Supplement A1: PBPK Modeling

A1.1 Rat Model

The PBPK model of Poet et al. (2010) for describing the toxicokinetics of NMP in rats and humans was revised for use in deriving an occupational exposure limit (OEL). These PBPK models were evaluated by the U.S. EPA to support their TSCA risk assessment (USEPA, 2015). In this update, additional data were considered to further calibrate and validate the model, and reviewer suggestions were considered. Model calibration consists of using data to optimize parameters when those parameters are unknown or approximated, validation is used to show the fits of the model to other datasets. **Table 1** (main body of the manuscript) summarizes studies and how they were used for model parameterization and optimization. The model described in 2010 was modified slightly; fetal compartment concentration calculations were changed and re-organized to improve mass-balance during growth, and dosing input methodology was improved. These modifications to the model resulted in modest changes in model output. Additional data were used to calibrate and validate the intravenous, oral and dermal routes of exposure in rats. While plasma and urinary excretion data for major metabolite (5HNMP) have also been reevaluated, primary attention has been paid to NMP, since the dose measure of interest are for the parent chemical. Model parameters for rats and humans are shown in **Table A1-1**.

A1.1.1 Intravenous Data

All available intravenous data were obtained from studies that administered radiolabeled NMP. Most of the available studies only provided peak measured concentration and pharmacokinetic parameters. The study chosen to calibrate the model was that described by Payan et al (2002), in which nulliparous rats were exposed to NMP doses ranging from 0.1 to 500 mg/kg. However, the authors only reported plasma NMP data for the lowest dose. This time-course data set was used to optimize metabolic rate parameters (VmaxC and Km) to describe the clearance of NMP from plasma. Unchanged NMP has only been found at very low levels in rat urine, so urinary elimination was set at a nominal value of 0.001 hr^{-1}.

Payan et al. (2002) estimated the post-distribution metabolic rates of NMP from the disappearance of NMP from plasma in their studies. These estimated rates (Km=200 mg/L and VmaxC=1.5 $mg/hr/kg^{0.75}$) were used as the seed values for the optimization carried out using the optimization routines supplied in acslX (v3.0.2.1; The AEgis Technologies Group, Inc, Huntsville, AL) in which the model was created. By starting with these values, it was hoped that the dose-range in that study would be represented and the optimized model would fit across doses. The final optimized parameters were Km= 225 mg/l and VmaxC=9 $mg/hr/kg^{0.75}$. (Wells and Digenis, 1988) administered an intravenous dose of 45 mg/kg to rats, which is 450x higher

than the dose used for optimization, and this was used to validate the metabolic rates over a large range (**Figure A1-1**).

A1.1.2. Oral Data

All available oral exposure data were obtained from studies that administered radiolabeled NMP. The most valuable data sets are those that specifically measured NMP in blood (dose measure used in the assessment). NMP is highly metabolized and generally not found in urine as unchanged NMP. The study chosen to calibrate the oral absorption rate was that described by Midgely et al. (1992). In this study, male and female rats received an oral gavage of 105 mg/kg (22.5 mg in rats weighing 192-239 g) NMP, co-exposed with 2-pyrrolidinone in a water vehicle. The authors concluded that 94.5% of the administered radiolabel was absorbed. It was assumed that the fraction absorbed applied to both NMP and 2-pyrrolidinone.

The data indicate a rapid uptake and a slow elimination of NMP from plasma. Using the metabolic rate constants optimized to fit the intravenous dosing, and the oral bioavailability measurements of Midgely et al (1992), the model estimates of plasma NMP clearance resulted in a much higher AUC than the data indicated (**Figure A1-2**). There is no suggestion of extra-hepatic (e.g. intestinal) metabolism, so another mechanism to describe this absorption pattern was investigated. NMP is readily absorbed across membranes (see dermal absorption data discussion below), and for some chemicals absorption has been proposed to occur either in the stomach or quickly in the intestine, then more slowly during later phases of transport (Levitt et al., 1997; Staats et al., 1991; Timchalk et al., 2002). Dual absorption was included in the PBPK model to describe oral absorption following the description from Staats et al. (1991). The resulting model predictions are vastly improved (**Figure A1-2**). Using dual oral absorption results in ~75% of the dose absorbed via the faster process and the remaining ~25% is more slowly absorbed.

Because developmental endpoints are of primary concern, the female rat oral exposure data of Ghantous (1995) were also modeled. Only the high dose (50 mg/kg) plasma NMP data were available to validate the oral absorption, and these data exhibit a high degree of variability compared to other studies (**Figure A1-2**). Following the 50 mg/kg exposures, ≥93% of the total dose was recovered in the animal or urine in female rats, so 94.5% oral absorption value obtained from Midgely et al. (1992) was assumed to be appropriate for all oral exposures.

A1.1.3 Dermal Data

Developmental studies for NMP have been conducted by the dermal route (Becci et al., 1982). In the original PBPK model publication (Poet et al., 2010), the dermal route was assessed using a permeability coefficient (Kp) of 4.7×10^{-3} cm/hr that was

approximated from *in vitro* studies (Payan et al., 2003). For the current assessment, the *in vivo* dermal exposure studies described by Payan et al. (2003) were used to optimize Kp. In this study, rats were exposed to 200 µl of neat NMP. According to Payan et al., by 24 hours after dosing, 80% of the NMP applied had penetrated the skin. The Kp value optimized to these data was estimated to be 4.3×10^{-3} cm/hr, which is consistent with the range of Kp values estimated from the *in vitro* studies (from 2.0×10^{-3} to 7.7×10^{-3}cm/hr: (Payan et al., 2002)) (**Figure A1-3**).

A1.1.4 Inhalation

No parameters were optimized to simulate the inhalation exposures of female rats to 104 ppm NMP for 6 hr (Ghantous et al., 1995), 100% inhalation bioavailability was assumed. These data, like the oral exposure data from the same source, appear to be more variable than from other studies. The model fits to the data are shown in **Figure 1** (main body of manuscript).

A1.2 Human Model

The dosimetry of NMP is of primary interest, and the submodel for 5HNMP has been reduced to a single compartmental model. Human exposures to NMP will be primarily via the inhalation route with some contribution from the dermal route (vapors or liquid). Ingestion of NMP is not expected to be a significant pathway in human populations. Both controlled and occupational human exposure data are available from the published literature. Controlled human biomonitoring studies were used to calibrate NMP and 5HNMP metabolic rates, and a workplace exposure assessment study was used to validate the model and exposure scenarios. The exposure scenarios are summarized in **Table A1-2**.

A1.2.1 Inhalation Data

A study conducted by the Hannover Medical School, the University of Dortmund, Germany (Bader, 2006) was used to calibrate inhalation parameters model. In this study, 8 healthy, non-smoking, male volunteers were exposed to 10, 40, or 80 mg/m^3 NMP in an environmental chamber. Over the course of several weeks, each volunteer was exposed sequentially to all 3 concentrations. The 8 volunteers were separated into 2 groups of 4 and each group was exposed in a shared chamber. The exposures were carried out in ascending concentrations, with a 1-week period between each session. Volunteers wore slacks and T shirts, and thus had arms exposed to vapor. Blood was collected from each volunteer in the middle of the 6-hour exposure period, at the end of exposure (6 hour), and 1, 2, 3, 18, and 42 hours

after the end of exposure. Urine was also collected from each volunteer at times up to 42 hours after the end of exposure. Because it is relatively rare to have blood and urine data for multiple exposure levels and multiple time points in individuals, efforts were made to ensure the exposure scenarios for these data were modeled as accurately as possible.

To collect the mid-exposure blood samples, volunteers left the chamber one at a time, and moved to another room to have blood drawn and to give a urine sample. The data are consistent with a sharp drop in concentration for the mid-exposure blood sampling. In the report, the time taken to leave the chamber, walk to the new room, donate blood and urine was suggested to be about 10 minutes. However, exact times were not recorded, and the exact time is unknown (personal communication, Dr. Bader). The notes indicate that the time between blood collection and urine collection was at least 5 minutes. In addition, the time from first collected sample to last (the first and fourth volunteers to leave the chamber) was up to 55 minutes for the recorded times for collection of blood from the first to the last volunteer to leave the chamber. If the times were equivalent for each subject, and the volunteers only left the chamber as the previous volunteer returned, this would indicate 18 minutes was needed for sample collection. Accordingly, the data from all volunteers was averaged and the model was used to optimize this estimated break in the middle of exposure. The model-optimized average break time was 20 minutes. The impact on dose metrics [maximum blood concentration (Cmax) or area under the concentration curve (AUC)] of the 20-minute break compared to a 10-minute break is negligible (**Table A1-3**)

The fraction inhalation uptake was assumed to be 100% of alveolar respiration, consistent with assumptions used for modeling the rat data. Initial rates of NMP and 5HNMP metabolism (Vmax, and Km) and saturable urinary elimination were optimized by first fitting NMP in plasma, then NMP in urine, then 5HNMP in plasma, then 5HNMP in urine. The study design with 8 volunteers each exposed to 3 doses permitted an assessment of inter-individual variability, so rate constants were optimized for each volunteer individually (**Figure 2,** main body of manuscript; **Table A1-4**). Modeling each volunteer individually also affords the opportunity to compare internal metric predictions in this population (**Table 3**; main body of the manuscript). The fit of the model do the average plasma NMP, 5HNMP, and urinary NMP and 5HNMP using an average of all rate constants is shown in **Figure A1-4**.

A1.2.2 Dermal Data: Vapor and Liquid

Volunteers in the study described by Akesson and Paulsson (1997) wore shorts and t-shirts, and thus also had dermal (vapor) exposures, as well as inhalation exposures, to NMP. The exposure concentrations for this study were similar to those of Bader (2006). With only inhalation exposures, the model under-predicted plasma NMP by about 25%, a vapor permeability coefficient, which accounts for both the skin permeability and the vapor/skin surface interaction, ($Kp_{(vapor)}$ of 22

cm/hr was optimized to fit these data, and is nearly equivalent to the previously optimized value (Poet et al., 2010) (**Figure A1-5**).

Akesson et al (2004) exposed 12 volunteers (6 male and 6 female) to 300 mg NMP either neat or diluted 50:50 in an aqueous solution. Blood and urine 5HNMP concentrations were monitored for up to 9 days. The plasma 5HNMP concentration was extracted from the figure using DigitizIt [Braunschweig, Germany). Urinary 5HNMP concentrations were extrapolate to total amount eliminated using the assumption that the average urinary flow for an adult is 18 ml/kg-day (Heffernan et al., 2013). Dilution resulted in a slower time to reach peak plasma 5HNMP and a reduction in peak plasma concentration. Optimized liquid $Kp_{(liquid)}$ for neat NMP was 2.0×10^{-3} cm/hr (**Figure A1-6**). To fit the data from the diluted exposures, a lower $Kp_{(liquid)}$ of 5.1×10^{-4} was needed (**Figure A1-6**). These liquid dermal permeability coefficients were in agreement with Kp values reported from in vitro studies (HLS, 2002). HLS (2002) evaluated the in vitro dermal absorption of NMP (neat and in aqueous solution) in human skin. In this study, finite doses (10 µl/cm2) or infinite doses (400 µl/cm2) of 14C-NMP were applied under semi-occlusive conditions (carbon filter 2 cm above skin surface) to dermatomed skin (~300 µm thick) maintained at 32°C in flow through (2 ml/h) diffusion cells. Kp values for liquid NMP in human skin were determined to be 2.2×10^{-3} cm/hr and 2.5×10^{-4} cm/hr for neat NMP and NMP in a 30% aqueous solution, respectively.

A1.2.3 Occupational exposures to NMP

In a biomonitoring study, Xiaofei et al (2000), followed 4 workers and 5 observers in a lens manufacturing facility. The workers washed lenses with NMP, working 11-hour shifts with a 1-hour lunch break (total 12 hours within the facility). Exposures were measured for each worker and observer using an activated charcoal sampler. The exposures over 5 days to those 4 individuals indicated the daily TWA of 0.09 to 0.69 ppm for their 12-hour shift, and the workers were assumed to be under light work, with increase respiration and heart rate as described in the published literature (Andersen et al., 1987). The weakly TWA mean was 0.33 ± 0.20 ppm for all 5 workers. The PBPK model underestimated average plasma NMP concentrations from this study by ~1.8x (data not shown) when 0.33 ppm inhalation and dermal exposure is assumed. However, droplets of NMP were noted on the lenses as the workers were moving those lenses to drying racks. A very low liquid splatter rate of 1.0 ml/hr onto the workers skin results in the model fits shown in **Figure A1-7**. The observers did not have direct contact with the lenses, but were in the same room as the workers for a total of 9 hours, thus it was assumed that their dermal exposure to liquid was half the rate of the workers with direct contact (**Figure A1-7**)

