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Physiologically-Based Pharmacokinetic Model to Predict Propellant Levels in Exhaled Air from Pressurized Medical Inhaler Use

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14. ABSTRACT

Bronchodilator asthma medications, like albuterol, are utilized to treat acute asthmatic exacerbations. They also have been utilized to improve lung function testing and athletic performance in individuals without asthma. These inhaled drugs are commonly administered using pressurized metered dose inhalers (pMDI). Hydrofluorocarbons HFC-134a and HFC-227ea are used as propellants in pMDIs and can be detected in exhaled breath. A physiologically-based pharmacokinetic (PBPK) model was used to simulate inhalation kinetics of the propellants HFC-134a and HFC-227ea, in order to determine the window of detection in exhaled breath for subjects who have inhaled albuterol from a pMDI. The model was developed and validated with data from published studies to predict propellant exhaled breath and venous blood concentrations. The PBPK model was then run to simulate exposure to a single puff from an asthma inhaler using HFC-134a as the propellant, to describe the exposures in a separately reported Air Force Research Laboratory study. On average, the model predicted detection of asthma inhaler use for approximately 16 hours following dosing for an analytical limit of detection (LOD) of 1 part per billion (ppb) or 65 hours post inhalation for a LOD of 1 part per trillion (ppt).

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Albuterol, hydrofluorocarbon, pressurized meter dose inhalers, PBPK, asthma

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PREFACE

This research was funded by the U.S. Air Force School of Aerospace Medicine, Department of Internal Medicine (USAFSAM/FECI). Principal investigators for the project this modeling report supports were Lt Col Dara D. Regn, MD (USAFSAM/FECI) and Jennifer A. Martin, PhD (AFRL/RH).

The majority of data discussed within this report were sourced from published works, as cited. Additional human data utilized were generated through the Air Force Research Laboratory (AFRL) Institutional Review Board protocol, "Investigation of Detection Strategies for Inhaled Asthma Medication of Air Force Pilot Candidates", FWR2020013H v1.00 (valid 22 January 2021 through 21 January 2026). The study is referred to within the report as the AFRL study. The data were fully de-identified prior to receipt. The full methods and results of this study will be reported elsewhere.

The portion of the research reported herein was conducted under cooperative agreements FA8650-15-2-6608 and FA8650-21-2-6250 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF). The work was also performed under UES, Inc. subcontract S-145-404-001. Dr. Jeffery Gearhart retired from HJF in July 2021 and Dr. Matt Linakis left 711 HPW/RHBB in May 2021.

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1.0 SUMMARY

Bronchodilator asthma medications, like albuterol, are utilized to treat acute asthmatic exacerbations. They also have been utilized to improve lung function testing and athletic performance in individuals without asthma. Routine methods and strategies for detecting or confirming bronchodilator use to improve lung function have not been developed. Albuterol and other asthma drugs are known to be excreted in urine but the lag time between dosing and excretion, relatively high limits of detection, and number of possible drugs make urine a poor medium for biomonitoring. Inhaled drugs are commonly administered using pressurized metered dose inhalers. Hydrofluorocarbons HFC-134a and HFC-227ea are used as propellants in pressurized metered dose inhalers and can be detected in exhaled breath.

The primary objective of this project was to use physiologically-based pharmacokinetic (PBPK) modeling to simulate inhalation kinetics of albuterol and the propellants HFC-134a and HFC-227ea in order to determine the window of detection for exhaled breath in subjects who have inhaled albuterol from a pressurized metered dose inhaler, and to model urinary excretion of albuterol. The study results from the AFRL Institution Research Board protocol "Investigation of Detection Strategies for Inhaled Asthma Medication of Air Force Pilot Candidates" (FWR2020013H v1.00), referred to herein as the AFRL study, did not detect albuterol in urine. The investigation of albuterol did not progress past the data mining phase to locate published information on existing PBPK models, kinetic datasets compatible with model development, and additional relevant information. This literature review is summarized in Appendix A of this report to provide a starting point should albuterol kinetic data be collected in a future study.

The PBPK model used for this work is a modification of a previously published PBPK model consisting of flow-limited compartments for brain, fat, liver, skin, rapidly perfused and slowly perfused tissues, plus first order urinary excretion from blood. The model was developed and validated with data from published studies to predict exhaled breath and venous blood concentrations. Additionally, while the AFRL study did not include exposure to HFC-227ea, simulations of HFC-227ea data were conducted for comparison as this propellant is commonly used in medical inhalers. The PBPK model then was run to simulate exposure to a single puff from an asthma inhaler using HFC-134a as the propellant, to describe the exposures in the AFRL study. The full methods and results of the AFRL study will be reported elsewhere.

The PBPK model and study data were in agreement for half of the ten AFRL subjects, while the simulated dose required optimization for the remaining half and for the average to achieve model predictions within two standard deviations (SDs) of the data. This is not unexpected as study participants, and the public in general, are known to be variable in compliance with proper inhaler use. These variances likely resulted in differences in the actual inhaled dose.

The model was utilized to predict the window of detection for HFC-134a in exhaled air based on the limit of detection (LOD) for the analytical method. On average, the model predicted detection of asthma inhaler use for approximately 16 hours following dosing for an analytical LOD of 1 parts per billion or 65 hours post inhalation for a LOD of 1 parts per trillion.

2.0 INTRODUCTION

Bronchodilator asthma medications, primarily albuterol, are commonly utilized to treat acute asthmatic exacerbations (Shin *et al.*, 2015). These drugs also have been utilized to improve lung function testing and athletic performance in individuals without asthma (Morton and Fitch, 1992). Data gathered in 2017 indicated that 7.9 percent of the general U.S. population was considered asthmatic (Stern *et al*., 2020). Thus, inhalers are fairly prevalent and may be borrowed or purchased from family or friends that are asthmatic. Conversely, suboptimal medication compliance is commonplace; an estimated 30 to 70 percent of asthmatics don't achieve good inhaler compliance. About a quarter of acute exacerbation events experienced by asthmatics are attributed solely to non-compliance (Shin *et al.*, 2015). Routine methods and strategies for detecting bronchodilator use or compliance have not been developed.

Detecting asthma medication use is not a simple task. Albuterol and other asthma drugs are known to be excreted in urine but the lag time between dosing and excretion, plus relatively high LODs make urine a poor medium for monitoring use immediately following a sporting event, lung function test, or supervised medical compliance administration. Additionally, the number of asthma drugs, aside from albuterol, make the prospect of chemical analysis daunting. Albuterol and other inhaled drugs are commonly administered using pressurized metered dose inhalers (pMDI) to minimize systemic absorption (Boulet *et al.*, 1999; PDR, 2021). Prior to the Montreal Protocol, which phased out ozone depleting substances, propellants in pMDIs were chlorofluorocarbons (CFCs). Modern propellants (used since 2005) are hydrofluoroalkanes (HFA): HFA-134 and HFA-227 (Dolovich, 1999); these may also be referred to as a hydrofluorocarbon (HFC), specifically HFC-134a and HFC-227ea, respectively. The terms HFC-134a and HFC-227ea will be used in the remainder of this report. Detection of the carrier gas in breath has been shown to help verify medicine administration compliance (Shin *et al*., 2015) and would allow for simple, short-term monitoring of asthma drug use.

The primary objective of this project was to use a PBPK model to simulate inhalation kinetics of albuterol and the propellants HFC-134a and HFC-227ea in order to determine the window of detection for these propellants in exhaled breath of subjects who have inhaled albuterol from a pMDI, and to model urinary excretion of albuterol. As the study related to the AFRL Institutional Review Board protocol "Investigation of Detection Strategies for Inhaled Asthma Medication of Air Force Pilot Candidates" (FWR2020013H v1.00) (referred to herein as the AFRL study) did not detect albuterol in urine. The investigation of albuterol did not progress past the data mining phase to locate published information on existing PBPK models, kinetic datasets compatible with model development, and additional relevant information. This literature review is summarized in Appendix A of this report to provide a starting point should albuterol kinetic data be collected in a future study.

3.0 METHODS

3.1 Literature Review for Propellant Information

Halon replacements were extensively researched for a variety of uses following the 1991 Montreal Protocol and its amendments, which pushed the phaseout of ozone-depleting CFCs (Emmen *et al*., 2000). For the purpose of replacing propellants in pMDIs, two HFCs were chosen based on their lack of toxicity, tastelessness (or lack of strong taste), and similarity in chemical properties (density, vapor pressure, boiling point) to CFCs that were already in use. HFC-134a was designated as a replacement for CFC-12 (dichlorodifluoromethane) and HFC-227ea replaced CFC-114 (dichlorotetrafluorethane) (Dolovich, 1999). HFC-134a is the simplified name for 1,1,1,2-tetrafluoroethane (CAS number 811-97-2), also called norflurane; its molecular weight is 102.03 gram per mole (g/mol) (PubChem, 2021a). HFC-227ea is 1,1,1,2,3,3,3-heptafluoropropane (CAS number 431-89-0, 170.03 g/mol) or apaflurane (PubChem, 2021b). Containing no chlorine, these molecules are not thought to contribute significantly to ozone breakdown (Emmen *et al*., 2000).

3.1.1 Pharmacological Properties and Activity. Both HFC-134a and HFC-227ea show little toxicity. As reviewed by Emmen *et al*. (2000), the propellants were tested in animals in both acute and long-term (two-year) studies, with minimal adverse results. Extensive clinical trials found these propellants safe for use in pMDIs. One study (Vinegar *et al*., 1997) indicated potential toxicity but this toxicity was not seen in another study (Emmen *et al*., 2000) using similar doses and longer exposures, suggesting that the issue may have been related more to the delivery method than the doses and exposure times. The Vinegar *et al*. (1997) study effects will be discussed below (Section 3.3.3).

Sellers (2017) suggested that HFC-134a can possess anesthetic agent properties, based on its similarity to halothane and a 1967 study in dogs, cats and monkeys, in which HFC-134a was administered at 50 percent with oxygen to anesthetize the animals. HFC-227ea is chemically similar to the anesthetic isoflurane, but without discernable anesthetic effect found. Fluorinated anesthetics and similar compounds act as calcium channel blockers and relax smooth muscles, including those in vasculature and bronchi. At appropriate clinical concentrations, this slight effect of HFC-134a most likely is not seen as negative for a patient.

3.1.2 Published PBPK Models and Parameters for Propellants. For a PBPK model of a pMDI propellant, the model should take into account a "normal" daily intake for a prescribed inhaler user. Graepel and Alexander (1991) consider that to be 0.0555 hours (200 seconds) daily, based on ten daily doses consisting of inhalation (five seconds), breath holding (ten seconds) and exhalation (five seconds). This slow inhalation-hold-exhalation pattern is the recommended method for maximal uptake of a drug like albuterol. Improper use of inhalers is common and can lead to exacerbated asthma symptoms due to poor drug uptake (Levy *et al*., 2013; Shin *et al*., 2015; Velsor-Friedrich *et al*., 2009).

To determine dose volume, if needed, a study by Sellers (1997) documented canister product weight and associated puff volume that could be extrapolated to provide estimates for commercially available inhalers of interest. Sellers studied the dose of HFC-134a and HFC-227ea delivered per puff by standard inhalers found in the United Kingdom (UK). A small albuterol inhaler (Salamol by IVAX pharmaceuticals, Waterford, Ireland; containing 7.88 g product) and a large albuterol inhaler (Ventolin by Allen and Hanburys, Uxbridge, UK; containing 17.32 g product) delivered 7 and 16 milliliters (mL) HFC-134a per puff, respectively.

3.1.2.1 HFC-134a Models and Parameters. Ernstgard *et al*. (2010) published measured human partition coefficients (PCs) for several halon replacements determined from a modified vial equilibration procedure and pooled blood samples from healthy volunteers (five men and five women). The HFC-134a blood:air (PB) PC was measured as 0.36 in this study and a comparison value of 0.56 was cited in Gunnare *et al*. (2006).

Gunnare *et al*. (2006) developed a PBPK model based on data from a human kinetics study they conducted. The study was also reported as a thesis (Gunnare, 2007). The model consisted of six compartments and describes HFC-134a kinetics in lung, rapidly perfused, fat, working muscle, resting muscle and liver tissues. It was used to simulate two-hour exposures with simultaneous exercise (50 Watts) for the entire exposure on computer-controlled exercise bicycles. Exhaled breath and venous blood concentrations for HFC-134a were predicted for various time points up to approximately 25 hours from the start of exposure. Although model code was not included in the publication, the model parameter values are presented in tables and were used in the modeling efforts presented in this report.

While PBPK models for halons and halon replacements are presented by Vinegar and Jepson (1995, 1996) and Vinegar *et al.* (2000), no simulations with corresponding data included HFC-134a. The publications also do not present either a schematic for the models or model code. Physiological parameter values used in the models are listed but no chemical-specific parameter values were included for HFC-134a.

3.1.2.2 HFC-227ea Models and Parameters. Creech *et al*. (1995) documented a rat PBPK model simulating gas uptake studies of HFC-227ea. The model structure included lung, gut, liver, fat, slowly perfused and rapidly perfused tissues. While model code was not included in the publication, parameter values were given. Unfortunately, human data were not simulated; therefore, this model was not of direct use. It is notable that rat PCs were measured for this propellant using a modified vial equilibration method. Average HFC-227ea tissue:air PC values were measured as 0.45, 0.42, 1.58, and 0.45 for the blood, liver, fat and gut (stomach plus small intestine), respectively. Similarly, metabolic parameters were determined from gas uptake studies in closed chambers.

Vinegar *et al*. (2000) published a human model for simulating very short exposures (zero to five minutes), which included a respiratory tract dead space and a pulmonary exchange sub-compartment. These additions improved predictions of kinetic data available from some other halons and HFCs modeled, but human kinetic data for HFC-227ea were limited by the toxic

effects seen in Vinegar *et al*. (1997). Again, model parameter values were presented but model code was not.

3.2 Propellant PBPK Modeling

3.2.1 PBPK Model Structure. The PBPK model used for this work is a modification of a previously published PBPK model describing the pharmacokinetics from exposure to isopropanol (IPA) and its metabolite, acetone (Clewell *et al*., 2001), which has flow-limited compartments for brain, fat, liver, skin and the remaining rapidly and slowly perfused tissues, and first order urinary excretion from blood. The original model provides the capability for simulating exposure via intravenous injection, intraperitoneal administration, oral gavage, drinking water, inhalation, and dermal application. Due to the high water solubility of IPA and acetone, Clewell *et al*. (2001) assumed that some absorption in the upper respiratory tract could occur during inhalation, with subsequent desorption during exhalation.

For the purposes of this work, coding for non-inhalation routes of exposure was removed. Code for the brain was also removed given that concentrations and effects in the brain are not of interest for this work; additionally, brain compartments and parameters are not included in the published models from which chemical specific parameters are obtained. The metabolite sub-model code was also removed as the metabolite pharmacokinetics of the propellants were not a target of this work. As it is assumed that there is no absorption of the propellants in the upper respiratory tract, code to simulate this absorption was also removed. The schematic for the resulting model is shown in Figure 1. The model was used to simulate inhalation exposure to HFC-134a and HFC-227ea by changing the chemical-specific values of the model parameters. It was executed using the Gear algorithm in acslX (AEgis Technologies Group, Inc., Huntsville, AL). The acslX model code can be found in Appendix B and the script files (*i.e.*, M files) necessary to run the code are located in Appendices D through E.

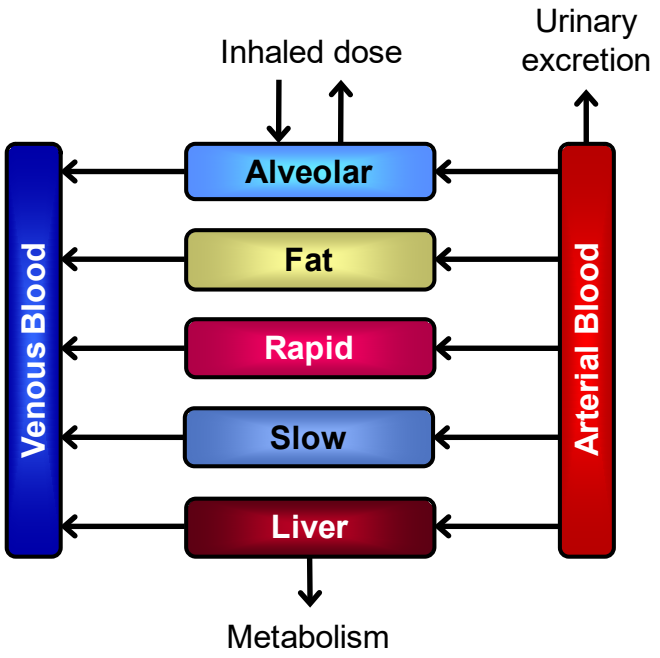

Figure 1. Schematic of Modified PBPK Model. This schematic shows the modified version of the Clewell *et al*. (2001) model used in this project.

3.2.2 Model Parameters. Chemical-specific parameters are taken from papers describing published PBPK models for HFC-134a and HFC-227ea (Gunnare *et al*., 2006; Vinegar and Jepson, 1995, 1996; Vinegar *et al*., 2000). Along with physiological parameters, relevant data from these papers were used to validate predictions of endpoints with the modified IPA model following exposure to HFC-134a (Emmen *et al*., 2000; Gunnare *et al*., 2006) and HFC-227ea (Emmen *et al*., 2000; Vinegar *et al*., 1997). Simulations of the AFRL study exhaled breath concentrations for HFC-134a use physiological parameters from Clewell *et al*. (2001), chemical-specific parameter values from Gunnare *et al*. (2006) and subject specific body weights for the AFRL subjects. These parameter values are summarized in Tables 1 and 2 and have been adjusted as necessary to be in the correct units for the model used here. Parameters not used for the simulations are set to have no effect on the model predictions and to not cause issues such as division by zero.

Table 1. Physiological Parameter Values

Parameter	Gunnare *et al.* (2006)	Vinegar & Jepson (1995, 1996)		Vinegar *et al.* (2000)	Clewell *et al.* (2001)
		Resting	Moderate Activity		
Body weight (kg)	72.5	70.0	70.0	70.0	70.0
Cardiac output (L/hr/kg$^{0.75}$)	25.65[a]	12.9	20.7	17.4	12.89
Pulmonary ventilation (L/hr/kg$^{0.75}$)	76.77[a]	25.97[e]	64.33[e]	25.97[e]	27.75
Fractional Tissue Blood Flows (fraction of cardiac output)					
Fat	0.0782[b]	0.0288	0.04	0.029	0.052
Liver	0.147[b]	0.3077[f]	0.2695[f]	0.308[f]	0.227
Rapidly perfused compartment	0.310[b]	0.4616	0.3188	0.461	0.533[h]
Slowly perfused compartment	0.465[b,c]	0.2019	0.4319	0.202	0.188
Fractional Tissue Volumes (fraction of body weight)					
Fat	0.204[b]	0.215	0.215	0.215	0.214
Liver	0.0217[b]	0.027	0.027	0.027	0.026
Rapidly perfused compartment	0.0514[b,d]	0.063[g]	0.063[g]	0.063[g]	0.056[h]
Slowly perfused compartment	0.508[b,c]	0.575	0.575	0.575	0.536

Notes:

[a]Converted from liters per minute (L/min) to liters per hour per kilogram (L/hr/kg$^{0.75}$) using a body weight of 72.5 kilogram (kg). These values are for a work load of 50 Watts.

[b]Fractional tissue blood flows are calculated from tissue blood flow in L/min divided by the cardiac output in L/min. Fractional tissue volumes are calculated from tissue volumes in L divided by body weight in L (assumes a specific gravity of 1.0).

[c]The fractional blood flow for the slowly perfused tissue is the sum of the values in Gunnare *et al*. (2006) for resting and working muscle.

[d]The fractional volume for the rapidly perfused tissue is the sum of the values in Gunnare *et al*. (2006) for the lung and the rapidly perfused tissue as there is no separate lung compartment in this model.

[e]Alveolar ventilation rates are converted to total pulmonary ventilation rates by dividing by 0.67 for input into the model.

[f]The fractional blood flow for the liver is the sum of the values in Vinegar and Jepson (1995, 1996) and Vinegar *et al*. (2000) for liver and gut as there is no gut compartment in this model.

[g]The fractional tissue volume for the rapidly perfused tissue is the sum of the values in Vinegar and Jepson (1995, 1996) and Vinegar *et al*. (2000) for rapidly perfused tissues and gut as there is no gut compartment in this model.

[h]The modified model used here doesn't include a brain compartment so for Clewell *et al*. (2001) the fractional brain values are added to the rapidly perfused values to maintain mass balance.

Table 2. Chemical-Specific Parameter Values[a]

Parameter	HFC-134a	HFC-227ea	
	Gunnare *et al.* (2006)	Vinegar & Jepson (1995, 1996)	Vinegar *et al.* (2000)
Molecular weight (g/mol)	102.03	170.0	170.03[c]
Partition Coefficients (unitless)			
Blood/air	0.56	0.225	0.033
Fat	7.96	7.022[b]	10.52[b]
Liver	0.87	1.867[b]	0.9394[b]
Rapidly perfused compartment	0.96	1.867[b]	0.9394[b]
Slowly perfused compartment	1.07	1.6[b]	0.6364[b]
Metabolism Parameters			
Maximum reaction rate ($L/min/kg^{0.75}$)	0.0	0.0	0.0
Michaelis-Menten affinity constant (mg/L^{d})	1.0	1.0	1.0
First order rate constant ($kg^{0.75}$/minute)	0.0	0.0	0.0
Uptake and Clearance Parameters ($L/min/kg^{0.75}$)			
Urinary clearance	0.0	0.0	0.0

Notes:
[a]The following parameters are not used for these chemicals and are therefore set to 0.0 or 1.0 to negate any effect and avoid division by zero: fractional volume of mucus, liquid/air partition, mucus/air partition, upper respiratory tract uptake, endogenous production parameters, and oral uptake parameters.
[b]Tissue/blood partitions are calculated from the blood/air partition and tissue/air partitions.
[c]Value from Vinegar *et al*. (1997).
[d]milligrams/liter

3.2.3 Model and Parameter Value Validation. The purpose of the validation figures for HFC-134a and HFC-227ea is to demonstrate whether the model is capable of adequately simulating the published data for these two chemicals (Emmen *et al*., 2000; Gunnare *et al*., 2006; Vinegar *et al*., 1997). Additionally, validation figures help determine which parameter sets best simulate the data and thus should be used for predicting the AFRL data. Studies from which data were used for validation are detailed below; digitized data utilized for validation are included in the script files in Appendix C.

Emmen *et al*. (2000) exposed eight healthy volunteers (four male and four female) to 1000, 2000, 4000, and 8000 parts per million (ppm) of HFC-134a for one hour each in a single-person whole-body exposure chamber. The study design included two air exposures and exposures to HFC-134a, HFC-227ea or CFC 12 (1000 and 4000 ppm). Propellant exposures were separated by at least a week. Exposures to the lower concentrations of HFC-134a were performed first, in case of adverse effects, of which there were none. Chamber concentrations were maintained within two percent of the target value. Blood samples were drawn from the arm via an indwelling cannula at intervals up to 60 minutes post-exposure; a final draw was performed at 24 hours post-exposure. Data were displayed graphically with male volunteers showing higher maximal blood concentrations than females. Increases for HFC-134a exposures (23 and 21 percent higher) were only significant during the 4000 and 8000 ppm exposures, respectively.

