood.

A PBPK model was developed for two aromatics (m-xylene and ethylbenzene) and its metabolic interactions evaluated as a binary mixture and then as components in the vapor phase of vaporized JP-8 (not aerosolized JP-8). We estimated that about 20% of the hydrocarbons identified in the vapor phase of JP-8 were metabolized by a common isoform of the P450 system, namely CYP2E1. With a DoD instrumentation grant we purchased and TDS A (autosampler) and Agilent 6890N GC with micro-ECD and FID detectors, 7683 liquid autosampler and a Varian CP-3800 GC with a Saturn 2200 Ion Trap MSD and a CombiPAL autosampler (liquid, headspace, and SPME injection capability.
Key findings from this study were:

1) A sensitive method for the measurement of hydrocarbons in tissues was developed using SPME and the ion trap mass spectrometer. This allowed for the measurement of prominent individual hydrocarbons in tissues which represent only 1-6% of the total fuel concentration in the chamber atmosphere.

2) Competitive inhibition in metabolism (caused by multiple hydrocarbons being metabolized through the same metabolic pathway at the same time) was evaluated using m-xylene and ethylbenzene as aromatic biomarkers. In summary, for vaporized fuel concentrations that were at or above 1100 mg/m^3, a modest competitive inhibition in metabolism occurred for ethylbenzene and m-xylene. For concentrations 400 mg/m^3 and below the kinetics and metabolism of m-xylene and ethylbenzene appeared to be similar to its behavior in the absence of vaporized JP-8 or no observable competitive inhibition in metabolism.

Publications

Smith, A.Q., J.L. Campbell, D.A. Keys, and J.W. Fisher. 2005. Rat tissue and blood partition coefficients for n-alkanes (C_8-C_{12}). International Journal of Toxicology, 24, 31-41.

Perleberg, U.R., D.A. Keys and J.W. Fisher. 2004. Development of a physiologically based pharmacokinetic model for decane, a constituent of jet propellant-8. Inhalation Toxicology, 16, 771-783.

Campbell, J. L. and J. W. Fisher. 200x Physiologically based pharmacokinetic model for m-xylene and ethylbenzene inhalation in JP-8 vapor. Inhalation Toxicology, in press.

Anand, Sathanandam S., Jerry L. Campbell and Jeffrey W. Fisher. RAT HEPATIC METABOLISM OF N-ALKANES, NONANE, DECANE AND TETRADECANE, IN VITRO. *Submitted* to Drug Metabolism and Disposition.

OTHER STUDIES NOT PUBLISHED

Preliminary dosimetry studies were carried out at the University of Arizona with aerosolized JP-8 and rats to determine lung and blood hydrocarbon burdens during and after exposure to aerosolized JP-8. Tables 1 and 2 below depict the corresponding concentrations of 23 hydrocarbons in lung and blood of rats at the end exposure using a whole body vaporized sample of JP-8 (Table 1) or a nose only aerosolized JP-8 exposure system (Table 2). The chamber concentrations of individual hydrocarbons are also reported. A direct comparison of the lung and tissue dosimetry can not be carried out because of differences in the exposure system, length of exposure and total hydrocarbon chamber concentration. However, qualitatively the two systems can be compared. There is a pronounced increase in heavier hydrocarbons (eg., pentadecane, tetradecane, tridecane, dodecane) in the vapor phase in the aerosolized JP-8 chamber atmosphere corresponding with the aerosol droplet composition. Consequently there are in increased concentrations of these hydrocarbons in the blood and lung of aerosol/vapor exposed rats. These findings may eventually help explain the differences noted between the toxicology findings with JP-8 at UA with aerosolized JP-8 and the earlier toxicity tests conducted by whole body exposures to JP-8 vapor only at WPAFB, OH.

Table 1. Chamber and tissue concentrations of individual components in samples collected at UGA after vapor exposure (1086 mg/m^3) to JP-8 for 4 hour.