A1.3 References

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A1.4. Pregnant Rat PBPK Model Code

```
PROGRAM NMP.ACSL

!PBPK MODEL FOR N-METHYL PYRROLIDONE
!FINAL RAT MODEL (5/09)
!T.S. POET,P HINDERLITER. CHEMICAL DOSIMETRY GROUP, PNNL, RICHLAND,
WA
!MODEL TRANSFERRED FROM SIMUSOLV TO ACSLXTREME FORMAT IN 08
!MODEL CONFIGURED FOR INHALATION (OPEN, WHOLE BODY/NOSE ONLY)
!   IV, ORAL,  DERMAL, AND IP  ROUTES OF ADMINISTRATION.

!MODEL TRACKS DISPOSITION OF NMP AND 5-HNMP.
!ASSUMPTIONS:
!    (1) FLOW-LIMITED (ALL COMPARTMENTS)
!    (2) METABOLISM OF NMP BY A SAT PATHWAY TO FORM 5HNP
!    (3) METABOLISM OF HNP BY SATURABLE PATHWAY TO ETC.
!    (5) METABOLISM OCCURS ONLY IN THE LIVER
!    (6) TISSUE:BLOOD PART. COEFF.   = HUMAN = KRISHNAN EQN
!          UPDATED IN CMD FILE TO MEASURED IN-HOUSE
!    (7) 5HNP ELIMIN FROM MIXED VENOUS - 1ST ORDER
!     THIS DIFFERS FROM 02: URINE BY *GFR CLEARANCE FROM KIDNEY
!   METAB RATE CONST. FROM REPORT - UPDATED WITH LIT VALUES IN CMD
FILE
!   PREG ADDED - OTHER PARAMETERS CHANGED NOMINALLY TO HARMONIZE
WITH FETAL IPA MODEL OF
!     GENTRY ET AL. REGU TOX PHARM 36:51-68, 2002

INITIAL
! MODEL UNITS
! CONCENTRATION, MG/L
! FLOW, L/HR
! BODY WT, KG

 CONSTANT  BWINIT=0.       ! PRE-PREGNANCY BODY WEIGHT (KG)
 CONSTANT  RATS=1.         !NUMBER OF ANIMALS IN EXPT. NOT USED IN
HUMAN MODEL
 CONSTANT  MWNMP=99.13      !MOL. WT. NMP, MG/MMOL
 CONSTANT  MWHP= 116.14     !MOL. WT. 5-HNP, MG/MMOL

!BLOOD FLOWS
!FROM BROWN ET AL TOX IND HEALTH 97
!AND/OR FROM IPA MODEL OF GENTRY ET AL.,
! BLOOD FLOWS (FRACTION OF CARDIAC OUTPUT)
  CONSTANT     QCC = 0        ! CARDIAC OUTPUT (L/HR FOR 1 KG
ANIMAL)
  CONSTANT     QPC = 0       ! ALVEOLAR VENT. RATE

  CONSTANT   QFATC = 0        ! FAT (NON-PREGNANT)
  CONSTANT   QLIVC = 0        ! LIVER
  CONSTANT   QMAMC = 0        ! MAMMARY TISSUE (NON-PREGNANT)
  CONSTANT   QSKNC = 0        ! SKIN
  CONSTANT   QUTRC = 0        ! UTERUS (NON-PREGNANT)
```

```
  CONSTANT   QRAPC = 0        ! RAPID USE STATIC RAPID FOR RATS
(MUST BE CHANGED FOR HUMAN)

! PERMEABILITY-AREA PRODUCT (L/HR)
  CONSTANT    PAFC = 0.1          ! DIFFUSION ON FETAL SIDE OF
PLACENTA
! NOTE 0.1 IS THE VALUE SUPPLIED BY ENVIRON AND USED FOR IPA, IT IS
UNSURE WHERE THE VALUE COMES FROM
! GRAPHING OUT TRANSPORT TO FETUS, 0.1 RESULTS IN A MAX FOR NMP,
MAYBE FOR IPA AS WELL

! TISSUE VOLUMES (FRACTION OF BODY WEIGHT)
!FROM BROWN ET AL TOX IND HEALTH 97 FOR RATS
!OR FROM GENTRY ET AL
  CONSTANT    VLUC = 0        ! LUNG
  CONSTANT   VFATC = 0         ! FAT (NON-PREGNANT)
  CONSTANT   VLIVC = 0        ! LIVER
  CONSTANT   VMAMC = 0         ! MAMMARY TISSUE (NON-PREGNANT)
  CONSTANT   VRAPC = 0        ! RAPIDLY PERFUSED
  CONSTANT   VUTRC = 0        ! UTERUS (NON-PREGNANT)
  CONSTANT    VBLC = 0       ! TOTAL BLOOD


! FOR PARENT MODEL, SKIN COMPARTMENT IS ONLY DEFINED AS DOSED SKIN
  CONSTANT    VSKC = 0.19           ! SKIN
  CONSTANT      SA = 0.01         !SURFACE AREA EXPOSED, SQ.CM
           TSA = 906.*BWINIT**(2./3.)   !TOTAL BODY SURFACE AREA,
SQ.CM.
                                            !MCDOUGAL ET AL. T.A.P.
85(1996)286
   IF (CONCL.GT.0.0) THEN
           VSKCC = VSKC*SA/TSA
      QSKCC = QSKNC*SA/TSA
   ELSE
           VSKCC = VSKC*SA/TSA
      QSKCC = QSKNC*SA/TSA
   ENDIF

! SLOWLY PERFUSED (DEFINED AS BALANCE OF TISSUES AND FLOWS)
 VSC = 0.91 - (VLUC + VFATC + VLIVC + VMAMC + VRAPC + VUTRC + VBLC +
VSKCC)
       ! NOTE: 0.91 IS APPROX WHOLE BODY LESS BONE
 QSC = 1. - (QFATC + QLIVC + QMAMC + QRAPC + QUTRC + QSKCC)

! SCALED BLOOD FLOWS (L/HR)
    QCINIT = QCC * (BWINIT**0.75)
     QFATI = QFATC * QCINIT
      QLIV = QLIVC * QCINIT
     QMAMI = QMAMC * QCINIT
      QRAP = QRAPC * QCINIT
      QSKN = QSKCC * QCINIT
      QSLW = QSC * QCINIT
     QUTRI = QUTRC * QCINIT
```

```
! SCALED TISSUE VOLUMES (L)
       VLU = VLUC * BWINIT
     VFATI = VFATC * BWINIT
      VLIVI = VLIVC * BWINIT
      VRAP = VRAPC * BWINIT
      VSLW = VSC * BWINIT
     VMAMI = VMAMC * BWINIT
     VUTRI = VUTRC * BWINIT
       VSK = VSKCC * BWINIT
       VBL = VBLC * BWINIT     ! TOTAL BLOOD
      VA = 0.25*VBL          !ARTERIAL BLOOD
           VV = 0.75*VBL          !VENOUS BLOOD

! PREGNANCY PARAMETERS
  CONSTANT  NUMFET = 7.0          ! NUMBER OF FETUSES (NOT USED FOR
HUMAN, ASSUME 1)
  CONSTANT   PUPBW = 4500.        ! BIRTH WEIGHT (MG)
  CONSTANT VFETD18 = 1051.254     ! VOLUME OF  FETUS AT DAY 18 ( )
OF PREGNANCY

! CONVERSION FACTORS
  CONSTANT    MGKG = 1.0E6        ! CONVERSION FACTOR FROM MG TO KG

!PARTITION COEFFICIENTS
!EXPERIMENTALLY MEASURED VALUES
   CONSTANT PB=0.          !NMP BLOOD:AIR
   CONSTANT PF=0 !NMP FAT:BLOOD - MEASURED
   CONSTANT PL=0 !MEASURED
   CONSTANT PR=0 !MEASURED LIVER
   CONSTANT PS=0 !NOT MEASURED MUSCLE - CORRECTED FOR FILTER ERROR
USING SKIN PROPORTIONALITY
   CONSTANT PSKL=0     !MEASURED
   CONSTANT PLU=0        !NMP LUNG:BLOOD
   CONSTANT PSKA= 0      !NMP SKIN:AIR
            CONSTANT PSKB=0  ! NMP SKIN:BLOOD
   CONSTANT PM=0       !MAMMARY, ESTIMATED FORM LIVER
   CONSTANT PPLA=0
   CONSTANT PUTR=0


!EXPERIMENTALLY MEASURED VALUES
   CONSTANT PLHNP=0             ! LIVER MEASURED
   CONSTANT PBHNP=0           !ESTIMATED AVG OF "OTHER" TISSUES
   CONSTANT PFHNP=0             !MEASURED
   CONSTANT PPLHNP=0

!METABOLIC RATE CONSTANTS
     CONSTANT  KM=0          !MICHAELIS CONSTANT, MG/L
     CONSTANT  VMAXC=0 !MAX. ENZ. ACT., MG/HR/L
     VMAX1 = VMAXC*BWINIT**0.75

  !5HNP TO OTHER METABS
     CONSTANT  KM2=0         !MICHAELIS CONSTANT, MG/L
     CONSTANT  VMAX2C=0           !MAX. ENZ. ACT., MG/HR/L
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     VMAX2 = VMAX2C*BWINIT**0.75

!URINARY ELIMINATION OF 5-HNMP - CLEARED FROM BLOOD
!NOTE FIRST ORDER RATE COMMENTED OUT, SATURABLE FITS BETTER
  CONSTANT KLC=0
  KL=KLC/(BWINIT**0.25)
 CONSTANT KLNC=0             !URINARY LOSS OF NMP, L/HR
      KLN=KLNC/(BWINIT**0.25)


!FRACTIONAL ABSORPTION
    CONSTANT  FRACIN = 1     !FRACTIONAL UPTAKE OF NMP BY
INHAL,START AT 65%
                                    !OF ALVEOLAR - AS IN AKESSON ET
AL 1997
    CONSTANT  FRACOR = 1.0      !FRACTION ABSORBED ORALLY, INITALLY
100%
    CONSTANT FRACF=1


! INITIAL CONDITIONS FOR CLOSED CHAMBER INHALATION
  CONSTANT    VCHC = 9E9   ! VOLUME OF CLOSED CHAMBER (L),START
LARGE FOR OPEN
  CONSTANT   KLOSS = 0.0   ! CHAMBER LOSS RATE /HR


!TIMING COMMANDS

      CONSTANT  TCHNG=6.0    !END OF INHAL EXPOSURE, HR
    CONSTANT  TSTOP=24.0    !END OF EXPERIMENT/SIMULATION, HR
      CONSTANT MAXT=0.01           !MAXIMUM STEP SIZE, HR THIS MAY
NEED SET LOWER FOR NEW VERSION OF ACSL TO RUN
      CONSTANT MINT=1E-7
      CONSTANT CINT = 0.2    !DATA LOGGING RATE /HR
    CONSTANT  GDDAYS=0.0   ! OFFSET FOR GESTATIONAL DAY SIMULATION
    CONSTANT  GDMONTHS=0.0  !OFFSET FOR HUMAN GD SIMULATION

!INITIAL EXPOSURE CONDITIONS
  ! EXPOSURE CONDITIONS BASED ON USER DEFINED INITIAL AMOUNTS OF
CHEMICAL (MG)
    CONSTANT  CONCPPM = 0.0                  !AIR CONCENTRATION IN
PPM!
           constant concmgs = 0.0
      ! Used to set air conc'n as mg/m3, PMS, 8-13-13
          VCH = VCHC-(RATS*BWINIT)         !VOLUME OF OCCUPIED
CHAMBER
          CONCMG = CONCMGS/1000 + CONCPPM*MWNMP/24451.    !CONVERT
PPM TO MG/LITER!
          CONSTANT   DOSEINTERVAL=24                              !TIME
BETWEEN DAILY DOSES
                constant concchppm0 = 0      ! Initial ppm in
closed chamber
                conchmg0= concchppm0*MWNMP/24451.
```

```
          ACHO = conchmg0 * VCH              !INIT. AMT IN CHAMBER,
MG!
   !ORAL
      CONSTANT  KAS=1.0          !1ST ORDER RATE CONST FOR ORAL ABS
from stomach,HR-1
                CONSTANT KAI=1
      !1ST ORDER RATE CONST FOR ORAL ABS from intestines,HR-1
      CONSTANT KSI=1
      CONSTANT  DOSE=0.0         !ORAL DOSE IN MG/KG BW
      ODOSE = FRACOR*DOSE*BWINIT        !CONVERT MG/KG BW TO MG
TOTAL(ORAL)
                        ! ODOSE multiplied by FRACOR to reduce oral
bioavailability
                        constant    dose2=0.0                    !
ORAL Dose in mg/kg BW, but total dose increases w/ BW; PMS 9-16-13
                gavds=dose2*FRACOR*BWINIT    ! Initial value for
this dose; PMS 9-16-13
   !FEED
      CONSTANT  KASF=1.0          !1ST ORDER RATE CONST FOR ORAL
ABS,HR-1
      CONSTANT  DOSEF=0.0         !ORAL DOSE IN MG/KG BW in feed
     !IV
      CONSTANT IVDOSE=0.0        !IV DOSE, MG/KG NMP
   !DERMAL
      CONSTANT CONCL = 0.0       !CONC OF NMP IN LIQUID, MG/L
      CONSTANT KPL = 0.0         !PERM COEFF FOR LIQUID, CM/HR
      CONSTANT VLIQ = 1.0E-99       !INITIAL VOLUME APPLIED, L
      CONSTANT DENSITY=     1.03
                constant DSK=0.0 ! Initial amount (mg/kg BW) rubbed
into skin; pms 8-14-13
                        ASKO=DSK*BWINIT  ! PMS, 8-14-13
                        DDNX=CONCL*VLIQ
!CONCL2=CONCL*FAD
                constant twash=8.0     ! Wash time in Becci et al.
(1982) exposures
       CONSTANT FAD=0.78  !FRAC no absorbed in Payan et al
          !IN VITRO HUMAN VAN DYK ET AL. AIHA J 56: 651-660
        !START WITH SMALL SA SO VSKE IS NON-ZERO (USED IN
DENOMINATOR OF CSK CALCULATION)

    !IP
      CONSTANT IPDOSE = 0.0      !IP DOSE, MG/KG NMP
      CONSTANT KIP=1.0           !1ST ORDER RATE OF ABS, HR-1
      PDOSE = IPDOSE*BWINIT       !TOTAL IP DOSE, MG

!DOSING SCHEDULE
           if (DSK.GT.0.0) then
                schedule SKWASH.AT.TWASH
           ENDIF
     if (CONCL.GT.0.0) then
                schedule DERMOFF.AT.TWASH
           ENDIF
if (CONCL.GT.0.0) then
                schedule SKWASH.AT.TWASH
```

```
           ENDIF
     SCHEDULE OFFD.AT.TCHNG     !TURN OFF EXPOSURE AT TCHNG
     CIZONE = 1.0               !START WITH INHALATION ON
     IVZONE = 1.0               !START WITH IV ON
DZONE = 1.0
           ! DZONE2=1.0
      !START WITH DERMAL ON
IF (CONCL.GT.0.0) THEN
     DZONE = 1.0             !START WITH DERMAL ON
ELSE
           DZONE = 0.0
ENDIF
constant tstart=0.2    ! offset start-time for gavage dosing
                 schedule GAVD.at.TSTART
     ALGORITHM IALG=2          !GEAR ALGORITHM

END

DYNAMIC

DERIVATIVE
!===============FETAL AND BW CHANGES
W/PREGNANCY=======================
    GDMONTH=GDMONTHS/0.64    !TO WAG GROWTH
                                              HOURS = T
   MINUTES = T * 60.0
      DAYS = T / 24.0 + GDDAYS +GDMONTH


! VOLUME OF FAT (L)
      VFAT = VFATI * (1.0 + (0.0165 * DAYS))

! VOLUME OF FETUS (KG)
     IF (DAYS.LT.10.0) THEN
         VFET = (1.0e-8 + NUMFET * ((0.1206 * DAYS)**4.53)) / MGKG
     ELSE IF (DAYS.LT.17.0) THEN
         VFET = (1.0e-8 + NUMFET * ((1.5 * (DAYS - 9))**2.8)) / MGKG
     ELSE
         VFET = (1.0e-8 + NUMFET * (VFETD18 + (((PUPBW - VFETD18) /
4.0) * (DAYS - 17)))) / MGKG
     ENDIF

! VOLUME OF   MAMMARY TISSUE (L)
      VMAM = VMAMI * (1.0 + (0.27 * DAYS))

! VOLUME OF   PLACENTA (L)
     IF (DAYS.LT.6.0) THEN
         VPLA = 1.0e-8
     ELSE IF (DAYS.LT.10.0) THEN
         VPLA = (1.0e-8 + NUMFET * (8. * (DAYS - 6.))) / MGKG
     ELSE
         VPLA = (1.0e-8 + NUMFET * ((32 * EXP(-0.23 * (DAYS - 10)))+
(40 * (EXP(0.28 * (DAYS - 10)) - 1)))) / MGKG
     ENDIF
```

```
! VOLUME OF   UTERUS (L)
     IF (DAYS.LE.3.0) THEN
         VUTR = VUTRI
     ELSE
         VUTR = VUTRI * (1.0 + (0.077 * ((DAYS - 3.)**1.6)))
     ENDIF

!VOLUME OF LIVER INCREASE   !Corley et al CRC 03,BUELKE-SAM ET AL
'82 AND OTHERS
IF (DAYS.LT.5.0) THEN
VLIV=VLIVI
ELSE
VLIV= VLIVI * (1.0 + (0.0455 * ((DAYS - 5.0))))
ENDIF

! INCREASE IN   BODY WEIGHT (KG)
               BW = BWINIT + (VFAT - VFATI) + VFET + (VMAM - VMAMI)
+ VPLA + (VUTR - VUTRI)+(VLIV - VLIVI)

! SCALED   ALVEOLAR VENTILATION (L/HR)
        QP = QPC * ((BW-VFET-VPLA)**0.75)
! INCREASE IN   BLOOD FLOWS (L/HR)
      QFAT = QFATI * (VFAT / VFATI)
      QMAM = QMAMI * (VMAM / VMAMI)
      QUTR = QUTRI * (VUTR / VUTRI)
!!!!!! NOTE THAT THE BLOOD FLOWS NO LONGER BALANCE. QP HAS INCREASED
BY THE ADDITIONAL
!!!!!! FETAL AND PLACENTAL VOLUMES BUT THE COMPARTMENTAL FLOWS HAVE
NOT CHANGED.
!!!!!! QRECOV WILL START AT 100 AND DECREASE THRU PREGNANCY (PMH 25-
APR-2007)


! TOTAL BODY FOR HNMP
           QB = QRAP+QSLW+QSKN+QMAM+QUTR !
      VB = VRAP+VSLW+VLU+VSK+VMAM+VUTR !


!   BLOOD FLOW TO PLACENTA (L/HR)
     IF (DAYS.LT.6.0) THEN
         QPLA = 0.0
     ELSE IF (DAYS.LT.10.0) THEN
         QPLA = (NUMFET * (0.55 * (DAYS - 6.0))) / 24
     ELSE IF (DAYS.LE.12.0) THEN
         QPLA = (NUMFET * (2.2 * EXP(-0.23 * (DAYS - 10)))) / 24
     ELSE
         QPLA = (NUMFET * ((2.2 * EXP(-0.23 * (DAYS - 10)))+
((0.1207 * (DAYS - 12.0))**4.36))) / 24
     ENDIF

! INCREASED   CARDIAC OUTPUT (L/HR)
               QC = QFAT+QLIV+QSLW+QRAP+QSKN+QMAM+QPLA+QUTR   !
SCALED PERMEABILITY-AREA PRODUCT
```

```
       PAF = PAFC * (VFET**0.75)


!==================FIRST MODEL FOR TRACKING
NMP=========================

!EQUATIONS FOR ORAL GAVAGE DOSING
!note this is an oral model that expects some direct absorption to
liver and some transfered to intestine for absorption to liver
!this structure explains early peak and slow elimination observed in
Midgely oral exposure data. Validated by Ghantous data
    RAOA = -(KAS * AO)-(KSI*AO)
     AO = ODOSE+ INTEG(RAOA,0.0)  !AMT REMAINING TO BE ABS, MG!
   RAO=KAS*AO
        OABS = INTEG(RAO,0.0)
RINTC=(KSI*AO)-(KAI*AINTC)  !RATE OF CHANGE IN INTESTINES
AINTC=INTEG(RINTC,0.0)
RAINTEST=KAI*AINTC  !TRANSFER TO LIVER
OIBS=INTEG(RAINTEST,0.0)


!EQUATIONS FOR FEED DOSING
      FDOSE = DOSEF*FRACF*BW        !CONVERT MG/KG BW TO MG
TOTAL(ORAL)
                      RAF = KASF * AF    !*FRACF
     AF = FDOSE - INTEG(RAF,0.0)  !AMT REMAINING TO BE ABS, MG!
   FABS = INTEG(RAF,0.0)

!AL = AMOUNT NMP IN LIVER COMPARTMENT (MG)
      RAL = QLIV*(CA - CVL)+ RAIP + RAO + RAF - RAML+RAINTEST
       AL = INTEG(RAL, 0.0)
      CVL = AL/(VLIV*PL)

     RAML = (VMAX1*CVL)/(KM+CVL)    !SATURABLE METABOLISM, MG/HR
      AML = INTEG(RAML,0.0)       !AMT NMP METAB BY SATURABLE PATH,
MG
    AML1B = RATS*AML*MWHP/MWNMP  !TOT AMT HNP PRODUCED IN LIVER, MG

!EQUATIONS FOR IP DOSING
    RAIP = KIP * AIP
     AIP = INTEG(-RAIP,PDOSE)  !AMT REMAINING TO BE ABS, MG!
        IPABS = INTEG(RAIP,0.0)

!EQUATIONS FOR IV INFUSION
    IVR = IVZONE*IVDOSE*BW/Tchng  !RATE OF INFUSION, MG/HR ! Using
Tchng instead; pms 8-29-13
    TIV = INTEG(IVR,0.0)         !TOTAL AMOUNT INJECTED, MG

! ARTERIAL BLOOD
     RAAB = (QC * (CVLU - CA))-RAUNP
      AAB = INTEG(RAAB, 0.0) !AMOUNT, MG
       CA = AAB / VA        !CONCENTRATION, MG/L
    AAUCB = INTEG(CA, 0.0)   !AUC, HR*MG/L
     RAUNP = KLN*CA*VV            !FIRST ORDER RATE OF LOSS (URINE
```

```
           AUNP = INTEG(RAUNP,0.0)

! CHAMBER CONCENTRATION (MG/L)
     RACH = (RATS * QP * CLEX) - (FRACIN * RATS * QP * CI) - (KLOSS
* ACH)
      ACH = INTEG(RACH, ACHO)


! THE FOLLOWING CALCULATION YIELDS AN AIR CONCENTRATION EQUAL TO
! THE CLOSED CHAMBER VALUE IF A CLOSED CHAMBER RUN IS IN PLACE AND
! A SPECIFIED CONSTANT AIR CONCENTRATION IF AN OPEN CHAMBER RUN IS
IN PLACE

      CCH = (ACH / VCH)! * CIZONE) + (CONCMG * (1.0 - CLON))

    CCPPM = CCH *24451/MWNMP
    CLOSS = INTEG(KLOSS * ACH,0.0)
CI = CCH*PULSE(0., DOSEINTERVAL,TCHNG) + CIZONE*CONCMG   ! MG/L !
Added CIZONE*CONCMG, PMS, 8-13-13

! LUNGS
        RALU = (QP * ((FRACIN * CI) - CLEX)) + RVV - (QC * CVLU)
      !
        ALU = INTEG(RALU, 0.0)
      CLU = ALU / VLU             !CONCENTRATION, MG/L
     CVLU = CLU / PLU           !EXITING CONCENTRATION, MG/L

!  AMOUNT INHALED
     RINH = FRACIN * QP * CCH *CIZONE
     AINH = INTEG(RINH, 0.0)   ! MG PER
    AINHC = AINH * RATS        ! MG FOR A GROUP OF RATS

!  AMOUNT EXHALED
     CLEX = CV / PB           ! CONCENTRATION, MG/L
     RAEX = QP * CLEX
      AEX = INTEG(RAEX, 0.0)   ! AMOUNT, MG PER
     AEXC = AEX * RATS          ! AMOUNT, MG, FOR A GROUP OF RATS

!ASK = AMOUNT NMP IN SKIN TISSUES (MG) AND DERMAL DOSING
      RASK = QSKN*(CA - CSKV) + RADL    !
       ASK = INTEG(RASK,ASKO)      ! Initial value, ASKO, added for
Becci et al. (1982) exposures; pms 8-14-13
       CSK = ASK/VSK              !'NMP IN SKIN, MG/L'
                CSKV = CSK/PSKB                          ! NMP IN
VENOUS BLOOD, PMS 8-22-13
     CVSK3 = CSK*1000/MWNMP     !'NMP IN CVSK, MICROMOL/L'


RADL = (KPL*(SA/1000))*(CSURF - (CSK/PSKB))*DZONE!*DZONEDAY
  ! Net rate of delivery to "L" skin from liquid, when liquid is
there
      ADL = INTEG(RADL, 0.0)
DDN=INTEG(-RADL,DDNX) ! Aount in liquid.  DDN is the initial amount.
!CSURF=DELAY(DDN/VLIQ,0,DLAY,10,999)
```

```
CSURF = DDN/VLIQ


            ! AMOUNT RECOVERED FOR EACH STUDY WITH AMOUNT (CONC)
ORIGINALLY APPLIED
       ! "LOSS" OR STICKING PROBABLY ESSENTIALLY IMMEDIATE AND NOT
KINETIC
       ! REPORTS OF ~11-24% STICKING TO DRESSING

! AMOUNT IN FAT (MG)
     RAFAT = QFAT * (CA - CVFAT)
      AFAT = INTEG(RAFAT, 0.0)
      CFAT = AFAT / VFAT
     CVFAT = CFAT / PF

! AMOUNT IN FETUSES (MG)
     RAFET = PAF * (CPLA - CFET)
      AFET = INTEG(RAFET, 0.0)
                 CFET = AFET / VFET     !
   AUCCFET = INTEG(CFET, 0.0)

! AMOUNT IN UTERUS (MG)
     RAUTR = QUTR * (CA - CVUTR)
      AUTR = INTEG(RAUTR, 0.0)
      CUTR = AUTR / VUTR
     CVUTR = CUTR / PUTR

! AMOUNT IN MAMMARY TISSUE (MG)
     RAMAM = QMAM * (CA - CVMAM)
      AMAM = INTEG(RAMAM, 0.0)
      CMAM = AMAM / VMAM
     CVMAM = CMAM / PM

! AMOUNT IN PLACENTA (MG)
     RAPLA = (QPLA * (CA - CVPLA)) + (PAF * (CFET - CPLA))
      APLA = INTEG(RAPLA, 0.0)
           CPLA = APLA / VPLA ! PMS, 8-13-13
     CVPLA = CPLA / PPLA

!AS = AMOUNT IN SLOWLY PERFUSED TISSUES (MG)
       RAS = QSLW*(CA - CVS)
        AS = INTEG(RAS, 0.0)
       CVS = AS/(VSLW*PS)
        CS = AS/VSLW

!AR = AMOUNT IN RAPIDLY PERFUSED TISSUES (MG)
       RAR = QRAP*(CA - CVR)
        AR = INTEG(RAR, 0.0)
       CVR = AR/(VRAP*PR)
        CR = AR/VRAP

!MIXED VENOUS BLOOD
      RVV = QC*CV ! PMS, 8-13-13
```

```
      RV=(QFAT*CVFAT+QLIV*CVL+QSLW*CVS+QRAP*CVR+QSKN*CSKV+CVMAM*QMAM
+CVPLA*QPLA+QUTR*CVUTR+IVR)-RVV    !
      AV=INTEG(RV,0.0)
      CV=AV/VV
      AUCBB=INTEG(CV,0.0)    !AUC, HR*MG/L

!-----------MASS BALANCE NMP --------------
  BODY = (AFAT+AR+AS+AL+ASK+AV+ALU+AAB+APLA+AMAM+AUTR)
  TMASS = RATS*(BODY + AML + AEX+AUNP+AFET)!COMPARE TO
                                  !AINH FOR OC MASS BAL
                                  !OR OABS FOR ORAL MASS BAL
                                  !OR TIV FOR IV MASS BAL
                                  !OR ADL FOR DERMAL LIQUID
 MASBAL=TMASS/(AINH+OABS+TIV+ADL+OIBS+1E-9)

! CHECK BLOOD FLOWS
      QTOT = QFATI + QLIV + QRAP + QSKN + QSLW + QUTRI +QMAM+QPLA
    QRECOV = 100.0 * (QTOT / QC)

!===============SECOND MODEL FOR TRACKING HNP====================

!ALHP = AMOUNT HNMP IN LIVER COMPARTMENT (MG)
  RALHP = QLIV*(CAHP-CVLHP)+ RAML1 - RAMLH
  RAML1=RAML*MWHP/MWNMP
  AML2B=INTEG(RAML1,0.0)
   ALHP = INTEG(RALHP,0.0)   !AMT IN MG HNMP, CORRECTED FOR MW
  CVLHP = ALHP/(VLIV*PLHNP)  !TOTAL HNMP

    RAMLH = (VMAX2*CVLHP)/(KM2+CVLHP)   !SATURABLE METABOLISM, MG/HR
     AMLH = INTEG(RAMLH,0.0) !AMT HNMP METAB BY SATURABLE PATH, MG
      rdose=ramlh/(BW**0.75)
      tdose=integ(rdose,0.0)

!ABHP = AMOUNT HNMP IN TISSUES (MG)
  RABHP = QB*(CAHP - CBSHP)
   ABHP = INTEG(RABHP,0.0)
  CBSHP = ABHP/(VB*PBHNP)

!AFHP = AMOUNT HNMP IN FAT (MG)
    RFSHP = QFAT*(CAHP - CVFHP)
     AFHP = INTEG(RFSHP,0.0)
  CVFHP = AFHP/(VFAT*PFHNP)

!CVHP = MIXED VENOUS BLOOD CONC TOTAL HNMP (MG/L)
  CRHP = (QLIV*CVLHP + QB*CBSHP + QFAT*CVFHP + QPLA*CVPLHP)-QC*CVHP-
RAUHP
  AVHP = INTEG (CRHP,0.0)
  CVHP = AVHP/VBL
  CAHP = CVHP
  CVHP2 = CVHP*1000/MWHP      !VENOUS BLOOD TOT CONC HNMP IN MICROM

  AUCVHP = INTEG(CVHP2,0.0)  !AUC HNMP VEN. BLOOD, MICROMOL*HR/L

  ! AMOUNT IN PLACENTA (MG)
```

```
     RAPLHP = (QPLA * (CAHP - CVPLHP)) + (PAF * (CFETHP - CPLHP))
      APLHP = INTEG(RAPLHP, 0.0)
     !IF (DAYS.GT.6.0) CPLHP = APLHP / VPLA
                 CPLHP = APLHP / VPLA
     CVPLHP = CPLHP / PPLHNP

  ! AMOUNT IN FETUSES (MG)
      RAFETHP = PAF * (CPLHP - CFETHP)
             AFETHP = INTEG(RAFETHP, 0.0)
           ! IF (DAYS.GT.6.0) CFETHP = AFETHP / VFET
                            CFETHP = AFETHP / VFET !
        AUCFETHP = INTEG(CFETHP, 0.0)


 !RATE OF ELIM IN THE URINE, RAUHP, FROM MIXED BLOOD
   RAUHP = KL*CAHP*VA              !FIRST ORDER RATE
      AUHP = INTEG(RAUHP,0.0)    !CUMULATIVE AMT HNMP IN URINE (MG),
NOT MGEQ

!-----------MASS BALANCE--------------
!-----------MASS BALANCE 5-HNMP SUBMODEL--------------
!COMMENT OUT EQUATIONS WHEN NOT USING TO ELIM. UNESSESARY INTEG
  BODYHP = (AFHP+ABHP+ALHP+AVHP+AFETHP+APLHP)*RATS  !+AABH
  TMASHP = RATS*(AUHP + BODYHP +AMLH)         !COMPARE TO AML1B

! CHECK BLOOD FLOWS 5HNMP COMPARTMENT
      QTOTH = QLIV + QFAT + QB+QPLA
    QRECOVH = 100.0 * (QTOTH / QC)

TERMT(T .GE. TSTOP)    !----STATEMENT TO STOP EXECUTION---

END     !END OF DERIVATIVE

! The following discrete block allows for repeated gavage dosing,
but with
! the total dose (gavds) only updated every 3 days, per the protocol
of
! Becci et al. (1982) and Saillenfait et al. (2002); PMS 9-16-13
      discrete GAVD
            IF (ROUND(DAYS).EQ.9.0)      gavds=FRACOR*dose2*BW
            IF (ROUND(DAYS).EQ.12.0)     gavds=FRACOR*dose2*BW
            IF (ROUND(DAYS).EQ.15.0)     gavds=FRACOR*dose2*BW
            IF (ROUND(DAYS).EQ.18.0)     gavds=FRACOR*dose2*BW
            ODOSE=ODOSE+gavds
            schedule GAVD .at. (T+24.0)
      end

!EXPOSURE CONTROL
DISCRETE SKWASH  ! PMS, 8-14-13
      ASK = 0.0   ! Assume skin washing in Becci et al. (1982)
removes all NMP from skin
      if (DAYS.LT.15.0) SCHEDULE REAPPLY.AT.(T+DOSEINTERVAL-TWASH)
END
DISCRETE DERMOFF !
```

```
      DDN = 0.0   ! Assume skin washing in Becci et al. (1982)
removes all NMP from skin
      if (DAYS.LT.21.0) SCHEDULE REAP.AT.(T+DOSEINTERVAL-TWASH)
END
DISCRETE REAPPLY ! PMS, 8-14-13
      IF (ROUND(DAYS).EQ.9.0)      ASKO=DSK*BW
      IF (ROUND(DAYS).EQ.12.0)     ASKO=DSK*BW
      IF (ROUND(DAYS).EQ.15.0)     ASKO=DSK*BW
      ASK = ASK + ASKO
      SCHEDULE SKWASH.AT.(T+TWASH)
END
DISCRETE REAP
      IF (ROUND(DAYS).EQ.21.0)     DDNX=CONCL/VLIQ*FAD
DDN=DDNX+DDN
      SCHEDULE DERMOFF.AT.(T+TWASH)
END

CONCL2=CONCL*FAD
DISCRETE OFFD
  IVZONE=0.0      !TURN IV OFF
  CIZONE=0.0      !TURN INHAL EXPOSURE OFF
  DZONE=0.0 !TURN OFF DERMAL
  !DZONE2=0.0
      SCHEDULE OND.AT.(T+DOSEINTERVAL-TCHNG)  ! PMS, 8-13-13
END
DISCRETE OND      ! PMS, 8-13-13
  CIZONE=1.0      !TURN INHAL EXPOSURE ON
DZONE=1.0
      !SCHEDULE OFFD.AT.(T+TCHNG)
END

END     !END OF DYNAMIC
END     !END OF PROGRAM212121
```

WESITG=0; WEDITG=0;
VCHC=1e9, KLOSS=0.0, DOSE=0,PDOSE=0,DOSE2=0, CONCCHPPM0 = 0
  CONCL=0, IVDOSE=0, TCHNG=999.0,TSTOP=120,CONCPPM=0,CONCMGS=0,DSK=0
FAD=0.82 % Payan estimated 76% urine+carcas+tissues, MIDGELY ESTIMATE - 18%+ RECOVERED ON DRESSING
  GDDAYS=0, SA=0.00001,DOSEINTERVAL=720,CINT=1, VLIQ=1e-99,
FRACIN=1 %
NUMFET=0.01 % Added to minimize impact on other volumes; Paul Schlosser (PS), U.S. EPA, 05-01-2103
FRACOR=0.945, KAS=1.5, KAI=0.006,KSI=0.85 %
%FRACOR calculated from Ghantous data by PMS - Fecal elimaination in females averages 94%
KPL=4.3e-3 %
  VLIVC=0.0366
  VLUC=0.005
  VFATC=0.09
  VRAPC=0.071
  QLIVC=0.183
  QPC=16.0
  QCC=16.0
  QFATC=0.07
  QSKNC=0.058
  QMAMC=1e-5
  QUTRC=1e-5
  KM=225
  VMAXC=9
  KM2=4.9
  VMAX2C=0.09
  QSC=0.14
  BWINIT=0.23
  GDDAYS=0
  GDMONTHS=0
  KLC=1.61
  KLNC=0.0001
  PAFC=0
  MAXT=1e-2
  MINT=1E-9

QFATC = 0.072
QLIVC = 0.183
QMAMC = 0.001
QSKNC = 0.058
QUTRC = 0.001
QRAPC = 0.512

VLUC = 0.007
VFATC = 0.10
VLIVC = 0.034
VMAMC = 0.01
VRAPC = 0.071
VUTRC = 0.002
VBLC = 0.067

PB=450.0

PF=0.62
PL=1.02
PR=1.02
PS=0.74
PLU=0.10
PSKL = 1 % MEASURED % 450 % Value for Poet 5-16-13 Payanderm.m file; PS 5-17-13
PSKB = 0.12 % SKIN:SALINE/BLOOD:SALINE
PSKA = 55 % SKIN:SALINE*BLOOD:AIR/BLOOD:SALINE
PM=1.0
PPLA=0.309
PUTR=0.34
PLHNP=3.0
PBHNP=0.73
PFHNP=0.40
PPLHNP=01.07