Male blood concentrations of HFC-227ea were 37, 50, 39 and 31 percent higher during 1000, 2000, 4000 and 8000 ppm exposures, respectively. Alpha- and beta-phase half-lives for HFC-134a were the same for both sexes: 9 and 42 minutes, respectively, while alpha- and beta-phase half-lives for HFC-227ea were different between sexes: 4.7 and 7.9 minutes for males and females, respectively. The beta-phase for HFC-227ea differs by sex and exposure concentration (low to high): 37, 62, 92 and 37 minutes for males, and 22, 42, 42 and 22 minutes for females. Blood samples taken 24-hours post-exposure were below the limit of quantitation (LOQ) of 0.011 microgram per milliliter (μg/mL) for HFC-134a and 0.010 μg/mL for HFC-227ea for all volunteers except one who had an HFC-134a concentration of 0.364 μg/mL.

Gunnare *et al*. (2006) completed a human kinetics study to facilitate development of a PBPK model. The study was performed with ten male volunteers with an average age and weight of 30 years (range of 22 to 40 years) and 83.5 kg (range of 22.3 to 34.5 kg), respectively. Target exposures were 500 ppm for two hours; exercise (50 Watts) was performed during the entire exposure on computer-controlled exercise bicycles. Exhaled breath was sampled at four points during the exposure and seven times post-exposure (six times up to six hours post-exposure and at 22.9 hours post-exposure the following day). Gunnare *et al*. (2006) calculated a mixed exhaled air concentration in their model as 0.89 times the arterial blood concentration divided by PB; this is equivalent to 0.89 times the alveolar blood concentration in the model used here. Capillary blood from the hand was collected at nine time points during exposure and at 11 times points during 23.3 hours post-exposure. Urine spot samples were gathered once before exposure and at 2, 4 and 6 hours after the start of exposure. Capillary blood and mixed exhaled air concentrations were reported for each individual in graphic form and average urine concentrations were summarized in a table.

The Vinegar *et al*. (1997) study supplies a limited amount of kinetic data. Exposures were well below safe levels determined by animal studies (rat, mouse and dog) and within the bounds of concentrations accepted for use in pMDIs. Exposures were to male volunteers via a face mask system and resulted in physical complications in all subjects exposed to HFC-134a or HFC-227ea; therefore, all exposures were stopped prior to scheduled completion. The first exposure to HFC-134a was for 4000 ppm for 4.5 minutes and resulted in loss of consciousness combined with loss of pulse and blood pressure; these effects were quickly reversed. Persistent issues for at least six weeks with dizziness and balance were reported. At the time of the exposure, the response was determined to be a vasovagal reflex and the study continued. A second subject was also exposed to 4000 ppm but exhibited a rapid rise in blood pressure and pulse approximately ten minutes into exposure; therefore, exposure ceased at 10.5 minutes. After an hour of breathing room air, this second subject was exposed to 2000 ppm. Following just 2.5 minutes of exposure, the subject's pulse and blood pressure again rose rapidly and the exposure was terminated. This subject also reported persistent dizziness and balance issues, a day-long headache, intermittent chest tightness, and fluttering sensation in the chest lasting two weeks, plus persistent tinnitus. Similarly, a high concentration of HFC-227ea (6400 ppm) resulted in a rapid rise in heart rate in a third subject after three minutes of exposure; exposure ceased at 3.5 minutes and the study was terminated. Exposure to HFC-227ea resulted in chest tightness for approximately three days plus dizziness and balance issues for six or seven days (Vinegar *et al*., 1997). For all three subjects, venous blood samples from the antecubital vein were collected at 30-second intervals until post-exposure. Blood concentrations were presented graphically.

Shin *et al*. (2015) studied whether exhaled breath concentrations of HFC-134a could be used to determine proper pMDI technique and medication delivery. Ten healthy volunteers (five males and five females, 25 to 48 years of age) were exposed to two puffs of an inhaled corticosteroid (Flovent, Glaxo Smith Kline, Research Triangle Park, NC) or albuterol (Proventil, Merck and Co., Inc., Whitehouse Station, NJ). Exhaled breath concentrations were gathered prior to and immediately following exposure, and at intervals from 2 to 48 hours post-administration. The study demonstrated that HFC-134a levels in the breath were still measurable and generally in excess of ambient room concentrations 24 hours following inhalation. The study LOD and LOQ were not expressly stated but the lowest measurements were in the 100 parts per trillion (ppt) by volume range. Peak concentrations increased six to seven times the lowest values. HFC-134a levels were displayed graphically for each individual subject; however, they were reported as a mixing ratio (*i.e.*, the ratio of the number of HFA-134a molecules to air molecules in a unit volume) and, therefore, were not used for validation. Data on changes in lung function were also reported graphically and in tables.

3.2.4 Prediction of Air Force Data. The PBPK model was run to simulate exposure to a single puff from an asthma inhaler, using HFC-134a as the propellant, to describe the exposures in the AFRL study. Simulations used chemical-specific parameters from Table 2 of Gunnare *et al*. (2006), physiological parameters from Table 1 of Clewell *et al*., (2001), and subject specific body weights from Table 3 (below). Gunnare *et al*. (2006) was the only paper with HFC-134a chemical specific parameters, but those subjects were exercising during the exposure; thus, those physiological parameters were deemed inappropriate for the AFRL data as these subjects were at rest. Some of the physiological parameter values presented in Vinegar and Jepson (1995, 1996) and Vinegar *et al*. (2000) are not consistent with what the authors of this work have typically seen; therefore, physiological parameters from Clewell *et al*. (2001) are used. The data from the AFRL study were not labeled as being either end- or mixed-exhaled air concentrations; based on the protocol for the AFRL study on how breath samples were to be collected, model predictions are for end-exhaled.

Table 3. Subject-Specific Body Weights

Subject Number	Gender	Body Weight	
		(pounds)	(kg)
Average	--	170.1	77.15
Subject 01 (A)	Female	149	67.57
Subject 02 (B)	Male	147	66.67
Subject 03 (C)	Male	153	69.39
Subject 04 (D)	Male	178	80.73
Subject 05 (E)	Female	137	62.13
Subject 06 (F)	Female	163	73.92
Subject 07 (G)	Male	200	90.70
Subject 08 (H)	Female	173	78.46
Subject 09 (I)	Male	180	81.63
Subject 10 (J)	Male	200	90.70

The exposure in ppm used for simulations was estimated based on the study specific inhalers, each of which contained a net weight of 8.5 grams (g) for an estimated 200 doses. The weight of albuterol and ethanol in the inhaler was considered negligible; therefore, 8.5 g is divided by 200 to get a per dose weight of 0.0425 g or 42.5 milligrams (mg). This dose is converted to moles using the molecular weight of HFC-134a (Table 2) and then converted to mL of gas by multiplying by the molecular weight of air at standard temperature, pressure and density (22.4 Liters/mole or 22.4 milliliters/mmole). A further adjustment is also made to account for the gas being at room temperature (20 degrees Centigrade or 293 kelvins (K)) by multiplying by the ratio of the room temperature to standard temperature (293 K/273 K). This gives 10.014 mL of HFC-134a per dose. This volume equates to 0.5 percent of a tidal volume of 2000 mL (Graepel and Alexander, 1991) or approximately 5000 ppm.

Simulations to reproduce the data were first run using an estimated dose of 5000 ppm but were repeated as necessary using adjusted doses until an adequate fit was achieved. As the length of time for which exhaled breath concentrations could be detected post-exposure is a priority for biomarker monitoring, the PBPK model was rerun using the best dose for each subject to determine the time at which the predicted exhaled breath reached the LOD. This was done for LODs of both 1 parts per billion (ppb) and 1 ppt.

3.2.5 Monte Carlo Analysis. A Monte Carlo analysis was conducted to demonstrate the potential impact in estimations for the time for end-exhaled breath samples to be below the LOD for HFC-134a for both 1 ppb and 1 ppt. Distributions were defined for both the physiological and chemical-specific parameters (Table 4) that are used for simulations of the AFRL subjects. SDs were calculated from means and each coefficient of variation (CV). The CVs were based on those used in published analyses (Covington *et al*., 2007; David *et al*., 2006). Parameters that were not included in the published analyses are given CVs consistent with similar parameters. Due to the uncertainty in the inhaled HFC-134a concentration and the lack of a basis for any value being more likely than any other to occur, a uniform distribution was used with upper and lower bounds equal to the highest and lowest estimated inhaled concentrations for the AFRL subjects. The analysis was performed using both a generic distribution for body weight based on the body weight in Clewell *et al*. (2001) and the CVs from the published analyses as well as using a body weight and corresponding CV determined from an Air Force biometric database (Choi *et al*., 2014; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI). Bounds for all distributions were calculated as two SDs except for when the body weight distribution was based on the Air Force biometric database, and then the maximum and minimum values from the database were used. The distributions were sampled to generate 5000 parameter sets; the same parameter sets were used for all estimations with the exception of body weight which uses information from either Clewell *et al*. (2001) or the Air Force biometric database. For each parameter set, the time to reach the specified LOD was saved and figures were created to show the results of this analysis.

Table 4. Monte Carlo Settings for Model Parameters[a]

Parameter	Mean	Coefficient of Variation (%)	Distribution	Bounds[b] Upper	Bounds[b] Lower
Body weight	70.0 / 76.71[d]	30 / 17.12[d]	Normal	112.0 / 129.5[d]	28.0 / 46.9[d]
Cardiac output	12.89	9	Normal	15.21	10.57
Ventilation perfusion ratio[c]	2.153	14	Log-Normal	2.7556	1.550
Inhaled concentration			Uniform	500.0	8000.0
Fractional Tissue Blood Flows (fraction of cardiac output)					
Fat	0.052	30	Normal	0.0832	0.0208
Liver	0.227	35	Normal	0.3859	0.0681
Rapidly perfused	0.533	20	Normal	0.7462	0.3198
Slowly perfused	0.188	15	Normal	0.2444	0.1316
Fractional Tissue Volumes (fraction of body weight)					
Fat	0.214	30	Normal	0.3424	0.0856
Liver	0.026	5	Normal	0.0286	0.0234
Rapidly perfused	0.056	10	Normal	0.0672	0.0448
Slowly perfused	0.536	30	Normal	0.8576	0.2144
Partition Coefficients (unitless)					
Blood/air	0.56	10	Log-Normal	0.672	0.448
Fat	7.96	30	Log-Normal	12.736	3.184
Liver	0.87	20	Log-Normal	1.218	0.522
Rapidly perfused	0.96	20	Log-Normal	1.344	0.576
Slowly perfused	1.07	20	Log-Normal	1.498	0.642

Notes:
[a]Means for physiological parameters are based on Clewell *et al*. (2001) and for chemical specific parameters are based on Gunnare *et al*. (2006) unless otherwise noted. Values not given in this table are not varied.
[b]Values shown are calculated as +/- two SDs.
[c]Ventilation perfusion ratio is the pulmonary ventilation rate divided by cardiac output and is used instead of varying the ventilation rate to maintain the relationship between cardiac output and ventilation rate.
[d]Body weight values from Clewell *et al*. (2001) and an Air Force biometric database (Choi *et al*., 2014), respectively; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI. CV of 17.12 is calculated from the mean and SD from the Air Force biometric database. Maximum and minimum values used with the body weight of 76.71 kg are from the Air Force biometric database (Choi *et al*., 2014).

4.0 RESULTS

4.1 Validation of PBPK Model and Parameter Values

Blood concentrations of HFC-134a from Emmen *et al*. (2000) for exposure to 1000, 2000, 4000 or 8000 ppm in four male and four female subjects are consistently over-predicted by the PBPK model using the physiological parameter values in Clewell *et al*. (2001) and the chemical specific parameter values from Gunnare *et al*. (2006) (Figure 2); physiological parameter values from Gunnare *et al*. (2006) were not used as they were representative of an exercise state and the subjects here were at rest during exposure. Using a smaller PB value from Ernstgard *et al*. (2010) (0.36 versus 0.56) improves the fits somewhat but the simulations still over-predict the data. While using resting physiological parameter values from Vinegar and Jepson (1995, 1996) or Vinegar *et al*. (2000) resulted in predictions that were similar to those using physiological parameter values from Clewell *et al*. (2001), they were not an improvement and thus are not shown in Figure 2. Neither individual data for the subjects nor SDs were given in the paper for additional comparison. As no information was given on physiological characteristics of the male and female subjects (*e.g.*, body weight), data for both males and females are shown with the same simulation.

Model predictions for capillary blood concentration of HFC-134a (Figure 3) following a 2-hour exposure to 500 ppm match the Gunnare *et al*. (2006) data well. Actual exposure concentrations for the ten male subjects ranged from 469 to 498 ppm with an average concentration of 486 ppm. While mean measured capillary blood concentrations from the exposure time frame are slightly over-predicted, the predictions are all well within two SDs of the data. Post-exposure data points are better predicted and are within two SDs for all but three post-exposure points. The predictions are also closer to most of the data points post-exposure than during the exposure period. Gunnare *et al*. (2006) referred to the exhaled air data as mixed air and calculated mixed exhaled air concentration in their model as 0.89 times the arterial blood concentration divided by PB. This is equivalent to 0.89 times the alveolar blood concentration in the model used here; therefore, the model predictions in Figure 3 for exhaled air are for 0.89 times the alveolar blood concentration. Model predictions for exhaled air concentration are also quite good with the simulation falling with two SDs for all data points.

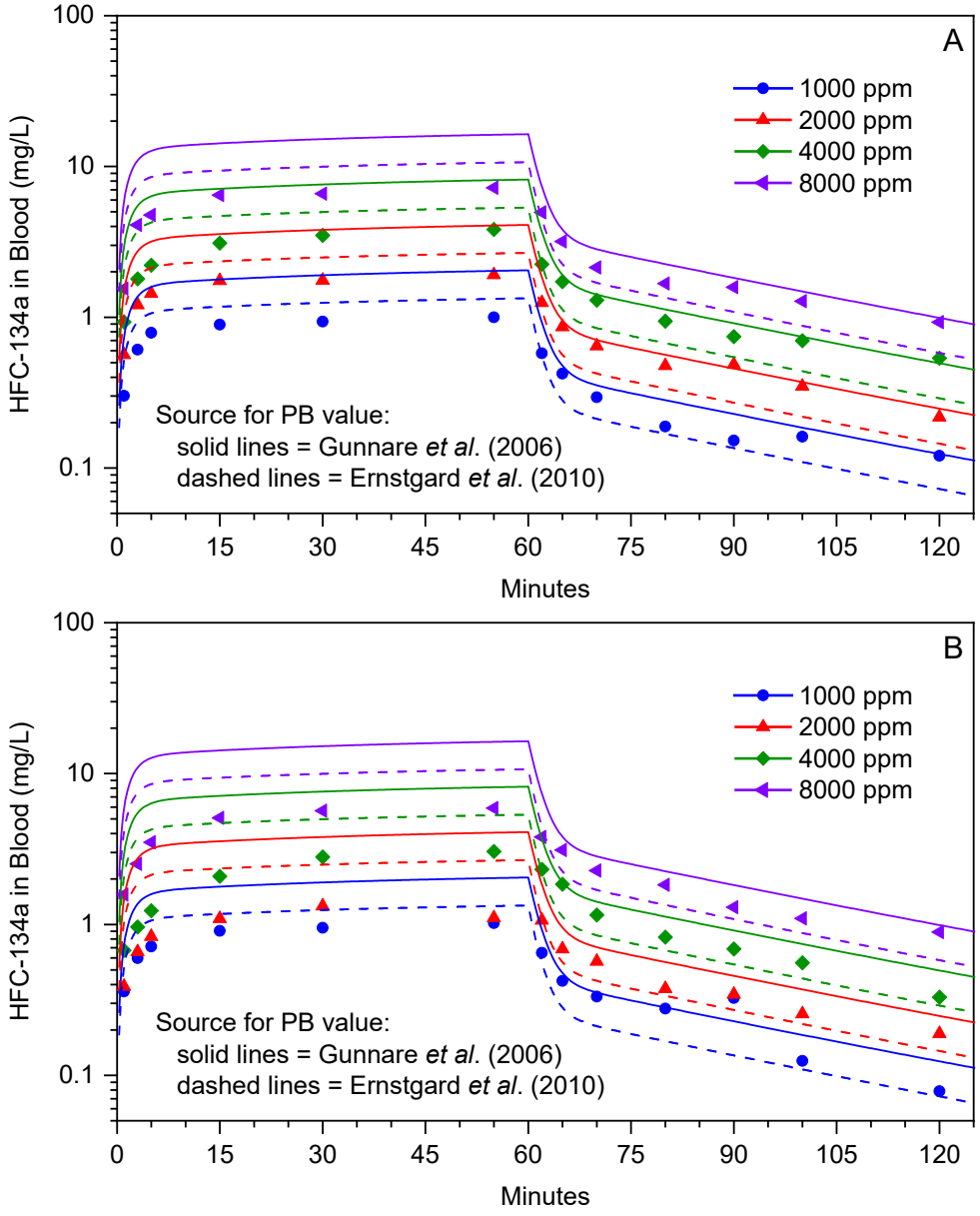

Figure 2. Emmen *et al*. (2000) HFC-134a Data. Predictions (lines) of blood concentrations of HFC-134a in four male (A) or four female (B) subjects during and following a one-hour inhalation exposure to 1000, 2000, 4000 or 8000 ppm HFC-134a using different sources for PB values as noted on the figure. Data (symbols) are from Emmen *et al*. (2000).

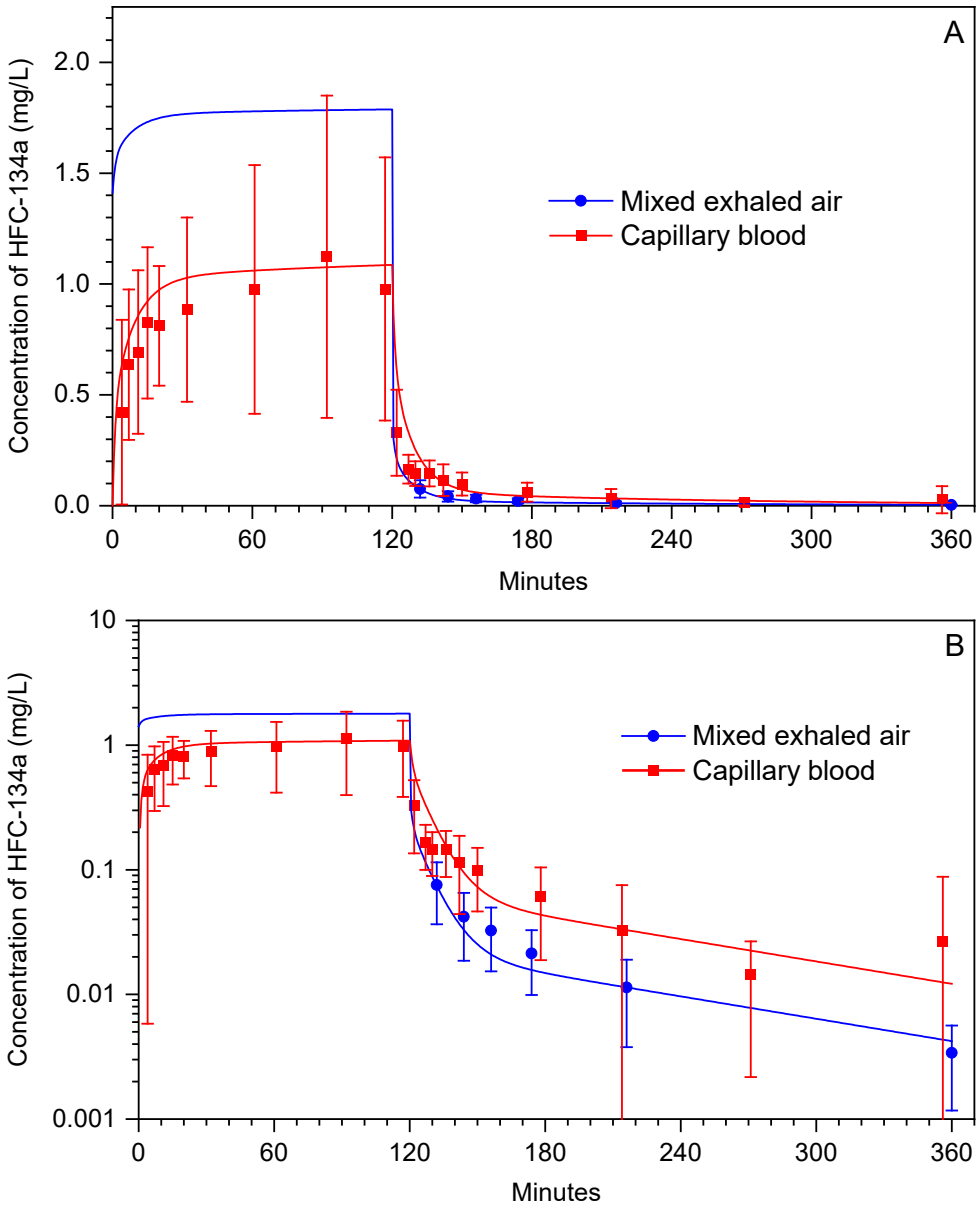

Figure 3. Gunnare *et al.* (2006) HFC-134a Data. Predictions of the average mixed exhaled air (blue lines) and capillary blood (red lines) concentrations of HFC-134a in ten male subjects during and following a two-hour inhalation exposure to an average of 486 ppm HFC-134a on a linear (A) and log (B) scale. Symbols are the mean ± two SDs of the data from Gunnare *et al.* (2006).

All combinations of physiological parameter values yield significant over-predictions of the HFC-134a data from Vinegar *et al.* (1997). Simulations use resting physiological parameter values from Vinegar and Jepson (1995, 1996) and physiological parameter values from Vinegar *et al.* (2000) and Clewell *et al.* (2001); Gunnare *et al.* (2006) physiological parameter values and moderate activity parameter values from Vinegar and Jepson (1995, 1996) were not used as the

subjects in this study were at rest during the exposure. Chemical specific parameter values for HFC-134a are only available from Gunnare *et al*. (2006) but simulations using both the PB value from Gunnare *et al*. (2006) and Ernstgard *et al*. (2010) were generated. Data from Vinegar *et al*. (1997) are for male subjects exposed to 4000 ppm for 4.5 or 11.5 minutes or to 2000 ppm for 2.5 minutes. The results of the various simulations are shown in Figures 4 and 5.