Compound	Chamber Concentration (mg/m^3)	Tissue Concentration at End of Exposure (ng/g)	
	Vapor	Lung	Blood
Heptane	67.0	<LOQ	25.7 ± 7.7
Methylcyclohexane	76.0	<LOQ	47.3 ± 10.6
Toluene	45.3	9.0 ± 1.6	59.8 ± 12.5
2-Methylheptane	46.3	<LOQ	22.5 ± 5.5
Octane	77.5	<LOQ	47.8 ± 10.2
Ethylbenzene	13.6	3.8 ± 0.2	36.8 ± 6.6
m-Xylene	25.2	7.4 ± 1.7	100.8 ± 18.3
p-Xylene	7.7	<LOQ	30.0 ± 3.3
o-Xylene	11.0	7.0 ± 0.3	58.4 ± 7.7
Nonane	67.7	<LOQ	46.8 ± 9.0
Propylcyclohexane	16.0	<LOQ	50.3 ± 9.6
1,3,5-Trimethylbenzene	4.8	<LOQ	18.0 ± 1.3
o-Ethyltoluene	6.1	3.7 ± 0.4	31.4 ± 2.1
1,2,4-Trimethylbenzene	10.6	5.6 ± 0.4	73.0 ± 4.0
Decane	37.1	5.3 ± 1.8	74.8 ± 10.0
1,2,3-Trimethylbenzene	7.5	2.7 ± 0.4	21.6 ± 0.7
Butylcyclohexane	4.0	2.2 ± 0.2	16.4 ± 3.0
Undecane	16.6	5.8 ± 1.5	57.0 ± 6.4
Naphthalene	0.2	2.6 ± 0.7	5.3 ± 1.0
Dodecane	4.8	6.4 ± 1.8	<LOQ
Tridecane	0.7	4.8 ± 1.9	<LOQ
Tetradecane	0.1	3.2 ± 1.4	<LOQ
Pentadecane	<LOQ	<LOQ	<LOQ

Table 2. Chamber concentrations of individual components in samples collected at UA after aerosolization of JP-8 (1751 mg/m^3) for 1 h.

Compound	Chamber Concentration (mg/m^3)		Tissue Concentration at End of Exposure (ng/g)	
	Vapor	Aerosol	Lung	Blood
Total Conc.	(1586 mg/m^3)	(165 mg/m^3)		
Heptane	2.72	ND	<LOQ	<LOQ
Methylcyclohexane	4.72	ND	<LOQ	<LOQ
Toluene	2.16	ND	<LOQ	<LOQ
2-Methylheptane	4.30	ND	<LOQ	<LOQ
Octane	12.1	ND	<LOQ	<LOQ
Ethylbenzene	4.21	ND	6.1 ± 0.4	4.8 ± 0.8
m-Xylene	6.07	ND	12.5 ± 0.4	8.3 ± 1.5
p-Xylene	1.35	ND	<LOQ	3.4 ± 0.6
o-Xylene	2.82	ND	11.6 ± 0.4	5.6 ± 1.1
Nonane	35.3	ND	<LOQ	11.4 ± 2.7
Propylcyclohexane	8.62	ND	<LOQ	14.9 ± 3.1
1,3,5-Trimethylbenzene	4.46	ND	15.8 ± 0.4	10.0 ± 1.6
o-Ethyltoluene	3.71	ND	15.7 ± 0.5	12.9 ± 2.1
1,2,4-Trimethylbenzene	10.6	<LOQ	53.1 ± 2.6	38.3 ± 6.8
Decane	80.6	0.37	17.2 ± 6.8	30.6 ± 6.1
1,2,3-Trimethylbenzene	4.12	<LOQ	35.7 ± 1.4	21.8 ± 3.9
Butylcyclohexane	11.0	ND	6.5 ± 2.4	17.3 ± 2.1
Undecane	80.0	2.03	102.2 ± 21.2	67.9 ± 5.2
Naphthalene	<LOQ	<LOQ	31.6 ± 7.7	28.0 ± 7.6
Dodecane	34.8	4.16	407.2 ± 25.9	106.9 ± 11.9
Tridecane	12.14	5.59	896.5 ± 68.1	143.4 ± 33.7
Tetradecane	4.03	6.20	1134 ± 98	127.2 ± 46.3
Pentadecane	1.21	6.05	1196 ± 134	57.2 ± 25.6

ANALYTICAL METHOD DEVELOPMENT

A significant effort was undertaken during this grant to develop and validate analytical methods for the identification and quantification of individual hydrocarbons found in the chamber atmosphere and in biological tissues from laboratory animals exposed to JP-8. Below are a citation for one paper in press and another in preparation by a Ph.D. student who will graduate this year.