A.5. Pregnant Human PBPK Model Code

```
 PROGRAM NMPHUMPG.ACSL

!PBPK MODEL FOR N-METHYL PYRROLIDONE IN PREGNANT WOMEN

!T.S. POET,P HINDERLITER. CHEMICAL DOSIMETRY GROUP, PNNL, RICHLAND, WA
!FIRST CREATED 8.8.08
!FINAL REPORT FROM INITIAL RAT MODEL DEVELOPMENT SUBMITTED 9.02
!Updates for 2014 publication - T.S. Poet and P.M. Schlosser
!MODEL CONFIGURED FOR INHALATION and DERMAL exposures
!MODEL TRACKS DISPOSITION OF NMP AND 5-HNMP.
!ASSUMPTIONS:
!   (1) FLOW-LIMITED (ALL COMPARTMENTS)
!   (2) METABOLISM OF NMP BY A SAT PATHWAY TO FORM 5HNP
!   (3) METABOLISM OF HNP BY SATURABLE PATHWAY TO ETC.
!   (5) METABOLISM OCCURS ONLY IN THE LIVER (NMP) and in single compartment for 5HNMP
!   (6) TISSUE:BLOOD PART. COEFF. RAT = HUMAN, measured in human blood and rat blood/tissues
!   (7) 5HNP ELIMIN FROM MIXED VENOUS - 1ST ORDER
! Pregnancy Description from  GENTRY ET AL. REGU TOX PHARM 36:51-68, 2002
!~~~~~~~~
! GENTRY MODEL NOTES:
! -CODING FOR PREGNANCY IS FROM MEHGFAT.CSL WITH SOME MINOR CHANGES
! -PHYSIOLOGICAL PARAMETERS ARE FROM MEHGFAT.CSL (AJUSTED AS NEEDED)
! -NON-PREGNANT MAMMARY TISSUE AND UTERINE VOLUME IS FROM ICRP
! -NON-PREGNANT MAMMARY TISSUE AND UTERINE BLOOD FLOWS ARE BASED ON THE
! - RATIOS OF MAMMARY AND UTERINE TISSUE VOLUMES TO RAPIDLY PERFUSED
! - TISSUE VOLUME AND BLOOD FLOW TO RAPIDLY PERFUSED TISSUE WHERE RAPIDLY
! - PERFUSED TISSUE INCLUDES LIVER, LUNG, ETC.
! - ((VMAMC/VRAPC)*QRAPC) AND ((VUTRC/VRAPC)*QRAPC)
! -DATA USED TO FIT CURVE FOR GROWING RAPIDLY PERFUSED TISSUE IN
! - MEHGFAT.CSL WAS REFIT SEPARATELY TO FIT CURVES FOR GROWING UTERUS
! - AND MAMMARY TISSUE IN THIS MODEL
! -BODY WEIGHT AND CARDIAC OUTPUT ARE CALCULATED AS THE INITIAL VALUES
! - PLUS THE CHANGE IN THE GROWING COMPARTMENTS
! -INCREASE IN BLOOD FLOW TO FAT, MAMMARY TISSUE, AND UTERUS ARE MODELED
! - AS BEING PROPORTIONAL TO THE INCREASE IN VOLUME IN THOSE COMPARTMENTS
! - BASED ON THE DATA IN THORESEN AND WESCHE, 1988 (UTERUS AND MAMMARY
! - TISSUE)
!~~~~~~~~~~

INITIAL


! HUMAN TOTAL PULMONARY VENTILATION RATE (L/HR FOR 1 KG ANIMAL)
  CONSTANT    QPC = 19

! HUMAN BLOOD FLOWS (FRACTION OF CARDIAC OUTPUT)
  CONSTANT    QCC = 16      ! CARDIAC OUTPUT (L/HR FOR 1 KG ANIMAL)
  CONSTANT   QFATC = 0.052      ! FAT (NON-PREGNANT FEMALE)
  CONSTANT   QLIVC = 0.227      ! LIVER
  CONSTANT   QMAMC = 0.027      ! MAMMARY TISSUE (NON-PREGNANT FEMALE)
  CONSTANT   QRAPC = 0.325     ! RAPIDLY PERFUSED
  CONSTANT   QSKC = 0.058      ! SKIN
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 CONSTANT   QUTRC = 0.0062      ! UTERUS (NON-PREGNANT FEMALE)
! GENTRY MODEL HAS 0.249, BUT ADDING THESE =0.944, SO BE AWARE CAN REPLACE WITH EQN

! PERMEABILITY-AREA PRODUCT (L/HR)
 CONSTANT    PAFC = 0.01        ! DIFFUSION ON FETAL SIDE OF PLACENTA-  GENTRY et al

! HUMAN TISSUE VOLUMES (FRACTION OF BODY WEIGHT)
 CONSTANT  BWINIT = 67.8       ! PRE-PREGNANCY BODY WEIGHT (KG)
 CONSTANT   VALVC = 0.0079      ! ALVEOLAR BLOOD
 CONSTANT VBLC=0.06
 CONSTANT   VFATC = 0.273       ! FAT (NON-PREGNANT FEMALE)
 CONSTANT   VLIVC = 0.026       ! LIVER
 CONSTANT   VMAMC = 0.0062      ! MAMMARY TISSUE (NON-PREGNANT FEMALE)
 CONSTANT   VRAPC = 0.1044      ! RAPIDLY PERFUSED
 !CONSTANT   VSLWC = 0.35        ! SLOWLY PERFUSED IN GENTRY MODEL, IN THIS MODEL IS
CALCULATED BELOW
 CONSTANT   VUTRC = 0.0014      ! UTERUS (NON-PREGNANT FEMALE)
 CONSTANT VSKC=0.19

! HUMAN DERMAL EXPOSURE PARAMETERS
 CONSTANT      KPL = 0.002        ! PERMEABILITY CONSTANT (KP) (CM/HR)
 CONSTANT    KPV=22.0                                              ! PERMEABILITYT CONSTANT
(CM/HR) FOR VAPOR


 !FOR PARENT MODEL, SKIN COMPARTMENT IS ONLY DEFINED AS DOSED SKIN
  CONSTANT     SAL = 0.01       !SURFACE AREA EXPOSED TO LIQUID, SQ.CM
  CONSTANT     SAVC = 0.25       !FRACTION SURFACE AREA EXPOSED TO GAS/VAPOR, SQ.CM
  CONSTANT      HT=170.0                    !HEIGHT (OR LENGTH) OF REFERENCE MAN
   TSA = 71.81*(BWINIT**0.425)*(HT**0.725)      !FOR HUMANS, DUBOIS AND DUBOIS, 1916, AS
REPORTED IN REFERENCE MAN
                  SAV = SAVC*TSA       ! SURFACE AREA EXPOSED TO GAS/VAPOR, SQ.CM
          VSKLC = VSKC*SAL/TSA
          QSKLC = QSKC*SAL/TSA
          VSKVC = VSKC*SAV/TSA
          QSKVC = QSKC*SAV/TSA
CONSTANT FAD = 0.0  !FRACTION ABSORBED - FROM BADER ET AL, CALCULATE FROM AMNT
REMAINING ON GAUZE

 ! SLOWLY PERFUSED (DEFINED AS BALANCE OF TISSUES AND FLOWS)
  VSLWC = 0.91 - ( VFATC + VLIVC + VMAMC + VRAPC + VUTRC + VSKVC + VSKLC)
           ! NOTE: 0.91 IS APPROX WHOLE BODY LESS BONE
   QSLWC = 1.0 - (QFATC + QLIVC + QMAMC + QRAPC + QUTRC + QSKVC + QSKLC)

! MOLECULAR WEIGHTS
 CONSTANT  MW=99.13     !MOL. WT. NMP, MG/MMOL
CONSTANT  MW1= 116.14    !MOL. WT. 5-HNP, MG/MMOL

   STOCH = MW1/MW

! HUMAN NMP/BLOOD PARTITION COEFFICIENTS
!EXPERIMENTALLY MEASURED RAT VALUES, HUMAN BLOOD
 CONSTANT    PB = 450.0       ! BLOOD/AIR
 CONSTANT    PFAT = 0.61        ! FAT
 CONSTANT    PLIV = 1.00        ! LIVER
```

```
 CONSTANT   PMAM = 1.0       ! MAMMARY TISSUE, ESTIMATED FROM LIVER
 CONSTANT   PPLA = 0.31      ! PLACENTA
 CONSTANT   PRAP = 1.0       ! RAPIDLY PERFUSED TISSUE, LIVER
 CONSTANT   PSLW = 0.30      ! SLOWLY PERFUSED TISSUE, MUSCLE
 CONSTANT   PUTR = 0.34      ! UTERUS
 CONSTANT   PSKL = 0.42          ! MEASURED SKIN;LIQUID (RAT)
 CONSTANT   PLU= 0.1             ! LUNG:BLOOD

!METABOLIC RATE CONSTANTS
 !NMP TO 5HNP
  CONSTANT  KM=0                 !MICHAELIS CONSTANT, MG/L
  CONSTANT  VMAXC=0  !MAX. ENZ. ACT., MG/HR/L


! HUMAN 5HNMP VOLUME OF DISTRIBUTION
          CONSTANT VOD5HC = 0.3          ! VOLUME-OF-DISTRIBUTION, PMS 9-11-14
                   VOD5H = VOD5HC*BWINIT
 !NO FETAL COMPARTMENT FOR METABOLITE, NMP IS CONSIDERED THE ACTIVE MOIETY, THIS
WILL HAVE MINIMAL EFFECT ON NMP OVER PREGNANCY - TSP.


!5HNP TO OTHER METABS
 CONSTANT  KM2=22.8             !MICHAELIS CONSTANT, MG/L
   CONSTANT  VMAX2C=1.0         !MAX. ENZ. ACT., MG/HR/L


 ! HUMAN UPTAKE AND CLEARANCE PARAMETERS
CONSTANT KAS=5.0 !ORAL UPTAKE - FROM RAT, NOT USED FOR HUMAN.
 !URINARY ELIMINATION OF 5-HNMP - CLEARED FROM BLOOD
 CONSTANT  KME=3.83             !FIRST-ORDER CONSTANT FOR 5HNMP IN URINE (L/HR)
  CONSTANT KMNE=0.182                       !FIRST-ORDER CONSTANT FOR NMP IN URINE
(L/HR)

! INITIALIZE HUMAN CONCENTRATIONS IN TISSUES (MG/L)
 CONSTANT   ICART = 0.0       ! BLOOD
 CONSTANT   ICFAT = 0.0       ! FAT
 CONSTANT   ICLIV = 0.0       ! LIVER
 CONSTANT   ICRAP = 0.0       ! RAPIDLY PERFUSED
 CONSTANT   ICSKN = 0.0       ! SKIN
 CONSTANT   ICSLW = 0.0       ! SLOWLY PERFUSED
   ICMAM = ICSLW           ! MAMMARY TISSUE
   ICUTR = ICRAP           ! UTERUS

! DOSING PARAMETERS
 CONSTANT    CONCPPM = 0.0       ! INHALED CONCENTRATION (PPM)
 CONSTANT   CONCMGM = 0.0                                ! INHALED CONCENTRATION (MG/M3)
 CONSTANT  IVDOSE = 0.0       ! IV DOSE (MG/KG)
 CONSTANT   PDOSE = 0.0       ! ORAL DOSE (MG/KG)
          CONSTANT PDOSE2=0.0
          CONSTANT PDOSE3=0.0
 CONSTANT  PDRINK = 0.0       ! DRINKING WATER DOSE (MG/KG/DAY)
 CONSTANT   TCHNG = 24.0      ! LENGTH INH. EXPOSURE OR IV INJ.(HRS)
 CONSTANT  DAYSWK = 5.0       ! NUMBER OF EXPOSURE DAYS PER WEEK
 CONSTANT    TMAX = 24.0      ! MAXIMUM TIME FOR EXPOSURES
```

```
CONSTANT S2=0.0
        !INHALATION ON
CONSTANT P2=3.0
        !INHALATION EXPOSURE
CONSTANT S3=3.16
        !INHALTION RESUME (HANOVER STUDY)
CONSTANT P3=3.0
        !SECOND DAILY EXPOSURE PERIOD
CONSTANT ON3=1.0       ! SET TO ZERO TO TURN OFF 2ND DAILY PULSE; pms 8-20-13
CONSTANT FULLWEEK=168.0     ! HRS IN A FULLWEEK; pms 8-20-13
HRSWEEK=24.0*DAYSWK          ! HRS/WEEK IN WORKPLACE; pms 8-20-13
        TABLE RESLVL, 1, 1441 / 1441*0.0, 1441*0.0 /
        CONSTANT        PREGTIME = 0.0             ! GESTATION DAY START

! STARTDS IS ADDED TO TCHNG TO ALLOW FOR DOSING THAT DOES NOT START AT T=0
!INITIAL EXPOSURE CONDITIONS
 !DERMAL
    CONSTANT CONCL = 0.0      !CONC OF NMP IN LIQUID, MG/L
                              CONSTANT SRATE = 0.0                          ! MG/HR DELIVERED TO SKIN BY
SPRAY APPLICATION; PMS 8-20-13
    CONSTANT VLIQ0 = 1.0E-99     !INITIAL VOLUME APPLIED, L
   CONSTANT RESID=0.0        !AMOUNT STICKING TO EXPOSURE SYSTEM, MG
                              CONSTANT BRUSH=0.0                            ! SET TO 1.0 FOR
!BRUSH/LIQUID EXPOSURE; PMS 8-20-13
    DDN = (CONCL - 1.0)*VLIQ0*FAD ! SUBTRACT 1 MG/L, ~ 1 PPM, FROM !INITIAL CONC. TO AVOID
VLIQ --> 0
        ! NOTE, FOR APPLICATION OF 100% NMP, IT IS NOT POSSIBLE FOR CSURF TO DROP
BELOW 100%.
! 100% NMP IS NOT DILUTED IN ANYTHING, SO THE "SOLUTION" CAN'T BECOME LESS DILUTE.
! THE VOLUME (VLIQ) WOULD ACTUALLY DECREASE UNTIL IT'S ALL ABSORBED.
! UNLESS THE EXPERIMENT RUNS LONG ENOUGH FOR 100% ABSORPTION, TREAT VLIQ AS
! EXTREMELY LARGE, ~ 10^9, FOR 100% NMP.
! BUT CHECK THAT YOU DON'T PREDICT MORE ABSORPTION THAN WAS ACTUALLY APPLIED! PMS
9-16-14



 ! EXPOSURE CONDITIONS BASED ON USER DEFINED INITIAL AMOUNTS OF CHEMICAL (MG)
   IF (CONCPPM.EQ.0.0) THEN
   CONCMG=CONCMGM/1000.0                                                !CONCERT MG/M3 TO
MG/L
 ELSE
    CONCMG = CONCPPM*MW/24451.   !CONVERT PPM TO MG/LITER!
 ENDIF

!CONSTANT CONCMG=0
        !HANNOVER STUDY UNIT MG/M3 SO CONCMG /1000(L/M3)
CONSTANT   DOSEINTERVAL=24.0                                             !TIME BETWEEN DAILY
DOSES

! SIMULATION CONTROL PARAMETERS
 CONSTANT STARTDS = 0.0        ! TIME FIRST DOSE IS GIVEN (HRS)
 CONSTANT   TSTOP = 6480.0     ! RUN SIMULATION FOR ABOUT 9 MONTHS
 CONSTANT   CINTC = 0.1
```

```
! SCALED HUMAN PULMONARY VENTILATION RATE (L/HR)
      QP = QPC * (BWINIT**0.75)
     QALV = 0.67 * QP

! SCALED HUMAN BLOOD FLOWS (L/HR)
   QCINIT = QCC * (BWINIT**0.75)
   QFATI = QFATC * QCINIT
   QLIV = QLIVC * QCINIT
   QMAMI = QMAMC * QCINIT
                    QPLAI = 58.5 * VPLAI       ! VALUE FOR 'DAYS'=0 PER CALCULATION BELOW; PMS,
8-20-13
   QRAP = QRAPC * QCINIT
   QSLW = (QSLWC * QCINIT) - QPLAI
   QUTRI = QUTRC * QCINIT
   QSKL = QSKLC * QCINIT
   QSKV = QSKVC * QCINIT


! SCALED HUMAN TISSUE VOLUMES (L)
     VALV = VALVC * BWINIT
     VFATI = BWINIT*(VFATC+(0.09*EXP(-12.90995862*EXP(-0.000797*24.0*PREGTIME))))
     VFETI = 3.50 * (EXP(-16.081*EXP(-5.67E-4*24.0*PREGTIME))+ EXP(-140.178*EXP(-7.01E-
4*24.0*PREGTIME)))
      VMAMI = BWINIT*(VMAMC+(0.0065*EXP(-7.444868477*EXP(-0.000678*24.0*PREGTIME))))
     VPLAI = 0.85*EXP(-9.434*EXP(-5.23E-4*24.0*PREGTIME))
     VUTRI = BWINIT*(VUTRC+(0.02*EXP(-4.715669973*EXP(-0.000376*24.0*PREGTIME))))
     VLIV = VLIVC * BWINIT
     VRAP = VRAPC * BWINIT
    VSKL = VSKLC * BWINIT
    VSKV = VSKVC * BWINIT
      VBL=VBLC * BWINIT
      VSLW = (VSLWC * BWINIT) !

! SCALED HUMAN METABOLISM PARAMETERS
      VMAX = VMAXC * (BWINIT**0.75)
VMAX1 = VMAX2C * (BWINIT**0.75)


! INITIALIZE HUMAN NMP AMOUNTS IN TISSUES
    IAART = ICART * VALV
    IAFAT = ICFAT * VFATI
    IALIV = ICLIV * VLIV
    IAMAM = ICMAM * VMAMI
    IARAP = ICRAP * VRAP
    IASKL = ICSKN * VSKL ! VSKCC ! PMS 8-20-13
    IASKV = ICSKN * VSKV
    IASLW = ICSLW * VSLW
    IAUTR = ICUTR * VUTRI
  INITTOT = IAART + IAFAT + IALIV + IAMAM + IARAP + IASKL + IASKV + IASLW + IAUTR


! INITIALIZE STARTING VALUES
      BW = BWINIT
    DRINK = (PDRINK * BW) / 24.0     ! DRINKING WATER DOSE (MG/HR)
```

```
    CINT = CINTC
     IV = 0.0
   DAYEXP = 1.0
    CINH = 0.0
    CONSTANT  FRACIN = 1
   CONSTANT  FRACOR = 1.0     !FRACTION ABSORBED ORALLY, INITALLY 100%

! CONVERT ORAL DOSE FROM UG/KG TO UMOLES
! MODIFY DOSE TO ACCOUNT FOR FRACTIONAL ABSORPTION

ODOSE1= PDOSE * BW * FRACOR    ! UMOLES
ODOSE2= PDOSE2* BW * FRACOR    ! UMOLES
ODOSE3= PDOSE3* BW * FRACOR    ! UMOLES

DZONE = 1.0       ! START WITH EXPOSURE ON
SCHEDULE OFFD.AT.P2
SCHEDULE OND2.AT.24.0
IF (ON3) SCHEDULE OND3.AT.S3

END             ! END OF INITIAL


DYNAMIC
 ALGORITHM  IALG = 2           ! GEAR STIFF METHOD needed for pregnancy and growth

 DISCRETE DOSE1
 ODOSE = ODOSE+ODOSE1
 SCHEDULE DOSE2 .AT. (TIME+TIME2)
 END

 DISCRETE DOSE2
 ODOSE = ODOSE+ODOSE2
 SCHEDULE DOSE3 .AT. (TIME+TIME3)
 END

 DISCRETE DOSE3
 ODOSE = ODOSE+ODOSE3
 SCHEDULE DOSE1 .AT. (TIME+REPTM-TIME2-TIME3)
 END

DISCRETE DOSEON    ! START DOSING
 INTERVAL DOSEINT = 24.0       ! INTERVAL TO REPEAT DOSING
 SCHEDULE DOSEOFF .AT. T + TCHNG
 IF ((T.GE.STARTDS) .AND. (T.LT.TMAX)) THEN
                    IF (T.LE.(STARTDS+TCHNG)) THEN
                                IF (IVDOSE.GT.0.0) CINT = MIN(CINTC, (TCHNG/10.0))
   IV = (IVDOSE*BW) / TCHNG    ! RATE OF INTRAVENOUS DOSING (MG/HR)
                    ENDIF
          ENDIF
END ! DOSEON

DISCRETE DOSEOFF
 CINH = 0.0
 CINT = CINTC
 IV = 0.0
```

```
END

DISCRETE OND2
        DZONE=1.0
        SCHEDULE OND2.AT.(T+24.0)
        SCHEDULE OFFD.AT.(T+P2)
END

DISCRETE OND3
        DZONE=1.0
        SCHEDULE OND3.AT.(T+24.0)
        SCHEDULE OFFD.AT.(T+P3)
END

!EXPOSURE CONTROL
DISCRETE OFFD
        DZONE=0.0       !TURN OFF DERMAL
END

DERIVATIVE


! VOLUME OF HUMAN FAT (L)
    VFAT = BWINIT*(VFATC+(0.09*EXP(-12.90995862*EXP(-0.000797*(T + PREGTIME*24.0)))))

! VOLUME OF HUMAN FETUS (L)
    VFET = 3.50 * (EXP(-16.081*EXP(-5.67E-4*(T + PREGTIME*24.0)))+ EXP(-140.178*EXP(-7.01E-
4*(T + PREGTIME*24.0))))

! VOLUME OF HUMAN MAMMARY TISSUE (L)
    VMAM = BWINIT*(VMAMC+(0.0065*EXP(-7.444868477*EXP(-0.000678*(T +
PREGTIME*24.0)))))

! VOLUME OF HUMAN PLACENTA (L)
    VPLA = 0.85*EXP(-9.434*EXP(-5.23E-4*(T + PREGTIME*24.0)))

! VOLUME OF HUMAN UTERUS (L)
    VUTR = BWINIT*(VUTRC+(0.02*EXP(-4.715669973*EXP(-0.000376*(T + PREGTIME*24.0)))))

! INCREASE IN HUMAN BODY WEIGHT (KG)
     BW = BWINIT + (VFAT - VFATI) + VFET + (VMAM - VMAMI) + VPLA + (VUTR - VUTRI)

! SCALED HUMAN ALVEOLAR VENTILATION (L/HR)
     QP = QPC * (BW**0.75)
    QALV = 0.67 * QP

! INCREASE IN HUMAN BLOOD FLOWS (L/HR)
    QFAT = QFATI * (VFAT / VFATI)
    QMAM = QMAMI * (VMAM / VMAMI)
    QUTR = QUTRI * (VUTR / VUTRI)

! HUMAN BLOOD FLOW TO PLACENTA (L/HR)
    QPLA = 58.5 * VPLA

! INCREASED HUMAN CARDIAC OUTPUT (L/HR)
```

```
    QC = QCINIT + (QFAT - QFATI) + (QMAM - QMAMI) + (QPLA - QPLAI) + (QUTR - QUTRI)


! SCALED PERMEABILITY-AREA PRODUCT
     PAF = PAFC * (VFET**0.75)


! ------------------ HUMAN NMP MODEL -------------------------

! AMOUNT EXHALED (MG)
    RAEXH = QALV * CALV
     AEXH = INTEG(RAEXH, 0.0)

CI = CONCMG*CZONE + RESLVL(T)
! FOR A 5 DAY/WK EXPOSURE, CHANGE FIRST PULSE TO PULSE(0,7*24,5*24)
! FOR DAILY, PULSE(0,1E6,24)

                        TORAL= ODOSE1 - AO          !AMT ABSORBED ORALLY, MG!
RSTOM = -KAS*AO
RAO = KAS*AO    ! CHANGE IN STOMACH (UMOLE/HR)
AO=ODOSE1+INTEG(RSTOM,0.0)    ! AMT IN STOMACH (UMOLE)

! AMOUNT IN FAT (MG)
    RAFAT = QFAT * (CART - CVFAT)
     AFAT = INTEG(RAFAT, IAFAT)
     CFAT = AFAT / VFAT
    CVFAT = CFAT / PFAT

! AMOUNT IN FETUS (MG)
    RAFET = PAF * (CPLA - CFET)
     AFET = INTEG(RAFET, 0.0)
     CFET = AFET / VFET
  AUCCFET = INTEG(CFET, 0.0)

! AMOUNT IN LIVER (MG)
    RALIV = (QLIV * (CART - CVLIV)) + RAO + DRINK - RAMET1
     ALIV = INTEG(RALIV, IALIV)
     CLIV = ALIV / VLIV
    CVLIV = CLIV / PLIV

! AMOUNT METABOLISED IN LIVER -- SATURABLE (MG)
   RAMET1 = (VMAX * CVLIV) / (KM+CVLIV)
    AMET1 = INTEG(RAMET1, 0.0)

! AMOUNT IN MAMMARY TISSUE (MG)
    RAMAM = QMAM * (CART - CVMAM)
     AMAM = INTEG(RAMAM, IAMAM)
     CMAM = AMAM / VMAM
    CVMAM = CMAM / PMAM

! AMOUNT IN PLACENTA (MG)
    RAPLA = (QPLA * (CART - CVPLA)) + (PAF * (CFET - CPLA))
     APLA = INTEG(RAPLA, 0.0)
     CPLA = APLA / VPLA
    CVPLA = CPLA / PPLA
```

```
! AMOUNT IN RAPIDLY PERFUSED TISSUE (MG)
    RARAP = QRAP * (CART - CVRAP)
     ARAP = INTEG(RARAP, IARAP)
     CRAP = ARAP / VRAP
    CVRAP = CRAP / PRAP

!ASKl = AMOUNT NMP IN liquid-exposed SKIN TISSUES (MG) AND DERMAL DOSING (from vapor)
            ! Equations below set for liquid-exposed skin, pms 8-21-13
     RASKl = QSKl*(CArt - CvSKl) + RADL
      ASKL = INTEG(RASKL,0.0)
        CSKL = ASKL/VSKL !
     CvSKL = CSKL/PSKL              !'NMP IN SKIN, MG/L'
  czone = pulse(0.0,fullweek,hrsweek)*DZONE ! pms 8-20-13
! for a 5 day/wk exposure, use fullweek=7*24, hrsweek=5*24 (Dayswk=5)
! for a single day, fullweek=1e16, hrsweek=24 (Dayswk=1)
sdeliv=srate*czone          ! Constant-rate spray delivery; pms 8-20-13
            ! Spray-dermal exposures, assumed simultaneous with inhalation (unless FRACIN = 0)
                                    ! RADVL allows absorption/desorption from spray or
                                    ! brushing dermal exposure, when both sdeliv and czone are zero;
this used to mix liquid and vapor - TS
                                    ! When BRUSH=0 but Czone=1, assumes gloves are on s RADVL=0
unless sdeliv>0.

ASURF=INTEG(RASURF,0.0)+DDN
CSURF=ASURF/VLIQ0
RASURF=-((((KPL*SAL/1000)*CSURF))*CZONE)

RADL=(((((KPL*SAL/1000)*CSURF))*CZONE)*(sdeliv.eq.0.0)+sdeliv)!-RADLL - TSP 10-14
ADL=INTEG(RADL,0.0)
!

RADLL=ADL*PSKL                                          !RATE OF ABSORPTION
ADLT=INTEG(RADLL,0.0)   !TOT ABSORBED


!ASKv = AMOUNT NMP IN vapor-exposed SKIN TISSUES (MG) AND DERMAL DOSING (from vapor);
pms 8-21-13
     RASKv = QSKv*(CArt - CvSKv) + RADVv
      ASKv = INTEG(RASKv,0.0)
     CSKv = ASKv/VSKv     !
      CvSKv = CSKv/PSKL             !'NMP IN SKIN, MG/L'
    RADVv = (KPV*SAv/1000.0)*CI
      ADVv = INTEG(RADVv,0.0)     !'AMT NMP ABSORBED DERMAL,MG'




! AMOUNT IN SLOWLY PERFUSED TISSUE (MG)
    RASLW = QSLW * (CART - CVSLW)
     ASLW = INTEG(RASLW, IASLW)
     CSLW = ASLW / VSLW
    CVSLW = CSLW / PSLW

! AMOUNT IN UTERUS (MG)
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```
    RAUTR = QUTR * (CART - CVUTR)
     AUTR = INTEG(RAUTR, IAUTR)
     CUTR = AUTR / VUTR
    CVUTR = CUTR / PUTR

! BLOOD VENOUS ARTERIAL (C)
CVEN=(QFAT*CVFAT + QLIV*CVLIV + QMAM*CVMAM + QPLA*CVPLA + QRAP*CVRAP +
QSLW*CVSLW &
        + QUTR*CVUTR + QSKV*CVSKV + QSKL*CVSKL + IV) / QC
IVTOT=INTEG(IV, 0.0)


! AMOUNT IN ARTERIAL BLOOD (MG)
            RAINH = QALV*(CI*FRACIN - CALV)
    RABLD = RAINH + QC*(CVEN-CART) - RAUNP
            INHALTOT = INTEG(RAINH, 0.0)
     ABLD = INTEG(RABLD, IAART)
     CART = ABLD / VBL
     CALV = CART / PB
  CALVPPM = CALV * 24450.0 / MW
  AUCCBLD = INTEG(CART, 0.0)

! AMOUNT IN URINE (MG)
  RAUNP = KMNE*CART                         !FIRST ORDER RATE OF LOSS (URINE
            AUNP = INTEG(RAUNP,0.0)

! ---------- HUMAN 5HNMP MODEL ----------

! AMOUNT IN BODY (MG)
   RA5H = (RAMET1*STOCH) - RAMETM1 - RAUHP
    A5H = INTEG(RA5H, 0.0)
    CVEN1 = A5H / VOD5H

! AMOUNT METABOLISED [IN LIVER] -- SCALED TO BW^0.75 BECAUSE IT'S 1 COMPARTMENT
MODEL, NOT LIVER (MG)
                    RAMETM1 = (VMAX1*CVEN1)/(KM2+CVEN1)
   AMETM1 = INTEG(RAMETM1, 0.0)

! AMOUNT IN URINE (MG)
  RAUHP = KME*CVEN1
AUHP = INTEG(RAUHP,0.0)

! ---------- CHECK MASS BALANCE ----------
  INTOT=INTEG((QALV*CI*FRACIN), 0.0)
! NEW SKIN TERMS ADDED BELOW; PMS 8-21-13
  TDOSE = INTOT  + AO + INITTOT+TORAL+ADL+ADVV !+ INTEG(IV, 0.0)
  NMPTOT = ABLD + AFAT + AFET + ALIV + AMAM + APLA + ARAP + ASKL + ASKV + ASLW + AUTR +
AEXH + AUNP + AMET1
    MASSBAL = TDOSE/(NMPTOT+0.000000000001)

TERMT(T.GT.TSTOP, 'SIMULATION FINISHED')

END             ! END OF DERIVATIVE

TERMINAL
```

```
  DAUCCBLD = AUCCBLD * 24.0 / TSTOP
  DAUCCFET = AUCCFET * 24.0 / TSTOP
END

END             ! END OF DYNAMIC
END             ! END OF PROGRAM
```

```
! .m files

WESITG=0; WEDITG=0;
SRATE=0; RESLVL=[zeros(1,1441) (0:1440)];
QPC     =       16;
QLIVC   =       0.25;
QSKC    =       0.058;
BWINIT  =       70;
VFATC   =       0.23;
VRAPC   =       0.042;
HT      =       180;
KAS     =       1.36;
ICFAT   =       0;
ICSKN   =       0;
CONCMGM         =       0;
PDOSE2  =       0;
TCHNG   =       8;
S2 = 0; P2      =       8;
S3      =       6720; P3        =       6720; ON3=0;
FULLWEEK=168
VLIQ    =       1.00e-19; BRUSH=0;
STARTDS         =       0;
FRACIN  =       1;
TIME1   =       8;
REPTM   =       24;
NSTP    =       10;
QCC     =       16;
QMAMC   =       0.027;
QUTRC   =       0.005;
VALVC   =       0.0079;
VLIVC   =       0.031;
VUTRC   =       0.0014;
KPV     =       22;
MW      =       99.13;
KM2=61 % avg of optimized individuals
VMAX2C=4.48% avg of optimized individuals
VOD5HC=0.32 %VOL DIS 5hnmp
VMAXC=44% avg of optimized individuals
KM=68% avg of optimized individuals
KME     =       2.8;
KMNE    =       0.1;
ICLIV   =       0;
ICSLW   =       0;
IVDOSE  =       0;
PDOSE3  =       0;
DAYSWK          =       1; % Days per week of exposure, eg 5 for workplace; PMS 8-28-13

CONCL   =       1E-5;
RESID   =       0;
TSTOP   =       0.1;
FRACOR          =       0.68;
TIME2   =       6720;
CINT    =       0.01;
MAXT    =       0.001;
QFATC   =       0.05;
```

```
QRAPC  =        0.48;
PAFC   =        0.1;
VBLC   =        0.06;
VMAMC  =        0.0062;
VSKC   =        0.19;
SAL    =        0.0001;
SAV = 0.001;
MW1    =        116.14;
ICRAP  =        0;
CONCPPM        =        0;
PDOSE  =        0;
PDRINK =        0;
TMAX   =        24;

KPL=2.0e-3; % to rat optimzed = 4.3e-3
DOSEINT=999;
CINTC  =        0.1;
TIME   =        0;
TIME3  =        6720;
PREGTIME=0.0001
PB     =        450;
PMAM   =        0.49;
PSLW   =        0.46;
PSKL   =        0.42 %skin/blood
PLIV   =        0.82;
PRAP   =        0.1;%lung
PLIV1  =        2.5;
PRAP1  =        0.43;  %lung
ICART  =        0;
PSLW1  =        0.33; %fat
PFAT   =        0.49;
PPLA   =        0.1;
PUTR   =        0.1;
PLU    =        0.1;
PB1    =        1;
PFAT1  =        0.33;
FAD = 1 % Value used in Poet 5-16-13 BADER_DRM.m and AkkesDerm.m; PMS 5-17-13
SAL=1;
SAVC=0.001;
VCHC=1.0e+9; KLOSS=0.0; % From human simulation scripts; use as default for human; PMS 8-28-13
start @nocallback;
```