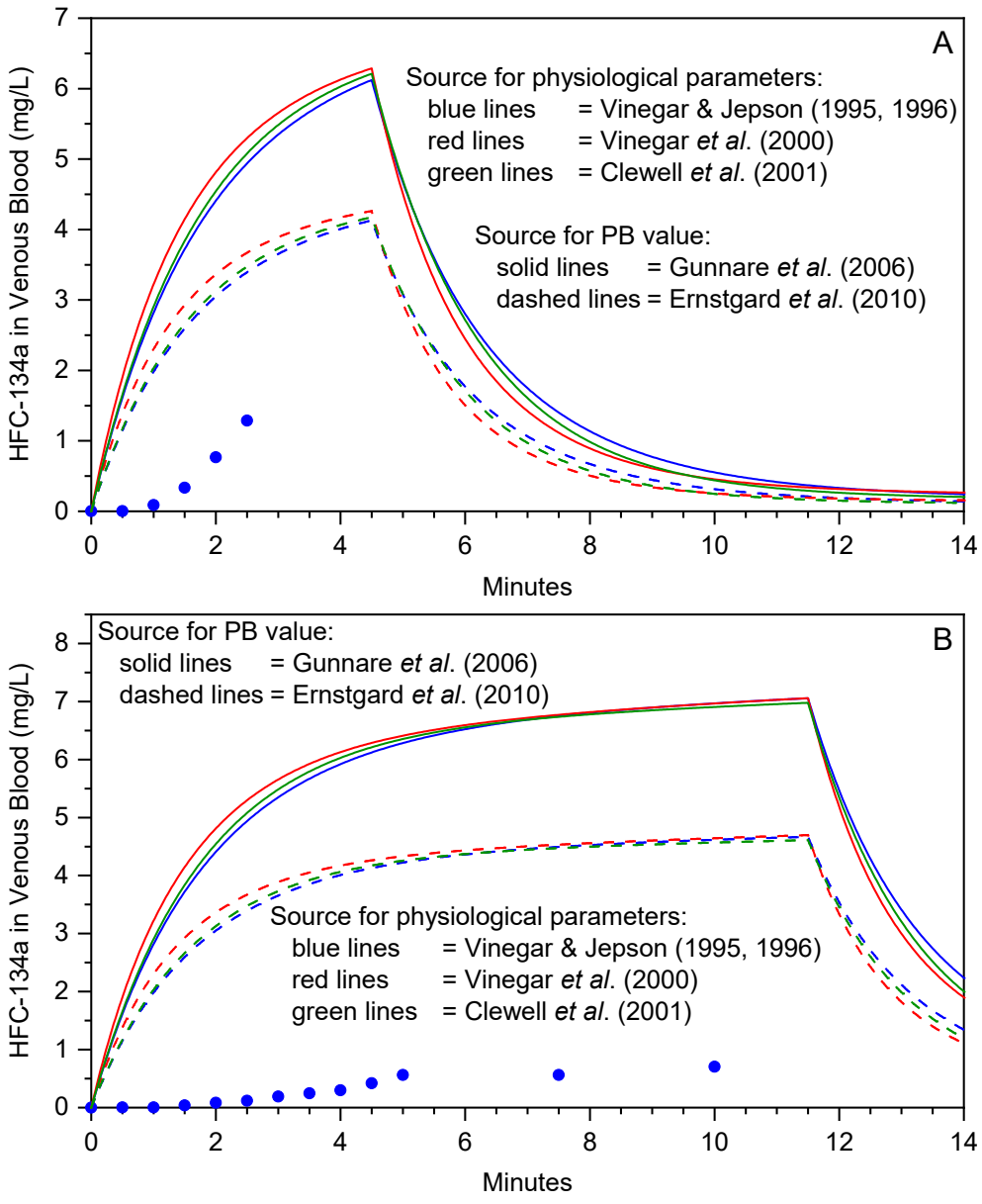

Figure 4. Vinegar *et al.* (1997) HFC-134a Data – 4000 ppm. Predictions (lines) of venous blood concentration of HFC-134a in one male subject during and following inhalation exposures to 4000 ppm HFC-134a for (A) 4.5 or (B) 11.5 minutes using different sources for the PB value and physiological parameters as noted on the figure. Data (symbols) are from Vinegar *et al*. (1997).

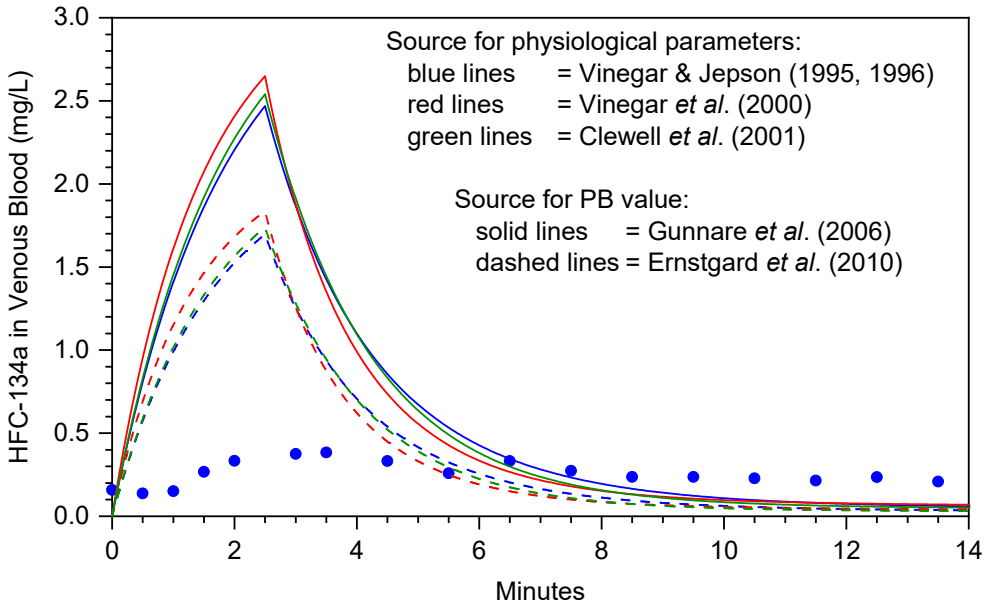

Figure 5. Vinegar *et al.* (1997) HFC-134a Data – 2000 ppm. Predictions (lines) of venous blood concentration of HFC-134a in one male subject during and following inhalation exposures to 2000 ppm HFC-134a for 2.5 minutes using different sources for the PB value and physiological parameters as noted on the figure. Data (symbols) are from Vinegar *et al*. (1997).

All blood concentrations of HFC-227ea from Emmen *et al*. (2000) for exposure to 1000, 2000, 4000 or 8000 ppm in four male and four female subjects are consistently over-predicted by the PBPK model when using chemical specific parameter values from Vinegar and Jepson (1995, 1996) regardless of the source for the physiological parameter values. All simulations using the chemical specific parameter values from Vinegar *et al*. (2000) are adequate up to about 80 minutes for males and 70 minutes for females regardless of the source for the physiological parameter values. Figure 6 shows only the simulations using chemical specific parameter values from Vinegar *et al*. (2000), but simulations using three sources for physiological parameter values (Clewell *et al*., 2001; Vinegar and Jepson, 1995, 1996 (resting); Vinegar *et al*., 2000) are shown. Gunnare *et al*. (2006) parameter values and moderate activity parameter values from Vinegar and Jepson (1995, 1996) were not used as the subjects in this study were at rest during the exposure. Note that the lines in Figure 6 mostly overlap during the exposure period for all physiological parameter value sources and continue to overlap for simulations using the Vinegar and Jepson (1995, 1996) (solid lines) and Clewell *et al*. (2006) (short dashed lines) parameter values until about 100 minutes after exposure starts. At this time, the simulations using the Clewell *et al*. (2006) parameter values result in slightly higher predictions than those using the Vinegar and Jepson (1995, 1996) parameter values. As with the figures for HFC-134a, data for both males and females are shown with the same simulation.

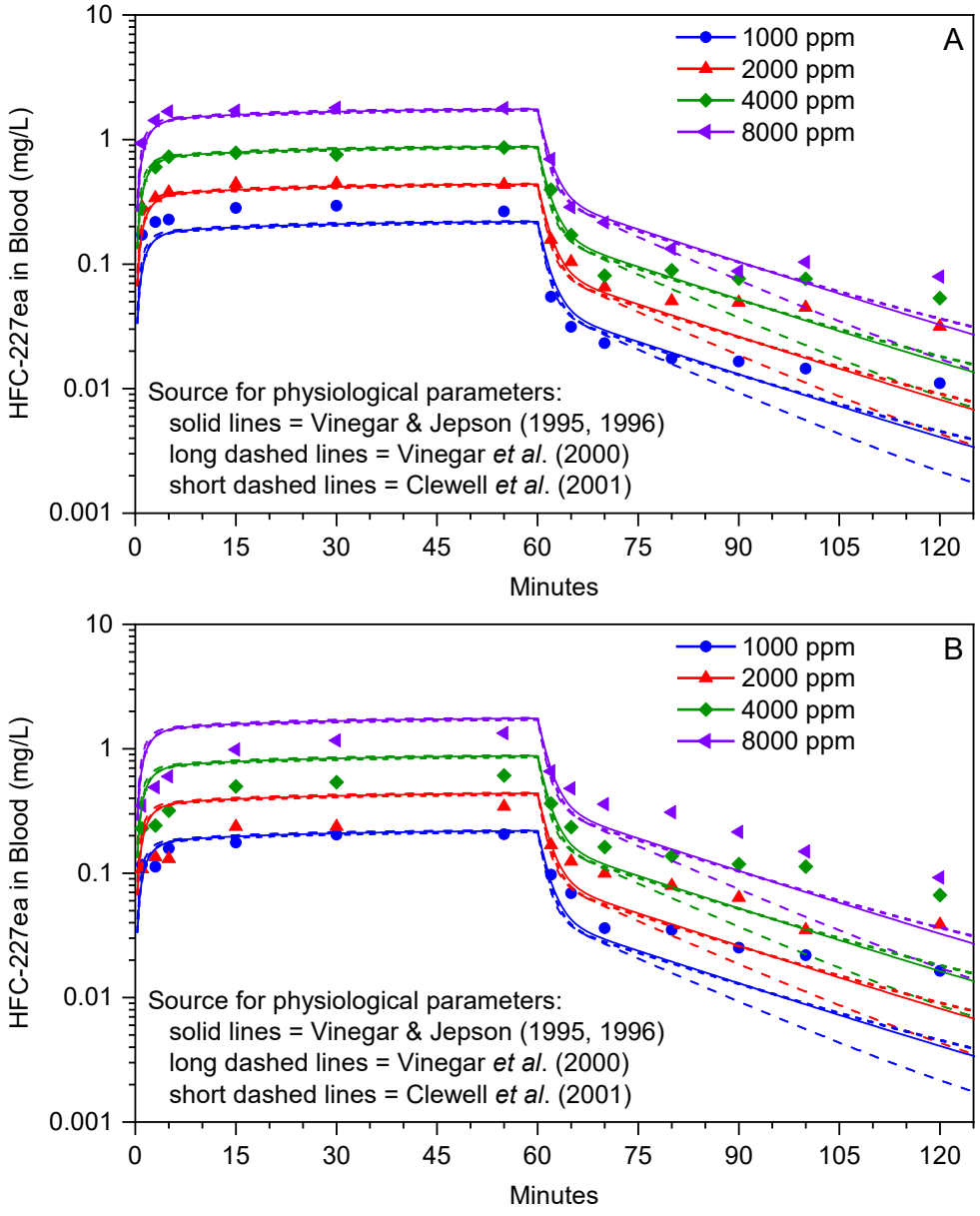

Figure 6. Emmen *et al.* (2000) HFC-227ea Data. Predictions (lines) of blood concentration of HFC-227ea in four male (A) or four female (B) subjects during and following a one-hour inhalation exposure to 1000, 2000, 4000 or 8000 ppm HFC-227ea using different sources for PB as noted on the figure. Data (symbols) are from Emmen *et al*. (2000).

As with HFC-134a, all combinations of physiological or chemical specific parameter values over-predict the HFC-227ea data from Vinegar *et al*. (1997). Simulations were made using resting physiological parameter values from Vinegar and Jepson (1995, 1996), physiological parameter values from Vinegar *et al*. (2000) and Clewell *et al*. (2001), and chemical specific parameter values for HFC-227ea from Vinegar and Jepson (1995, 1996) and Vinegar *et al*. (2000). Gunnare *et al*. (2006) physiological parameter values and moderate activity parameter

values from Vinegar and Jepson (1995, 1996) were not used as the subjects in this study were at rest during the exposure. Data from Vinegar *et al*. (1997) are for a male subject exposed to 6400 ppm for 3.5 minutes. Again, the exposure was stopped early due to physical complications displayed by the subject early in his exposure. The results of the various simulations are shown in Figure 7.

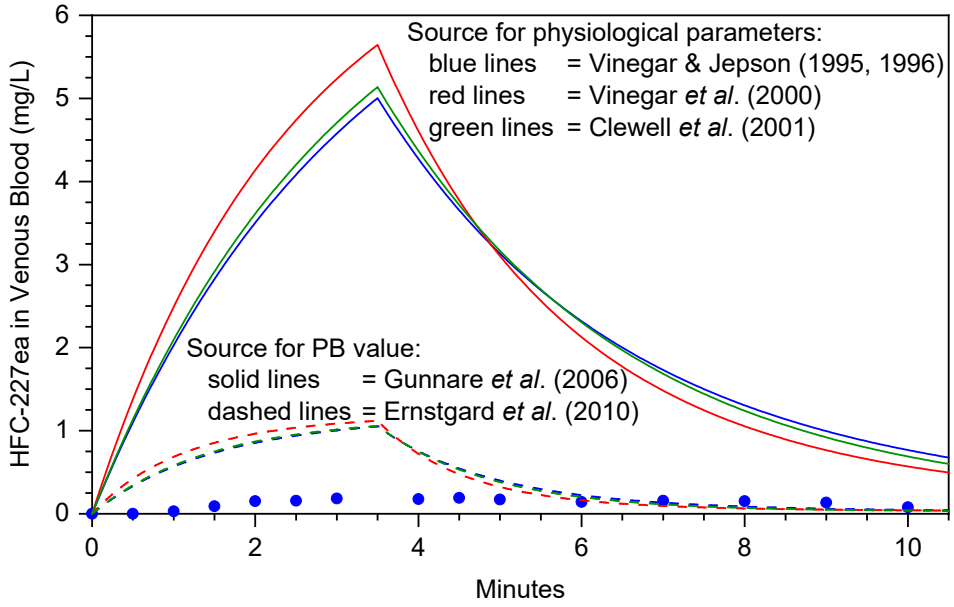

Figure 7. Vinegar *et al*. (1997) HFC-227ea Data. Predictions (lines) of venous blood concentration of HFC-227ea in one male subject during and following inhalation exposures to 6400 ppm HFC-134a for 3.5 minutes using different sources for the PB value and physiological parameter values as noted on the figure. Data (symbols) are from Vinegar *et al*. (1997).

4.2 Prediction of AFRL Data

For half of the ten AFRL subjects and the average of the ten subjects, the estimated concentration of 5000 ppm either noticeably over- or under-predicted the data for end-exhaled air concentration of HFC-134a following a single puff of asthma medication from an inhaler. In these cases, the dose was adjusted until the simulation better matched the data. The concentrations used for each subject are summarized in Table 5. Figure 8 shows the PBPK model prediction of end-exhaled air concentration as compared to the average of the data from ten subjects. Figures 9 through 18 show model predictions with the data for each individual subject. Times to reach the LODs of 1 ppb and 1 ppt are also shown in Table 5. While plots of the simulation out to the time to reach the LOD are not shown for all of the subjects, Figure 19 shows the simulation using the average exposure concentration of 3900 ppm as an example.

Table 5. Exposure Concentrations and Times to Reach LOD

Subject Number	Concentration (ppm)	Time to Reach LOD (hours)	
		1 ppb	1 ppt
Average	3900	20.34	73.27
Subject 01 (A)	5000	21.81	73.18
Subject 02 (B)	5000	21.76	72.95
Subject 03 (C)	5000	21.90	73.61
Subject 04 (D)	500	5.130	58.26
Subject 05 (E)	8000	24.93	75.23
Subject 06 (F)	1500	12.98	65.52
Subject 07 (G)	2500	17.34	72.63
Subject 08 (H)	5000	22.35	75.67
Subject 09 (I)	5000	22.49	76.35
Subject 10 (J)	1500	13.25	68.54

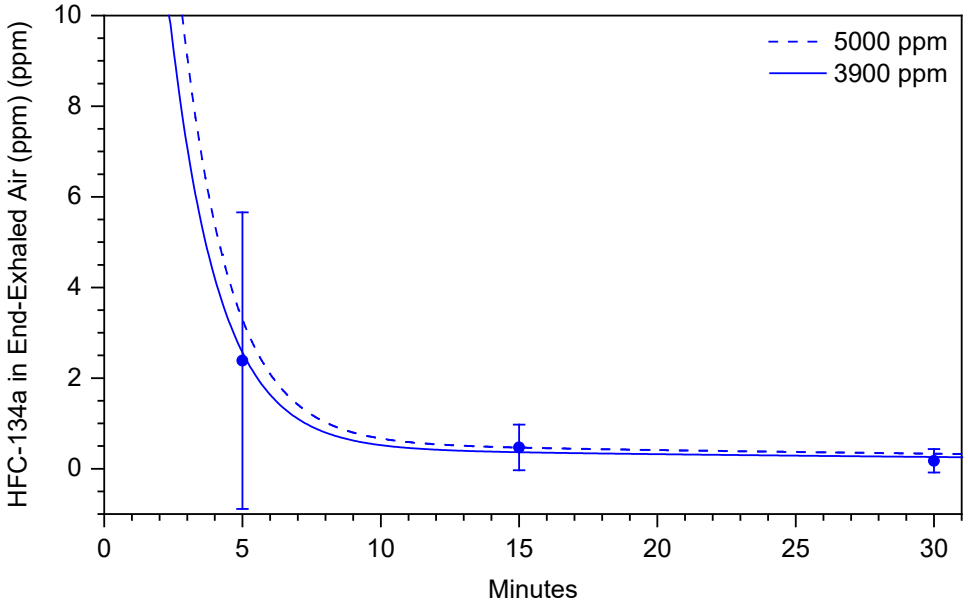

Figure 8. Average End-Exhaled Air HFC-134a Concentration. Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the average of the inhaled concentrations of 3900 ppm and the initial estimated concentration of 5000 ppm. Mean (symbols) ± two SDs (bars) are for the ten subjects from the AFRL study.

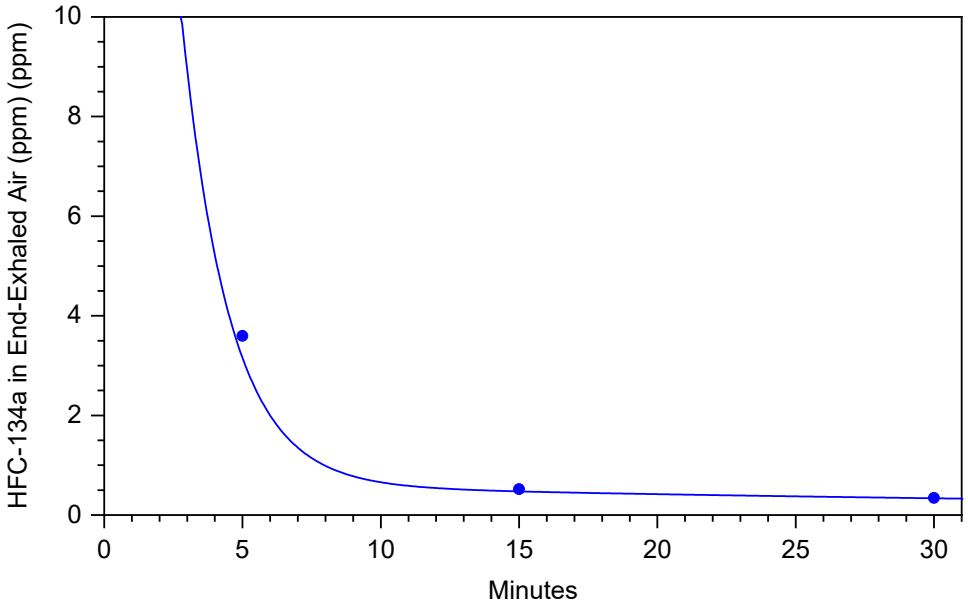

Figure 9. End-Exhaled Air HFC-134a Concentration for AFRL Subject 01 (A). Prediction of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the initial estimated concentration of 5000 ppm.

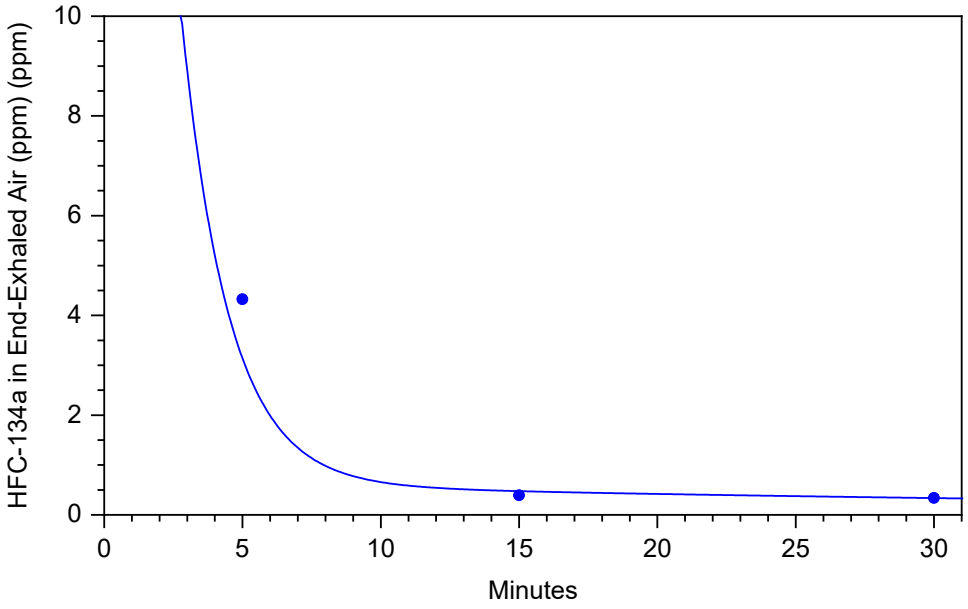

Figure 10. End-Exhaled Air HFC-134a Concentration for AFRL Subject 02 (B). Prediction of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the initial estimated concentration of 5000 ppm.

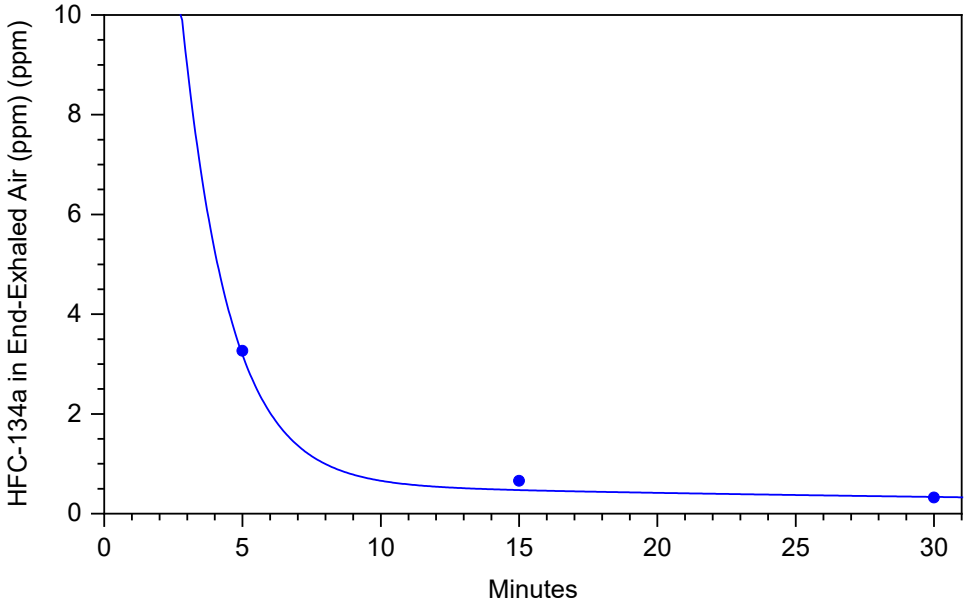

Figure 11. End-Exhaled Air HFC-134a Concentration for AFRL Subject 03 (C). Prediction of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the initial estimated concentration of 5000 ppm.