S.D. Gregg, J.L Campbell, J.W. Fisher and M.G. Bartlett, "Analytical Methods for the Characterization of Generated Jet Fuel: Vapor and Aerosol Samples." In Press, Biomedical Chromatography

S.D. Gregg, S. Muralidhara, J.L. Fisher and M.G. Bartlett, "Determination of Twelve Major Components of Jet Propellent-8 from Rat Blood and Liver by Solid Phase Microextraction Gas Chromatography Mass Spectrometry." In Prep, Analytical Chemistry.

Below is acslXtreme code written by Dr. Jerry Campbell to study the analysis of metabolic interactions of two aromatic hydrocarbons (M-xylene and ethylbenzene) in the rat exposed to vaporized JP-8 fuel.

```
CODE FOR JP-8 INTERACTION MODEL (Campbell et al., in press)
PROGRAM JP8int
!Created by Jerry Campbel on 09/29/2005
!Modified last: 08/03/2006

INITIAL

 CONSTANT  QPC = 15.0          !Alveolar ventilation rate (L/hr/kg)
 CONSTANT  QCC = 15.0          !Cardiac output (L/hr)
 CONSTANT  BW  = 0.265 !Body weight (kg)

!Blood Flows (Fraction of QC, Delp 1998 for F344 rats)
 CONSTANT  QLC = 0.25          !Liver
 CONSTANT  QFC = 0.09          !Fat
 CONSTANT  QBC = 0.027         !Brain
 CONSTANT  QLUC = 0.015        !Lung (Brown et al, 1997)

!Tissue Volumes (Fraction of BW, Schoeffner et al., 1999)
 CONSTANT VLC  = 0.04   !Liver tissue Schoeffner et al, 1999
 CONSTANT VBC  = 0.0076        !Brain tissue Schoeffner et al, 1999
 CONSTANT VFC  = 0.07   !Fat

!Partition Coefficients (m-Xylene, Tardif et al, 1997)
 CONSTANT  PWBmx = 46.0        !Blood:Air
 CONSTANT  PLmx = 1.98 !Liver:Blood
 CONSTANT  PFmx = 40.4 !Fat:Blood
 CONSTANT  PBmx = 1.98 !Brain:Blood (set to PR)
 CONSTANT  PSmx = 0.91 !Slowly Perfused:Blood
 CONSTANT  PRmx = 1.98 !Rapidly Perfused:Blood

!Partition Coefficients (ethylbenzene, Tardif et al, 1997)
 CONSTANT  PWBebz = 42.7       !Blood:Air
 CONSTANT  PLebz = 1.96!Liver:Blood
 CONSTANT  PFebz = 36.4!Fat:Blood
 CONSTANT  PBebz = 1.41        !Brain:Blood (set to PR)
 CONSTANT  PSebz = 0.61 !Slowly Perfused:Blood
 CONSTANT  PRebz = 1.41        !Rapidly Perfused:Blood
```

```
!Partition Coefficients (Lumped Aromate component of Fuel vapor)
      !Used Average Partition for m-Xylene and Ethylbenzene
 CONSTANT  PWBlp = 44.4       !Blood:Air
 CONSTANT  PLlp = 1.97  !Liver:Blood
 CONSTANT  PFlp = 38.4  !Fat:Blood
 CONSTANT  PBlp = 1.8         !Brain:Blood (set to PR)
 CONSTANT  PSlp = 0.76  !Slowly Perfused:Blood
 CONSTANT  PRlp = 1.8         !Rapidly Perfused:Blood

!Metabolic Parameters (Vmaxc=mg/kg-h and Km=mg/kg)

!m-Xylene
 CONSTANT Vmaxmxc = 8.75
 CONSTANT Kmmx = 0.87