Supplement A.2. USEPA Revisions to the Poet et al. (2010) PBPK Model Used to Support TSCA Risk Assessment for NMP.

A2.1 Rat PBPK Model

Exposure control

Because both Becci et al. (1982) and Saillenfait et al. (2002) explicitly stated that the animal body weights were measured every 3rd day of gestation, and the dermal/oral doses were adjusted accordingly on those days (as weight increases during pregnancy), corresponding conditional (if/then) statements were added to the 'GAVD' and 'REAPPLY' discrete blocks, to re-calculate the doses on those days.
The code for the dermal discrete blocks follows. ASK0 is the absolute amount applied on each day; DSK is the dose/kg BW. Because Becci et al. (1982) rubbed the material into the skin, it is assumed to be added directly into the skin compartment (ASK), rather than as a liquid on top. Hence the dose is given as an addition of ASK0 (mg/day applied) to ASK.

```
DISCRETE SKWASH       ! PMS, 8-14-13
    ASK = 0.0       ! Assume skin washing in Becci et al. (1982) removes all NMP
    from skin
    if (DAYS.LT.15.0) SCHEDULE REAPPLY.AT.(T+DOSEINTERVAL-TWASH)
END
DISCRETE REAPPLY      ! PMS, 8-14-13
    IF (ROUND(DAYS).EQ.9.0)   ASKO=DSK*BW
    IF (ROUND(DAYS).EQ.12.0) ASKO=DSK*BW
    IF (ROUND(DAYS).EQ.15.0) ASKO=DSK*BW
    ASK = ASK + ASKO
    SCHEDULE SKWASH.AT.(T+TWASH)
END
```

Also, because Becci et al. (1982) washed the skin area exposed to dermal application at the end of a set time interval, a "SKWASH" discrete block was introduced at which time the amount in that patch of skin was assumed to be momentarily reduced to zero. During periods of dermal application, transport from the liquid to the skin was turned on using the pulse function, DZONE. After removal of the liquid it was assumed that NMP in the skin patch could volatilize into the otherwise clean air, with the rate defined by the same permeability constants, but using the skin:air partition coefficient.
The rate of transfer to/from the skin area is then defined by:

```
RADL=(KPL*SA/1000.0)*((CSURF-(CSK/PSKL))*DZONE - (1.0-
    DZONE)*(CSK/PSKA))
! 2ND term, (1.0-DZONE)*(CSK/PSKA), allows for evaporative loss when
    DZONE=0
```

Finally, a constant, CONCMGS, was introduced so that the air concentration could be set directly in mg/m^3. This is converted to the concentration in mg/L (CONCMG) in the code and added to the inhalation exposure, turned on and off using the switch, CIZONE, which is turned on and off using SCHEDULE/DISCRETE statements:

```
CI = CCH*PULSE(0., DOSEINTERVAL,TCHNG) + CIZONE*CONCMG   ! MG/L !
    Added CIZONE*CONCMG, PMS, 8-13-13
```

Skin compartment

Corrections to the mass balance equations for the rat skin are as indicated in the commented code copied below. It includes the initial condition, ASK0, for the initial dermal application, but is otherwise now the standard format for PBPK models. As received the code had multiplied CSK rather than CSKV (skin venous blood concentration) by the blood flow (QSKN) for the rate of efflux in blood, and had not separately calculated CSKV.

```
RASK = QSKN*(CA - CSKV) + RADL       ! NOW MINUS CSKV, NOT CSK; PMS 8-21-
    13
ASK = INTEG(RASK,ASKO)  ! Initial value, ASKO, added for Becci et al. (1982)
    exposures; pms 8-14-13
CSK = ASK/VSK        !'NMP IN SKIN, MG/L'
CSKV = CSK/PSKB          ! NMP IN VENOUS BLOOD, PMS 8-22-13
```

The corresponding flow term for transfer from the skin to the mixed venous blood compartment was also corrected (ie, to use CVSK instead of CSK).

While these changes to the skin compartment equations initially degraded the fits to the dermal exposure considerably, it also appeared that the associated partition coefficients were not consistent with the measured values reported by Poet et al. (2010), Table 5. They were recalculated as follows:

Skin:liquid, PSKL = 0.42: value as measured for skin:saline, vs. 450

Skin:blood, PSKB = 0.12: (skin:saline)/(blood:saline)

Skin:air, PSKA = 55: (skin:saline)*(blood:air)/(blood:saline) = (skin:blood)*(blood:air)

Blood flows

Since the placenta is a separate compartment for the 5HNMP compartment, its blood-flow and volume were removed from the sums used for the 'rest of body' for 5HNMP. Also, the term for blood flow from the placenta was added to the mixed-venous blood mass balance for 5HNMP.

To assure flow mass balance, instead of calculating cardiac output (QC) as an initial amount plus the change from initial for each compartment, it was just calculated as the sum over all the compartments:

```
! QC = QCINIT + (QFAT - QFATI) + (QMAM - QMAMI) + QPLA+ (QUTR - QUTRI)
QC = QFAT+QLIV+QSLW+QRAP+QSKN+QMAM+QPLA+QUTR          ! pms, 8-13-
13
```

Parameter Consolidation

In the provided files, some physiological and chemical-specific parameter were set in separate scripts; e.g., skin transport parameters in the dermal exposure scripts. This approach creates the potential for inconsistent parameters between different exposure simulations. Therefore all parameters are now set in the ratparam.m script except those which are experimental control variables (eg., air concentration, duration of exposure). The final set of parameters used and any inconsistencies with previous values in ratparam.m that may have differed are noted in that script.

A2.2 Human PBPK Model

Model Structure

NMP metabolism and urinary elimination

Since the human PK data were consistent with a nearly linear model (first-order kinetics, including metabolism) estimation of a metabolic saturation constant, Km, using the traditional Michaelis-Menten equation for metabolism of NMP, was difficult. In particular as estimates of Km became larger, model fits became less sensitive to variation in its value. Therefore equation was changed from the standard form, rate = Vmax*C/(Km + C), where C is the concentration of NMP in the liver, to the equivalent form, rate = VK1*C/(1 + AF1*C), where VK = Vmax/Km and AF1 = 1/Km. The affinity constant, AF1, can be easily bounded to be non-negative and possibly converge to zero, corresponding to an indeterminately large Km. Since VK represents hepatic metabolism, it was assumed to scale with BW the same as Vmax; i.e., VK1 = VK1C*$BW^{0.75}$.

The urinary elimination of NMP was assumed to be first order, rather than saturable, using a rate constant (KUMNE) that was *not* scaled by BW.

5-HNMP

Since 5-HNMP is not being considered as an internal metric for toxicity and its volume-of-distribution (VOD) appeared to be over-estimated using the original PBPK model structure and measured tissue partition coefficients, it's description was replaced with a classical one-compartment PK model. Further, as the metabolism of 5-HNMP also appeared to be linear and the data for estimating a Km value even weaker, a transformation of its metabolic rate equation like that for NMP described just above was assumed, but with the affinity assumed to be effectively zero, resulting in a first-order metabolic rate equation. As with NMP, the urinary elimination of 5-HNMP was also assumed to be first-order. The resulting model then becomes:

d A5H/dt = RAMET1*STOCH – RAMETM1 – RAUHP (rate of change of amount of 5-HNMP)

CVEN1 = A5H/VOD5H (concentration of 5-HNMP in venous blood)

VOD5H = VOD5HC*BW (volume of distribution assumed to scale with BW)

RAMETM1 = CVEN1 *VK2, where VK2 = VK2C*$BW^{0.75}$ (rate of metabolism of 5-HNMP)

RAUHP = KME*CVEN1 (rate of urinary elimination of 5-HNMP)

RAMET1 = rate of NMP metabolism to 5-HNMP (mg NMP metabolized/h)

STOCH = ratio of 5-HNMP to NMP molecular weights.

Exposure and Timing Control

To support the exposure assessment for the TSCA risk assessment, a table function, RESLVL, was added as a place-holder for reading in defined (residential) inhalation exposure time-courses; specifically from U.S. EPA exposure assessment modeling. A constant, GDstart, the day of gestation on which the simulation starts, and a variable Gtime, the hours into gestation, were added to facilitate separating exposure control from gestation timing.

A second set of DISCRETE/SCHEDULE blocks were added to allow for split exposure scenarios (morning/afternoon worker exposure; dual-episode residential exposures).
DZONE, set in the DISCRETE/SCHEDULE blocks, controls the time within a day when discontinuous exposure occurs. Czone is the product of DZONE and a pulse function used to control for days/week exposure in workplace scenarios:

```
Czone = pulse(0.0,fullweek,hrsweek)*DZONE ! pms 8-20-13
! for a 5 day/wk exposure, use fullweek=7*24, hrsweek=5*24 (Dayswk=5)
! for a single day, fullweek=1e16, hrsweek=24 (Dayswk=1)
```

A binary constant, BRUSH, was added to set exposure scenarios dermal contact with liquid occurs. For workplace scenarios, exposure to vapor and liquid are assumed to be simultaneous; i.e., the worker leaves the location with NMP vapor and washes his/her hands when he/she has finished applying the material. The rate for delivery from a liquid film to the 'SKL' skin compartment (also see further below) is then defined by:

```
RADL=(PVL*SAL/1000.0)*(CSURF-(CSKL/PSKL))*Czone*BRUSH
    ! Net rate of delivery to "L" skin from liquid, when liquid is there
```

The equations for transfer of vapor (air concentration = CI) to the SKL compartment, which occurs during periods with no liquid/spray contact for the SKL compartment are similarly:

```
RADVL = (PV*SAL/1000.0)*(CI - (CSKL/PSKA))*(1.0-Czone*BRUSH)
! Net rate of delivery to "L" skin from air, when liquid not present
```

Since the dermal exposures are to neat or highly concentrated preparations of NMP, it would not be appropriate to assume that the residual volume on the skin remains constant as absorption occurs. Further assuming that water penetration of the skin is minimal, the amount of water in the liquid solution is assumed to remain constant. The initial volume on the skin is defined by a new constant VLIQ0 and the density of NMP at 40C (~ skin temperature) = DENSITY = 1.02×10^6 mg/L. To avoid potential divide-by-zero errors, the nominal initial concentration (CONCL) is reduced by 1 mg/L (1 ppm) when computing the initial amount of NMP and water in the liquid:

DDN = (CONCL - 1.0)*VLIQ0*FAD ! Subtract 1 mg/L, ~ 1 ppm, from initial conc. to avoid VLIQ --> 0
AH20 = (DENSITY+1.0-CONCL)*VLIQ0 ! ... and add it to H20. pms 9-16-14

A mass-balance equation was then added to attract the remaining amount **and** volume on the skin surface, which are then used to calculate the concentration:

ASURF = INTEG(-RADL, DDN) ! Amount in liquid. DDN is the initial amount.
VLIQ = (AH20 + ASURF)/DENSITY
CSURF = ASURF/VLIQ

This volume balance is important for analysis and calibration of the dermal PK studies were small volumes (5 or 10 ml) were applied at the beginning of the exposure and not replenished. However in workplace and residential user exposures, it's assumed that fresh liquid is constantly replacing any NMP that is absorbed, keeping the surface concentration essentially constant. Therefore the initial volume, VLQ0, is set to a large value (10^6 L) for those scenarios.

Skin compartment

As for the rat, and noted in the main report, corrections were made to the human skin transport and PK (equations not shown here, but same as for rat). The partition coefficients were also recalculated as was done for the rat, with rat parameters for skin:saline and blood:air, but human blood:saline.

The original skin compartment which is coded to include uptake from liquid-dermal contact was renamed by adding "L" to the end, SK → SKL, and second skin compartment to account for concurrent vapor-skin uptake, SKV, was added. This was done because when the human model was calibrated for inhalation exposure, an exposed skin surface area of 6700 cm^2 was used. When this surface are is reduced to ~ 0, predicted blood levels of NMP shown in the upper panel of Figure 4 in the QA report are reduced ~ 45%. Thus vapor uptake through the skin is a significant component of inhalation exposure and there is no reason to assume, *a priori*, that this uptake (or desorption) does not occur through a similar area of exposed skin during workplace and residential exposures, except for any area that would have liquid contact or otherwise be occluded (e.g., by wearing rubber gloves). So the SKV compartment allows for simultaneous absorption of vapor through skin that does not have liquid contact, and from areas of skin with liquid contact. The surface area of SKV and SKL are SAV and SAL, respectively, and can be set for different exposure scenarios.

To account for variations with individual BW, a parameter for the fraction of skin area exposed to vapor was introduced: SAVC, with SAV = SAVC*TSA, where TSA is the total body surface area. For EPA simulations, SAVC was set to 0.25, representing the head, neck, arms, and hands, minus any area assumed to have liquid contact, or covered with protective gloves or a face-mask.

Tissue and blood-flow mass balances

The model had been previously coded with an alveolar blood compartment (ALV), but this was commented out by the author in the DYNAMIC section. Therefore this volume fraction should not be subtracted when calculating the slowly-perfused volume. The fraction of blood-flow to slowly perfused tissue was updated to also

account for the SKV compartment; on the other hand a separate skin compartment is not used for 5HNMP, so the skin blood flow is NOT subtracted for the metabolite-slowly-perfused compartment (SLW5). These have all been corrected.
QSKCC (original fractional flow to the skin) had been subtracted twice, both in calculating QSLWC and then in the calculation of QSLW. The 2nd subtracted created a mass balance error and hence was removed. On the other hand, placental blood flow is now subtracted, so the total flow to slowly-perfused continues to total cardiac output minus all other tissue/group flows.
For tissues that change with gestation day, the initial values were corrected to match the calculation in the DYNAMIC section, which would apply at the first time-step.
In the dynamic section, the calculation of QC was corrected to include the *increase* in placental flow (QPLA – QPLAI) rather than the total placental flow (QPLA), since QCINIT includes QPLAI. QSLW5 and VSLW5 (5HNMP slow compartment flow and volume) are now calculated in the DYNAMIC section by subtraction.

Parameter Consolidation

As for the rat model, the human model physiological and biochemical parameters are now primarily set in a single script, human_params.m. These are parameters obtained by fitting Bader et al. (2006) inhalation data with the exception of the high-concentration data from one individual, but the data otherwise grouped without distinction between individuals (see below). An alternate set of fitted parameters was obtained by fitting the data for each individual separately, focused on the low-concentration data, and then calculating the average of each parameter across the individually-fitted values. This subset of parameters is selected by using human_avg_params.m. Since further analysis of the dermal absorption of liquid NMP showed that this uptake differed between neat (100%) NMP and diluted NMP, separate value of PVL were obtained for neat vs. diluted (also see below). Hence only constants which define specific exposure scenarios (include skin areas exposed) and PVL are defined in the specific simulation scripts.

Re-evaluation of Human Data and Re-calibration of Fitted Parameters

Based on a careful review of the data tables in Bader et al. (2006) and personal communication with Dr. Michael Bader and Dr. Christoph van Thiel, it was determined that each subject entered and left the exposure chamber at slightly different times and were likely not sampled at exactly the same time after the beginning and end of each exposure segment. While the total exposure time for each subject was monitored and kept to exactly 6 h on each exposure day, based on the timing of the blood and urine samples (taken outside the exposure chamber), it is clear that the study design was not exactly followed. In particular, while the morning and afternoon exposures were supposed to be 3 h each, the time between the mid-day and first afternoon blood samples was less than 3 h for some individuals in some exposures (and the mid-day sample was taken much later after noon than for such samples). In these cases it seemed likely that the individual spent slightly more than 3 h in the chamber in the morning, and slightly less in the afternoon, for that exposure. Based on the recorded data and communications, the

exposure timing used for modeling and simulation was set to 3.1 h for the morning exposure, a mid-day break of 0.2 h, and 2.9 h for the afternoon exposure. Since individual subjects did not (could not have) entered and exited the chamber at exactly the same time, the time of their entrance to the chamber for each exposure was estimated based on the recorded times of the blood and urine samples. The sample times used for modeling were then calculated relative to the estimated entry times.

It was also clear that a number of the measurements, especially those of 5-HNMP for the low-concentration exposure, were recorded as the limit-of-detection (LOD), when the measured value fell below this limit. This was confirmed with Dr. Bader (personal communication). Therefore all measurements at/below the LOD were removed from the data set to avoid the bias they would otherwise introduce.

It also appeared that the high-concentration-exposure (80 mg/m^3) for one subject deviated significantly from the other subjects; see Figure A2-1. Since the blood concentration at 6 h was well below those of the other subjects, and that at 24 h well above (4 subjects had levels below the LOD), this high concentration set was excluded from analysis of the grouped data. Blood concentrations at the middle and low exposure for this individual were among the range of the other subjects, hence included in the group data set.

With this one data set removed, the revised model was fit to the group data for exposures at 9.7 and 80 mg/m^3, by adjusting the following parameters: PV, VK1C, AF1, KUMNE, VK2C, VOD5HC, and KME. Since the data for the 40 mg/m^3 exposure were consistent with the 80 mg/m^3, but the data for 9.7 mg/m^3 appeared not to be, and it was considered especially important to describe low-concentration exposures, there 40 mg/m^3 data were excluded from this exercise. The resulting parameter values are as follows, with model fits to the group data shown in Figure A2-2, left side. These fits are compared to ones obtained by fitting the data for each individual separately, where possible using only the low-concentration exposure data, and then calculating the average across the individual fits for each parameter (right side of Figure A2-2; details below).

Parameters fitted to group data for 9.7 and 80 mg/m^3 exposures	**Average of parameters fit to data for each individual separately, primarily 9.7 mg/m^3**
PV = 1.6 (cm/h)	PV = 16.4 (cm/h)
VK1C = 0.47 (L/(h*kg$^{0.75}$))	VK1C = 0.386 (L/(h*kg$^{0.75}$))
AF1 = 0.02 (L/mg)	AF1 = 0.02 (L/mg) [fixed at group-fit value]
VK2C = 0.035 (L/(h*kg$^{0.75}$))	VK2C = 0.0359 (L/(h*kg$^{0.75}$))
VOD5HC = 0.26 (L/kg)	VOD5HC = 0.243 (L/kg)
KME = 2.3 (L/h)	KME = 2.75 (L/h)
KUMNE = 0.092 (L/h)	KUMNE = 0.103 (L/h)

In their summary statistics, Bader et al. (2006) reported group-averages of the peak NMP blood levels as being 0.293 mg/L for the 9.7 mg/m^3 and 1.585 mg/m^3. The ratio of these two (1.585/0.293 = 5.4), is considerably less than one would expect assuming linearity with exposure level (80/9.7 = 8.25) and is the opposite of what

one would expect due to metabolic saturation of the conversion of NMP to 5-HNMP. This is not true for the ratio peak 5-HNMP levels in blood (8.08), however, which is comparable to the relative exposure level. If the nonlinearity in NMP blood levels were due to more efficient metabolism at the higher exposure level, then ratio of 5-HNMP blood levels would have been greater than expected.

Since the mechanism for the nonlinearity in blood NMP levels is unclear, and it would be undesirable to under-estimate NMP blood levels and hence human risks at lower exposure levels, it was decided to estimate parameters using only the low-exposure data, if possible, or with minimal use of the high-exposure data. (For two of the subjects the blood levels of 5-HNMP did not rise above the LOD for the low exposure, making it impossible to estimate VOD5HC for them. Hence the 80 mg/m^3 blood 5-HNMP data also needed to be used to estimate their parameters.) Given the observation that the high-exposure data for one subject was disparate from the other subjects, it also seemed possible that the apparent nonlinearity in the average PK data was due to the mixing of data from the 8 subjects in the study. Therefore fits focused on the low-exposure data were conducted separately for each subject. Since limiting to the low-exposure data would provide almost no information on metabolic saturation, and the affinity (AF1) obtained from the fits to the group data was quite low (0.02 L/mg), AF1 was held at that group-fit value for this exercise. The resulting parameter values are listed in Table A2-1 and fits to the individual data shown in Figure A2-3. In order to allow one to see the fit to the low concentration and otherwise compare the fits across individuals, the y-axis scale was held constant for each analyte across the individuals, though this meant that the simulation curves for the higher exposure data sometimes went off the top of the plot.

It is interesting to note that for half of the subjects (#12, #14, #16, and #25), the fits and data for NMP in blood show that the data are quite consistent with the essentially linear PBPK model, while for the other half the simulations with parameters fitted to the low-concentration data over-predict the high-concentration NMP data.

Supplement B: Benchmark Dose Modeling for Developmental Toxicity Endpoints of N-Methyl-2-Pyrrolidone (NMP)

B.1 Window of Susceptibility Assessment

Because the study designs for the key developmental toxicity studies are varied and provide coverage for different portions of the gestation period (early, mid, and late), the data set for NMP can support an evaluation of the window of susceptibility for fetal/pup body weight changes. To evaluate an early-stage window of susceptibility, daily AUC values were averaged across an exposure window defined as gestation day 1 (GD1) through gestation day X (GDX), where X was allowed to vary from 2-20. Similarly, to evaluate a late-stage window internal dose was averaged across an exposure window defined as GDX-GD20, where X is allowed to vary from 1-19. For the skeletal malformations data, the evaluation was restricted to GD6-20, since none of the three studies included exposures during GD1-5. In addition, due to the presence of a threshold for this endpoint (e.g., hockey stick shape for dose-response curve), the correlation was restricted to data points with a non-zero incidence value. After each adjustment in the exposure window, the correlation coefficient for consistency in the dose-response relationship across all five data sets was recorded. The window of susceptibility was identified as the time period for which optimal correlation across data sets was achieved. For benchmark dose (BMD) modeling of fetal/pup body weight changes, daily AUC values were averaged across the window of susceptibility.