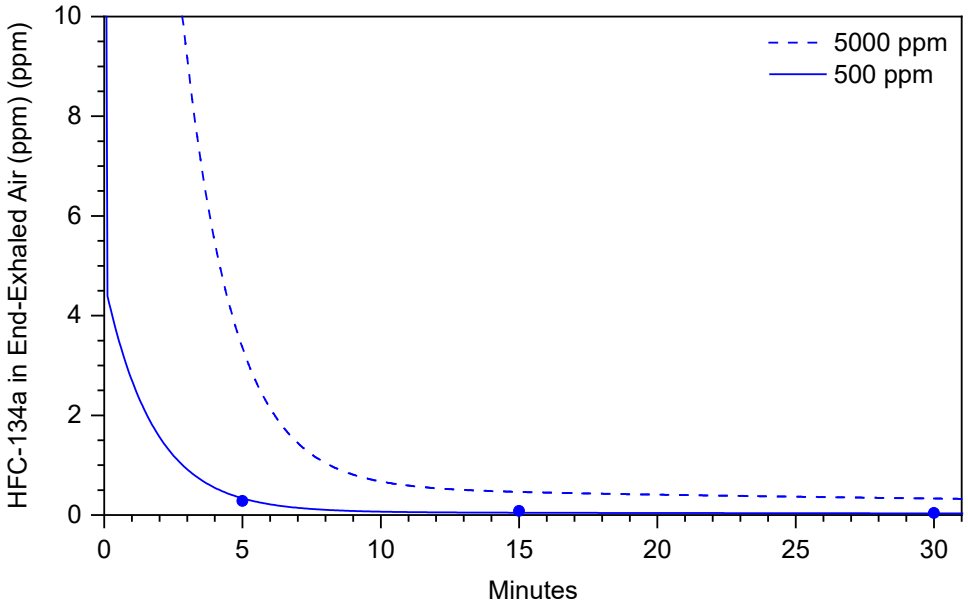

Figure 12. End-Exhaled Air HFC-134a Concentration for AFRL Subject 04 (D). Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using a fit inhaled concentration of 500 ppm and the initial estimated concentration of 5000 ppm.

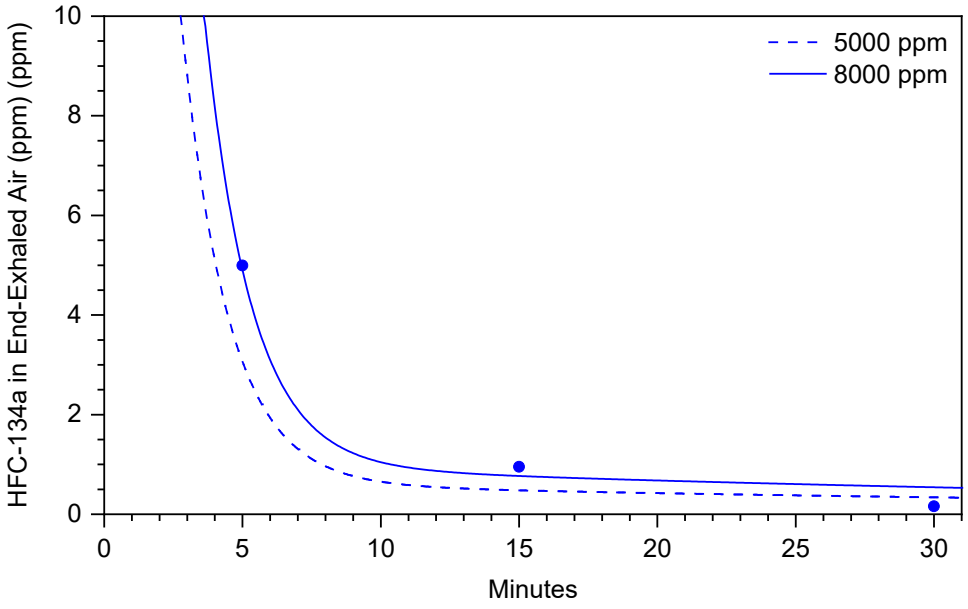

Figure 13. End-Exhaled Air HFC-134a Concentration for AFRL Subject 05 (E). Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using a fit inhaled concentration of 8000 ppm and the initial estimated concentration of 5000 ppm.

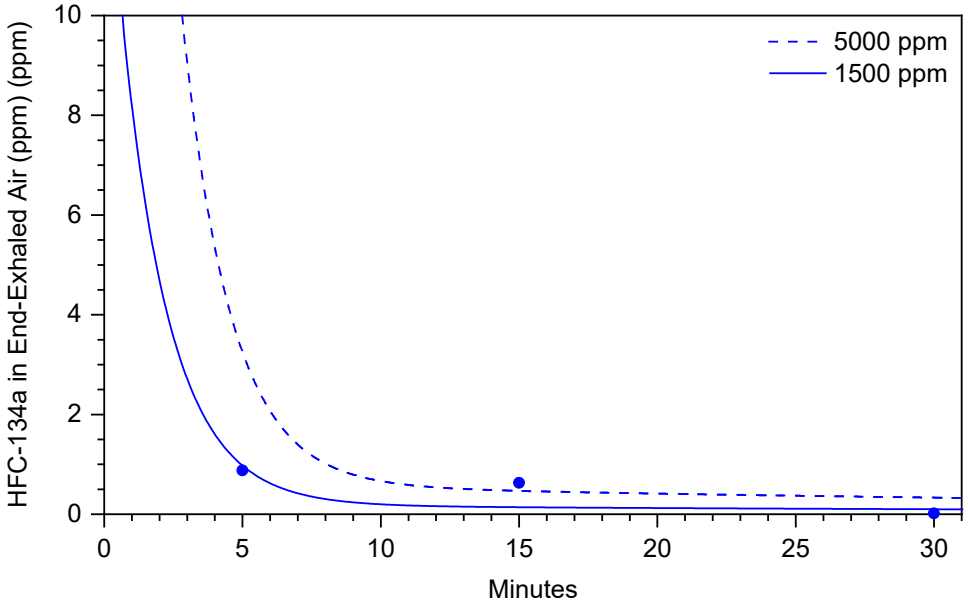

Figure 14. End-Exhaled Air HFC-134a Concentration for AFRL Subject 06 (F). Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using a fit inhaled concentration of 1500 ppm and the initial estimated concentration of 5000 ppm.

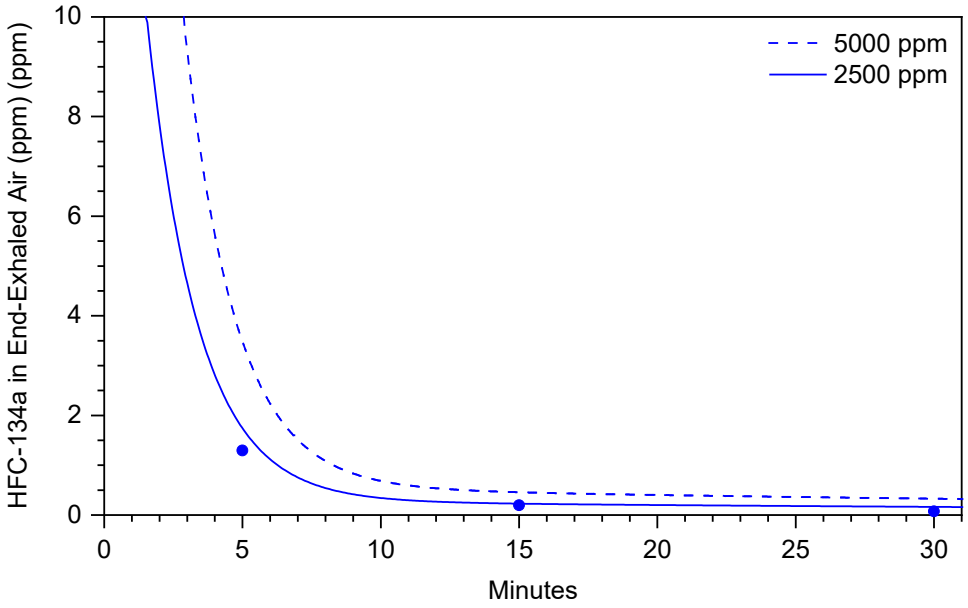

Figure 15. End-Exhaled Air HFC-134a Concentration for AFRL Subject 07 (G). Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using a fit inhaled concentration of 2500 ppm and the initial estimated concentration of 5000 ppm.

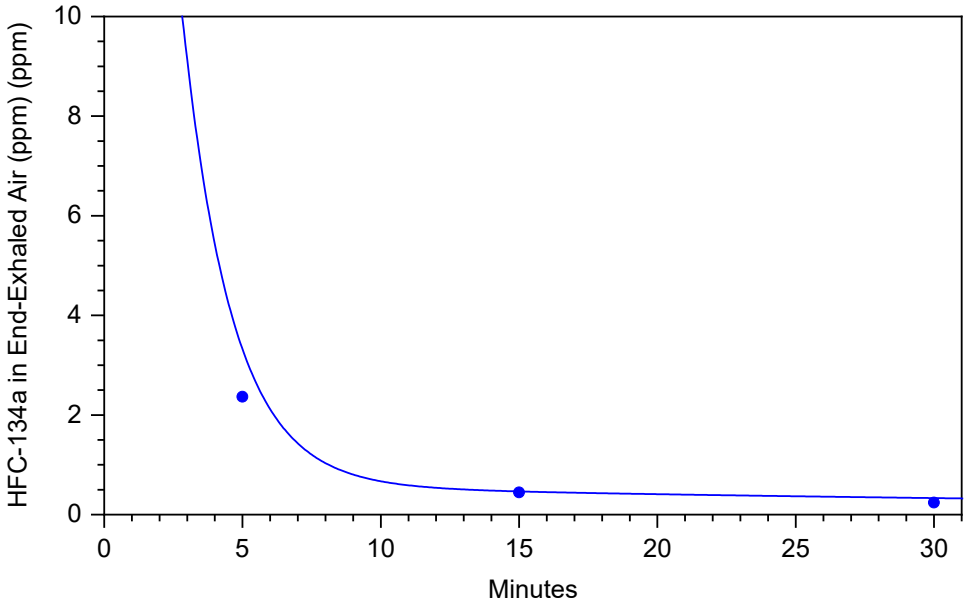

Figure 16. End-Exhaled Air HFC-134a Concentration for AFRL Subject 08 (H). Prediction of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the initial estimated concentration of 5000 ppm.

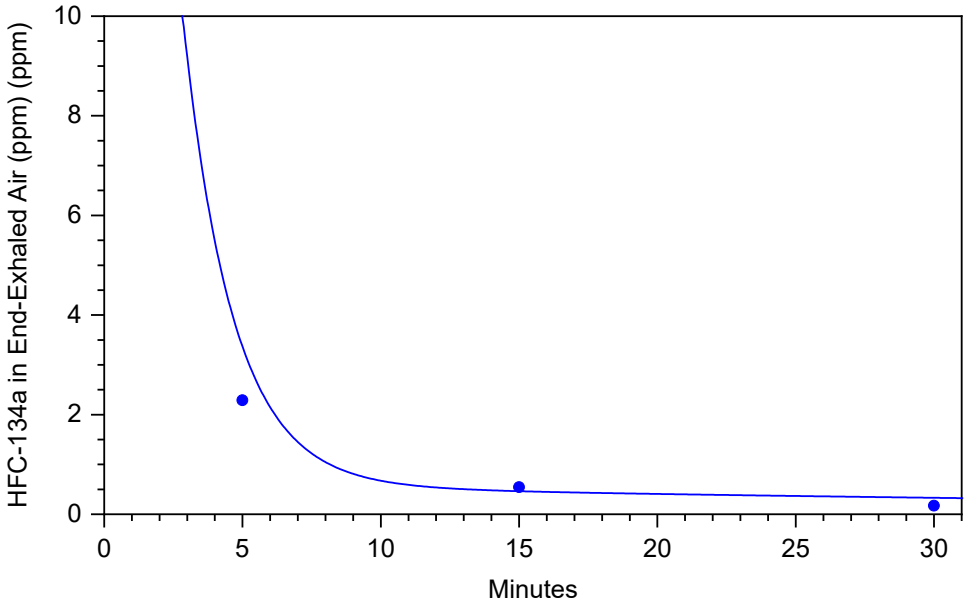

Figure 17. End-Exhaled Air HFC-134a Concentration for AFRL Subject 09 (I). Prediction of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using the initial estimated concentration of 5000 ppm.

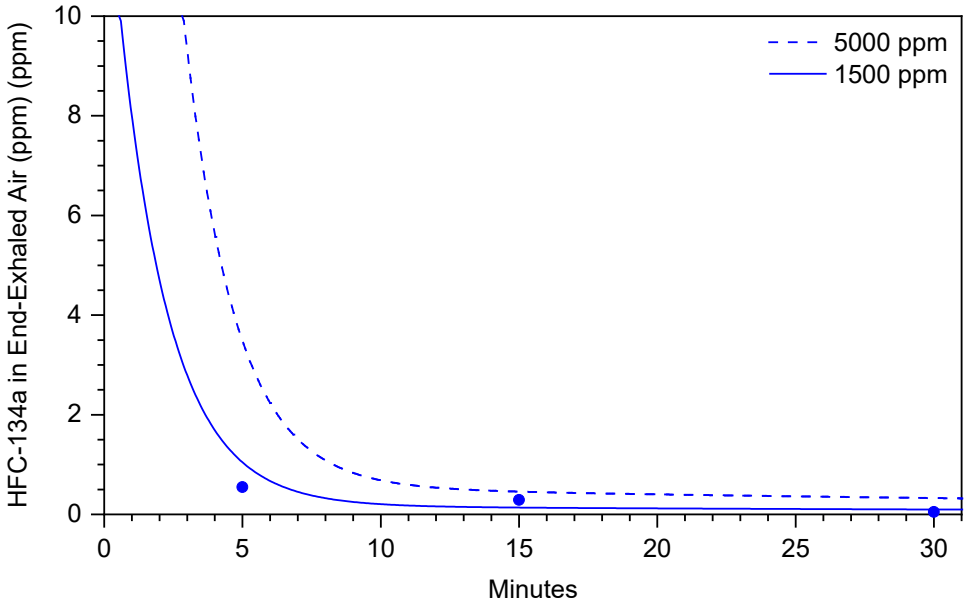

Figure 18. End-Exhaled Air HFC-134a Concentration for AFRL Subject 10 (J). Predictions of HFC-134a concentration in end-exhaled air following inhalation of HFC-134a in one puff of asthma medication using a fit inhaled concentration of 1500 ppm and the initial estimated concentration of 5000 ppm.

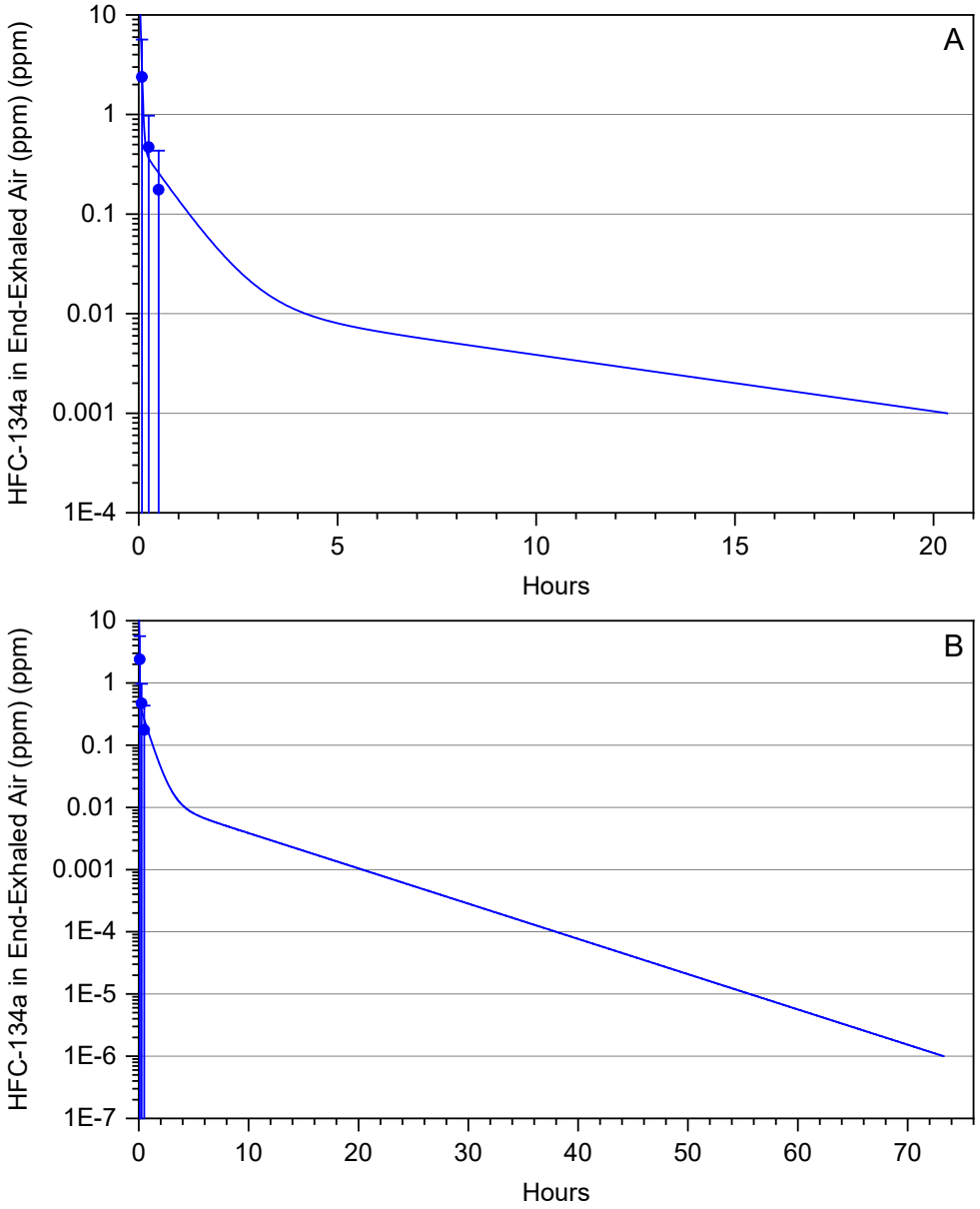

Figure 19. Time to Reach a LOD. Prediction of the time to reach a LOD of 1 ppb (A) or 1 ppt (B) following inhalation exposure of HFC-134a in one puff of asthma medication using the average of the inhaled concentrations of 3900 ppm. Mean (symbols) ± two SDs (bars) are for the ten subjects from the AFRL study.

4.3 Monte Carlo Analysis

The Monte Carlo analysis generated 5000 sets of parameters. In addition to compiling the predicted times to reach the LODs of 1 ppb and 1 ppt, the resulting distribution of inhaled concentrations used for these estimates was compiled (Figure 20). Distributions for the times to reach the LOD using either the body weight information from Clewell *et al*. (2001) or from the

Air Force biometric database (Choi *et al*., 2014; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI) are shown in Figures 21 and 22. Parameter sets for these figures are the same with the exception of the body weight as these came from two different definitions for the body weight distribution. Lines representing normal, lognormal and Weibull distributions are superimposed over the histograms of times to demonstrate what type of distribution best represents the output. The distribution that seems to best represent the output is shown with a solid line while the remaining two distributions are shown with dashed lines.

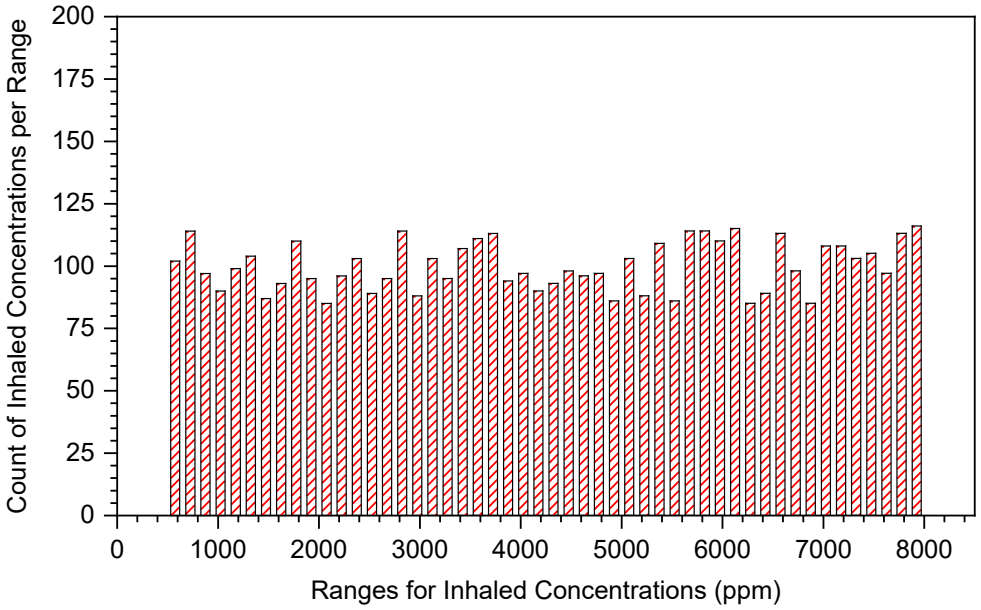

Figure 20. Distribution of Inhaled Concentrations from Monte Carlo. The parameter for inhaled concentration was defined to be uniform with a lower limit of 500 ppm and an upper limit of 8000 ppm.

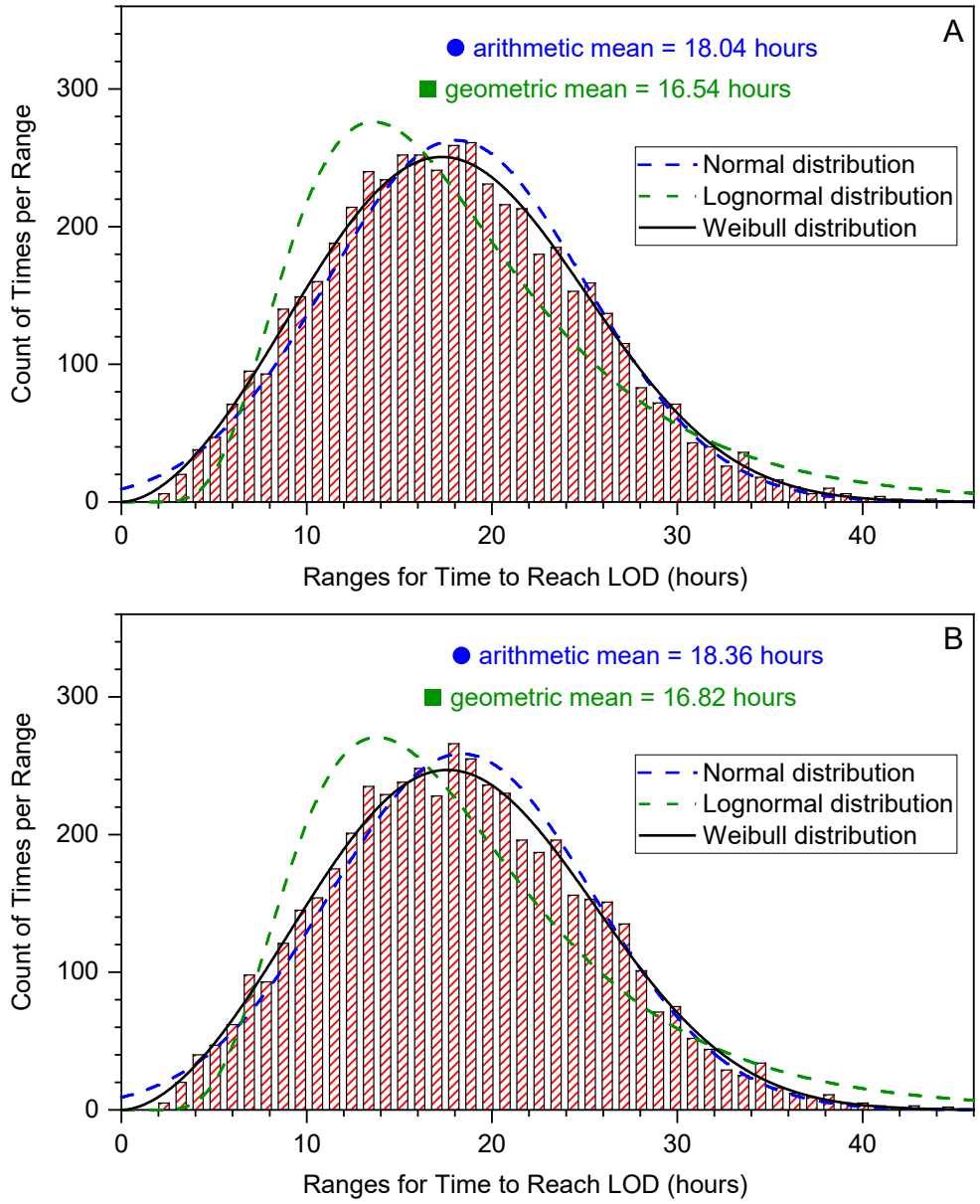

Figure 21. Distribution of Times to Reach a LOD of 1 ppb. The body weight distribution is defined with (A) a mean from Clewell *et al*. (2001) or (B) a mean, CV and bounds based on the Air Force biometric database (Choi *et al*., 2014; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI).