!Ethylbenzene
 CONSTANT Vmaxebzc = 6.01
 CONSTANT Kmebz = 0.67

!Lumped vapor fraction
 constant vmaxlpc = 10.22
 constant Kmlp = 1.61

!Inhalation Dosing Parameters
 CONSTANT TCHNG = 4.0 !Length of inhalation exposure (hrs)
 CONSTANT  WDAYS=5.
 CONSTANT  WEDAYS=2.
 CONSTANT  DAYS=7.
 CONSTANT  PDAYS=0.
 CONSTANT EXPTIM = 240        !length of simulation (hr)

!Scaled parameters

!Scaled Blood Flows
      QC = QCC*BW**0.75
      QP = QPC*BW**0.75
      QL = QLC*QC
      QF = QFC*QC
      QB = QBC*QC
      QR = 0.78*QC-QL-QB
      QS = 0.22*QC-QF

!Scaled Tissue Volumes
      VL = VLC*BW
      VF = VFC*BW
      VB = VBC*BW
```

```
      VS = 0.82*BW-VF
      VR = 0.09*BW-VL-VB

 !Metabolic Parameters
      Vmaxmx = Vmaxmxc*BW**0.75
      Vmaxebz = Vmaxebzc*BW**0.75
      Vmaxlp = Vmaxlpc*BW**0.75

 ! Dosing Parameters

 !constant inhalation parameters
  CONSTANT concmx2=0
  CONSTANT concebz2=0
  CONSTANT conclp2=0

  CONSTANT MWmx = 106.16      !m-xylene Molecular weight (g/mol)
  CONSTANT MWebz = 106.16     !ethylbenzene Molecular weight (g/mol)
  CONSTANT MWlp = 115.3       !Lump fuel Molecular weight (g/mol)

  CONSTANT TSTOP = 6.0
  CONSTANT sflag = 1.0

END !Initial

DYNAMIC

      ALGORITHM IALG = 2
      CINTERVAL CINT = 0.01


DERIVATIVE

 !Inhalation Timing command (Start and length of exposure)
  pflag =pulse(0.,exptim,tchng)*pulse(0.,(wdays+wedays)*24.,wdays*24.)

 !Concentration Inhaled (ppm)
      CImx = (concmx2*pflag*MWmx/24450)
      CIebz = (concebz2*pflag*MWebz/24450)
      CIlp = conclp2*pflag  !Entered as mg/L in air instead of ppm

!Model Code

!CA = Concentration in venous blood supply perfusing lung at site of
 !gas exchange (mg/l)

      CAmx = (QC*CVmx+QP*CImx)/(QC+(QP/PWBmx))
```

```
AUCWBmx = INTEG(CAmx,0.)

CAlp = (QC*CVlp+QP*CIlp)/(QC+(QP/PWBlp))
AUCWBlp = INTEG(CAlp,0.)

CAebz = (QC*CVebz+QP*CIebz)/(QC+(QP/PWBebz))
AUCWBebz = INTEG(CAebz,0.)


!CV = Mixed Venous Blood Concentration
CVmx = (QF*CVFmx+QL*CVLmx+QS*CVSmx+QR*CVRmx+QB*CVBmx)/QC
CVAUCmx= integ(cvmx,0.)

CVlp = (QF*CVFlp+QL*CVLlp+QS*CVSlp+QR*CVRlp+QB*CVBlp)/QC
CVAUClp= integ(cvlp,0.)

CVebz = (QF*CVFebz+QL*CVLebz+QS*CVSebz+QR*CVRebz+QB*CVBebz)/QC
CVAUCebz= integ(cvebz,0.)


!AX = Amount Exhaled
CXmx = CAmx/PWBmx
RAXmx = QP*CXmx
AXmx = INTEG(RAXmx,0.)
RAImx = QP*CImx
AImx =integ(RAImx,0.)
doseinhmx= aimx-axmx

CXlp = CAlp/PWBlp
RAXlp = QP*CXlp
AXlp = INTEG(RAXlp, 0.)
RAIlp = QP*CIlp
AIlp =integ(RAIlp,0.)
doseinhlp= ailp-axlp

CXebz = CAebz/PWBebz
RAXebz = QP*CXebz
AXebz = INTEG(RAXebz, 0.)
RAIebz = QP*CIebz
AIebz =integ(RAIebz,0.)