Results for the window of susceptibility evaluation for fetal/pup body weight changes are shown in **Figure B-1**. Correlation across data sets was poor for window defined as GD1-GD5 (r2=0.04), but gradually improved as the end of the window was extended stepwise from GD5 to GD20 (r2 improves from 0.04 to 0.70). Correlation across data sets was reasonable for window defined as GD-1-GD20 (r2=0.70), gradually improved as the start of the window was extended stepwise from GD1 to GD13 (r2 improves from 0.70 to 0.84), then decreased slightly as the start of the window was extended stepwise from GD13-GD20 (r2 decreases slightly from 0.84 to 0.78). Optimal correlation across data sets was achieved using a window of susceptibility defined as GD13-GD20. This window corresponds well with the time period during rapid weight gain in the fetus (**Figure B-3**).

Results for the window of susceptibility evaluation for skeletal malformations are shown in **Figure B-2**. Correlation across data sets was poor for window defined as GD6-GD9 (r2=0.03), but gradually improved as the end of the window was extended stepwise from GD10 to GD20 (r2 improves from 0.03 to 0.99). Correlation across data sets was reasonable for window defined as GD6-GD20 (r2=0.99), and slightly improved as the start of the window was extended stepwise from GD6 to GD7 (r2 improves from 0.99 to 0.999), then decreased slightly as the start of the window was extended stepwise from GD7-GD20 (r2 decreases slightly from 0.999 to 0.60). Optimal correlation across data sets was achieved using a window of susceptibility defined as GD7-GD20 (**Figure B-2**).

B2. Benchmark Dose Modeling of Skeletal Malformations

Benchmark Dose (BMD) modeling was performed using USEPA's BMD Software package (version 2.5), in a manner consistent with USEPA guidelines (USEPA, 2012). Dichotomous models were used to fit dose-response data for skeletal malformations. The data from the oral toxicity study (Saillenfait et al., 2002) were assessed alone, and combined with the data from the inhalation toxicity study (Saillenfait et al., 2003) and dermal toxicity study (Becci et al.,1982). Incidence data for fetal malformations were assessed two different ways: (1) on an affected litter basis (using data from column 8 in **Table 2** of the manuscript); and (2) on an affected fetus basis (using data from column 9 in **Table 2** of the manuscript). Daily peak NMP in maternal blood, averaged over the window of susceptibility defined above (GD7-20), was used as an appropriate dose measure for this endpoint. The best fitting model was selected based on Akaike information criterion (AIC; lower value indicates a better fit), chi-square goodness of fit p-value (higher value indicates a better fit), ratio of the BMC:BMCL (lower value indicates less model uncertainty), and visual inspection (**Figure B-4**). A comparison of model fits obtained for skeletal malformations is provided in **Table B-1**. The best-fitting models, based on the criteria described above, are indicated in bold italics.

B.3 Benchmark Dose Modeling of Fetal/Pup Body Weight Changes

BMD modeling was performed using USEPA's BMD Software package (version 2.5), in a manner consistent with USEPA guidelines (USEPA, 2012). Continuous models were used to fit dose-response data for fetal/pup body weight changes.
The five data sets indentified in Table 2 of the manuscript were assessed a pooled data set, and as separate data sets. Daily AUC for NMP in blood, averaged over the window of susceptibility defined above (GD13-20), was used as an appropriate dose measure for this endpoint. The best fitting model was selected based on Akaike information criterion (AIC; lower value indicates a better fit), chi-square goodness of fit p-value (higher value indicates a better fit), ratio of the BMC:BMCL (lower value indicates less model uncertainty), and visual inspection (**Figure B-5, B-6**). A comparison of model fits obtained for fetal/pup body weight changes is provided in **Table B-2**. The best-fitting models, based on the criteria described above, are indicated in bold italics.

B.4 Route-to-Route Concordance of Fetal/Pup Body Weight Changes

The dose-response data for the oral gavage toxicity study of Saillenfait et al. (2002) was considered to provide the best overall characterization of the dose-response relationship for NMP and fetal/pup body weight changes because: (1) of the five data sets included in this evaluation, this data set provides the largest range of internal doses; and (2) the PBPK model was specifically parameterized using oral gavage exposures (**Supplement A**). To address uncertainty in the internal dose predictions for other routes (inhalation, dermal) and media (feed) route-specific

adjustment factors were included and then optimized to provide the best overall correlation with the dose-response curve of Saillenfait et al. (2002). The results of this analysis are presented in **Figure B-6A**. Without the route-specific adjustment factors, the correlation coefficient is 0.84. Optimal correlation was achieved by including adjustment factor values of 3.3 and 0.6 for inhalation (whole-body) and dietary exposures, respectively. The optimized adjustment factor for dermal exposures was not appreciably different from 1 (i.e., no adjustment is necessary). With the inclusion of these route-specific adjustment factors, the overall correlation in the pooled data set improves to 0.94 (**Figure B-6B**). Based on this evaluation, to improve the dose-response relationships for fetal/pup body weight changes across routes of exposure, whole-body inhalation exposures (Saillenfait et al., 2003; Solomon et al., 1995) would need to produce internal doses of NMP that are approximately 3.3-fold higher than estimated by the current model. On the other hand, oral exposures to NMP in feed (Thornton, 1999) would need to produce internal doses that are approximately 40% lower (e.g., a bioavailability of approximately 60% in feed) than estimated by the current PBPK model. Additional data are required regarding the internal doses of NMP following whole-body inhalation and dietary exposures, to assess whether the magnitude of these differences are supportable.

Tab A1-1. PBPK Model Parameters

Parameter	Rat	Human	Reference
Tissue Volumes (% body weight)			
Fat	9	23	Brown et al. (1997) and Gentry et al. (2002)
Blood	6.7	6	
Liver	3.7	3.1	Brown et al. (1997) and Gentry et al. (2002)
Rapidly perfused	7.1	4.2	Brown et al. (1997) and Gentry et al. (2002)
Skin[a]	19.	5.1	Brown et al. (1997)
Flows (L/h)			
Alveolar ventilation[b]	4.83	362.5	Brown et al. (1997)
Cardiac output	4.83	362.5	Brown et al. (1997)
Blood Flows - Percent of cardiac output			
Liver	18.3	25.0	Brown et al. (1997) and Gentry et al. (2002)
Richly perfused	51.2	48.0	Brown et al. (1997) and Gentry et al. (2002)
Slowly perfused	14.0	19.0	Brown et al. (1997) and Gentry et al. (2002)
Fat	7.0	5.0	Brown et al. (1997) and Gentry et al. (2002)
Skin[a]	1.0	3.0	Brown et al. (1997) and Gentry et al. (2002)
Biochemical constants			
NMP: VmaxC (mg/h/kg0.75)	9	44	Optimized, See Table 1
NMP: Km (mg/l)	225	68	Optimized, See Table 1
5-HNMP: VmaxC (mg/h/kg0.75)	0.09	4.5	Optimized, See Table 1
5-HNMP: Km	4.9	61	Optimized, See Table 1
Urinary saturable elimination			
First-order urinary elimination			
NMP Kel	0.001	0.1	Optimized, See Table 1
5-HNMP Kel	1.6	2.8	Optimized, See Table 1
Absorption			
Dermal liquid: KP (cm/h)	4.3e-3	2.0e-3	Optimized, See Table 1
Dermal vapor: Kp (cm/h)	NA	22	Optimized, See Table 1
Oral to liver (h^{-1})	1.5		Optimized, See Table 1
Stomach to Intestine (h^{-1})	0.85		Optimized, See Table 1
Intestine to Liver (h^{-1})	0.006		Optimized, See Table 1

[a]Values are for total skin. The skin compartment in the PBPK model was comprised solely of the area of the skin exposed. The remainder of the skin was included in the slowly perfused compartment.
[b]Ventilation rates are body weight specific.

Table A1-2. Summary of Exposures for key human studies

Study	Inhalation	Dermal Vapor	Dermal Liquid	Comments
Bader, 2006	10, 40, 80 mg/m3	Lower Arms and head (10% Surface Area)	none	Dermal contributes 14% of total uptake, independent of exposure concentration
Akesson and Paulson	10, 25, 50 mg/m3	Lower limbs and head (50% Surface Area)	none	Dermal contributes 25% of total uptake, independent of exposure concentration
Akkesson et al, 2006	None	None	300 mg over 5 cm2	Diluted results in a 75% decrease in absorption
Xiaofei et al, 2004 - workers	9-42 ppm (TWA)	Full arms and head (30% surface area)	Splatter – 1 ml/h	Washing glass with NMP, splatter expected. Dermal Vapor contributes 4%, liquid contributes 40% of total absorbed NMP
Xiaofei et al. 2004 – observers	24-32 ppm (TWA)	Full arms and head (30% surface area)	Splatter – 0.5 ml/h –, assumed 50% splatter rate of the workers	Washing glass with NMP, splatter expected. Dermal Vapor contributes 27 %, liquid contributes 45% of total absorbed NMP

Table A1-3. Impact of Alternative Breaktime Assumptions (10 or 20 minutes) on Predicted Dose Measures in Volunteers

10 minute break between 3 h exposures			
Exposure	10 mg/m^3	40mg/m^3	80 mg/m^3
24 h AUC (mg/h×L)	1.36	5.44	10.9
Cmax (mg/L)	0.193	0.775	1.56
20 min break between 3 h exposures			
24 h AUC (mg/h/L)	1.36	5.44	10.9
Cmax (mg/L)	0.195	0.784	1.58
Difference between 10 and 22 minute break (%)			
AUC	0.0	0.0	0.0
Cmax	1.3	1.3	1.3

Table A1-4. Individual rate constants (Vmax/Km) for volunteers from Bader (2005)

	NMP			5HNMP		
Exposure Group 1	**VmaxC ($mg/h/kg^{0.75}$)**	**Km (mg/L)**	**VmaxC/Km ($L/h/kg^{0.75}$)**	**VmaxC ($mg/h/kg^{0.75}$)**	**Km (mg/L)**	**VmaxC/Km ($L/h/kg^{0.75}$)**
1	22.0	45.3	0.486	2.20	78.9	0.028
10	25.3	32.4	0.781	2.74	51.8	0.053
14	56.8	92.4	0.615	5.82	36.7	0.159
17	59.1	65.1	0.908	7.19	72.1	0.100
Exposure Group 2						
4	41.8	104	0.402	4.98	49	0.102
12	68.4	76.9	0.889	3.99	46.2	0.086
16	60.4	88.7	0.681	5.98	73.9	0.081
25	18.3	41.1	0.445	2.94	77.9	0.038
Mean	44.0	68.2	0.65	4.48	60.8	0.081
SD	19.8	26.5	0.20	1.79	16.6	0.042

19 **Table A2-1. PBPK Model Parameters Used to Support USEPA's TSCA Risk Assessment for NMP**

Parameter	Rat	Human	Reference
Tissue Volumes (% body weight)			
Fat	9	23	Brown et al. (1997) and Gentry et al. (2002)
Blood	6.7	6	
Liver	3.66	3.1	Brown et al. (1997) and Gentry et al. (2002)
Rapidly perfused	7.1	4.2	Brown et al. (1997) and Gentry et al. (2002)
Skin[a]	19.	5.1	Brown et al. (1997)
Mammary	1.0	0.62	Gentry et al. (2002)
Uterus	0.2	0.14	Gentry et al. (2002)
Flows (L/h/kg$^{0.75}$)			
Alveolar ventilation [b]	10.72, 10.05	10.72	0.67*16 L/h/kg$^{0.75}$ or 0.67*15 L/h/kg$^{0.75}$; Brown et al. (1997)
Cardiac output	16, 15	16, 15	Brown et al. (1997)
Blood Flows - Percent of cardiac output			
Liver	18.3	25.0	Brown et al. (1997) and Gentry et al. (2002)
Richly perfused	51.2	48.0	Brown et al. (1997) and Gentry et al. (2002)
Slowly perfused	-~ 0.231	17.35-18.8*	By mass balance: 100 - (QFatC + QLivC + QMamC + QRapC + QUtrC + QSKvC + QSKlC); includes non-exposed skin; QSKvC and QSKlC vary between ~ 0 and 1.45, depending on exposure scenario
Fat	7.2	5.0	Brown et al. (1997) and Gentry et al. (2002)
Skin[a]	5.8	5.8**	Brown et al. (1997) and Gentry et al. (2002); ** value for entire

			skin, a fraction of which is exposed to vapor or liquid
Mammary	0.1	2.7	Gentry et al. (2002)
Uterus	0.1	0.5	Gentry et al. (2002)
Biochemical constants			
NMP: VmaxC (mg/h/kg0.75)	9	44, 19.3	Optimized, See Table 1
NMP: Km (mg/l)	225	68, 50	Optimized, See Table 1
5-HNMP: VmaxC (mg/h/kg0.75)	0.09	4.5, --	Optimized, See Table 1
5-HNMP: Km	4.9	61, --	Optimized, See Table 1
5-HNMP: VKC (L/h/kg$^{0.75}$)		(0.0738). 0.0359	(VmaxC/Km from above); optimized
First-order urinary elimination			
NMP Kel	0.001	0.1, 0.103	Optimized, See Table 1
5-HNMP Kel	1.6	1.7, 2.75	Optimized, See Table 1
Absorption			
Dermal liquid: KP (cm/h)	4.6e-3	2.7e-3 (neat), 4.78e-4 (diluted)	Optimized, See Table 1; value used for NMP diluted in water ~ 50% or lower NMP fraction
Dermal vapor: Kp (cm/h)	NA	23, 16.4	Optimized, See Table 1
Oral to liver (h^{-1})	1.5	--	Optimized, See Table 1
Stomach to Intestine (h^{-1})	0.85	--	Optimized, See Table 1
Intestine to Liver (h^{-1})	0.006	--	Optimized, See Table 1

[a]Values are for total skin. The skin compartment in the PBPK model was comprised solely of the area of the skin exposed. The remainder of the skin was included in the slowly perfused compartment.

[b]Ventilation rates are body weight specific.

Table A2-2. Estimated Parameters for Each Subject of the Bader et al. (2006) PK Experiments Used for USEPA's PBPK Model

Subject	VK1C	KUMNE	PV	VK2C	KME	VOD5HC
1	0.25	0.11	19	0.017	3.2	0.2
4	0.17	0.042	34	0.004	3	0.14
10	0.22	0.069	35	0.027	2.8	0.12
12	0.63	0.046	12	0.044	1.9	0.39
14	0.57	0.2	10	0.08	2.5	0.4
16	0.45	0.06	0	0.08	1.9	0.2
17	0.38	0.2	20	0.02	4.3	0.26
25	0.42	0.1	1.5	0.015	2.4	0.23
average	**0.386**	**0.103**	**16.4**	**0.0359**	**2.75**	**0.243**

SUPPLEMENT B: Benchmark Dose Modeling

Tab B-1. Benchmark Dose Estimates for Skeletal Malformations in Rats Exposed to NMP

				Internal Dose (Peak NMP in Blood mg/L)		
Data Set (basis)	**Model***	**AIC**	**p-Value**	**BMD10**	**BMDL10**	**BMD/BMDL**
Saillenfait et al. (2002) (Litter)	Gamma	48.767	0.331	302	222	1.4
	Dichotomous-Hill	***45.348***	***1.000***	***390***	***315***	***1.2***
	Logistic	52.533	0.066	310	240	1.3
	LogLogistic	48.598	0.355	303	223	1.4
	Multistage 3	48.598	0.263	283	202	1.4
	Probit	51.400	0.109	307	234	1.3
	LogProbit	47.911	0.464	303	226	1.3
	Weibull	50.598	0.154	284	202	1.4
	Quantal-Linear	59.055	0.003	130	87	1.5
Saillenfait et al. (2002) (Fetus)	Gamma	110.467	0.994	419	373	1.1
	Dichotomous-Hill	***110.383***	***1.000***	***444***	***376***	***1.2***
	Logistic	112.825	0.486	422	380	1.1
	LogLogistic	110.748	0.947	418	372	1.1
	Multistage 4	110.506	0.713	391	356	1.1
	Probit	111.094	0.871	419	376	1.1
	LogProbit	110.402	0.999	421	373	1.1
	Weibull	111.047	0.882	414	368	1.1
	Quantal-Linear	135.287	0.000	326	228	1.4
Saillenfait et al. (2002, 2003); Becci et al. (1982) Combined (Litter incidence)	Gamma	80.140	0.891	305	246	1.2
	Dichotomous-Hill	***77.849***	***1.000***	***380***	***312***	***1.2***
	Logistic	85.669	0.365	326	278	1.2
	LogLogistic	79.881	0.909	310	248	1.2
	Multistage 3	81.604	0.653	273	234	1.2
	Probit	84.026	0.516	314	264	1.2
	LogProbit	78.958	0.960	305	247	1.2
	Weibull	83.364	0.583	288	230	1.3
	Quantal-Linear	105.922	0.000	139	102	1.4
Saillenfait et al. (2002, 2003); Becci	Gamma	204.780	1.000	424	388	1.1
	Dichotomous-Hill	206.822	1.000	433	391	1.1

et al. (1982) Combined (Fetal incidence)	Logistic	208.599	0.911	427	400	1.1
	LogLogistic	205.140	1.000	422	389	1.1
	Multistage 5	***203.713***	***1.000***	***414***	***385***	***1.1***
	Probit	205.600	0.999	424	394	1.1
	LogProbit	204.726	1.000	426	388	1.1
	Weibull	205.548	1.000	419	385	1.1
	Quantal-Linear	247.607	0.000	387	295	1.3

*Log forms of the Logistic and Probit models failed to return results for these data sets, and therefore are not included in this table.

Tab B-2. Benchmark Dose Estimates for Fetal/Pup Body Weight Changes in Rats Exposed to NMP

					Internal Dose (Average Daily AUC GD14-20, mg*h/L)		
Data Set	**Variance Model**	**Model**	**AIC**	**p-Value**	**BMDSD**	**BMDLSD**	**BMD/BMDL**
Pooled data sets (Saillenfait et al., 2002, 2003; Becci et al. 1982; Solomon et al., 1995; Thornton, 1999)	Homogenous Assumed*	Exponential (M3)	2531.14	<0.0001	3169	2679	1.2
		Hill	***2525.39***	***<0.0001***	***3073***	***2818***	***1.1***
		Linear	2535.56	<0.0001	2498	2283	1.1
		Power	2531.97	<0.0001	3145	2635	1.2
		Polynomial 2°	2533.48	<0.0001	3027	2523	1.2
Saillenfait et al. (2002)	Homogenous Assumed*	***Exponential (M3)***	***-107.17***	***0.425***	***1784***	***1390***	***1.3***
		Hill	-106.87	0.907	2058	1531	1.3
		Linear	-103.30	0.055	1248	1095	1.1
		Power	-103.92	0.084	1580	1202	1.3
		Polynomial 2°	-102.14	0.034	1375	1119	1.2
Saillenfait et al. (2003)	Homogenous Assumed*	***Exponential (M2)***	***-83.80***	***0.760***	***846***	***526***	***1.6***
		Hill	-80.35	NC	NC	NC	NC
		Linear	-83.78	0.753	843	533	1.6
		Power	-83.78	0.753	843	533	1.6
		Polynomial 2°	-83.78	0.753	843	533	1.6
Saillenfait et al. (2003) with historical controls		Exponential (M2)	-724.04	0.752	872	616	1.4
Saillenfait et al. (2002, 2003) combined	Homogenous Assumed	***Exponential (M3)***	***-185.06***	***0.0144***	***2199***	***1789***	***1.2***
		Hill	-184.72	0.0142	2580	1953	1.3
		Linear	-179.99	0.0017	1472	1330	1.1
		Power	-182.88	0.0061	1959	1557	1.3
		Polynomial 2°	-181.57	0.0036	1761	1455	1.2
Saillenfait et al. (2002, 2003) combined with historical controls		Exponential (M3)	-820.43	0.008	2118	1790	1.2
Becci et al. (1982)	Non-homogenous	Exponential (M2)	-121.82	0.000	NC	NC	NC
		Hill	-134.67	NC	3616	1347	2.7
		Linear	-126.19	0.001	1644	1248	1.3
		Power	***-136.67***	***0.371***	***3682***	***2314***	***1.6***
		Polynomial 2°	-69.54	<0.0001	NC	NC	NC

Solomon et al. (1995)	Homogenous	***Exponential (M2)***	***28.13***	***0.139***	***607***	***419***	***1.4***
		Hill	29.88	0.055	699	0.000013	53000000
		Linear	28.15	0.138	609	430	1.4
		Polynomial 2 (restricted)	28.15	0.138	609	430	1.4
		Power	28.15	0.138	609	430	1.4
Thornton (1999)	Non-homogenous	Exponential (M3)	21.89	0.1653	5698	3591	1.6
		Hill	23.87	N/A	2482	2070	1.2
		Linear	25.84	0.0195	3205	2540	1.3
		Power	21.89	0.1652	5806	3670	1.6
		Polynomial 3°	***19.89***	***0.3816***	***5868***	***4136***	***1.4***

Bolded/italicized rows indicate the best fitting model for each data set based on the criteria described in the text.

*Homogenous assumed = variance is nonhomogenous but use of a power variance model performs more poorly than an assumption of homogenous variance.

**Parameters for the polynomial model were restricted to be nonpositive; NC=not calculated

NMP OEL Figure Legends

Fig. A1-1. Plasma NMP following iv exposure of rats to NMP: (A) 0.1 mg/kg (Payan et al. 2002), triangles = arithmetic mean, solid line = PBPK model prediction; (B) 45 mg/kg (Wells and Digenis, 1988), circles = arithmetic mean (of measurements using 3 radiolabels), error bars = standard deviation, solid line = PBPK model prediction

Fig. A1-2. Plasma NMP following oral exposure of rats to NMP: solid circles = arithmetic mean (104 mg/kg; Midgely et al., 1992), hollow circles = arithmetic mean (53.5 mg/kg; Ghantous, 1995), error bars = standard deviation, solid lines = updated PBPK model predictions, dashed lines = PBPK model prediction with a single uptake rate.

Fig. A1-3. Plasma NMP following dermal exposure of rats to NMP: (A) 200 µl neat NMP (Payan et al., 2003); solid circles = arithmetic mean, solid line = PBPK model prediction using optimized Kp

Fig. A1-4. Pharmacokinetics of NMP and 5HNMP in inhalation exposures of humans to NMP: (A) Plasma NMP (Bader and van Thriel, 2006); (B) Plasma 5HNMP; (C) Urinary NMP; (D) Urinary 5HNMP. For all graphs, triangles= 20 ppm, circles = 10 ppm, diamonds = 2.5 ppm, lines = PBPK model predictions; Data is arithmetic mean, error bars = standard deviation of n=8 volunteers.

Fig. A1-5. Model fits to volunteers (Akesson and Paulsson, 1997). For all graphs, triangles = 20 ppm, circles = 10 ppm, diamonds = 2.5 ppm, dashed lines = PBPK model predictions from inhalation exposure only, solid lines= PBPK model predictions including dermal absorption of NMP vapor.

Fig. A1-6. Model fits to volunteers exposed to NMP via dermal exposure (Akesson et al., 2004). A) Solid squares = average plasma 5HNMP concentration in n=6 male volunteers, open triangles=average plasma 5HNMP in n=6 female volunteers, filled circles = average 5HNMP concentration in n=6 male volunteers exposed to diluted (50:50) NMP, solid line=model predicted concentrations in male volunteers, dashed lines=model predicted concentration in female volunteers, dotted line=model predicted concentration in male volunteers exposed to diluted NMP. B) Solid squares = average urinary 5HNMP in n=6 male volunteers, open triangles=average urinary 5HNMP in n=6 female volunteers, filled circles = average urinary 5HNMP in n=6 male volunteers exposed to diluted (50:50) NMP, solid line=model predicted concentrations in male volunteers, dashed lines=model predicted concentration in female volunteers, dotted line=model predicted concentration in male volunteers exposed to diluted NMP.

Fig. A1-7. Model fits to 4 workers or 5 observers in a lens cleaning factory (Xiaofei et al., 2000). A) Workers cleaned the lenses with NMP and personal air samplers were measured to estimate average air concentration of NMP, squares = arithmetic mean of n=4 individuals, error bars = standard deviation, solid lines=average of the TWA exposure estimates (0.09 – 0.69 ppm, mean 0.28 ppm), dashed line included the assumption that workers were in contact with small amounts of liquid NMP dermally. B) Observers watched as workers cleaned the lenses with NMP and personal air samplers were measured to estimate average air concentration of NMP, squares = arithmetic mean of n=5 individuals, error bars = standard deviation, solid lines=high and low exposure estimate (0.24 – 0.32 ppm, mean 0.28 ppm). It was assumed that the observers were exposed to half of the splatter that the workers encountered

NMP OEL Figure Legends

Figure A2-1: NMP blood concentration data from Bader et. (2006). Curves are simulations for 9.7, 40, and 80 mg/m3 exposures. Squares are individual blood concentration data for the 80 mg/m3 exposure. Solid squares are from the one individual with the highest BW and height (102 kg, 190 cm), compared to the other subjects (65-80 kg, 168-183 cm).

Figure A2-2: Alternate fits to collective data from Bader et al. (2006). Left panels show fits to the grouped data for 9.7 and 80 mg/m3 (data shown). Simulations in right panel used average of parameters fit to each individual separately, primarily for 9.7 mg/m3 (see text for details).

Figure A2-3: Model fits to Individual of Bader et al. (2006). Model fit separately to each subject. See text for details.

Fig. B-1. Window of Susceptibility for Fetal/Pup Body Weight Changes

Fig. B-2. Window of Susceptibility for Skeletal Malformations

Fig. B-3. Simulated tissue growth in pregnant rats. The window of susceptibility coincides with rapid fetal growth that occurs on GD 13-20.

Fig. B-4. Benchmark Dose Modeling for Skeletal Malformations

Fig. B-5. Benchmark Dose Modeling for Fetal/Pup Body Weight Changes Using Pooled Data Set

Fig B-6. Benchmark Dose Modeling for Fetal/Pup Body Weight Changes Using Individual Data Sets

Fig. B-7. Optimization of Dose-Response Correlation Across Routes of Exposure

Supplement A: PBPK Model Figures

Fig.A1-1.