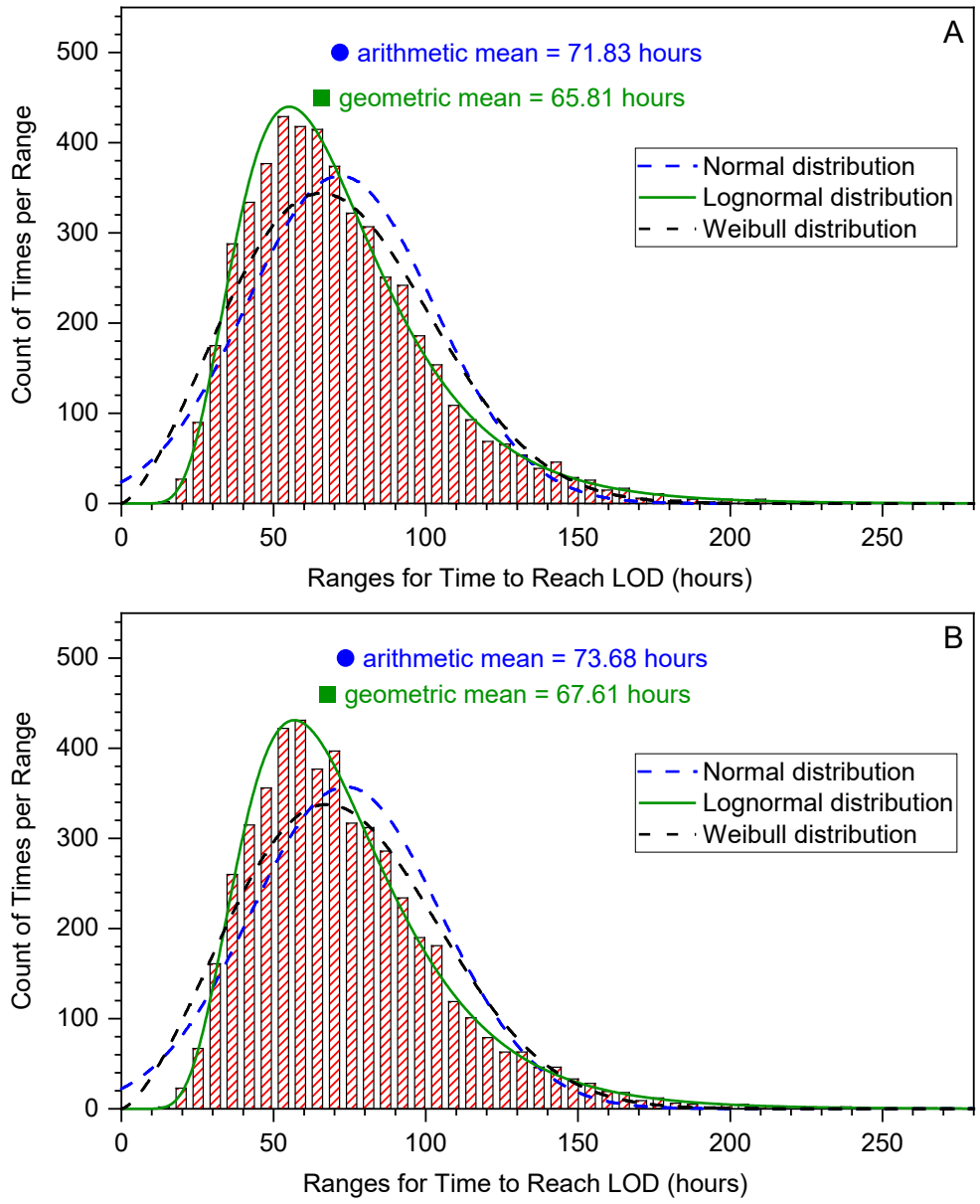

Figure 22. Distribution of Times to Reach a LOD of 1 ppt. The body weight distribution is defined with (A) a mean from Clewell *et al*. (2001) (A) or (B) a mean, CV and bounds based on the Air Force biometric database (Choi *et al*., 2014; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI).

5.0 DISCUSSION

5.1 Validation of Propellant PBPK Model and Parameter Values

Validation of propellant data sets resulted in mixed success. Individual data and variance descriptions (*e.g.*, SDs) for the Emmen *et al*. (2000) data were not given, so there is no way to determine if simulations would be captured within the inter-individual variation or whether data for some individuals might be better matched than for others. It is interesting to note, however, that the data for female subjects looked to be consistently lower than data for male subjects, suggesting the possibility of some gender differences in pharmacokinetics (*e.g*., body composition). It should also be noted that the doses and planned exposure times for the Vinegar *et al*. (1997) data fell within the range for the Emmen *et al*. (2000) data; however, there were no issues noted in Emmen *et al*. (2000) to necessitate stopping the exposures early. Vinegar and Jepson (1995, 1996) and Vinegar *et al*. (2000) presented a model for HFC-227ea, and Vinegar *et al*. (1997) collected data for exposure to HFC-134a; however, no simulations of a model were shown for the HFC-134a data in any of the located publications or tech reports related to work by Vinegar. While the data sets of Emmen *et al*. (2000) and Vinegar *et al*. (1997) were not well predicted by the model, they only included data for venous blood concentrations of HFC-134a while the data from the AFRL study are for exhaled air concentration.

Gunnare *et al*. (2006) is the only study simulated here that includes exhaled air concentrations and those data, along with Gunnare *et al*. (2006) data for venous blood, are well predicted by the parameter values. Hence, it was determined that the PBPK model was suitable to simulate exhaled air concentration using the chemical specific parameter values from Gunnare *et al*. (2006). As the AFRL subjects were not exercising, it was concluded that the physiological parameter values should come from another source that did not reflect an exercise condition. Physiological parameter values from Clewell *et al*. (2001) and study specific body weights for the ten AFRL subjects are thus used.

The AFRL study does not include exposure to HFC-227ea but, as HFC-227ea is also a common propellant in asthma medication and several of the publications located in the execution of this work included information on HFC-227ea, it was decided to also model the data for this chemical for comparison. With the exception of post-exposure clearance, model predictions of the HFC-227ea data from Emmen *et al*. (2000) were reasonably well predicted by the model when using chemical specific parameter values from Vinegar *et al*. (2000) and any of the physiological parameter values that were representative of a non-exercising state. Again, however, no combination of parameter sets could adequately model the data from Vinegar *et al*. (1997). As with the HFC-134a study, exposure had to be stopped earlier than planned; however, the dose and exposure time again fell within the range of concentrations and the exposure time from the Emmen *et al*. (2000) study for which no issues are noted. Also, as with the HFC-134a data, no model simulations could be located in any of the publications or tech reports related to work by Vinegar.

5.2 Prediction of Air Force Data

While it was necessary to optimize estimated inhaled concentrations for five of the AFRL subjects, concern over compliance with the dosing protocol had been noted by the AFRL study investigators and can also be an issue in the general public when using asthma inhalers (Levy *et al*., 2013; Velsor-Friedrich *et al*., 2009). In particular, the AFRL study investigators noted that Subject 04 (D) was taking shallow breaths when inhaling during dosing. This behavior would correspond to a much smaller inhaled concentration of 500 ppm being estimated for this subject. However, even though the inhaled concentration used to model the average data was adjusted to 3900 ppm to get a better fit, the fit using the initially estimated concentration of 5000 ppm is within two SDs of the data. Thus it was determined that, while dependent on the inhaled concentration, the PBPK model is able to adequately simulate exhaled air concentration using the chemical specific parameter values from Gunnare *et al*. (2006) and the physiological parameter values from Clewell *et al*. (2001).

Times to reach a LOD of 1 ppb ranged from about 5 hours up to almost 25 hours post-exposure; however, most were around 20 hours post exposure. Not surprisingly, the shortest time was for Subject 04 (D) who also had the smallest fit inhaled concentration, likely due to shallow breathing during dosing, and the longest time was for Subject 05 (E) who had the largest fit inhaled concentration. For a LOD of 1 ppt, times ranged from about 60 hours to about 76 hours with most around 73 hours. It should be noted, however, that none of the data used for validation of the PBPK model and parameter values extended more than six hours from the beginning of exposure or 4 hours post-exposure. The exposure times for these validation data were also much longer than for the AFRL study, but the inhaled concentrations estimated for the AFRL subjects were contained within the range of the validation study doses (500 ppm for Gunnare *et al*. (2006) and 1000 to 8000 ppm for Emmen *et al*. (2000)).

5.3 Monte Carlo Analysis

Figure 26 shows that the sampled inhaled concentrations are fairly well distributed across the range that was specified. More iterations or a different method for sampling from the distribution (*i.e.*, acslX does not use Latin hypercube methods which can better cover a distribution in fewer iterations) could result in more even coverage (Covington and Gearhart, 2020). Using body weights based on a mean from Clewell *et al*. (2001) versus body weights based on information from the Air Force biometric database (Choi *et al*., 2014; pilot specific data from personal communication with Jeffrey Hudson, 711 HPW/HPI) made very little difference in the estimates for the time to reach the LODs of both 1 ppb and 1 ppt. This is not surprising as the means were not very different (70 kg versus 76.71 kg) although the upper bound moved higher for the Air Force database body weight distribution definition and the lower bound was lower when based on Clewell *et al*. (2001). Times for an LOD of 1 ppb ranged from about 2 hours to about 45 hours with an arithmetic mean around 18 hours and a geometric mean around 16.5 hours. For an LOD of 1 ppt, the times ranged from about 12.5 hours to well over a week (just under 270 hours) with an arithmetic mean around 72 hours and a geometric mean around 66 hours. It is interesting to note that the times for a LOD of 1 ppb seem to follow a Weibull distribution better while those for a LOD of 1 ppt follow a lognormal distribution better.

The Monte Carlo analysis demonstrates the potential variability in predictions for the time to reach the LOD post-exposure. As mentioned above regarding Subject 04 (D), the effective absorbed dose of the chemical is likely not only dependent on the administered concentration but also on breathing rate during administration of the dose. While it should be noted that actual delivered dose would be dependent on breathing rate as well as the concentration of HFC-134a from the inhaler, these variations can be represented by randomly sampling from a distribution of inhaled concentrations.

5.4 Future Modeling Opportunities

Unfortunately for monitoring purposes, more inhaled drugs, including some asthma treatments, are now being packaged as dry powder inhalers (DPIs) without propellant. These drugs are compounded with a crystalline carrier particle in a capsule or divided dose disk (Morton and Fitch, 1992). Breath drawn through the inhalation device after loading the metered dose pulls the dry powder into the lungs. Since DPIs are activated by inhalation, not a pressurized release of propellant, good administration of the dose is not dependent on correctly timed inhalations; better lung delivery of several drugs has been achieved with DPIs verses pMDIs (Telko and Hickey, 2005). DPIs are also compact, many drugs are shelf stable for long periods in their dry form, and research is ongoing into better lung deposition using different inhaler designs (Wang *et al*., 2006).

The continued development and deployment of DPIs removes the propellant as a biomarker of drug use and compliance. If an alternative strategy is needed for detecting albuterol use or compliance, saliva monitoring should be considered. Saliva is often a good mirror of drug plasma concentration (Idkaidek, 2017). Therefore, modeling of albuterol kinetics in saliva would be a step toward its use as a biomarker.

6.0 CONCLUSIONS

The work detailed here demonstrated that an existing in-house PBPK model using parameter values from other published models for the chemical of interest was able to adequately simulate data from an AFRL study that collected end-exhaled air concentrations of HFC-134a following a single puff of asthma medication which used HFC-134a as the propellant. This allowed the authors to predict the time it would take for end-exhaled air concentrations to reach a specified LOD and to then expand that work to include the model in a Monte Carlo analysis to estimate times for a larger population. This analysis indicated that, on average, asthma inhaler use could be detected approximately 16 hours following dosing for a LOD of 1 ppb or up to 65 hours later for a LOD of 1 ppt.

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APPENDIX A: LITERATURE REVIEW FOR ALBUTEROL INFORMATION

A1.0 METHODS

A1.1 Literature Review for Albuterol Information

Albuterol is prescribed as albuterol sulfate (CAS number 51022-70-9), in either a solid or dissolved state; it has a molecular weight of 576.7 g/mol (PubChem, 2021c). However, most publications discuss the dosage as μg of albuterol base (the active ingredient); the base has a molecular weight of 239.31 g/mol (PubChem, 2021d). Also known as salbutamol in Europe and elsewhere, the terms "albuterol" or "albuterol base" were used throughout the current project.

Albuterol is the current first step in rescue treatment for intermittent asthma in teens (12 years old and up) and adults. Dosing for mild persistent asthma is typically two to four puffs every four to six hours (Cloutier *et al*., 2020). Treatments in the UK are administered as multiples of 100 μg of albuterol, while in the United States, the standard dose increment is 90 μg albuterol base (Pascoe *et al*., 2016). The recommended dose in the United States is 180 μg albuterol base in two puffs (FDA, 2018). At least 30 seconds between puffs is the standard recommendation (Boulet *et al*., 1999).

A1.1.1 Pharmacological Properties and Activity. Albuterol is a selective short-acting β2-agonist (Vaisman *et al*., 1987). Maximum effectiveness is reached within ten minutes after inhalation, and may last four to six hours if resting, or two hours during exercise (Morton and Fitch, 1992). The compound is 29 times more selective for β2-receptors versus β1-, leading to a much higher selectivity for the smooth muscle of the pulmonary system versus the β1-receptors prevalent in cardiac muscle (PubChem, 2021d). This comparatively low impact on heart muscle stimulation makes it a desirable alternative to previously used bronchodilators for a variety of airflow-obstructive lung diseases (Vaisman *et al*., 1987).

Albuterol most commonly consists of two enantiomers in equal quantities (Dhand *et al*., 1999); only (R)-albuterol is effective as a bronchodilator (Boger and Friden, 2019; Gumbhir-Shah *et al*., 1998). S-albuterol may contribute to adverse effects, such as additional respiratory events or asthma exacerbation, but are not well studied. R-albuterol may be administered alone and is known as levalbuterol, but in practice is cost prohibitive.

Although lung function improvement from albuterol in non-asthmatics is relatively small, some improvement has been shown in several studies (Bedi *et al*., 1988; Koch *et al*., 2015; Srichana *et al*., 2005). Koch *et al*. (2015) found that albuterol improved lung function (forced expiratory volume in one second; FEV_1) by 7.0 percent in athletes with exercise induced asthma and only 2.7 percent in athletes without the condition. In a different comparison of healthy volunteers and stable asthmatics, non-asthmatics improved their FEV_1 by approximately 4 percent (mean; range -2 to 25 percent) and their forced expiratory flow (FEF_{25-75}) by 13 percent (range -7 to 43 percent). Albuterol improved asthmatic lung function even more; FEV_1 improved 7 percent

(range -4 to 24 percent) and FEF_{25-75} increased 20 percent (range -2 to 46 percent) (Srichana *et al*., 2005). Goubalt *et al*. (2001) found that doses of 200 or 800 μg albuterol increased bronchodilation immediately following inhalation by normal cyclists, which may improve respiratory adaptation to exercise. However significant improvement in endurance was not seen when compared with placebo administration.

In a recent meta-analysis published by Riiser *et al*. (2020), supra-therapeutic doses of β2-agonists were shown to improve performance in sprint and strength (largely anaerobic) exercises. In one study, oral doses (12 mg/day for three weeks) were found to allow normal athletes a significantly longer time to muscle exhaustion. Exercise exhaustion periods increased 29 percent (23.7 to 30.5 minutes, Collomp *et al*., 2000). Single inhaled doses of 800 μg were found to improve cyclists' endurance by nearly 2 percent over an hour's work (van Baak *et al*., 2004).

As found in the Goubault *et al*. (2001) study discussed above, mild improvements in non-asthmatic lung function after using a clinically sanctioned dose of albuterol are often not associated with better endurance in athletes with or without asthma. Several studies reviewed by Morton and Fitch (1992) found no significant effect on performance variables when inhaled albuterol was tested in non-asthmatic athletes. These and other authors (Naranjo Orellana *et al*., 2006) have found albuterol to not be ergogenic to any significant extent. Therefore, for world-class athletic competition, a distinction is made between short-term inhaled albuterol administered at clinically recommended doses used by known asthmatics and the long-acting oral counterpart which might impart a significant advantage (Fitch, 2016).

A1.1.2 Albuterol Kinetic Studies. Kinetic data from inhaled albuterol studies in volunteers are available, although the low therapeutic doses historically have posed chemistry challenges for adequate detection in plasma following dosing. Therefore, many published kinetic studies follow higher doses than used therapeutically. Initial plasma concentrations from inhalation of the aerosol are augmented by gastrointestinal absorption of a portion of the dose, often making plasma time courses bi-phasic (Cazzola *et al*. 2002). Alternatively, some studies utilize activated charcoal (ActC) to prevent oral uptake, which then focuses the kinetics on the albuterol absorbed in the respiratory tract (Anderson *et al*., 1998; Logsdon *et al*., 1997; Silkstone *et al*., 2000; Srichana *et al*., 2005; Ward *et al*., 2000).

pMDIs may be used with and without spacing (holding) chambers. For portability, many asthma patients do not use the chamber. Using just a pMDI, approximately 80 percent of the dose deposits in the oropharynx and is swallowed. The swallowed dose affects plasma concentrations after approximately 60 minutes. If a chamber is used, deposition is decreased in the oropharynx but doesn't improve pulmonary deposition (Dhand *et al*., 1999).

Plasma half-life values were determined for pMDI-inhaled mixed enantiomer albuterol by Anderson *et al*. (1998). ActC was used to prevent oral absorption. Absorption, alpha and beta phases were found to be somewhat different for males and females. Five male healthy volunteers were reported to have half-life phases equaling 5.2, 20.5 and 4.9 minutes, respectively. The averages of five female half-life phases were 3.5, 15.3 and 3.9 minutes, respectively. Combined values, useful for modeling studies where the sex of the patients is

unknown, were 4.3, 17.9 and 4.4 minutes, respectively. The enantiomers have different clearance rates; R- and S-albuterol clearances were found to be 46.77 and 14.70 L/hour, respectively (Tripp *et al*., 2008).

*A1.1.2.1 Plasma and Urine Kinetics in Non-Asthmatics***.** Anderson *et al*. (1998) published time courses of plasma albuterol concentrations in both male and female healthy volunteers (five each) following a standard two-puff 180 μg (R,S)-albuterol base regimen using an inhaler and spacer device. ActC slurry was given by mouth to prevent oral absorption of albuterol. Volunteers were 26 ± 3 (mean ± SD) years of age; males weighed 78 ± 8 kg while females weighed 61 ± 7 kg. The Proventil pMDI was manufactured by Schering Corp. and contained a CFC propellant; the spacer was an Aerochamber from Monaghan. ActC (10 g) was administered two minutes pre- and post-dose and at one- and three-hours post-dose. Blood samples were drawn at intervals from 0 to 12 hours post-dosing. Data were graphically displayed for averaged males and females (mean ± SD) and for an individual male.

Harrison *et al*. (1996) compared the uptake of albuterol from HFC-134a containing inhalers (3M Health Care Ltd., Loughborough, UK) with older inhalers containing a CFC (Ventolin, Allen & Hanburys, Research Triangle Park, NC). Eight healthy males with a mean (± SD) age of 28 ± 4 years and weight of 78.7 ± 6.8 kg were recruited for this study. The exposure period began with the first puff of the inhaler. Two puffs, 30 seconds apart, delivered a total of 108 μg albuterol sulfate (90 μg albuterol base). The dose of HFC-134a was not reported. Whole blood concentrations were reported for HFC-134a over the first 16 minutes. Serum levels of albuterol administered using either propellant were only intermittently detectable over the first 60 minutes, given a LOQ of 1 ng/mL.

To verify their analytical technique for detecting albuterol using gas chromatography-mass spectrometry with selected-ion monitoring, Logsdon *et al* (1997) published the kinetic time course data for a single healthy subject who received 360 μg albuterol. The subject inhaled 90 μg albuterol base per puff (Proventil MDI, Schering, Kenilworth, NJ), four times with 30 seconds between puffs from a commercial pMDI. An Aerochamber spacer (Plattsburgh, NY) was used. ActC (10 g) was orally administered as a slurry two minutes pre- and post-dosing, as well as at one- and three-hours post dose, to prevent absorption of swallowed albuterol. Blood samples were drawn at 5, 10, 15, 30, and 45 minutes plus 1-, 2-, 3-, 4- and 6-hours post-inhalation. Plasma data from this single subject were presented graphically.

In a comparison of a standard pMDI and a novel modified metered-dose actuator device, Newnham *et al*. (1993) administered cumulative doses of 200, 600, 1400 and 2600 μg albuterol to ten healthy males with an average age of 24 years (standard error of 1 year). A single dose of 200 μg albuterol was administered; blood samples were drawn immediately prior to the dose and at 5-, 10-, 30- and 60-minutes post-dose. Plasma albuterol was reported for both inhaler types; the pMDI brand was not stated. Although the actuator device produced a higher maximal concentration, the areas under the curve (AUCs) of both delivery devices are not significantly different from each other, as depicted graphically.

Silkstone *et al*. (2000) investigated urinary excretion of albuterol following inhalation and oral administration. This study highlights the absorption of the dose from the lung, as inhaled concentrations were administered with and without ActC. Five female and five male healthy subjects, aged 28.1 ± 7.0 years (mean ± SD) and weighing 69.6 ± 11.2 kg, were exposed to each of four treatments, with a seven-day gap between test days. The four treatments were: oral albuterol (500 μg, supplier not stated), with and without a 5 g ActC slurry immediately following the doses, and five inhalations of 100 μg albuterol base from a Ventolin (Glaxo Wellcome, UK) pMDI, separated by eight minutes, again with and without AC immediately following. Study times were recorded from the first dose and urine was collected cumulatively over 120 minutes in four different intervals. Albuterol was not detectable in urine from oral albuterol administered with ActC. The authors presented the data in tabular form for the four accumulation intervals. Inhaled albuterol excretion over the first half hour was shown to be due to pulmonary absorption; differences between the with- and without-ActC administrations were apparent after 30 minutes due to absorption of the swallowed drug.

Srichana *et al*. (2005) exposed healthy and asthmatic volunteers to three albuterol dry powder formulas for efficacy comparison. The three test formulations were prepared using micronized albuterol sulfate (Glaxo-Wellcome, Ware, UK) and different sized particles of lactose; doses appear to have been calculated using weighed amounts of the sulfate, not the base compound. ActC (520 mg one hour prior to exposure) was used to prevent absorption from the GI tract. The authors also tested the efficacy of ActC administered prior to an oral albuterol dose; 260 mg of ActC allowed the absorption of approximately 2.5 percent of a 400 μg oral dose, so the ActC preventative was doubled for the inhalation study. The healthy group included six males and six females ranging from 20 to 41 years old (mean = 33 years); all were exposed to 100, 120 or 160 μg albuterol on different study days a week apart. Blood samples were drawn at intervals from 0 to 480 minutes after inhalation and the results were presented in graphical form.