doseinhebz= aiebz-axebz

!AS = Amount in Slowly Perfused
RASmx = QS*(CAmx-CVSmx)
ASmx = INTEG(RASmx,0.)
CVSmx = ASmx/(VS*PSmx)
```

```
      CSmx = ASmx/VS

      RASlp = QS*(CAlp-CVSlp)
      ASlp = INTEG(RASlp,0.)
      CVSlp = ASlp/(VS*PSlp)
      CSlp = ASlp/VS

      RASebz = QS*(CAebz-CVSebz)
      ASebz = INTEG(RASebz,0.)
      CVSebz = ASebz/(VS*PSebz)
      CSebz = ASebz/VS

!AR = Amount in Rapidly Perfused
      RARmx = QR*(CAmx-CVRmx)
   ARmx = INTEG(RARmx,0.)
   CVRmx = ARmx/(VR*PRmx)
   CRmx = ARmx/VR

      RARlp = QR*(CAlp-CVRlp)
   ARlp = INTEG(RARlp,0.)
   CVRlp = ARlp/(VR*PRlp)
   CRlp = ARlp/VR

      RARebz = QR*(CAebz-CVRebz)
   ARebz = INTEG(RARebz,0.)
   CVRebz = ARebz/(VR*PRebz)
   CRebz = ARebz/VR

!AF = Amount in Fat
      RAFmx = QF*(CAmx-CVFmx)
      AFmx=INTEG(RAFmx,0.)
      CVFmx =AFmx/(PFmx*VF)
      CFmx=AFmx/VF

      RAFlp=QF*(CAlp-CVFlp)
      AFlp=INTEG(RAFlp,0.)
      CVFlp=AFlp/(PFlp*VF)
      CFlp=AFlp/VF
      AUCFlp=INTEG(CFlp,0.)

      RAFebz = QF*(CAebz-CVFebz)
      AFebz=INTEG(RAFebz,0.)
      CVFebz=AFebz/(PFebz*VF)
      CFebz=AFebz/VF

!AB = Amount in Brain
```

```
RABmx = QB*(CAmx-CVBmx)
ABmx = INTEG(RABmx,0.)
CVBmx = ABmx/(VB*PBmx)
CBmx = ABmx/VB

RABebz = QB*(CAebz-CVBebz)
ABebz = INTEG(RABebz,0.)
CVBebz = ABebz/(VB*PBebz)
CBebz = ABebz/VB

RABlp = QB*(CAlp-CVBlp)
ABlp = INTEG(RABlp,0.)
CVBlp = ABlp/(VB*PBlp)
CBlp = ABlp/VB

!AL = Amount in Liver
RALmx = QL*(CAmx-CVLmx)-RAMmx
ALmx=INTEG(RALmx,0.)
CVLmx=ALmx/(PLmx*VL)
CLmx=ALmx/VL

RALebz = QL*(CAebz-CVLebz)-RAMebz
ALebz=INTEG(RALebz,0.)
CVLebz=ALebz/(PLebz*VL)
CLebz=ALebz/VL

RALlp = QL*(CAlp-CVLlp)-RAMlp
ALlp=INTEG(RALlp,0.)
CVLlp=ALlp/(PLlp*VL)
CLlp=ALlp/VL

!AM = Amount metabolized (includes metabolic interaction)
RAMmx=(Vmaxmx*CVLmx)/(Kmmx*chKmmxi+CVLmx)
AMmx=INTEG(RAMmx,0.)

RAMebz=(Vmaxebz*CVLebz)/(Kmebz*chKmebzi+CVLebz)
AMebz=INTEG(RAMebz,0.)

RAMlp=(Vmaxlp*CVLlp)/(Kmlp*chKmlpi+CVLlp)
AMlp=INTEG(RAMlp,0.)

!Metabolic Interaction Calculation (change in Km)
chKmmxi=1+(CVLebz/Kmebz+CVLlp/Kmlp)
chKmebzi=1+(CVLmx/Kmmx+CVLlp/Kmlp)!+CVLtl/Kite)
chKmlpi=1+(CVLmx/Kmmx+CVLebz/Kmebz)!+CVLtl/Kmtl)
```

```
!Mass Balance
        TotalCmx = AFmx+ALmx+ASmx+ARmx+ABmx
        Balmx=aimx-(TotalCmx+ammx+axmx)

        TotalCebz = AFebz+ALebz+ASebz+ARebz+ABebz
        Balebz=aiebz-(TotalCebz+amebz+axebz)

        TotalClp = AFlp+ALlp+ASlp+ARlp+ABlp+axlp
        Ballp=ailp-(TotalClp+amlp+axlp)

END ! DERIVATIVE

TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')

TERMINAL

END ! DYNAMIC
END ! TERMINAL
END ! PROGRAM
```