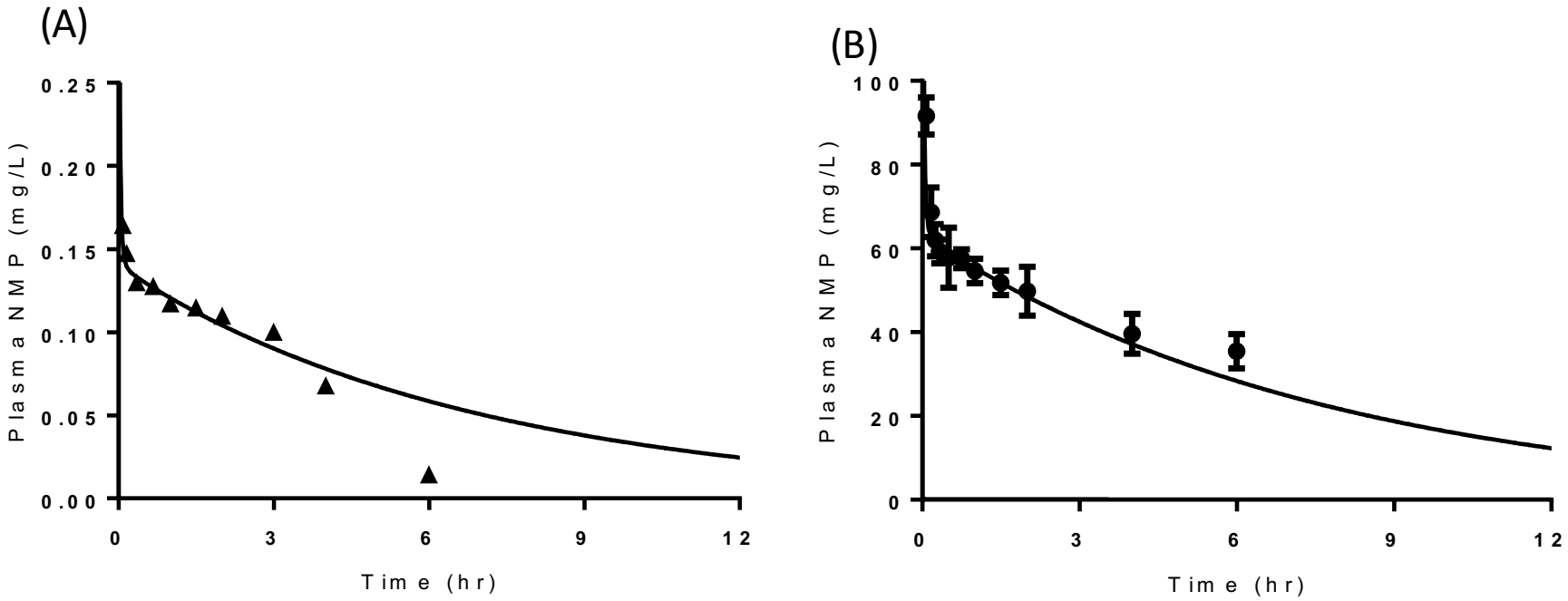

(A)
0.25
0.20
0.15
0.10
0.05
0.00
Plasma NMP (mg/L)
0
3
6
9
12
Time (hr)
(B)
100
80
60
40
20
0
Plasma NMP (mg/L)
0
3
6
9
12
Time (hr)

Fig.A1-2.

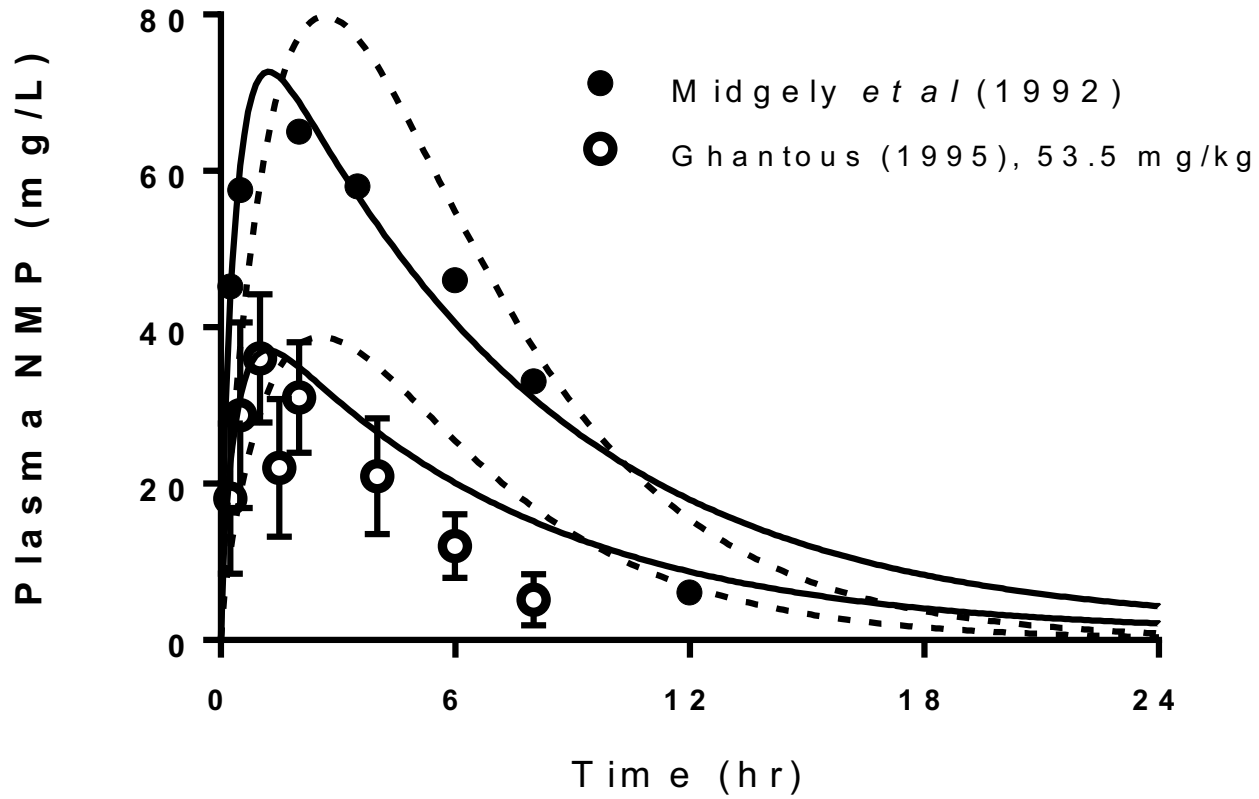

Plasma NMP (mg/L)
80
60
40
20
0
0
6
12
18
24
Time (hr)
Midgely *et al* (1992)
Ghantous (1995), 53.5 mg/kg

Fig.A1-3.

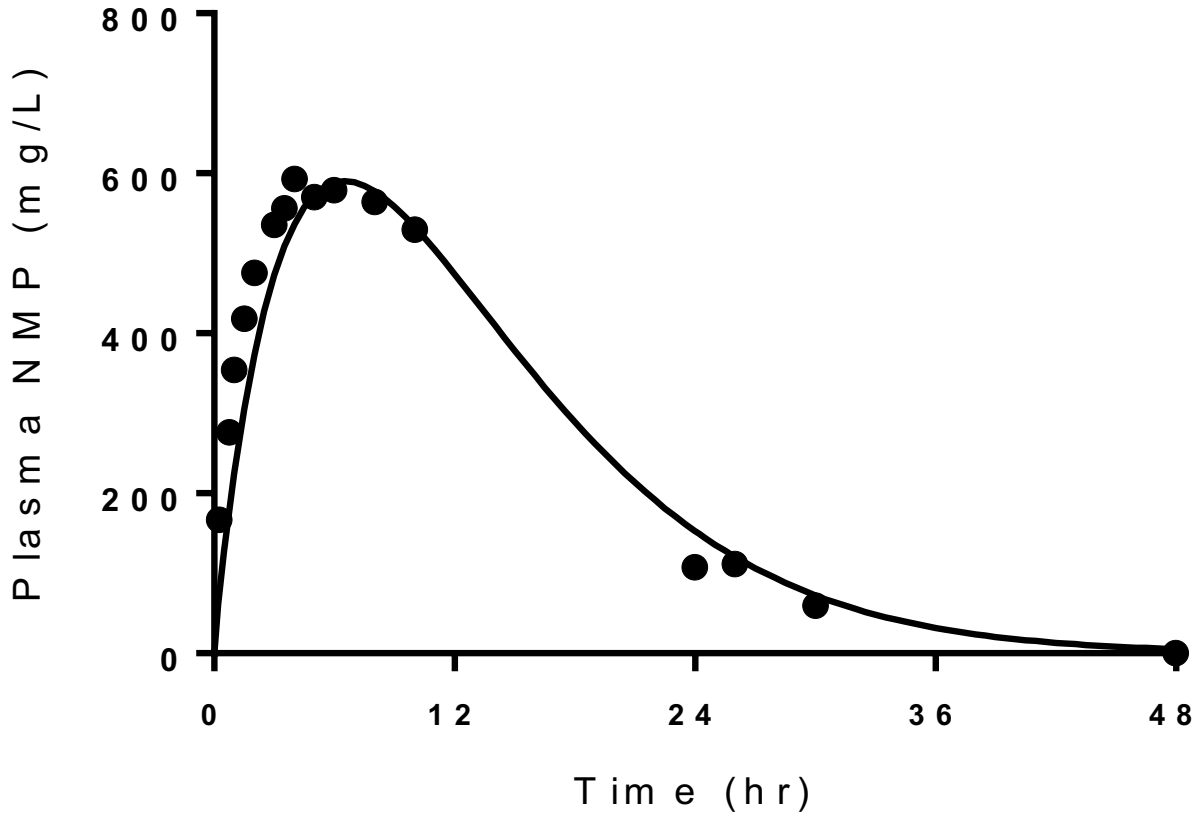
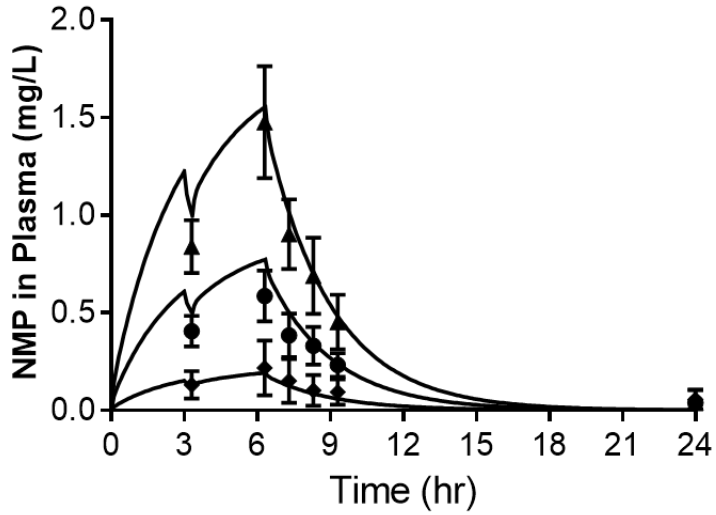
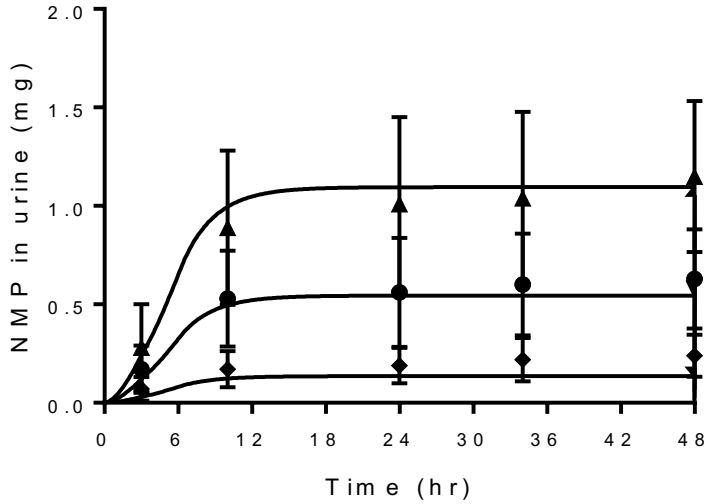
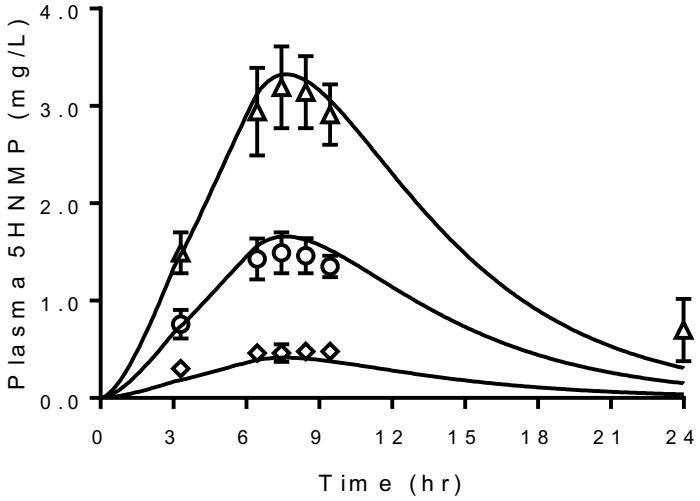


Fig. A1-4.


NMP in Plasma (mg/L)
2.0
1.5
1.0
0.5
0.0
0 3 6 9 12 15 18 21 24
Time (hr)


NMP in urine (mg)
2.0
1.5
1.0
0.5
0.0
0 6 12 18 24 30 36 42 48
Time (hr)


Plasma 5HNMP (mg/L)
4.0
3.0
2.0
1.0
0.0
0 3 6 9 12 15 18 21 24
Time (hr)

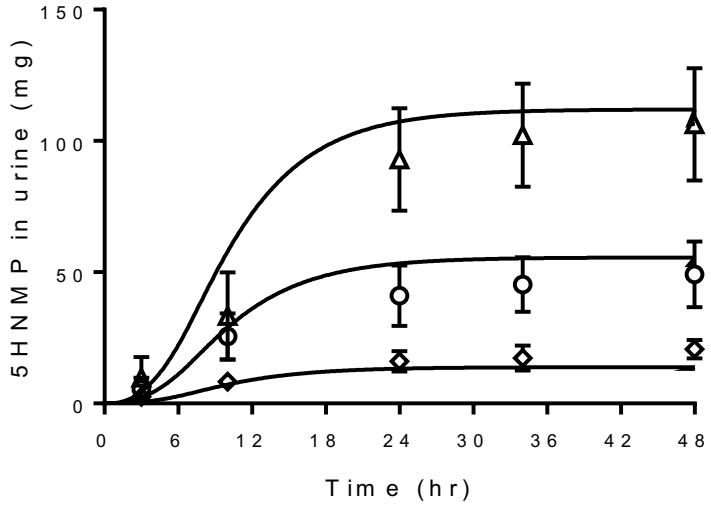

5HNMP in urine (mg)
150
100
50
0
0 6 12 18 24 30 36 42 48
Time (hr)

Fig. A1-5.

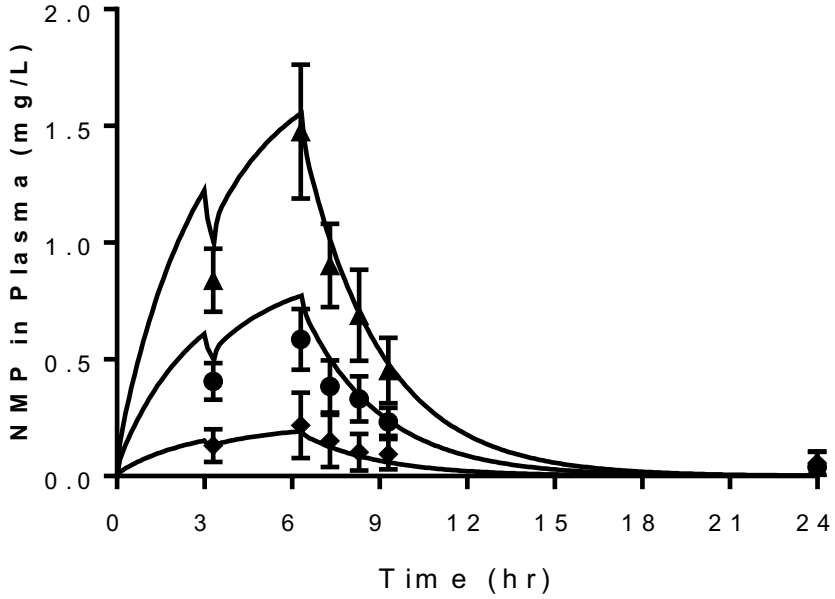


Fig. A1-6.

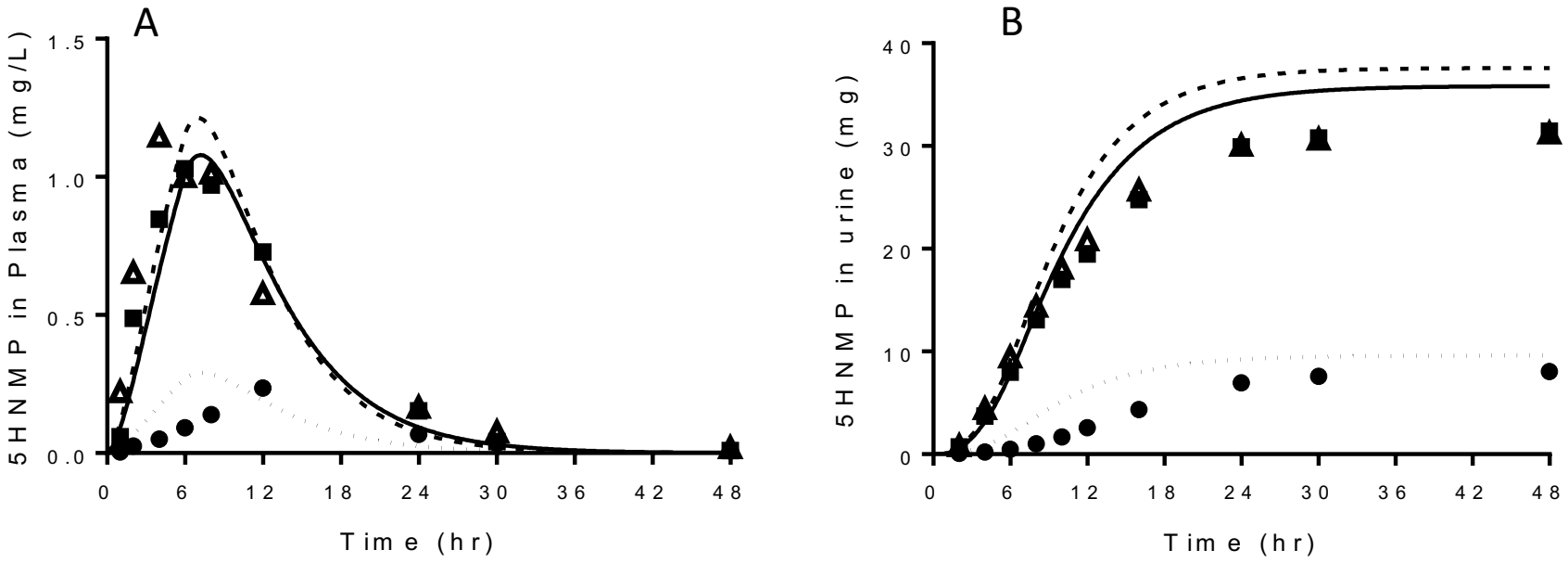

A
5HNMP in Plasma (mg/L)
1.5
1.0
0.5
0.0
0
6
12
18
24
30
36
42
48
Time (hr)
B
5HNMP in urine (mg)
40
30
20
10
0
0
6
12
18
24
30
36
42
48
Time (hr)

Fig. A1-7.

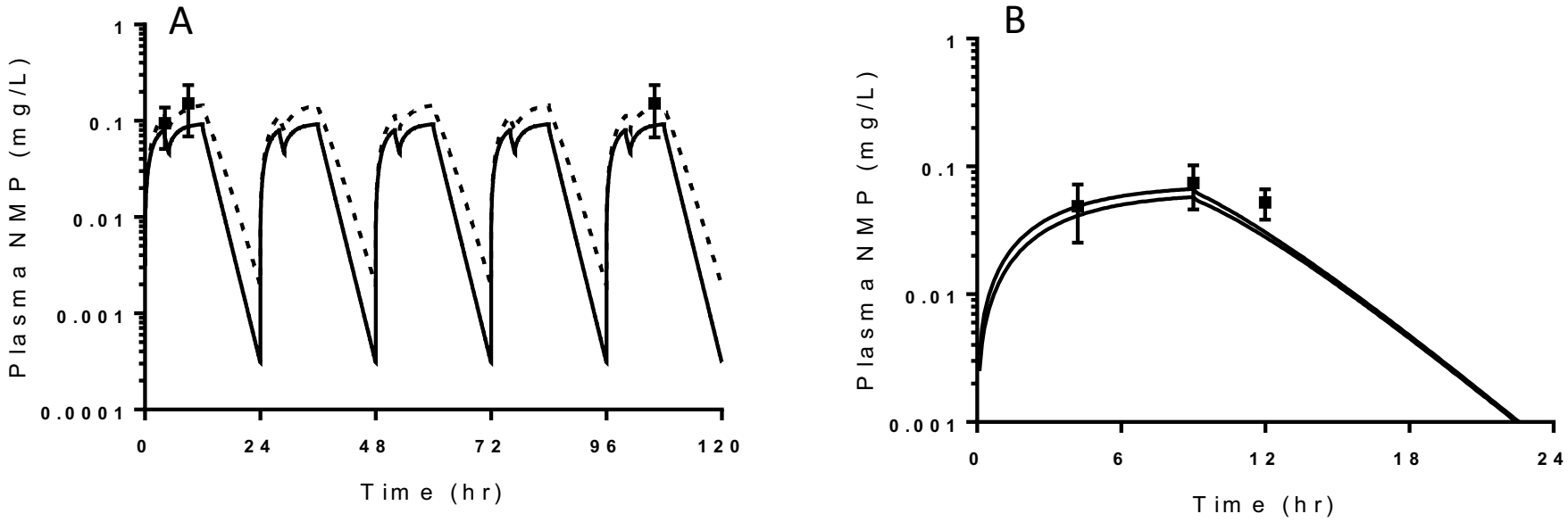

A
Plasma NMP (mg/L)
1
0.1
0.01
0.001
0.0001
0
24
48
72
96
120
Time (hr)
B
Plasma NMP (mg/L)
1
0.1
0.01
0.001
0
6
12
18
24
Time (hr)

Fig. A2-1.

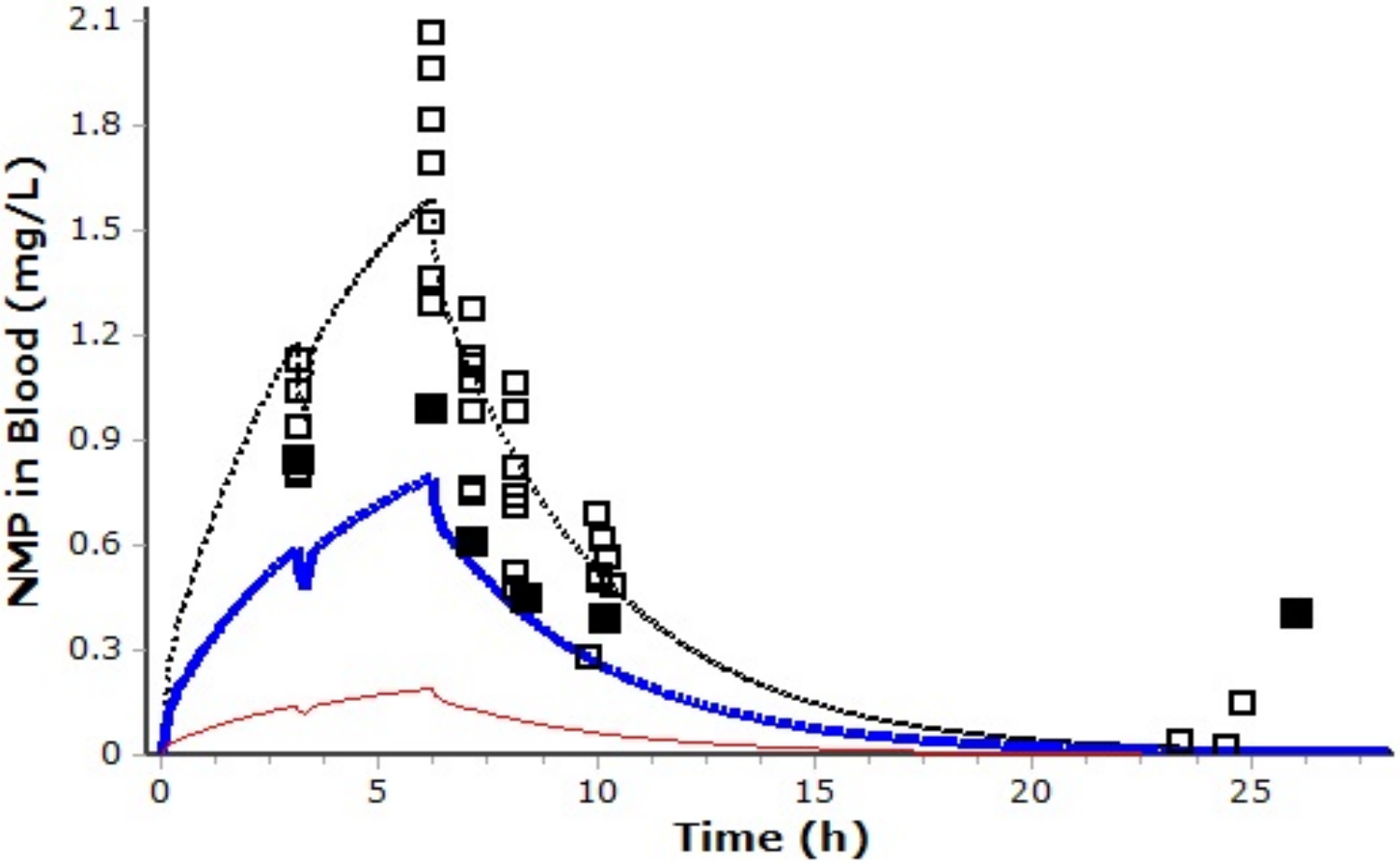


Fig. A2-2.