Tomlinson *et al*. (2003) concentrated on the interindividual variation in urinary excretion of albuterol between subjects and intraindividual variation of subjects when repeatedly exposed. The authors recruited eight women and seven men to test interindividual urinary excretion following single doses of 100, 200, 300, 400, or 500 μg administered as inhalation(s) of 100 μg per puff from a Ventolin (Glaxo Wellcome, UK) pMDI on separate study days. The number of days between doses was not specified. Study volunteers were 30.3 ± 6.8 years of age (mean ± SD) and weighed 68.5 ± 11.4 kg. Subjects were trained extensively on the consistent use of the pMDI. Cumulative urine was collected representing 0 to 30 minutes post-exposure, starting at the first inhalation of albuterol. Means and SDs were described in the text and individual data points were graphed. To examine intra- and interindividual variability, a subset including four women and three men (29.1 ± 4.5 years, 66.1 ± 14.4 kg) were exposed to 100, 300 or 500 μg, five times each. Individual means and SDs were reported in a table; overall statistics were described in the text. Finally, the authors tested the intra- and interindividual variability of five replicates of 100 μg albuterol/day using a breath-actuated pMDI (Easi-Breathe). Five individuals participated in this experiment (32.0 ± 2.9 years, 66.8 ± 14.1 kg). Their cumulative mean 0- to 30-minute urinary excretion using this pMDI was 2.52 ± 0.66 μg, comparable to the mean excretion amounts from both the interindividual and intraindividual experiments using the Ventolin pMDI.

Vaisman *et al*. (1987) compared the kinetics of inhaled albuterol in patients with cystic fibrosis and healthy adults. Five healthy subjects with an average age of 30.8 years (SE of 2 years; range of 27 to 37 years) and an average weight of 62.1 kg (SE of 5 kg; range of 52.6 to 80.5 kg) were exposed to 5 mg albuterol (0.5 percent solution, Allen and Hanburys, Ltd., Toronto, Canada) over seven minutes by nebulizer; the body weight adjusted average dose was 82.6 ± 6.2 μg/kg. Serum concentrations were presented in graphs at intervals from 15 to 120 minutes post-exposure. Although an additional group of five healthy volunteers received albuterol intravenously, the time course kinetics useful for the PBPK model were not reported. However, by comparing the intravenous and inhaled dose serum levels, Vaisman and coauthors were able to estimate the bioavailability of albuterol to be 2.3 percent.

A1.1.2.2 Plasma and Urine Enantiomer Kinetics in Non-Asthmatics. Gumbhir-Shah *et al*. (1998) published a comparison of plasma concentrations and cumulative urine amounts for volunteers exposed by nebulizer to (R)- or (R,S)-albuterol (Ventolin ampules; Allen and Hansburys, Greenford, UK). Group A volunteers were exposed to 1.25 mg of the (R)-enantiomer in both dosing scenarios. Group B subjects received 5 mg of the (R)-albuterol via 4-step nebulization of the same dosing capsules as used for Group A. Blood concentrations for both groups are reported on a graph with 14 overlapping individual lines for Group A and 13 lines for Group B. Additionally, although cumulative urine amounts of excreted albuterol were measured, only summary data were reported (percent recovered and excretion rate).

Schmekel *et al*. (1999) compared albuterol enantiomer kinetics over different routes of administration (inhalation, endotracheal instillation and oral). Healthy volunteers were dosed with albuterol (Ventolin brand formulations, Glaxo-Wellcome, Uxbridge, Middlesex, UK) via one or more routes, four to eight subjects per route. Oral doses consisted of a 4 mg racemic albuterol tablet; inhalation doses were 0.4 mg using a Diskhaler DPI, and endotracheal instillation of 0.2 mg albuterol occurred under full anesthetic during an orthopedic surgery involving an extremity. The endotracheal instillation kinetic study was designed to compare with the standard inhalation study, in order to determine what portion of the standard inhalation was absorbed in the lung. Blood collection occurred before dosing and at intervals up to 360 minutes post-dosing. Plasma concentrations of R- and S-albuterol are reported, as well as cumulative urine amounts from 0 to 6 hours and 6 to 24 hours.

Ward *et al*. (2000) also compared the metabolism of inhaled R- and S-albuterol. Fifteen healthy non-smokers were recruited. The seven females ranged in age from 23 to 40 years and weighed 50 to 90 kg. Eight males ranged from 21 to 43 years and 55 to 95 kg. Albuterol formulations were Ventolin brand (Glaxo-Wellcome Research & Development, Ltd, Greenford, UK). Each volunteer inhaled 1200 μg albuterol (12 inhalations from a pMDI at 20-second intervals), with and without ActC; 2 mg albuterol orally, with and without ActC; and 500 μg albuterol intravenously over five minutes; each of the five treatments was separated by at least seven days. ActC was given in a slurry by mouth at the following time points: 5 mg at two minutes pre-, 5 mg at two minutes post-, and 10 mg at 1-, 2-, and 3-hours post-albuterol administration. Blood samples were drawn at intervals ranging from 1 minute to 24 hours post-albuterol administration. Cumulative urine was collected in six intervals covering 0 minutes post-dose through 24 hours. The charcoal regimen was found to be 92 to 98 percent effective. Inhaled R- and S-albuterol

plasma concentrations were reported graphically and showed similar uptake from the lung for both enantiomers (group given ActC) and increased uptake of S-albuterol from the digestive tract (no ActC group). Unfortunately, intravenous plasma concentrations and cumulative urine amounts were not presented for any dose group.

A1.1.2.3 Plasma and Urine Kinetics in Asthmatics. Unprescribed use of albuterol may be attempted by both healthy individuals looking for an added boost in lung volume, or by former asthmatics who have outgrown most asthma symptoms but may still have lung deficits. An albuterol PBPK model should be able to replicate both healthy adults and those impacted by asthma in order to predict a range of drug uptake reflective of those with residual lung deficits. Albuterol pharmacokinetics are very different when the healthy and asthmatics are compared. The Srichana *et al*. (2005) data indicate at least a 46 percent lower peak plasma albuterol concentration in asthmatics given the same dose as the healthy subjects.

Janson (1991) ran a parallel intravenous and inhalation albuterol study in stable asthmatics who had been without acute symptoms for a minimum of two weeks. Patients included six men and six women, with an overall mean age of 54 years (33- to 71-year range). Patients were to abstain from oral and inhaled β2-agonists for 24 and 6 hours, respectively. Inhaled doses of 0.15 mg/kg (Ventoline, Glaxo) were administered by nebulizer over a mean duration of 13 minutes (10- to 15-minute range) and intravenous doses of 5 μg/kg (Ventoline, Glaxo) were infused over ten minutes. Blood samples were drawn at -1, 1, 15, 30, 45, 60, and 90 minutes. Plasma time courses for individual patients were presented to compare the routes of administration. Maximum concentrations plus AUCs were reported.

Newnham and Lipworth (1994) tested the effects of two nebulizers (Ventstream, Medic-Aid, Pagham, UK; and Hudson Updraft II, Hudson Respiratory Care Inc., CA) for delivery of three albuterol concentrations to stable asthmatics. Eight asthmatics with a mean (± SE) age of 41 (± 5) years (range = 24 to 69) without recent exacerbation abstained from relevant medications prior to testing. Albuterol doses (Ventolin Nebules, Allen and Hanburys, Uxbridge, UK) were 1.25, 2.5 and 5.0 mg. Nebulizer output (g/minute) and percent of dose measuring less than 5 μm (respirable particles) were recorded. The authors reported time courses for both nebulizers as mean and SE at each concentration.

In order to determine the effect of proper (slow), fast and late inhalation on the dose delivered using a pMDI, Tomlinson *et al*. (2005) studied urinary excretion and methacholine challenges in 12 mild asthmatics. Cumulative urinary excretion of albuterol was measured for 0 to 30 minutes post-inhalation following one or two puffs of 100 μg albuterol from an Easi-Breathe breath activated pMDI (IVAX, UK). Doses were separated by at least seven days. Descriptions of the subjects' disease states are similar to those described in other studies as "stable". The 12 people were an average (mean ± SD) of 21.1 ± 2.0 years of age and weighed 70.2 ± 17.2 kg. Cumulative excreted albuterol was reported as 2.25 ± 0.65 and 5.37 ± 1.36 μg for 100 and 200 μg doses, respectively. In an additional experiment, fast inhalation (approximately 60 L/minute) of 100 μg resulted in statistically less drug than slow inhalation (20 L/minute) of the same dose, whether the pMDI was actuated late (five seconds past optimal) or not. Mean and SD

cumulative urine values for fast, slow and late administrations were 1.90 ± 0.70, 2.67 ± 0.84, and 2.72 ± 0.67 μg, respectively.

A1.1.3 Published PBPK Models and Parameters for Albuterol. Boger and Friden (2019) recently published a complex PBPK-pharmacodynamic model for albuterol. This model's lung compartment was constructed of 24 airway generations plus an extrathoracic region. The model was built to predict the greater pharmacodynamic effect on forced expiratory volume of a 400 μg inhaled dose versus a 2 mg oral dose. A rat study accompanied this model to determine the kinetics of an intravenous versus intratracheal dose. The model assumes pulmonary permeability of albuterol is conserved between the species.

Albuterol served as a test compound for an *in vitro* system using a simulated respiratory tract lining fluid (RTLF) in order to predict dissolution and absorption or clearance of inhaled drugs. This RTLF study was combined with a PBPK model developed in GastroPlus (version 9.6, Simulations Plus, Lancaster CA) using its Pulmonary Compartmental Absorption and Transit (PCAT) module. Physicochemical and particle parameter values are listed in the publication, which are useful for modeling albuterol as a particulate. The model was used to predict absorption from the respiratory tract based on measured values of particle solubility (Radivojev *et al*., 2021).

Pinto *et al*. (2021) published a PBPK model for albuterol administered using a dry powder inhaler (DPI), in order to understand the effects of different carrier particles. The measured particulate properties measured in this study, and predicted deposition from Multiple-Path Particle Dosimetry (version 2.11, Applied Research Associates, Inc., Albuquerque NM), would not relate directly to doses from pMDIs. The PBPK model was also developed in GastroPlus 9.6 using the PCAT module; the article offered few parameter values to assist in modeling uptake of albuterol from a pMDI outside of this proprietary modeling software.

A2.0 DISCUSSION

A2.1 Albuterol Modeling Considerations

To predict blood, plasma and urine concentrations of albuterol, a PBPK model should include inhalation, oral and intravenous routes. Intravenous data sets such as those shown in Janson (1991) and Boger and Friden (2019) would assist in properly parameterizing a PBPK model. Oral albuterol formulation kinetics have been studied (*e.g.*, Powell *et al*., 1985; 1986); much of the albuterol dose (80.4 percent in the Newman *et al*., 1981 study) is deposited in the mouth and swallowed. Some studies administer ActC to absorb this portion of the dose in the stomach (Anderson *et al*., 1998; Logsdon *et al*., 1997), while other studies allow absorption, as would normally happen when a person uses a pMDI to administer albuterol. Therefore, the oral route is necessary in a PBPK model to capture the behavior of oral absorption simultaneous with inhalation in studies where ActC is not administered.

The enantiomers of albuterol have different kinetics. Although Gumbhir-Shah *et al*. (1998), Schmekel *et al*. (1991) and Ward *et al*. (2000) were effectively able to analyze the R- and S-enantiomers of albuterol, the development of a racemic PBPK model is not likely to be beneficial at this time. The differences between the enantiomer metabolic rates, however, may affect the PBPK model and alter the metabolic constants used in the parameterization.

Albuterol delivery is not straightforward. Deposition studies in the 1980s indicate that pMDIs deliver their doses of pressurized aerosols in relatively large propellant droplets traveling at initially high rates of speed. In eight patients with asthma and chronic bronchitis, the percent deposition of the dose was as follows: 8.8 lung, 80.4 mouth, 1.0 expired and 9.8 remained in the pMDI mouthpiece. The dose (mean ± SD) in the lung was broken down to 3.0 (± 3.1) percent deposited in the alveoli and 5.8 (± 2.6) percent in the conducting airways (Newman *et al*., 1981). Accurately assigning the delivery efficacy of a particular pMDI device for a published study is not possible and would have to be estimated (fit) for each study and probably each individual. The same is true of DPI delivery systems, as used in the Schmekel *et al*. (1999) study.

To add additional complexity to simulating albuterol kinetic data, older studies reported herein used pMDIs with a CFC propellant. In one study, the dose administered by a greener propellant (HFC-134a) was not found to differ from the older CFC inhaler (Harrison *et al*., 1996). In another non-albuterol study, desired effects from the greener propellant pMDIs were not different from the CFC counterparts (Chopra *et al*., 2005). Conversely, other studies indicate that greener propellants may actually deliver medications more effectively than CFC propellant inhalers. Used with a spacer, newer propellants deliver up to 75 percent of the dose to the lung tissue. The force of delivery with the greener formulations is less than with the CFCs, which, while giving patients the impression they aren't getting as much medication, actually assists in improved delivery of the dose to the target tissue (Velsor-Friedrich *et al*., 2009).

To further complicate modeling of inhaled albuterol, dose delivery is affected by patient technique. Incorrect technique has been shown to cause poor asthma control and increased need for short-term steroid prescriptions (Levy *et al*., 2013). Only 58 to 78 percent of patients using pMDI inhalers correctly perform all of the steps to properly receive the prescribed medication dose (Velsor-Friedrich *et al*., 2009).

APPENDIX B: ACSLX MODEL CODE

```
PROGRAM: Halon_Model.CSL -- Model to use for modeling HFC-134a and HFC_227ea

! Physiological parameter values here are from the published IPA model (Clewell et al., 2001)
! Chemical specific parameter values are set to -1.0 or 0.0 so must be set BEFORE running a
! simulation


INITIAL

! ----------------- CONTROL FLAGS ----------------------------------
! Flags to turn options on and off
  LOGICAL AdjFlows     ! NEEDED FOR MONTE CARLO - adjusts tissue blood flows
  LOGICAL AdjVols      ! NEEDED FOR MONTE CARLO - adjusts tissue volumes
  LOGICAL FindLODTime  ! Whether to find time when limit of detection (LOD) is found

! ----------------- PHYSIOLOGICAL PARAMETERS ------------------------
  CONSTANT            BW = 70.0        ! Body weight (kg)
  CONSTANT           QCC = 12.89       ! Cardiac output (L/hr/kg^0.75)
  CONSTANT           QPC = 27.75       ! Total pulmonary ventilation (L/hr/kg^0.75)
!  CONSTANT           VPR = 1.6774     ! Alveolar ventilation-perfusion ratio
  CONSTANT      AdjFlows = .FALSE.     ! Don't adjust tissue blood flows
  CONSTANT       AdjVols = .FALSE.     ! Don't adjust tissue blood volumes

! Fractional Blood Flows (fraction of cardiac output)
  CONSTANT         QFatC = 0.052       ! Fat
  CONSTANT         QLivC = 0.227       ! Liver
  CONSTANT         QRapC = 0.533       ! Rapidly perfused
  CONSTANT         QSlwC = 0.188       ! Slowly perfused

! Fractional Tissue Volumes (fraction of body weight)
  CONSTANT         VFatC = 0.214       ! Fat
  CONSTANT         VLivC = 0.026       ! Liver
  CONSTANT         VRapC = 0.056       ! Rapidly perfused
  CONSTANT         VSlwC = 0.536       ! Slowly perfused
  CONSTANT        VBodyC = 0.84        ! Sum of mean fractional volumes

! Simulation Control Parameters
  CONSTANT         TStop = 24.0        ! (hrs)
  CINTERVAL         CINT = 0.0001      ! (hrs)


! ----------------- DOSING PARAMETERS ------------------------------
! Inhalation Exposure Parameters
  CONSTANT          Conc = 0.0         ! Inhaled concentration of parent (ppm)
  CONSTANT         TChng = 0.1667      ! Length of inhalation exposure (hrs)
  CONSTANT           LOD = 0.001       ! Limit of detection (LOD) (ppb)
  CONSTANT   FindLODTime = .FALSE.     ! Flag to find LOD

! ----------------- CHEMICAL-SPECIFIC PARAMETERS --------------------
! Molecular Weight (g/mol)
  CONSTANT            MW = -1.0

! Tissue/Blood Partition Coefficients (unitless)
  CONSTANT            PB = -1.0        ! Blood/air
  CONSTANT          PFat = -1.0        ! Fat
  CONSTANT          PLiv = -1.0        ! Liver
  CONSTANT          PRap = -1.0        ! Rapidly perfused tissue
  CONSTANT          PSlw = -1.0        ! Slowly perfused tissue

! Saturable Metabolism Parameters
  CONSTANT         VMaxC = 0.0         ! Maximum reaction rate (mg/hr/kg^0.75)
  CONSTANT            KM = -1.0        ! Michaelis-Menten (mg/L)

! First-Order Metabolic Rate (kg^0.25/hr)
  CONSTANT           kFC = 0.0
```

```
! Uptake and Clearance Parameters
  CONSTANT         ClUrC = 0.0         ! Urinary clearance (L/hr/kg^0.75)
! ----------------- PHYSIOLOGICAL PARAMETER SCALING -----------------
! Alveolar Ventilation Rate (L/hr)
       QAlv = 0.67 * (QPC * (BW**0.75))

! Scaled Blood Flows (L/hr)
      QAdjus = 1.0
  IF (AdjFlows) QAdjus = QFatC + QLivC + QRapC + QSlwC
         QC = QCC * (BW**0.75)
       QFat = (QFatC / QAdjus) * QC                      ! Fat
       QLiv = (QLivC / QAdjus) * QC                      ! Liver
       QRap = (QRapC / QAdjus) * QC                      ! Rapidly perfused tissues
       QSlw = (QSlwC / QAdjus) * QC                      ! Slowly perfused tissues
         QC = QFat + QLiv + QRap + QSlw

! Scaled Tissue Volumes (L)
      VAdjus = 1.0
  IF (AdjFlows) VAdjus = VBodyC / (VFatC + VLivC + VRapC + VSlwC)
       VFat = (VFatC * VAdjus) * BW                      ! Fat
       VLiv = (VLivC * VAdjus) * BW                      ! Liver
       VRap = (VRapC * VAdjus) * BW                      ! Rapidly perfused tissues
       VSlw = (VSlwC * VAdjus) * BW                      ! Slowly perfused tissues

! ----------------- DOSING PARAMETER SCALING ------------------------
! Inhalation Exposure Parameters
      CIZone = 1.0           ! Switch to turn inhalation on and off

! ----------------- CHEMICAL-SPECIFIC PARAMETER SCALING -------------
! Scaled Maximum Metabolic Rates (mg/hr)
       VMax = VMaxC * (BW**0.75)

! Scaled First-Order Metabolic Rates (/hr)
         kF = kFC / (BW**0.25)

! Scaled Urinary Clearance Rates (L/hr)
       ClUr = ClUrC * (BW**0.75)

! ----------------- INITIALIZE STARTING VARIABLES -------------------
     LODTime = -1.0                       ! Time LOD is reached (hrs)
        CEnd = 0.0                        ! (mg/L)
      PerEnd = 0.0                        ! (percent)
        CMix = 0.0                        ! (mg/L)
      PerMix = 0.0                        ! (percent)

  SCHEDULE DoseOff .AT. T + TChng

END               ! End of Initial


DYNAMIC
  ALGORITHM  IALG = 2             ! Gear stiff method


DISCRETE DoseOff
  CIZone = 0.0
END


DISCRETE LODTimeReached
   LODTime = Hours
END


DERIVATIVE
     Hours = T
   Minutes = T * 60.0
      Days = T / 24.0

! Amount in Inhaled Air (mg)
       CInh = ((Conc * MW) / 24450.0) * CIZone
```

```
          CP = (CInh * 24450.0) / MW

! Amount Exhaled (mg)
       RAExh = QAlv * CAlv
        AExh = INTEG(RAExh, 0.0)

! Concentration in End-Exhaled Air (mg/L)
        CEnd = RAExh / QAlv
     CEndPPM = CEnd * (24450.0 / MW)
  IF (Conc .GT. 0.0) PerEnd = (CEnd / ((Conc * MW) / 24450.0)) * 100.0

! Save Time at which LOD is Reached
  SCHEDULE LODTimeReached .XN. CEndPPM - LOD

! Amount in Arterial Blood (mg)
        CArt = ((QAlv * CInh) + (QC * CVen)) / ((QAlv / PB) + QC + ClUr)
        CAlv = CArt / PB
     CAlvPPM = CAlv * (24450.0 / MW)
     AUCCArt = INTEG(CArt, 0.0)

! Amount in Urine (mg)
       RAUrn = ClUr * CArt
        AUrn = INTEG(RAUrn, 0.0)

! Amount in Fat (mg)
       RAFat = QFat * (CArt - CVFat)
        AFat = INTEG(RAFat, 0.0)
        CFat = AFat / VFat
       CVFat = CFat / PFat

! Amount in Liver (mg)
       RALiv = (QLiv * (CArt - CVLiv)) - RAMetTot
        ALiv = INTEG(RALiv, 0.0)
        CLiv = ALiv / VLiv
       CVLiv = CLiv / PLiv

! Amount Metabolised in Liver -- Saturable and 1st Order (mg)
       RAMet = (VMax * CVLiv) / (KM + CVLiv)
    RAMetTot = RAMet + (kF * CVLiv * VLiv)
     AMetTot = INTEG(RAMetTot, 0.0)

! Amount in Rapidly Perfused Tissue (mg)
       RARap = QRap * (CArt - CVRap)
        ARap = INTEG(RARap, 0.0)
        CRap = ARap / VRap
       CVRap = CRap / PRap

! Amount in Slowly Perfused Tissue (mg)
       RASlw = QSlw * (CArt - CVSlw)
        ASlw = INTEG(RASlw, 0.0)
        CSlw = ASlw / VSlw
       CVSlw = CSlw / PSlw

! Concentration in Mixed Venous Blood (mg/L)
        CVen = (QFat*CVFat + QLiv*CVLiv + QRap*CVRap + QSlw*CVSlw) / QC

! ----------------- CHECK MASS BALANCE ------------------------------
       TDose = INTEG((QAlv*CInh), 0.0)
      Parent = AFat + ALiv + ARap + ASlw + AExh + AUrn + AMetTot
     MassBal = TDose - Parent

TERMT((FindLODTime .AND. (LODTime.GT.0.0)) .OR. (T.GT.TStop), 'Simulation Finished')

END               ! End of Derivative
END               ! End of Dynamic
END               ! End of Program
```

APPENDIX C: UTILITY M FILES FOR SIMULATIONS

The following M files are called within various other M files utilized for this work.