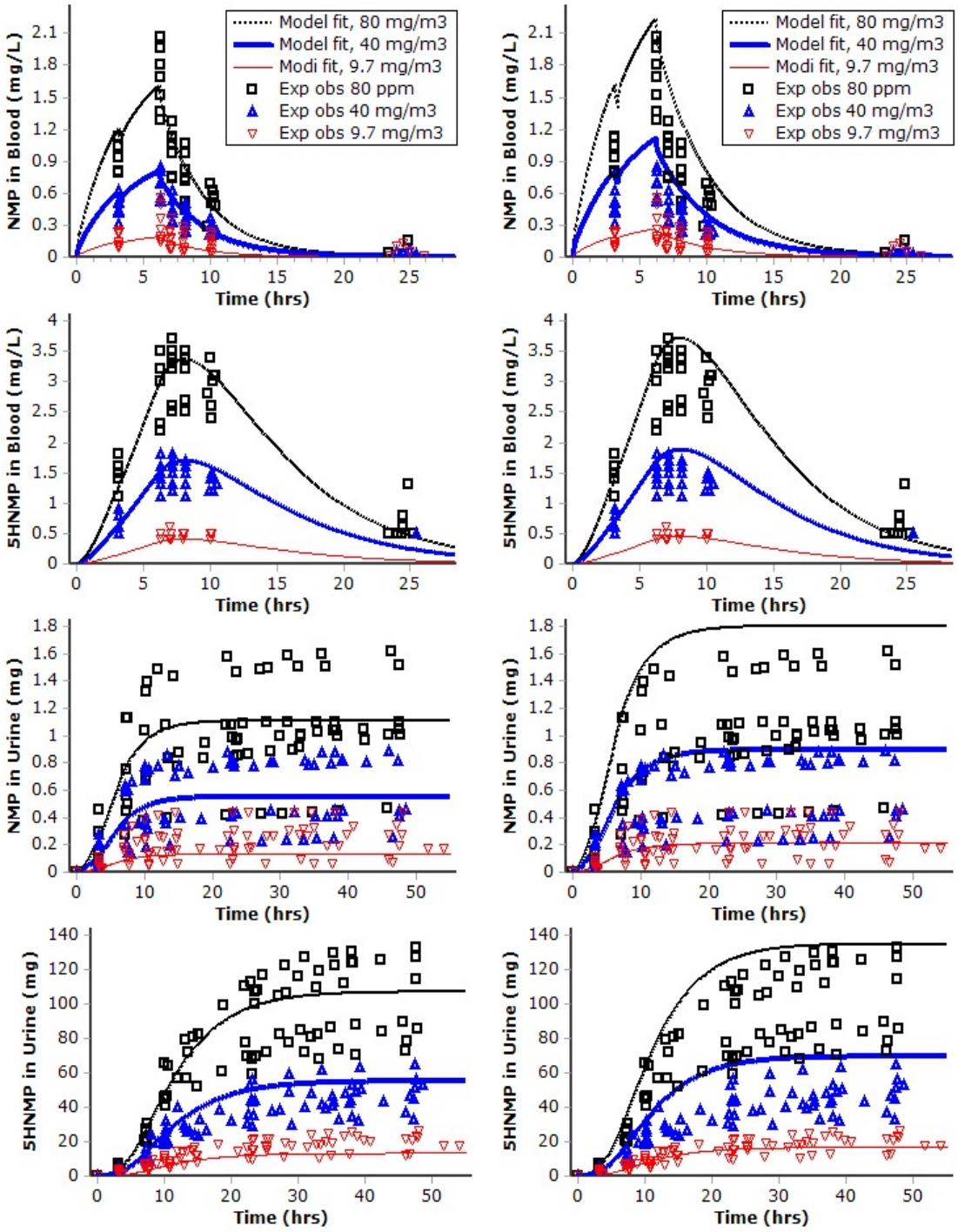

Model fit, 80 mg/m3
Model fit, 40 mg/m3
Modi fit, 9.7 mg/m3
Exp obs 80 ppm
Exp obs 40 mg/m3
Exp obs 9.7 mg/m3
NMP in Blood (mg/L)
5HNMP in Blood (mg/L)
NMP in Urine (mg)
5HNMP in Urine (mg)
Time (hrs)

Fig. A2-3.

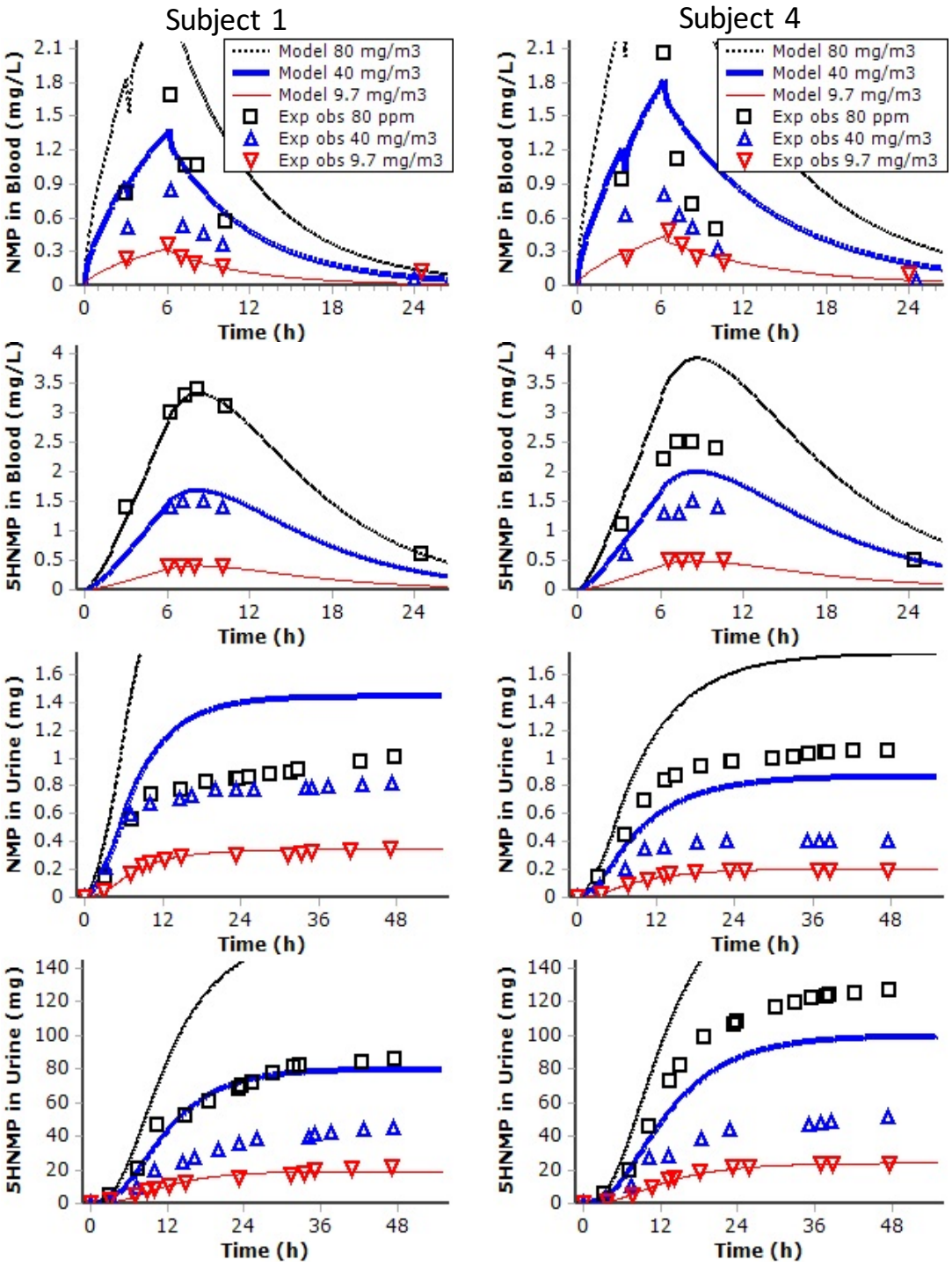

Subject 1
Subject 4
Model 80 mg/m3
Model 40 mg/m3
Model 9.7 mg/m3
Exp obs 80 ppm
Exp obs 40 mg/m3
Exp obs 9.7 mg/m3
NMP in Blood (mg/L)
SHNMP in Blood (mg/L)
NMP in Urine (mg)
SHNMP in Urine (mg)
Time (h)

Fig. A2-3 (cont'd).

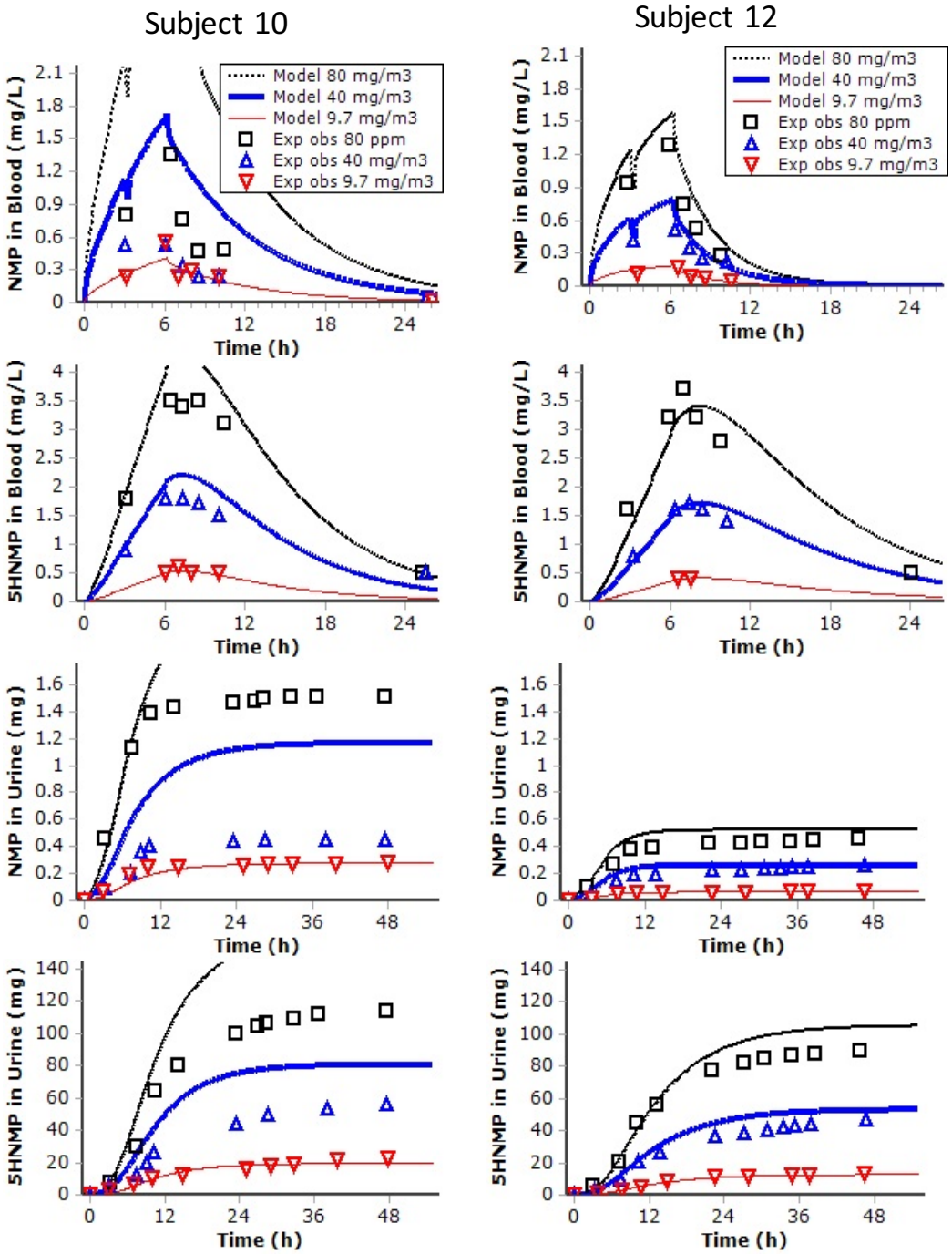


Fig. A2-3 (cont'd).

Subject 14 (also used 80 mg/m3 data)

Subject 16 (also used 80 mg/m3 data)

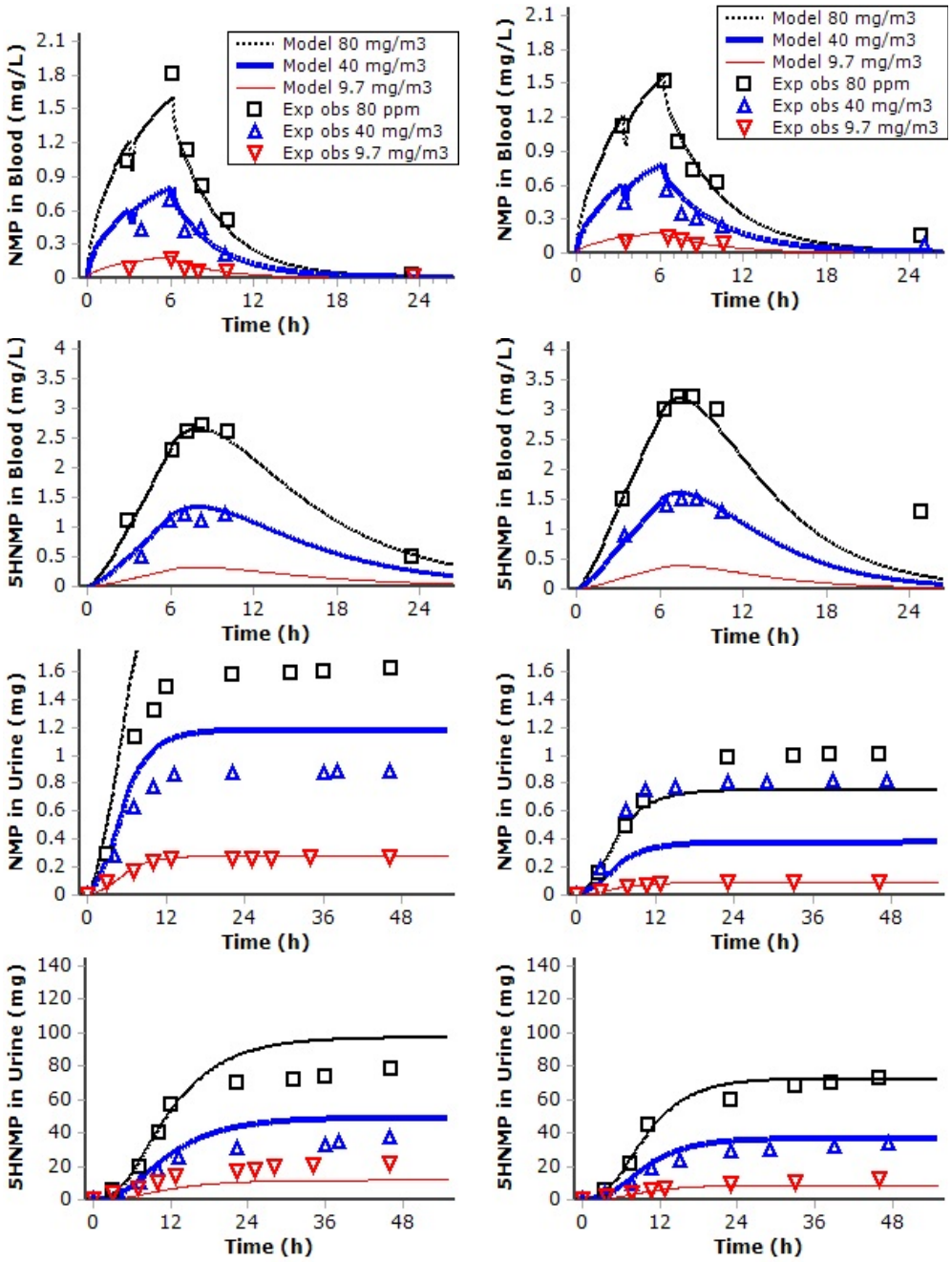
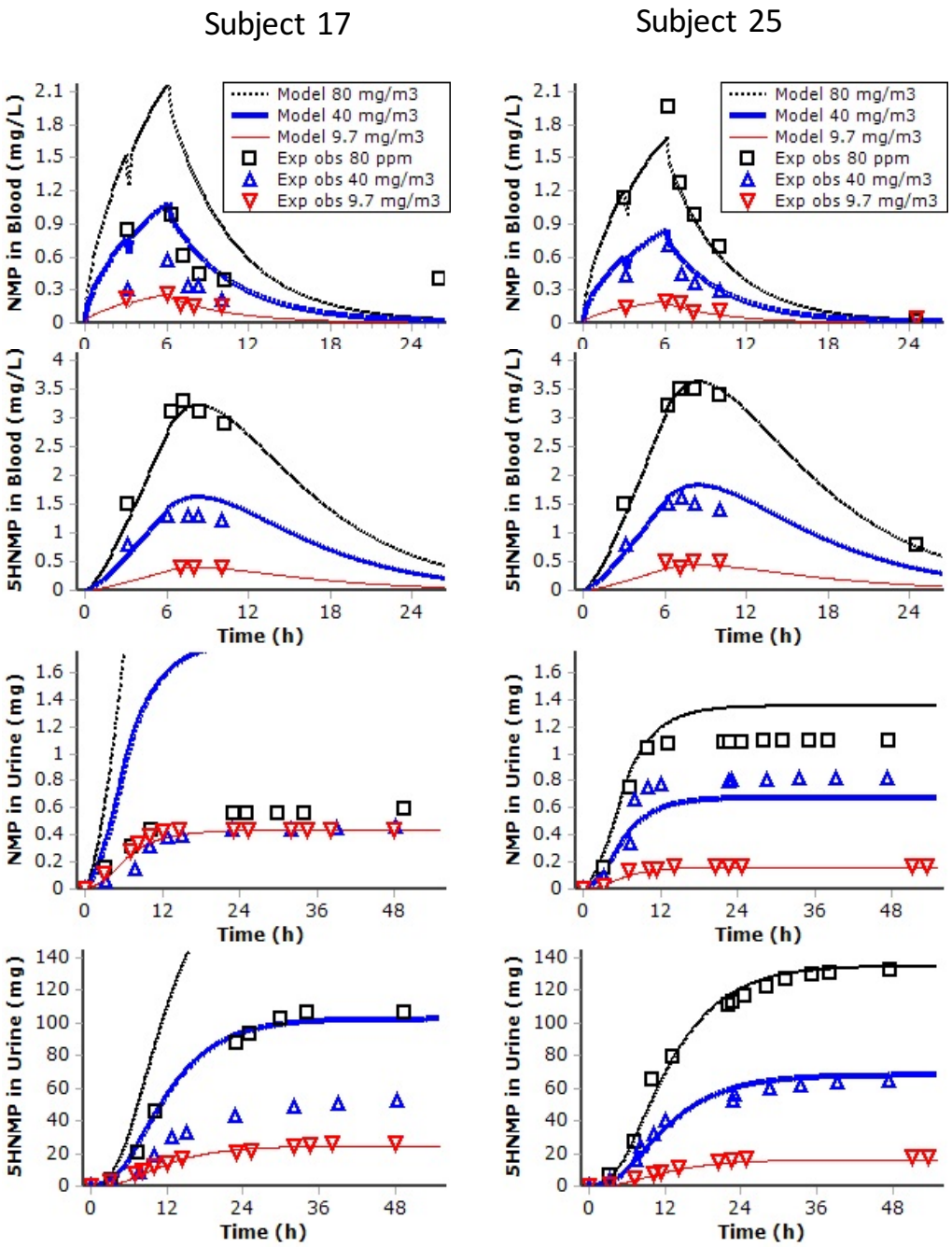


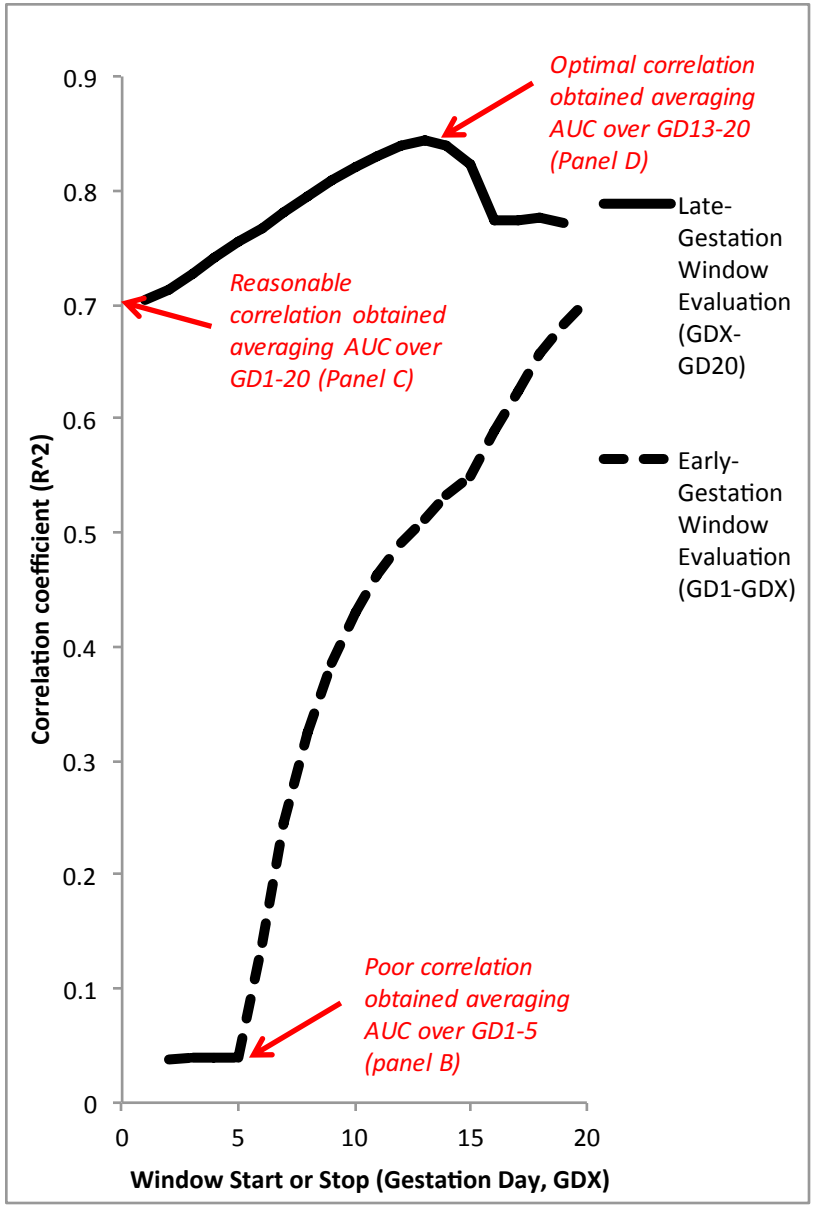
Fig. A2-3 (cont'd).



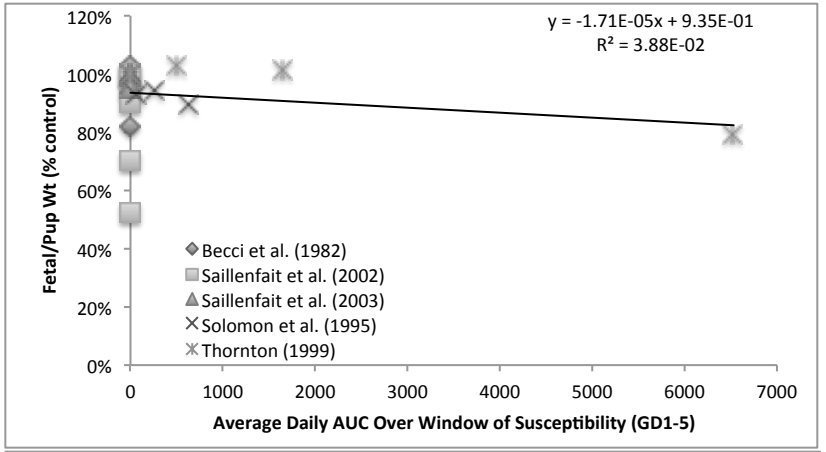
Supplement B: BMD Figures

Fig. B-1. Window of Susceptibility for Fetal Body Weight Effects

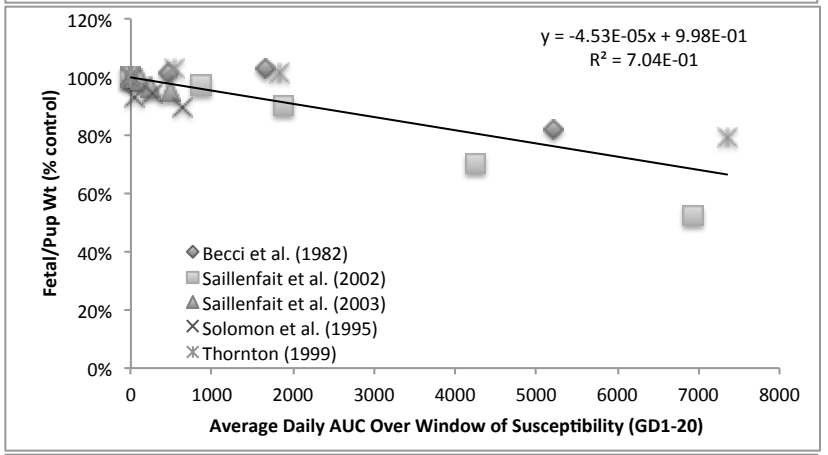
(A)



(B)



(C)



(D)