HFC134a Gunnare.m

```
% Values from Gunnare et al. (2006) and Gunnare (2007)
% Gunnare S, Ernstgård L, Sjögren B, and Johanson G.  2006.  Toxicokinetics of
%    1,1,1,2-tetrafluoroethane (HFC-134a) in male volunteers after experimental exposure.
%    Toxicology Letters 167:54-65.
% and
% Gunnare S.  2007.  Fluorinated hydrocarbons used as refrigerants -- toxicokinetics and effects
%    in humans.  Theisis.
% Text of paper (pg 59) states zero metabolism was assumed so all metabolic parameters are set to
%    turn off all metabolism (KM set to 1 to avoid division by 0)
% Urinary clearance is set to 0.0 since text of paper (pg 63) states that "excretion via urine
%    seems to be virtually negligible"
     MW=102.03;
     PB=0.56; PFAT=7.96; PLIV=0.87; PRAP=0.96; PSLW=1.07;
     VMAXC=0.0; KM=1.0; KFC=0.0;
     CLURC=0.0;
```

HFC227ea Vinegar.m

```
% Values from Vinegar et al. (2000)
% Gunnare S, Ernstgård L, Sjögren B% Vinegar A, Jepson GW, Cisneros M, Rubenstein R and Brock WJ.
%     2000.  Setting safe acute exposure limits for halon replacement chemicals using
%     physiologically based pharmacokinetic modeling.  Inhalation Toxicology 12:751-763.
% MW is from Wikipedia
% Tissue partitions are converted from tissue:air to tissue:blood by dividing by PB
% Text of paper (pg 754) states metabolism was set to 0.0 (KM set to 1 to avoid division by 0)
% Urinary clearance is not mentioned in paper so set to 0.0
     MW=170.03;
     PB=0.033; PFAT=0.347/PB; PLIV=0.031/PB; PRAP=0.031/PB; PSLW=0.021/PB;
     VMAXC=0.0; KM=1.0; KFC=0.0;
     CLURC=0.0;
```

HFC227ea Vinegar Jepson.m

```
% Values from Vinegar & Jepson (1995, 1996) for resting QCC, QPC and fractional tissue blood
%     flows
% Vinegar A and Jepson GW. 1995.  Relating blood concentration time courses to cardiac
%    sensitization thresholds during inhalation of halon replacement chemicals. Tech Report.
% and
% Vinegar A and Jepson GW. 1996.  Cardiac sensitization thresholds of halon replacement chemicals
%    predicted in humans by physiologically-based pharmacokinetic modeling.  Risk Analysis
%    16(4):571-579.
% Tissue partitions are converted from tissue:air to tissue:blood by dividing by PB
% Urinary clearance is not mentioned in paper so set to 0.0
     MW=170.0;
     PB=0.225; PFAT=1.58/PB; PLIV=0.42/PB; PRAP=0.42/PB; PSLW=0.36/PB;
     VMAXC=0.0; KM=1.0; KFC=0.0;
     CLURC=0.0;
```

Human Gunnare.m

```
% Values from Gunnare et al. (2006) and Gunnare (2007)
% Gunnare S, Ernstgård L, Sjögren B, and Johanson G.  2006.  Toxicokinetics of
%    1,1,1,2-tetrafluoroethane (HFC-134a) in male volunteers after experimental exposure.
%    Toxicology Letters 167:54-65.
% and
% Gunnare S.  2007.  Fluorinated hydrocarbons used as refrigerants -- toxicokinetics and effects
%    in humans.  Theisis.
% QCC was given in L/min (10.62) so is converted to L/hr/kg^0.75
% QPC is back calculated from the alveolar value (21.3 L/min converted to L/hr/kg^0.75)
% Tissue blood flows were given in L/min so converted to fraction of cardiac output (QC is 10.62
%    L/min)
% QSlwC is the sum of the values for resting (0.606 L/min) and working muscle (4.33 L/min)
```

```
% Tissue volumes were given in L so converted to fraction of body weight
% VArtC is the value from the published IPA model (Clewell et al., 2001)
% VRapC is the sum of the values for lungs (1.52 L) and rapidly perfused tissue (2.21 L)
% VSlwC is the sum of the values for resting muscle (18.4 L) and working muscle (18.4 L)
% VBodyC is set to 1 as the model is set to not adjust blood flows and tissue volumes (AdjFlows
%    and AdjVols set to 0)
% SUM OF TISSUE BLOOD FLOWS IS SLIGHTLY OFF FROM CARDIAC OUTPUT (10.616 vs 10.62)

  ADJFLOWS=0; ADJVOLS=0;
  GunnareQC=10.62;
  BW=72.5; QCC=(GunnareQC*60.0)/(BW^0.75); QPC=((21.3*60.0)/(BW^0.75))/0.67;
  QFATC=0.83/GunnareQC; QLIVC=1.56/GunnareQC; QRAPC=3.29/GunnareQC;
  QSLWC=(0.606+4.33)/GunnareQC;
  VFATC=14.8/BW; VLIVC=1.57/BW; VRAPC=(1.52+2.21)/BW; VSLWC=(18.4+18.4)/BW; VBODYC=1.0;
```

Human IPADefault.m

```
% Values from IPA published model -- Clewell et al. (2001)
% This version of the model doesn't have a brain compartment or URT scrubbing so QBrnC and VBrnC
%    are added to QRapC and VRapC, respectively, and VMucC is added to VRapC to maintain mass
%    balance

  ADJFLOWS=0; ADJVOLS=0;
  BW=70.0; QCC=12.89; QPC=27.75;
  QFATC=0.052; QLIVC=0.227; QRAPC=0.114+0.419; QSLWC=0.188;
  VFATC=0.214; VLIVC=0.026; VRAPC=0.02+0.0001+0.036; VSLWC=0.536; VBODYC=0.84;
```

Human Vinegar.m

```
% Values from Vinegar et al. (2000)
% Vinegar A, Jepson GW, Cisneros M, Rubenstein R and Brock WJ.  2000.  Setting safe acute
%    exposure limits for halon replacement chemicals using physiologically based pharmacokinetic
%    modeling.  Inhalation Toxicology 12:751-763.
% QPC is back calculated from the alveolar value (17.4 L/hr/kg^0.75)
% QLivC is the sum of the Gut (0.219) and Liver (0.089) values
% VArtC is the value from the published IPA model (Clewell et al., 2001)
% VRapC is the sum of the Gut (0.022) and Rapidly Perfused (0.041) values
% VBodyC is set to 1 as the model is set to not adjust blood flows and tissue volumes
%    (AdjFlows and AdjVols set to 0)

  ADJFLOWS=0; ADJVOLS=0;
  BW=70.0; QCC=17.4; QPC=17.4/0.67;
  QFATC=0.029; QLIVC=0.219+0.089; QRAPC=0.461; QSLWC=0.202;
  VFATC=0.215; VLIVC=0.027; VRAPC=0.022+0.041; VSLWC=0.575; VBODYC=1.0;
```

Human Vinegar Jepson ModActivity.m

```
% Values from Vinegar & Jepson (1995, 1996) for moderate activity QCC, QPC and fractional tissue
%    blood flows
% Vinegar A and Jepson GW. 1995.  Relating blood concentration time courses to cardiac
%    sensitization thresholds during inhalation of halon replacement chemicals. Tech Report.
% and
% Vinegar A and Jepson GW. 1996.  Cardiac sensitization thresholds of halon replacement chemicals
%    predicted in humans by physiologically-based pharmacokinetic modeling.  Risk Analysis
%    16(4):571-579.
% QPC is back calculated from the alveolar value (17.4 L/hr/kg^0.75)
% QLivC is the sum of the Gut (0.2093) and Liver (0.0602) values
% VArtC is the value from the published IPA model (Clewell et al., 2001)
% VRapC is the sum of the Gut (0.022) and Rapidly Perfused (0.041) values
% VBodyC is set to 1 as the model is set to not adjust blood flows and tissue volumes (AdjFlows
%    and AdjVols set to 0)
% SUM OF FRACTIONAL TISSUE BLOOD FLOWS IS OFF FROM CARDIAC OUTPUT (1.0602 vs 1.0)

  ADJFLOWS=0; ADJVOLS=0;
  BW=70.0; QCC=20.7; QPC=43.1/0.67;
  QFATC=0.04; QLIVC=0.2093+0.0602; QRAPC=0.3188; QSLWC=0.4319;
  VFATC=0.215; VLIVC=0.027; VRAPC=0.022+0.041; VSLWC=0.575; VBODYC=1.0;
```

Human Vinegar Jepson Rest.m

```
% Values from Vinegar & Jepson (1995, 1996) for resting QCC, QPC and fractional tissue blood
%    flows
% Vinegar A and Jepson GW. 1995.  Relating blood concentration time courses to cardiac
%    sensitization thresholds during inhalation of halon replacement chemicals. Tech Report.
% and
% Vinegar A and Jepson GW. 1996.  Cardiac sensitization thresholds of halon replacement chemicals
%    predicted in humans by physiologically-based pharmacokinetic modeling.  Risk Analysis
%    16(4):571-579.
% QPC is back calculated from the alveolar value (17.4 L/hr/kg^0.75)
% QLivC is the sum of the Gut (0.2192) and Liver (0.0885) values
% VArtC is the value from the published IPA model (Clewell et al., 2001)
% VRapC is the sum of the Gut (0.022) and Rapidly Perfused (0.041) values
% VBodyC is set to 1 as the model is set to not adjust blood flows and tissue volumes (AdjFlows
%    and AdjVols set to 0)

  ADJFLOWS=0; ADJVOLS=0;
  BW=70.0; QCC=12.9; QPC=17.4/0.67;
  QFATC=0.0288; QLIVC=0.2192+0.0885; QRAPC=0.4616; QSLWC=0.2019;
  VFATC=0.215; VLIVC=0.027; VRAPC=0.022+0.041; VSLWC=0.575; VBODYC=1.0;
```

Init.m

```
prepare @All
HVDPRN=0;
WESITG=0;
WEDITG=0;
```

ResetDoses.m

```
  FINDLODTIME=0; LOD=0.0;
  CONC=0.0; TCHNG=0.1667;
  CINT=0.01;
```

APPENDIX D: M FILES FOR VALIDATION FIGURES

The following M files are used to generate the simulations for the validation figures. Some lines were too long for the page width and were thus reformatted to fit the page; however, these additional line breaks and space may need to be removed for the M file to run correctly.

Emmen et al 2000 HFC134a.m

```
% Data from
% Emmen HH, Hoogendijk EM, Klöpping-Ketelaars WA, Muijser H, Duistermaat E, Ravensberg JC,
%    Alexander DJ, Borkhataria D, Rusch GM and Schmit B.  2000.  Human safety and
%    pharmacokinetics of the CFC alternative propellants HFC 134a (1,1,1,2-tetrafluoroethane) and
%    HFC 227 (1,1,1,2,3,3, 3-heptafluoropropane) following whole-body exposure.  Regul Toxicol
%    Pharmacol 32(1):22-35.
% Data from Figures 4 and 8
% 4 male and 4 female subjects exposed to 1000, 2000, 4000 or 8000 ppm HFC-134a or HFC-227 for 1
%    hour

% DATA Emmen_HFC134a_Male (Minutes, CVen at 1000, 2000, 4000 and 8000 ppm)
Emmen_HFC134a_Male = [ ...
    1.0   0.301532083   0.563392219   0.931166749   1.545106607
    3.0   0.609669573   1.208763019   1.795420113   4.088405325
    5.0   0.788297256   1.438315732   2.213823789   4.76953409
   15.0   0.893805885   1.744314058   3.107990133   6.461727523
   30.0   0.935882328   1.755533426   3.494441649   6.607199378
   55.0   0.998529235   1.910518297   3.817985431   7.218820044
   62.0   0.575750279   1.250246984   2.245386066   4.953783598
   65.0   0.422745969   0.861695043   1.722044345   3.179559924
   70.0   0.294789049   0.645228082   1.294548639   2.139592919
   80.0   0.188333597   0.477203387   0.942401104   1.672524644
   90.0   0.151954953   0.486256052   0.742529276   1.580854341
  100.0   0.161087673   0.348422148   0.699054227   1.27549546
  120.0   0.120440849   0.217161779   0.535219136   0.927604705];

% DATA Emmen_HFC134a_Female (Minutes, CVen at 1000, 2000, 4000 and 8000 ppm)
Emmen_HFC134a_Female = [ ...
    1.0   0.358950315   0.388540909   0.673815776   1.578991002
    3.0   0.598283907   0.657950235   0.962342169   2.519601678
    5.0   0.714967091   0.831099615   1.235009478   3.500087774
   15.0   0.906469197   1.091944229   2.082533004   5.077292957
   30.0   0.950124262   1.325179346   2.80149348    5.669976419
   55.0   1.019497259   1.107920658   3.041985306   5.89420759
   62.0   0.648914077   1.064652167   2.314003411   3.796509944
   65.0   0.423022507   0.688564992   1.846167044   3.114111243
   70.0   0.332169798   0.569251273   1.156690368   2.277023228
   80.0   0.27675076    0.375441069   0.822507158   1.830688592
   90.0   0.325441994   0.34399719    0.687998139   1.301774488
  100.0   0.12475157    0.255504298   0.557538225   1.097551708
  120.0   0.078432754   0.188963763   0.329006458   0.88913353];

  PlotOutput=[];  CheckOutput=[];
  ResetDoses
  Human_IPADefault
  HFC134a_Gunnare

% Subjects exposed to 1000 ppm HFC-134a for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i=1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-134a '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
```

```
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '
set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-134a for 1 hour (4 male subjects)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=20.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-134a for 1 hour (4 female subjects)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=20.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,5), 'm+', _minutes, _cven, 'm-')

% ------ HFC-134a (using PB from Ernstgard) ------------------------------------------------------
% Using lower PB value from Ernstgard et al. (2010)
  PB=0.36;

% Subjects exposed to 1000 ppm HFC-134a for 1 hour
  CONC=1000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-134a '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,2), 'b+', _minutes, _cven, 'b-')
```

```
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-134a for 1 hour (4 male subjects) (PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=15.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-134a for 1 hour (4 female subjects) (PB from Ernstgard)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=15.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1,1,Emmen_HFC134a_Male(:,1), Emmen_HFC134a_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2,1,Emmen_HFC134a_Female(:,1), Emmen_HFC134a_Female(:,5), 'm+', _minutes, _cven, 'm-')

  save PlotOutput @file='Emmen_HFC134a_Simulations.txt' @format=ascii
  save CheckOutput @file='CheckOutput_Emmen_HFC134a.txt' @format=ascii

set @preference=NoBackslashEscapes
```

Emmen et al 2000 HFC227ea.m

```
% Data from
% Emmen HH, Hoogendijk EM, Klöpping-Ketelaars WA, Muijser H, Duistermaat E, Ravensberg JC,
%    Alexander DJ, Borkhataria D, Rusch GM and Schmit B.  2000.  Human safety and
%    pharmacokinetics of the CFC alternative propellants HFC 134a (1,1,1,2-tetrafluoroethane) and
%    HFC 227 (1,1,1,2,3,3, 3-heptafluoropropane) following whole-body exposure.  Regul Toxicol
%    Pharmacol 32(1):22-35.
% Data from Figures 4 and 8
% 4 male and 4 female subjects exposed to 1000, 2000, 4000 or 8000 ppm HFC-134a or HFC-227 for 1
hour

% DATA Emmen_HFC227_Male (Minutes, CVen at 1000, 2000, 4000 and 8000 ppm)
Emmen_HFC227_Male = [ ...
    1.0   0.171923543   0.286574022   0.276380767   0.935243582
    3.0   0.21715506    0.339400008   0.600921433   1.421424141
    5.0   0.227939866   0.376897557   0.72906811    1.676669673
   15.0   0.2823978     0.439598274   0.781464131   1.705589602
   30.0   0.294435894   0.443820775   0.754816232   1.799872215
   55.0   0.264788477   0.434317838   0.864137828   1.78275372
   62.0   0.054737116   0.157060372   0.396216161   0.695894787
   65.0   0.031300349   0.104226272   0.170956567   0.286110949
   70.0   0.023159353   0.065127896   0.08093142    0.215995082
   80.0   0.017492621   0.050597152   0.089224617   0.132881303
   90.0   0.016484686   0.049241767   0.076344384   0.087888879
  100.0   0.014449885   0.044754673   0.076114606   0.103754814
  120.0   0.0110579     0.031600807   0.053314005   0.079399271];

% DATA Emmen_HFC227_Female (Minutes, CVen at 1000, 2000, 4000 and 8000 ppm)
Emmen_HFC227_Female = [ ...
    1.0   0.116848133   0.107953772   0.229026316   0.348433853
    3.0   0.112758424   0.134745073   0.242077349   0.489746506
    5.0   0.157863164   0.13054472    0.31811108    0.59930849
   15.0   0.17636741    0.236395371   0.497563069   0.982992362
   30.0   0.204187266   0.236395371   0.538557517   1.165401693
   55.0   0.205810273   0.342960065   0.611288771   1.333301497
   62.0   0.097395453   0.168185273   0.362503598   0.65903909
   65.0   0.069018971   0.12399659    0.234531172   0.480148259
   70.0   0.03605953    0.099342405   0.162298754   0.35822404
   80.0   0.03507403    0.079275758   0.137438651   0.308194735
   90.0   0.025152031   0.063513395   0.11824407    0.214120883
  100.0   0.021897833   0.03507403    0.113205674   0.149352341
  120.0   0.01640211    0.038722699   0.066603293   0.092144605];

% ------ HFC-227ea (all Vinegar&Jepson resting params) ----------------------------------------
  PlotOutput=[];  CheckOutput=[];
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC227ea_Vinegar_Jepson

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i=1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar&Jepson resting params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
```

```
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar&Jepson resting params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar&Jepson resting physiological and Vinegar chemical params) -----------
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC227ea_Vinegar

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar&Jepson physio and Vinegar chem.
     params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.5;")
```

```
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar&Jepson physio and Vinegar chem.
     params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar&Jepson moderate activity params) ----------------------------------
  ResetDoses
  Human_Vinegar_Jepson_ModActivity
  HFC227ea_Vinegar_Jepson

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes

  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar&Jepson moderate activity params)\";")
```

```
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar&Jepson moderate activity
     params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar&Jepson moderate activity physiological and Vinegar chem params) -----
  ResetDoses
  Human_Vinegar_Jepson_ModActivity
  HFC227ea_Vinegar

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
```

```
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar&Jepson mod activity physio and
     Vinegar chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar&Jepson mod activity physio and
     Vinegar chem params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar physiological and Vinegar&Jepson chem params) -----------------------
  ResetDoses
  Human_Vinegar
  HFC227ea_Vinegar_Jepson

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '
```

```
set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar physio and Vinegar&Jepson chem.
     params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar physio and Vinegar&Jepson chem.
     params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (all Vinegar params) ------------------------------------------------------
  ResetDoses
  Human_Vinegar
  HFC227ea_Vinegar

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
```

```
% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (Vinegar params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (Vinegar params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

set @preference=NoBackslashEscapes

% ------ HFC-227ea (IPA physiological and Vinegar&Jepson chem params) ---------------------------
  ResetDoses
  Human_IPADefault
  HFC227ea_Vinegar_Jepson

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
```

```
% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (IPA physio and Vinegar&Jepson chem.
     params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (IPA physio and Vinegar&Jepson chem.
     params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=12.0;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')
set @preference=NoBackslashEscapes

% ------ HFC-227ea (IPA physiological and Vinegar chem params) ----------------------------------
  ResetDoses
  Human_IPADefault
  HFC227ea_Vinegar

% Subjects exposed to 1000 ppm HFC-227 for 1 hour
  CONC=1000.0; TCHNG=1.0; TSTOP=2.1; CINT=0.005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
```

```
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Emmen et al., 2000. Inhalation HFC-227  '
% SET TITLE(41)='4 Male and 4 Female Subjects            '
% SET TITLE(81)='1000, 2000, 4000 or 8000 ppm for 1 hour '

set @preference=BackslashEscapes
  plot1=plot(0, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 male subjects) (IPA physio and Vinegar chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Emmen et al. (2000)  Inhalation of 1000, 2000, 4000 or
     8000 ppm HFC-227 for 1 hour (4 female subjects) (IPA physio and Vinegar chem params)\";")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.5;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=125.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  CONC=2000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,3), 'r+', _minutes, _cven, 'r-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,3), 'r+', _minutes, _cven, 'r-')

  CONC=4000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,4), 'g+', _minutes, _cven, 'g-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,4), 'g+', _minutes, _cven, 'g-')

  CONC=8000.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  plot(plot1, 1, Emmen_HFC227_Male(:,1), Emmen_HFC227_Male(:,5), 'm+', _minutes, _cven, 'm-')
  plot(plot2, 1, Emmen_HFC227_Female(:,1), Emmen_HFC227_Female(:,5), 'm+', _minutes, _cven, 'm-')

  save PlotOutput @file='Emmen_HFC227ea_Simulations.txt' @format=ascii
  save CheckOutput @file='CheckOutput_Emmen_HFC227ea.txt' @format=ascii

set @preference=NoBackslashEscapes
```

Gunnare et al 2006.m

```
% Data from
% Gunnare S, Ernstgård L, Sjögren B, and Johanson G.  2006.  Toxicokinetics of
%    1,1,1,2-tetrafluoroethane (HFC-134a) in male volunteers after experimental exposure.
%    Toxicology Letters 167:54-65.
% Data from Figure 3 -- averaged over 10 subjects
% 10 male subjects were exposed to 500 ppm HFC-134a (actual average of 486 ppm) for 2 hours
%    during a work load of 50W (actual average of 52 W)
% Average pulmonary ventilation during exposure was 22 L/min (20-26) and decreased to 15 L/min
%    (11-22) post-exposure

% DATA GunnareBlood (Minutes, CVen)
GunnareBlood = [ ...
    4.0   0.422013255
    7.0   0.636053871
   11.0   0.693374767
   15.0   0.824431659
   20.0   0.811529298
   32.0   0.884613045
   61.0   0.975831667
   92.0   1.123516901
  117.0   0.977756298
  122.0   0.329543659
  127.0   0.164764445
  130.0   0.144785265
  136.0   0.146051184
  142.0   0.115569858
  150.0   0.097991825
  178.0   0.061566517
  214.0   0.0323336
  271.0   0.014397205
  356.0   0.026873623];

% DATA GunnareAir (Minutes, 0.89*CAlv)
GunnareAir = [ ...
  132.0   0.075679034
  144.0   0.041952718
  156.0   0.03252914
  174.0   0.021334425
  216.0   0.011369597
  360.0   0.003400997];

  PlotOutput=[];  CheckOutput=[];
  ResetDoses
  Human_Gunnare
  HFC134a_Gunnare

  CONC=486.0; TCHNG=2.0; TSTOP=6.0; CINT=0.01;
  start @NoCallback
  i=1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _calv*0.89, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Gunnare et al., 2006. Inhalation HFC134a'
% SET TITLE(41)='10 Male Subjects - ~500 ppm for 2 hours '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes

  plot1=plot(0, GunnareBlood(:,1), GunnareBlood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Gunnare et al. (2006)  Inhalation of ~500 ppm HFC-134a
     (10 male subjects)\";")
  pltscript(plot1, "Chart.Axes.Left.LogarithmicBase=10;")
  pltscript(plot1, "Chart.Axes.Left.Logarithmic=true;")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
```

```
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"Average HFC-134a Concentration in Blood
     (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=360.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, GunnareAir(:,1), GunnareAir(:,2), 'b+', _minutes, _calv*0.89, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Gunnare et al. (2006)  Inhalation of ~500 ppm HFC-134a
     (10 male subjects)\";")
  pltscript(plot2, "Chart.Axes.Left.LogarithmicBase=10;")
  pltscript(plot2, "Chart.Axes.Left.Logarithmic=true;")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.25;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"Average HFC-134a Concentration in Exhaled Air
     (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=370.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

% Using lower PB value from Ernstgard et al. (2010)
  PB=0.36;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _calv*0.89, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

  plot1=plot(0, GunnareBlood(:,1), GunnareBlood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Gunnare et al. (2006)  Inhalation of ~500 ppm HFC-134a
     (10 male subjects) (PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.LogarithmicBase=10;")
  pltscript(plot1, "Chart.Axes.Left.Logarithmic=true;")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"Average HFC-134a Concentration in Blood
     (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=360.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  plot2=plot(0, GunnareAir(:,1), GunnareAir(:,2), 'b+', _minutes, _calv*0.89, 'b-')
  pltscript(plot2, "Chart.Header.Text = \"Gunnare et al. (2006)  Inhalation of ~500 ppm HFC-134a
     (10 male subjects) (PB from Ernstgard)\";")
  pltscript(plot2, "Chart.Axes.Left.LogarithmicBase=10;")
  pltscript(plot2, "Chart.Axes.Left.Logarithmic=true;")
  pltscript(plot2, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot2, "Chart.Axes.Left.Maximum=2.25;")
  pltscript(plot2, "Chart.Axes.Left.Title.Text=\"Average HFC-134a Concentration in Exhaled Air
     (mg/L)\";")
  pltscript(plot2, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot2, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Maximum=370.0;")
  pltscript(plot2, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  save PlotOutput @file='Gunnare_Simulations.txt' @format=ascii
  save CheckOutput @file='CheckOutput_Gunnare.txt' @format=ascii

set @preference=NoBackslashEscapes
```

Vinegar et al 1997 HFC134a.m

```
% Data from
% Vinegar A, Jepson GW, Cook RS, McCafferty JD and Caracci MC.  1997.  Human inhalation of Halon
%    1301, HFC-134a and HFC-227ea for Collection of Pharmacokinetic Data.  Wright-Patterson AFB
%    OH: Occupational and Environmental Health Directorate, Toxicology Division.
%    AL/OE-TR-1997-0116.
% Data from Figures 3 and 4
% Male subjects were exposed to 4000 ppm HFC-134a for 4.5 or 11.5 minutes
% or 2000 ppm HFC-134a for 2.5 minutes
% or 6400 ppm HFC-227ea for 3.5 minutes
% Subjects were in a sitting position for HFC-134a and were in a reclined position for HFC-227ea

% DATA Vinegar_134a_Blood_3 (Minutes, CVen)
Vinegar_134a_Blood_3 = [ ...
   0.0   0.001733989
   0.5   0.001796743
   1.0   0.088599432
   1.5   0.335007597
   2.0   0.76530287
   2.5   1.287547478];

% DATA Vinegar_134a_Blood_5_4000 (Minutes, CVen)
Vinegar_134a_Blood_5_4000 = [ ...
   0.0   0.001733989
   0.5   0.001796743
   1.0   0.001857846
   1.5   0.038353371
   2.0   0.081784027
   2.5   0.116543911
   3.0   0.189466427
   3.5   0.246778908
   4.0   0.297148826
   4.5   0.420383459
   5.0   0.564433398
   7.5   0.563007398
  10.0   0.703839548];

% DATA Vinegar_134a_Blood_5_2000 (Minutes, CVen)
Vinegar_134a_Blood_5_2000 = [ ...
   0.0   0.157868184
   0.5   0.137113155
   1.0   0.151052779
   1.5   0.267349803
   2.0   0.333333058
   3.0   0.375092479
   3.5   0.383828484
   4.5   0.331907884
   5.5   0.259168676
   6.5   0.33215312
   7.5   0.273291607
   8.5   0.236985996
   9.5   0.237109852
  10.5   0.228560458
  11.5   0.214805793
  12.5   0.235746606
  13.5   0.208113419];

% ------ HFC-134a (Vinegar&Jepson resting physio and Gunnare chem params) -----------------------
  PlotOutput=[];  CheckOutput=[];
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC134a_Gunnare

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i=1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
```

```
% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (Vinegar&Jepson physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=7.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
    _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 11.5 minutes (Subject 5) (Vinegar&Jepson physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=8.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (Vinegar&Jepson physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=3.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
```

```
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-134a (Vinegar&Jepson resting physio and Gunnare chem params, PB from Ernstgard) ----
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC134a_Gunnare

% Using lower PB value from Ernstgard et al. (2010)
  PB=0.36;

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (Vinegar&Jepson physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=5.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 11.5 minutes (Subject 5) (Vinegar&Jepson physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
```

```
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (Vinegar&Jepson physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-134a (Vinegar physio and Gunnare chem params) -------------------------------------
  ResetDoses
  Human_Vinegar
  HFC134a_Gunnare

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (Vinegar physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=7.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
     _cven, 'b-')
```

```
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 11.5 minutes (Subject 5) (Vinegar physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=8.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (Vinegar physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=3.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-134a (Vinegar physio and Gunnare chem params, PB from Ernstgard) -------------------
  ResetDoses
  Human_Vinegar
  HFC134a_Gunnare

% Using lower PB value from Ernstgard et al. (2010)
  PB=0.36;

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (Vinegar physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=5.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
```

```
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 11.5 minutes (Subject 5) (Vinegar physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (Vinegar physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-134a (IPA physio and Gunnare chem params) ----------------------------------------
  ResetDoses
  Human_IPADefault
  HFC134a_Gunnare

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
```

```
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (IPA physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=7.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes


% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
for 11.5 minutes (Subject 5) (IPA physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=8.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (IPA physio params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=3.0;")
```

```
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-134a (IPA physio and Gunnare chem params, PB from Ernstgard) -----------------------
  ResetDoses
  Human_IPADefault
  HFC134a_Gunnare

% Using lower PB value from Ernstgard et al. (2010)
  PB=0.36;

% Male subject exposed to 4000 ppm HFC-134a for 4.5 minutes
  CONC=4000.0; TCHNG=4.5/60.0; TSTOP=14.0/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 4.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0,Vinegar_134a_Blood_3(:,1), Vinegar_134a_Blood_3(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 4.5 minutes (Subject 3) (IPA physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=5.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% Male subject exposed to 4000 ppm HFC-134a for 11.5 minutes
  TCHNG=11.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 4000 ppm for 11.5 minutes'
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_4000(:,1), Vinegar_134a_Blood_5_4000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 4000 ppm HFC-134a
     for 11.5 minutes (Subject 5) (IPA physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes
```

```
% Male subject exposed to 2000 ppm HFC-134a for 2.5 minutes
  CONC=2000.0; TCHNG=2.5/60.0;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997. Inhalation HFC134a'
% SET TITLE(41)='Male Subject - 2000 ppm for 2.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_134a_Blood_5_2000(:,1), Vinegar_134a_Blood_5_2000(:,2), 'b+', _minutes,
     _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 2000 ppm HFC-134a
     for 3.5 minutes (Subject 5) (IPA physio params, PB from Ernstgard)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.001;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=2.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=14.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  save PlotOutput @file='Vinegar_HFC134a_Simulations.txt' @format=ascii
  save CheckOutput @file='CheckOutput_Vinegar_HFC134a.txt' @format=ascii

set @preference=NoBackslashEscapes
```

Vinegar et al 1997 HFC227ea.m

```
% Data from
% Vinegar A, Jepson GW, Cook RS, McCafferty JD and Caracci MC.  1997.  Human inhalation of Halon
%    1301, HFC-134a and HFC-227ea for Collection of Pharmacokinetic Data.  Wright-Patterson AFB
%    OH: Occupational and Environmental Health Directorate, Toxicology Division.
%    AL/OE-TR-1997-0116.
% Data from Figures 3 and 4
% Male subjects were exposed to 4000 ppm HFC-134a for 4.5 or 11.5 minutes
% or 2000 ppm HFC-134a for 2.5 minutes
% or 6400 ppm HFC-227ea for 3.5 minutes
% Subjects were in a sitting position for HFC-134a and were in a reclined position for HFC-227ea

% DATA Vinegar_227ea_Blood (Minutes, CVen)
Vinegar_227ea_Blood = [ ...
   0.0   0.00020949
   0.5   0.000545245
   1.0   0.029904832
   1.5   0.090179372
   2.0   0.153187071
   2.5   0.155837822
   3.0   0.183092719
   4.0   0.176824954
   4.5   0.19188293
   5.0   0.172661878
   6.0   0.143050984
   7.0   0.156975023
   8.0   0.152179398
   9.0   0.13413614
  10.0   0.079497224];

% ------ HFC-227ea (Vinegar&Jepson resting params) ------------------------------------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  PlotOutput=[];  CheckOutput=[];
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC227ea_Vinegar_Jepson
```

```
  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i=1;
  PlotOutput = addcolsj(PlotOutput, _minutes, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (Vinegar&Jepson resting params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar&Jepson resting physio and Vinegar chem params) ----------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  ResetDoses
  Human_Vinegar_Jepson_Rest
  HFC227ea_Vinegar

  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (Vinegar&Jepson resting physio and Vinegar chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=1.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar params) ---------------------------------------------------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  ResetDoses
  Human_Vinegar
  HFC227ea_Vinegar

  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
```

```
% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (Vinegar params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=1.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-227ea (Vinegar physio and Vinegar&Jepson chem params) -----------------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  ResetDoses
  Human_Vinegar
  HFC227ea_Vinegar_Jepson

  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (Vinegar physio and Vinegar&Jepson chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-227ea (IPA physio and Vinegar&Jepson chem params) ---------------------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  ResetDoses
  Human_IPADefault
  HFC227ea_Vinegar_Jepson

  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (IPA physio and Vinegar&Jepson chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
```

```
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=6.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
set @preference=NoBackslashEscapes

% ------ HFC-227ea (IPA physio and Vinegar chem params) ----------------------------------------
% Male subject exposed to 6400 ppm HFC-227ea for 3.5 minutes
  ResetDoses
  Human_IPADefault
  HFC227ea_Vinegar

  CONC=6400.0; TCHNG=3.5/60.0; TSTOP=10.5/60.0; CINT=0.0005;
  start @NoCallback
  i = i + 1;
  PlotOutput = addcolsj(PlotOutput, _cven, @Justification = 'begin');
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);

% SET TITLE='Vinegar et al., 1997.Inhalation HFC227ea'
% SET TITLE(41)='Male Subject - 6400 ppm for 3.5 minutes '
% SET TITLE(81)='                                        '

set @preference=BackslashEscapes
  plot1=plot(0, Vinegar_227ea_Blood(:,1), Vinegar_227ea_Blood(:,2), 'b+', _minutes, _cven, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"Vinegar et al. (1997)  Inhalation of 6400 ppm HFC-227ea
     for 3.5 minutes (Subject 1) (IPA physio and Vinegar chem params)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=1.5;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-227ea Concentration in Blood (mg/L)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=10.5;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")

  save PlotOutput @file='Vinegar_HFC227ea_Simulations.txt' @format=ascii
  save CheckOutput @file='CheckOutput_Vinegar_HFC227ea.txt' @format=ascii

set @preference=NoBackslashEscapes
```

APPENDIX E: M FILES FOR ANALYSIS OF AFRL STUDY DATA

The following M files are used to generate the simulations for the validation figures. Some lines were too long for the page width and were thus reformatted to fit the page; however, these additional line breaks and spaces may need to be removed for the M file to run correctly.

AF Data.m

```
% Post-inhaler concentrations from "HFA THz Concentrations.xlsx" not adjusted for pre-inhaler
%    concentrations

% DATA Average (Minutes, Hours, CEnd_PPM (mean, std, mean+2std, mean-2std))
Average = [ ...
   5.0   0.0833   2.384016771   1.635822827   5.655662425   -0.887628883
  15.0   0.25     0.470341588   0.251023571   0.972388731   -0.031705554
  30.0   0.5      0.175305759   0.129645212   0.434596184   -0.083984665];

% DATA Subject1 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject1 = [ ...
   5.0   0.0833   3.595792582   3.707905104   3.483680059
  15.0   0.25     0.516971766   0.551209051   0.482734481
  30.0   0.5      0.341382174   0.371056113   0.311708235];

% DATA Subject2 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject2 = [ ...
   5.0   0.0833   4.32426647    4.435824659   4.212708281
  15.0   0.25     0.39218675    0.423419538   0.360953963
  30.0   0.5      0.341101679   0.367795247   0.314408111];

% DATA Subject3 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject3 = [ ...
   5.0   0.0833   3.265288799   3.349895709   3.180681888
  15.0   0.25     0.656229932   0.690842886   0.621616978
  30.0   0.5      0.321096143   0.350411612   0.291780675];

% DATA Subject4 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject4 = [ ...
   5.0   0.0833   0.281789998   0.310810629   0.252769367
  15.0   0.25     0.079996125   0.108679117   0.051313132
  30.0   0.5      0.03941336    0.066413575   0.012413146];

% DATA Subject5 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject5 = [ ...
   5.0   0.0833   4.995829489   5.134046704   4.857612274
  15.0   0.25     0.952539214   0.990335349   0.914743079
  30.0   0.5      0.16065215    0.18794698    0.133357319];

% DATA Subject6 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject6 = [ ...
   5.0   0.0833   0.876931168   0.918497193    0.835365143
  15.0   0.25     0.629584794   0.668159329    0.591010259
  30.0   0.5      0.015412548   0.046136534   -0.015311437];

% DATA Subject7 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject7 = [ ...
   5.0   0.0833   1.296405241   1.401920096    1.190890386
  15.0   0.25     0.195316479   0.290177154    0.100455804
  30.0   0.5      0.07171563    0.158903119   -0.015471858];

% DATA Subject8 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject8 = [ ...
   5.0   0.0833   2.367910025   2.478900245   2.256919805
  15.0   0.25     0.447143189   0.525370537   0.368915841
  30.0   0.5      0.241876653   0.2835157     0.200237605];
```

```
% DATA Subject9 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject9 = [ ...
   5.0   0.0833   2.287397134   2.418147272   2.156646996
  15.0   0.25     0.543430633   0.60454523    0.482316036
  30.0   0.5      0.169617203   0.267687142   0.071547264];

% DATA Subject10 (Minutes, Hours, CEnd_PPM (conc, conc+error, conc-error))
Subject10 = [ ...
   5.0   0.0833   0.548556806   0.675367472    0.42174614
  15.0   0.25     0.290017      0.403571715    0.176462285
  30.0   0.5      0.050790052   0.178616773   -0.077036669];

  PlotOutput=[];  CheckOutput=[];
  saved_LODTimes=[];
  ResetDoses
  Human_IPADefault
  HFC134a_Gunnare
  LOD=0.001; FINDLODTIME=1;

% Average of 10 subjects (Conc is the average of the concentrations used for each subject)
  BW=76.19047619; CONC=3900.0; TCHNG=0.0013889; TSTOP=168.0; CINT=0.01;
  start @NoCallback

% SET TITLE='AF Subjects -- Inhalation HFC134a       '
% SET TITLE(41)='10 Subjects -- 4 puffs asthma medication'
% SET TITLE(81)='                                      '

set @preference=BackslashEscapes
  plot1=plot(0, Average(:,1), Average(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Average  3900 ppm
     (average of doses)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  plot(plot1, 1, Average(:,1), Average(:,4), 'b+')
  plot(plot1, 1, Average(:,1), Average(:,5), 'b+')
  i=1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 1 (A)
  BW=67.57369615;
  CONC=5000.0;
  start @NoCallback
  plot1=plot(0, Subject1(:,1), Subject1(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 1 (A)  5000
     ppm\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
```

```
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 2 (B)
  BW=66.66666667;
  CONC=5000.0;
  start @NoCallback
  plot1=plot(0, Subject2(:,1), Subject2(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 2 (B)  5000
     ppm\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 3 (C)
  BW=69.3877551;
  CONC=5000.0;
  start @NoCallback
  plot1=plot(0, Subject3(:,1), Subject3(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 3 (C)  5000
     ppm\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
```

```
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 4 (D)
% Note: during albuterol administeration, subject was making shallow breaths while inhaling the
%    albuterol
  BW=80.72562358;
  CONC=500.0;
  start @NoCallback
  plot1=plot(0, Subject4(:,1), Subject4(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 4 (D)  500 ppm
     (adjusted to fit data)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 5 (E)
  BW=62.13151927;
  CONC=8000.0;
  start @NoCallback
  plot1=plot(0, Subject5(:,1), Subject5(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 5 (E)  8000 ppm
     (adjusted to fit data)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;
```

```
% Subject 6 (F)
  BW=73.92290249;
  CONC=1500.0;
  start @NoCallback
  plot1=plot(0, Subject6(:,1), Subject6(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 6 (F)  1500 ppm
     (adjusted to fit data)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 7 (G)
  BW=90.70294785;
  CONC=2500.0;
  start @NoCallback
  plot1=plot(0, Subject7(:,1), Subject7(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 7 (G)  2500 ppm
     (adjusted to fit data)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 8 (H)
  BW=78.45804989;
  CONC=5000.0;
  start @NoCallback
  plot1=plot(0, Subject8(:,1), Subject8(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 8 (H)  5000
     ppm\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
```

```
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 9 (I)
  BW=81.63265306;
  CONC=5000.0;
  start @NoCallback
  plot1=plot(0, Subject9(:,1), Subject9(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 9 (I)  5000
     ppm\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;

% Subject 10 (J)
  BW=90.70294785;
  CONC=1500.0;
  start @NoCallback
  plot1=plot(0, Subject10(:,1), Subject10(:,3), 'b+', _minutes, _cendppm, 'b-')
  pltscript(plot1, "Chart.Header.Text = \"AF Data  Inhalation of HFC134a  Subject 10 (J)  1500
     ppm (adjusted to fit data)\";")
  pltscript(plot1, "Chart.Axes.Left.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Left.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Left.Maximum=10.0;")
  pltscript(plot1, "Chart.Axes.Left.Title.Text=\"HFC-134a Concentration in Exhaled Air (ppm)\";")
  pltscript(plot1, "Chart.Axes.Bottom.Automatic=false;")
  pltscript(plot1, "Chart.Axes.Bottom.Minimum=0.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Maximum=31.0;")
  pltscript(plot1, "Chart.Axes.Bottom.Title.Text=\"Minutes\";")
  i = i + 1;
  CheckOutput(i,1) = max(_massbal);
  CheckOutput(i,2) = min(_massbal);
```

```
  saved_LODTimes(i,1) = CONC;
  saved_LODTimes(i,2) = LODTIME;
  saved_LODTimes(i,3) = CENDPPM;
  LOD=0.000001;
  start @NoCallback
  PlotOutput = addcolsj(PlotOutput, _hours, @Justification = 'begin');
  PlotOutput = addcolsj(PlotOutput, _cendppm, @Justification = 'begin');
  CheckOutput(i,3) = max(_massbal);
  CheckOutput(i,4) = min(_massbal);
  saved_LODTimes(i,4) = LODTIME;
  saved_LODTimes(i,5) = CENDPPM;
  LOD=0.001;
  save PlotOutput @file='AF_Subjects.txt' @format=ascii
  save CheckOutput @file='CheckOutput_AF_Subjects.txt' @format=ascii
  save saved_LODTimes @file='LODTimes_AF_Subjects.txt' @format=ascii
set @preference=NoBackslashEscapes
```

MC_HFC134a.m

```
% MC analysis for HFC134a exposure

%  load @format=model @file=Vast

% Initialize arrays
  mb_maxmin_fail=[];
  fit_LODTimes=[];
  NumFails=0;

% Set parameters for simulations
  Init
  ResetDoses
  Human_IPADefault
  HFC134a_Gunnare

  TCHNG=0.0013889; TSTOP=336.0; CINT=0.001;
  LOD=0.001; FINDLODTIME=1;

% Initialize random seed
  seedrnd(250920880, 868189991);
%  seedrnd(969960349, 890917552);

% Define parameters for number of iterations for Monte Carlo
  numIts = 5000;  numIts2 = numIts*2;  NumSims = 0;

% Start Monte Carlo analysis
  for iter = [1 : numIts2]

%  MALE AF BW = 0 + 1 * normrnd(84.14, 11.12, 60.23, 129.5);
%  FEMALE AF BW = 0 + 1 * normrnd(67.76, 9.186, 46.90, 93.10);
%  COMBINED AF BW = 0 + 1 * normrnd(76.71, 13.13, 46.90, 129.5);
%  IPA BW    BW = 0 + 1 * normrnd(70, 21, 28.0, 112.0);
      BW = 0 + 1 * normrnd(70, 21, 28.0, 112.0);
     QCC = 0 + 1 * normrnd(12.89, 1.1601, 10.57, 15.21);
     VPR = 0 + 1 * lognrnd(0.75706, 0.13932, 1.55, 2.7556);
     QFATC = 0 + 1 * normrnd(0.052, 0.0156, 0.0208, 0.0832);
     QLIVC = 0 + 1 * normrnd(0.227, 0.07945, 0.0681, 0.3859);
     QRAPC = 0 + 1 * normrnd(0.533, 0.1066, 0.3198, 0.7462);
     QSLWC = 0 + 1 * normrnd(0.188, 0.0282, 0.1316, 0.2444);
     VFATC = 0 + 1 * normrnd(0.214, 0.0642, 0.0856, 0.3424);
     VLIVC = 0 + 1 * normrnd(0.026, 0.0013, 0.0234, 0.0286);
     VRAPC = 0 + 1 * normrnd(0.056, 0.0056, 0.0448, 0.0672);
     VSLWC = 0 + 1 * normrnd(0.536, 0.1608, 0.2144, 0.8576);
     PB = 0 + 1 * lognrnd(-0.58479, 0.099751, 0.448, 0.672);
     PFAT = 0 + 1 * lognrnd(2.0313, 0.29356, 3.184, 12.736);
     PLIV = 0 + 1 * lognrnd(-0.15887, 0.19804, 0.522, 1.218);
     PRAP = 0 + 1 * lognrnd(-0.060432, 0.19804, 0.576, 1.344);
     PSLW = 0 + 1 * lognrnd(0.048048, 0.19804, 0.642, 1.498);
     CONC = 0 + 1 * unifrnd(500.0, 8000.0);
```

```
      disp(sprintf("Starting MC Iteration #%d of %d", iter, numIts2));
      disp("----------------------------");

%     Run parameter set with fixed dose, exposure length and start time for exposure
      start @NoCallback

%     Check mass balances to make sure simulation is valid
      if (max(_massbal) < 0.00000001 & min(_massbal) > -0.00000001)
          NumSims = NumSims + 1;
          fit_LODTimes(NumSims,1) = CONC;
          fit_LODTimes(NumSims,2) = LODTIME;
          fit_LODTimes(NumSims,3) = CENDPPM;

          disp(sprintf("Finished MC Simulation #%d of %d", NumSims, numIts));
          disp("----------------------------");
      else
          mb_maxmin_fail(1,NumFails) = max(_massbal);
          mb_maxmin_fail(2,NumFails) = min(_massbal);
      end

%     If desired number of valid simulations have been completed, exit loop
      if (NumSims == numIts)
          break;
      end
  end

  disp(sprintf("Ran %d simulations to get output for %d simulations", iter, numIts));

% Save the LOD times
  save fit_LODTimes @file='mc_HFC134a_fit_LODTimes.txt' @format=ascii

% Save the minimum and maximum mass balance values
  save mb_maxmin_fail @file='mc_male_ipa_mb_fail.txt' @format=ascii

zznumbins = sqrt(250);
```

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

µg/mL	microgram per milliliter
ActC	activated charcoal
AFRL	Air Force Research Laboratory
AUC	area under the curve
CFCs	chlorofluorocarbons
CV	coefficient of variation
DPIs	dry powder inhalers
FEF_{25-75}	forced expiratory flow
FEV_1	forced expiratory volume in one second
g	grams
g/mol	Gram per mole
HFA	hydrofluoroalkane
HFC	hydrofluorocarbon
IPA	isopropanol
K	kelvins
kg	kilogram
$L/hr/kg^{0.75}$	liters per hour per 0.75 kilogram
L/min	liters per minute
LOD	limit of detection
LOQ	limit of quantitation
mg	milligrams
mL	milliliters
PB	blood:air partition
PBPK	physiologically-based pharmacokinetic
PCAT	Pulmonary Compartmental Absorption and Transit
PCs	partition coefficients
pMDI	pressurized metered dose inhaler
ppb	parts per billion
ppm	Parts per million
ppt	Parts per trillion
RTLF	respiratory tract lining fluid
SDs	standard deviations
UK	United Kingdom