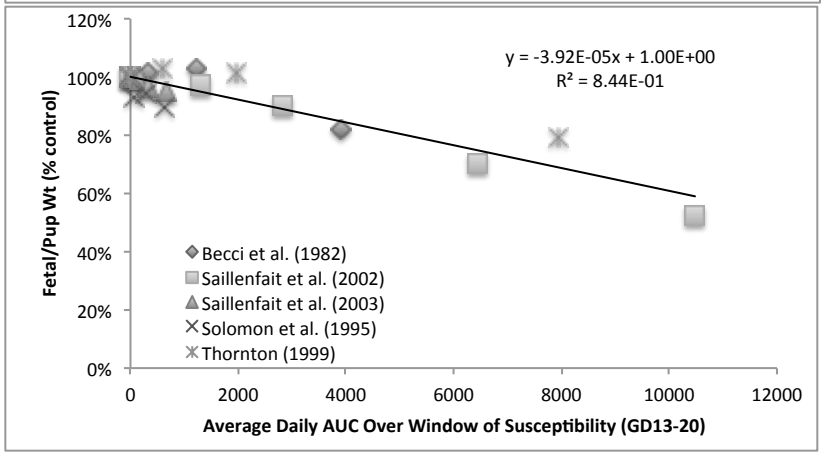
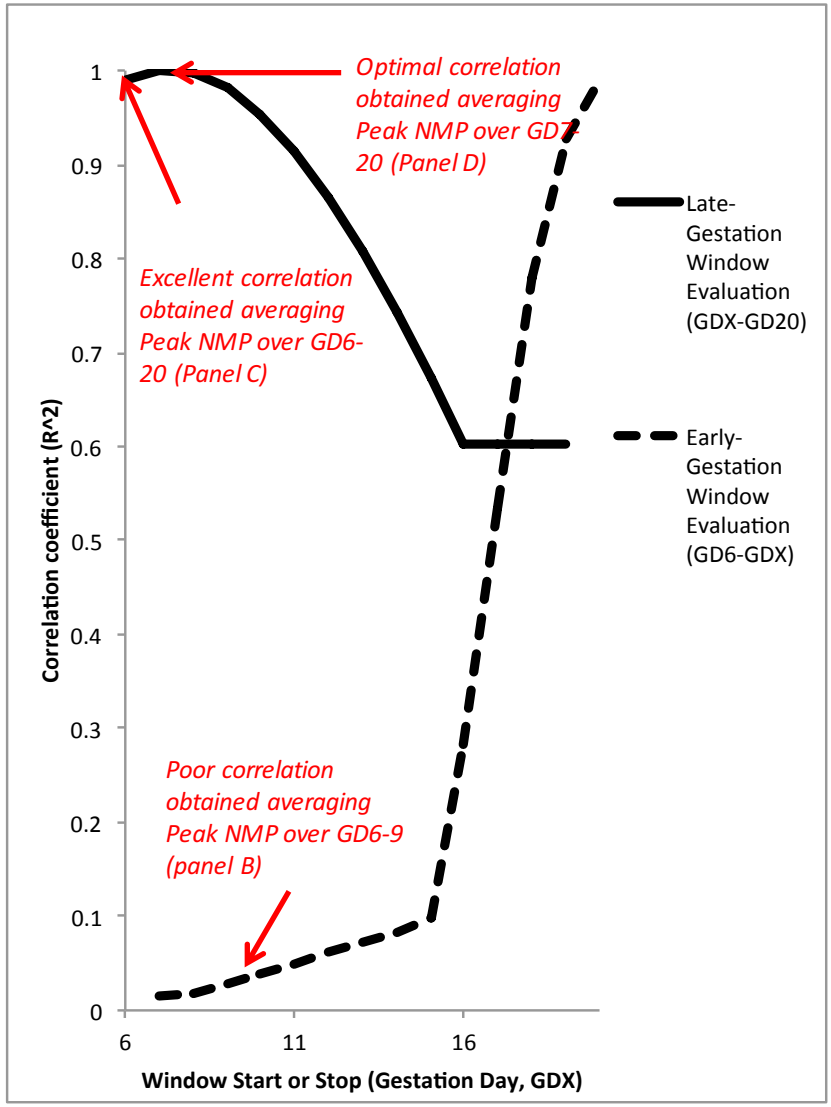
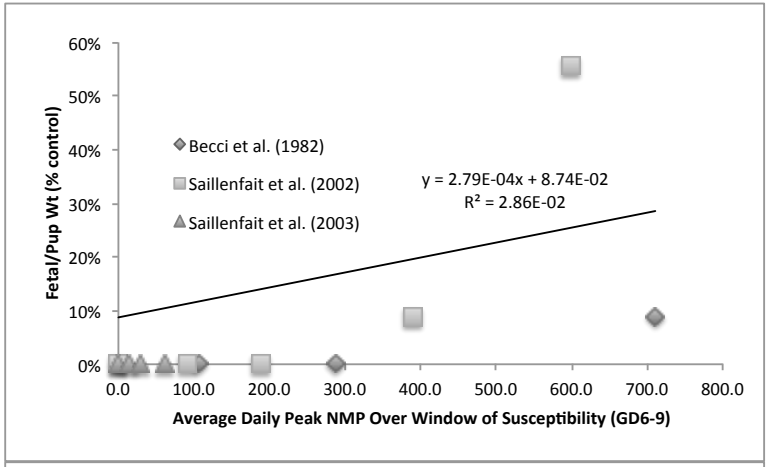


Fig. B-2. Window of Susceptibility for Skeletal Malformations

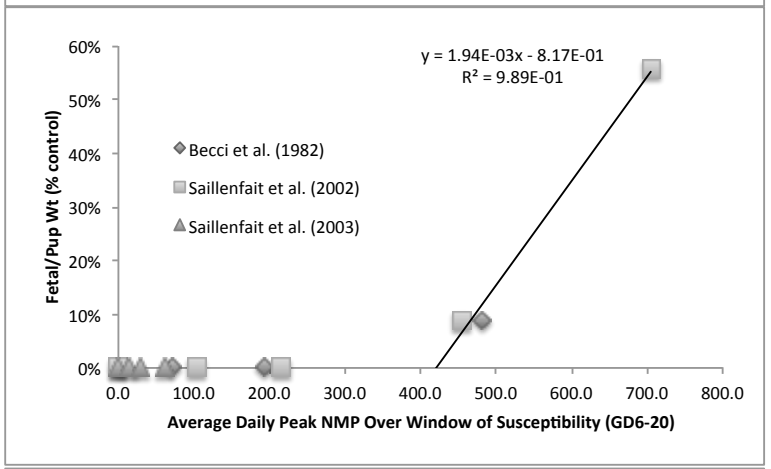
(A)



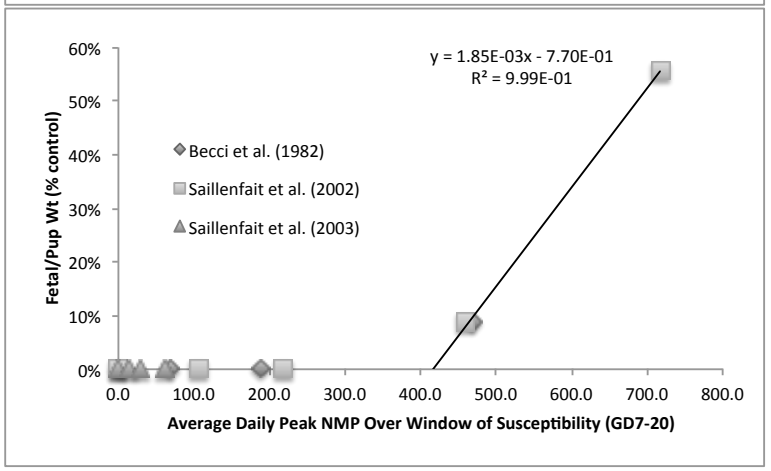
(B)



(C)



(D)



Alternate Fig. B-3. Fetal Growth over Gestation

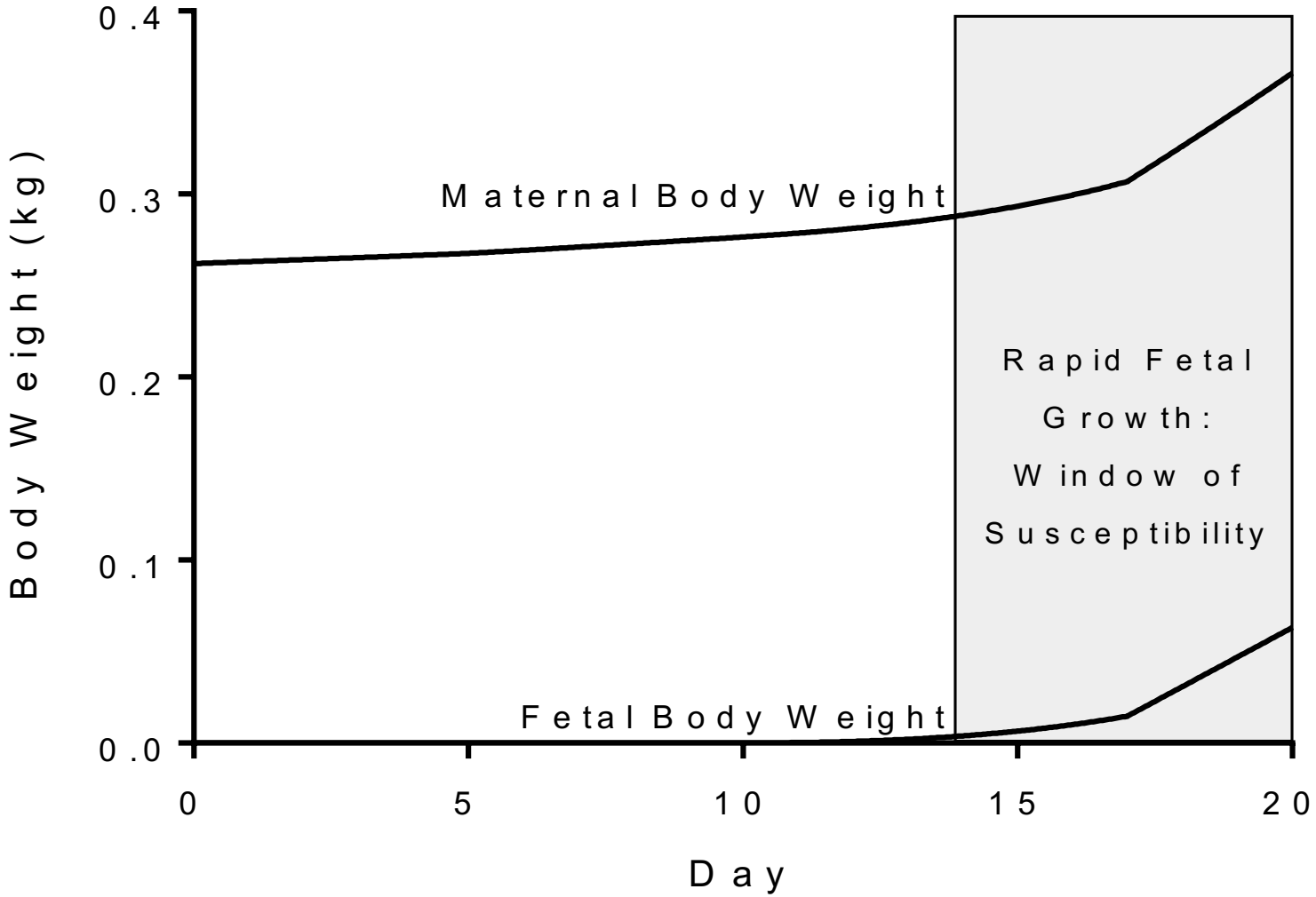
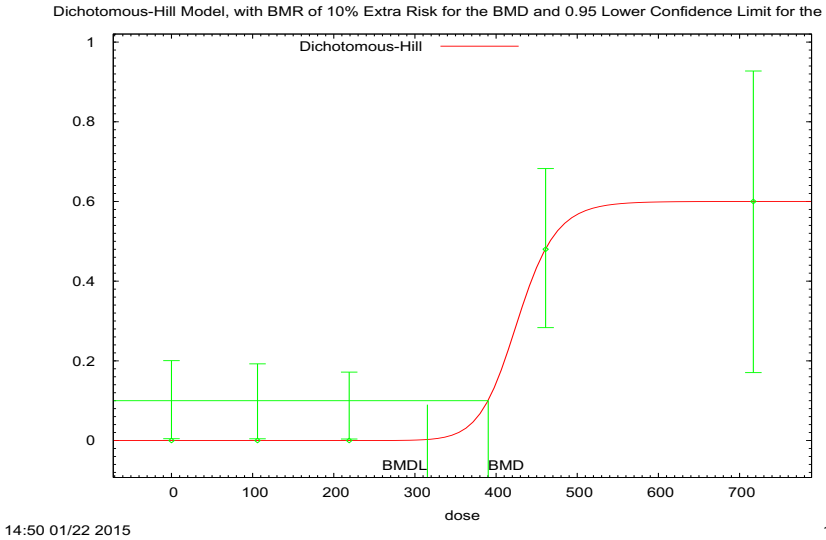


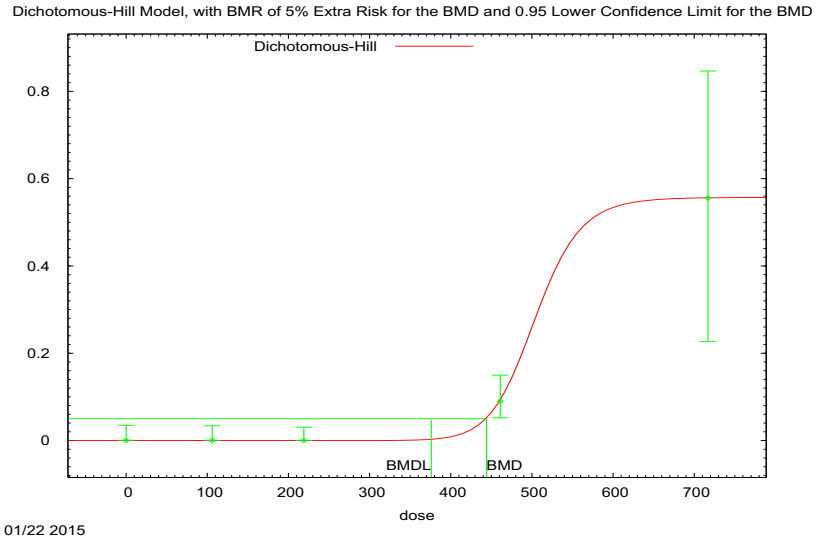
Fig.B-4. BMD Skeletal Malformations

Litter Incidence

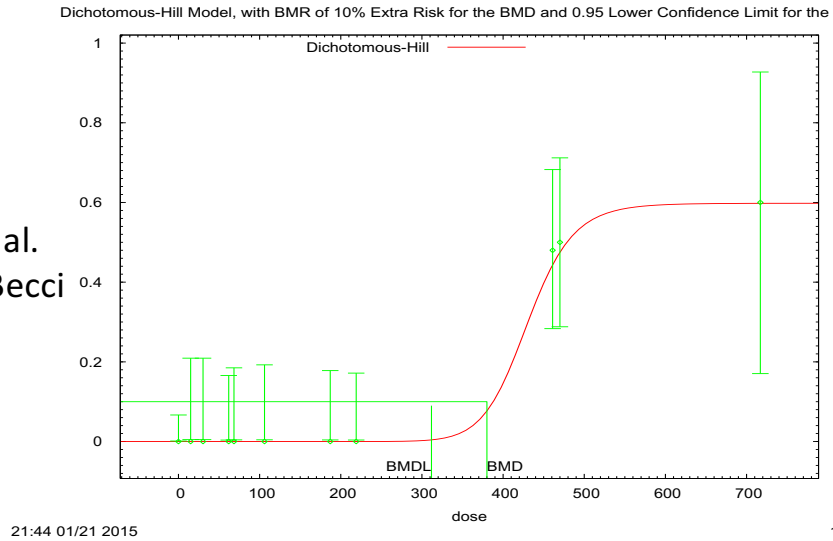
Fetal Incidence

Saillenfait et al. 2002





Pooled Data (Saillenfait et al. 2002, 2003; Becci et al. 1982) Combined



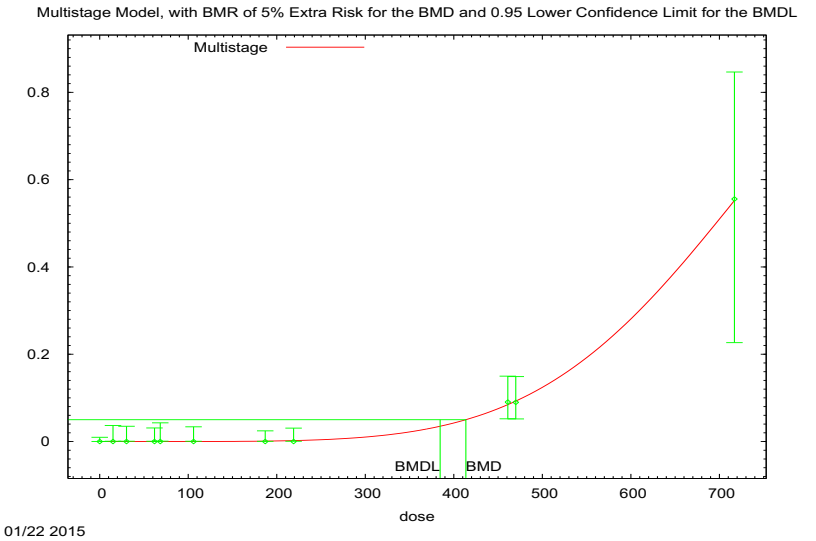


Fig.B-5. BMD Fetal/Pup Body Weight Changes Using Pooled Data

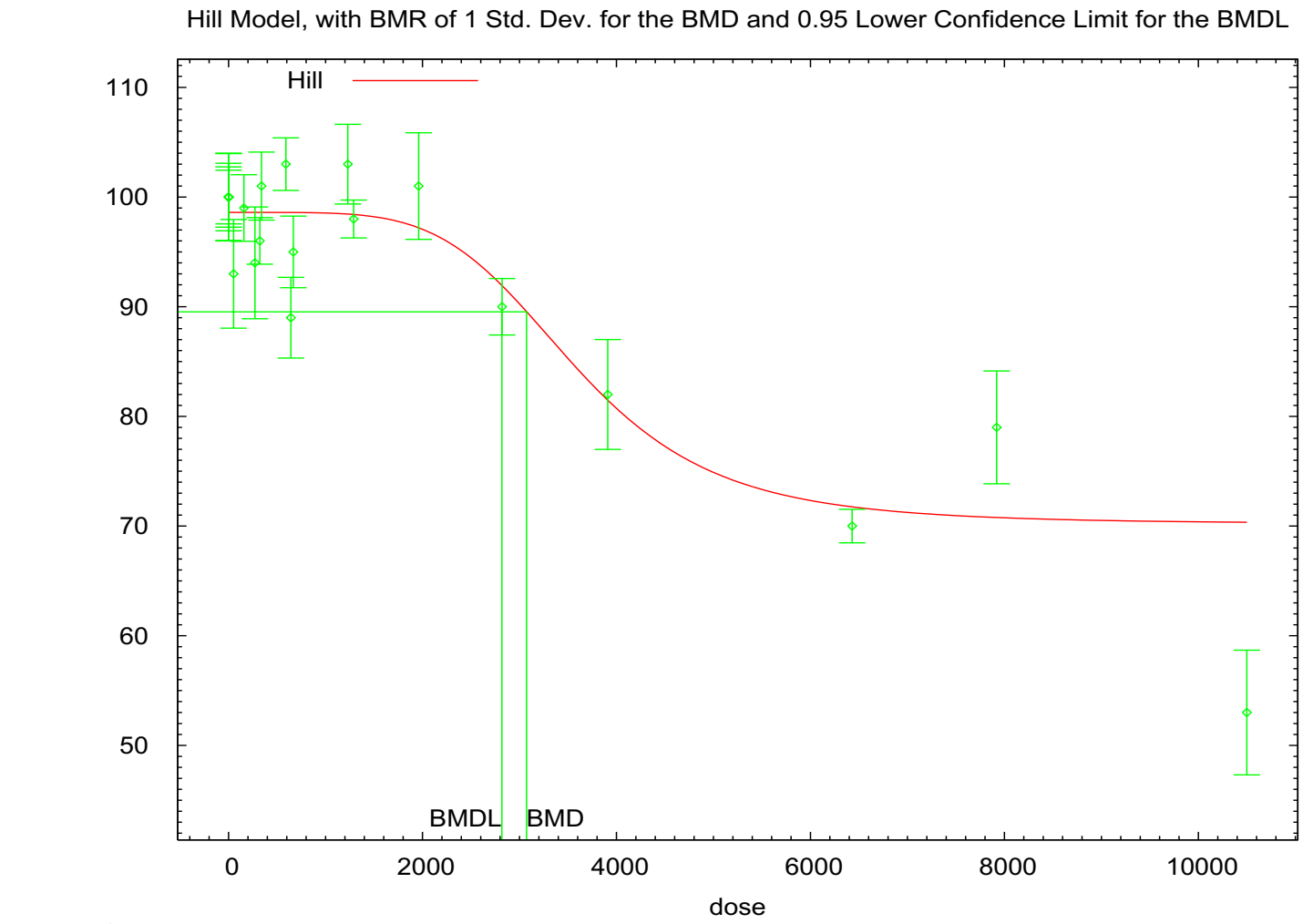
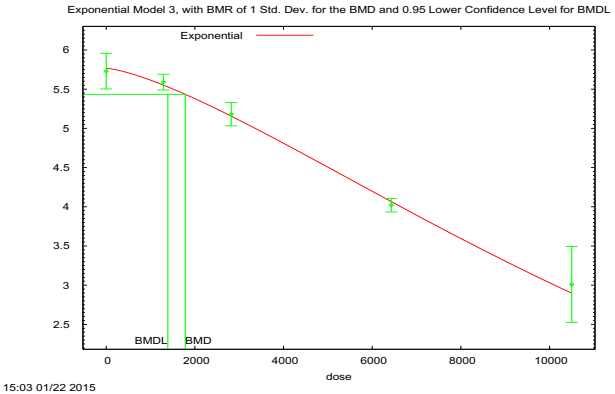
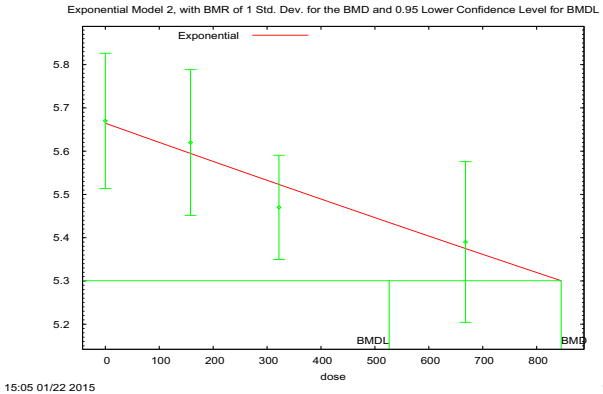


Fig.B-6. BMD Fetal/Pup Body Weight Changes

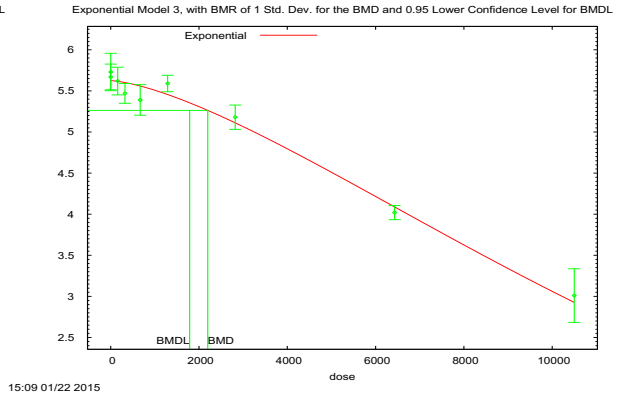
Saillenfait et al. 2002



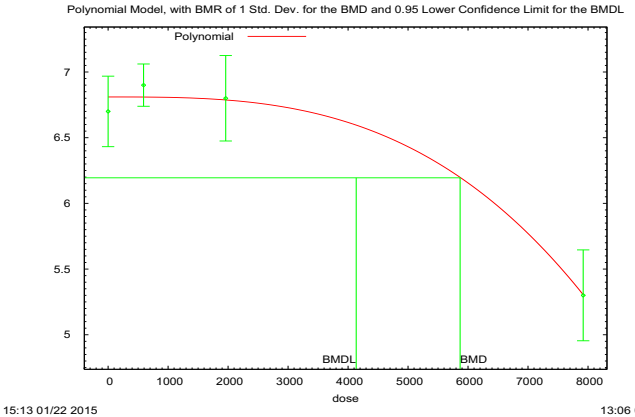
Saillenfait et al. 2003



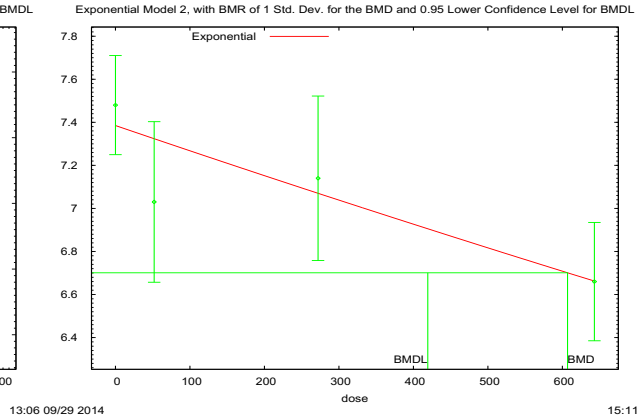
Saillenfait et al. 2002, 2003 Combined



Thornton 1999



Solomon et al. 1995



Becci et al. 1982

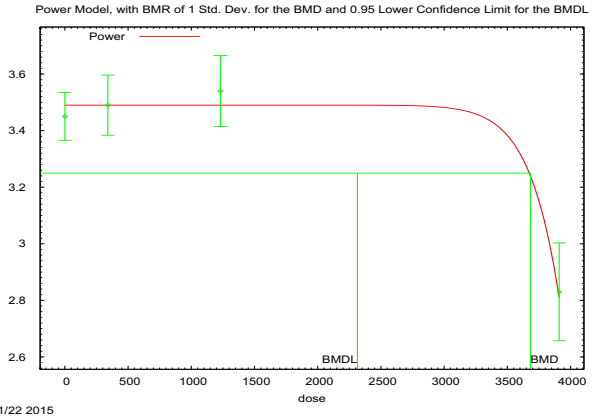
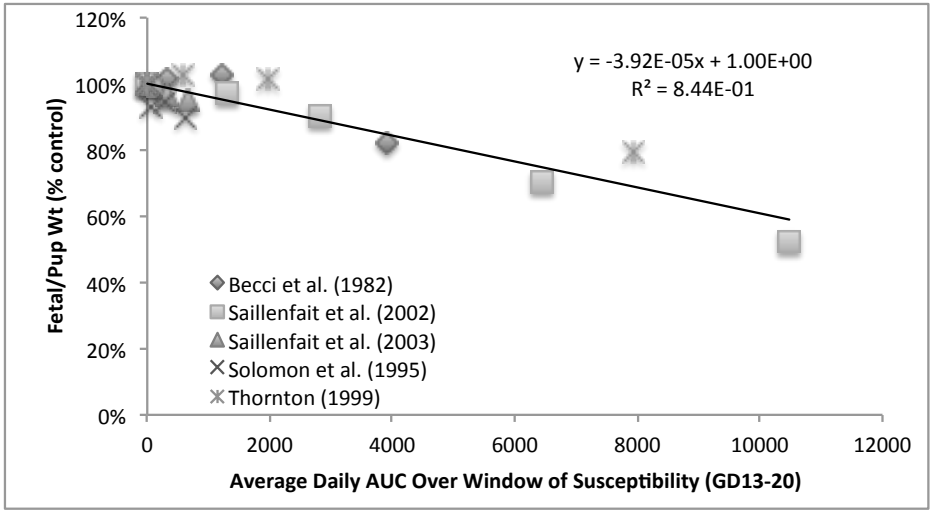


Fig. B-7. Optimization of Dose-Response Data Across Routes

(A) Unadjusted



(B) Adjusted for each exposure route