Supporting information

Evaluation of interindividual human variation in bioactivation and DNA adduct formation of estragole in liver predicted by physiologically based kinetic/dynamic (PBK/D) and Monte Carlo modeling

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Table S1: PBK model parameters used in the rat and human model

Physiological parameters[a]			**Tissue: blood partition coefficients**[b]		
	Rat	Human		Rat	Human
Body weight (kg)	0.2	70	estragole		
Percentage of body weight			Liver	2.4	6.5
Liver	3.4	2.6	Lung	2.4	na
Lungs	0.5	na	Kidney	2.4	na
Kidney	0.7	na	Fat	76.9	105
Fat	7.0	21.4	Rapidly perfused	2.4	6.5
Rapidly perfused	4.4	3.8	Slowly perfused	0.8	4.0
Slowly perfused	75	59.6	1'-hydroxyestragole		
Arterial blood	1.85	1.98	Liver	1.1	1.6
Venous blood	5.55	5.93			
			Kinetic constants	Km[1]/ Vmax[2]	Km[1]/ Vmax[2]
Cardiac output (L hr^{-1} ($BW^{0.74}$))	15.0	15.0	1'-hydroxylation estragole	116/ 1.48	21/ 0.73
Percentage of cardiac output			3'-hydroxylation estragole	93/ 1.05	350/ 35
Liver	17.4	22.7	epoxidation estragole	154/ 2.16	83/ 0.85
Kidney	14.1	17.5	*O*-demethylation estragole	458/ 0.85	290/ 1.35
Fat	7.0	5.2	sulfonation 1'-hydroxyestragole	33.4/ 0.2	See Table 2
Rapidly perfused	44.5	29.8	glucuronidation 1'-hydroxyestragole	51.5/ 203.4	708/ 0.3
Slowly perfused	17	24.8	oxidation 1'-hydroxyestragole	na	See Table 2
Absorption rate constant					
k_a[3]	1	1			

[1] µmol
[2] nmol min^{-1} mg S9 or microsomal $protein^{-1}$
[3] hr^{-1}

Code S1: PBK model code human model

{PBK model code for the human physiologically based biokinetic (PBBK) model using Berkeley Madonna Software (Copyright ©1993-2001 Robert I. Macey & George F. Oster - http://www.berkeleymadonna.com/):

The model is based on a previously developed PBK model for estragole in human by Punt et al., 2009 (Toxicol Sci. 2009 Aug; 110(2):255-69.)}

```
;==================================================================================
;Physiological parameters
;==================================================================================
;Tissue volumes
BW = 60 {Kg}                    ; body weight human
 VLc = 0.026                    ; fraction of  liver tissue
 VLuc = 0.008                   ; fraction of lung tissue
 VFc = 0.214                    ; fraction of fat tissue
 VAc =  0.02                    ; fraction of arterial blood: 0.079*1/4
 VVc = 0.059                    ; fraction of venous blood: 0.079*3/4
 VRc = 0.08-VLc-VLuc            ; fraction of richly perfused tissue
 VSc = 0.836-VFc-VAc-VVc        ; Fraction of blood flow to slowly perfused tissue
                                ; total of fractions = 0.916
VL = VLc*BW {L or Kg}
VLu= VLuc*BW
VF = VFc*BW
VR = VRc*BW
VS = VSc*BW
VA = VAc*BW
VV = VVc*BW
;----------------------------------------------------------------------------------------------------------------------------------
;Blood flow rates
QCc = 15          ; Info: QC=15*BW^0.74
QC = QCc*BW**0.74 {L/hr}        ; Info: QC=15*BW^0.74
QLc = 0.227                     ; Fraction of blood flow to liver
QFc = 0.052                     ; Fraction of blood flow to fat
QRc = 0.70-QLC                  ; Fraction of blood flow to richly perfused tissue
QSc = 0.30-QFC                  ; Fraction of blood flow to slowly perfused tissue
                                ; total of fractions = 1
QL = QLc*QC {L/hr}
QF = QFc*QC
QR = QRc*QC
QS = QSc*QC

;==================================================================================
;Partition Coefficients
;==================================================================================
;estragole
PLE = 6.5                       ; liver/blood partition coefficient
PLuE=6.5                        ; lung/blood partition coefficient
PKE=6.5                         ; kidney/blood partition coefficient
PFE = 105                       ; fat/blood partition coefficient
PRE = 6.5                       ; richly perfused tissues/blood partition coefficient
PSE = 4.1                       ; slowly perfused tissues/blood partition coefficient
;1'-hydroxyestragole
PLHE = 1.6                      ;liver/blood partition coefficient

;==================================================================================
```

```
;Biochemical parameters
;==========================================================================================

;Linear uptake rate (hr-1)
Ka = 1
;-----------------------------------------------------------------------------------------------------------------------------------
;Metabolism liver
;Scaling factors
S9PL=143;       Liver S9 protein yield (mg/gram liver)
MPL=32;         Liver microsomal protein yield (mg/gram liver)
L=VLC*1000;     Liver = 26 (gram/kg BW)

;metabolites of estragole, unscaled maximum rate of metabolism (nmol min-1 (mg protein)-1)
VmaxLHEc = 0.73                 ;HE = 1'-hydroxyestragole,
VmaxLAPc = 0.38                 ;AP = 4-allylphenol
VmaxLEEc = 0.85                 ;EE = estragole-2',3'-oxide
VmaxLHAc = 1.35;                ;HA = 3'-hydroxyanethole

;metabolites of estragole, scaled maximum rate of metabolism (umol hr-1)
VMaxLHE = VMaxLHEc/1000*60*MPL*L*BW
VMaxLAP = VMaxLAPc/1000*60*MPL*L*BW
VMaxLEE = VMaxLEEc/1000*60*MPL*L*BW
VMaxLHA = VMaxLHAc/1000*60*MPL*L*BW

;metabolites of estragole, affinity constants (umol/L)
KmLHE = 21
KmLAP = 290
KmLEE = 83
KmLHA = 350

;metabolites of 1'-hydroxyestragole, unscaled maximum rate of metabolism (nmol min-1 (mg protein)-1)
VmaxLHEGc = 0.3         ; HEG = 1'-hydroxyestragole glucuronide
{1'-sulfoxxyestragole by individual human liver microsomes}
        {Monte Carlo simulation}
        VmaxLHEScc = init(exp(normal(3.7,0.6)))
{nmol/min/mg microsomal protein, init is used to prevent Berkley Madonna to take a new value at each time point
during the run}
        VmaxLHESc  = IF VmaxLHEScc > 6.69 AND VmaxLHEScc < 244.69 THEN VmaxLHEScc  ELSE 100001
{Values higher or lower than 3 times the SD are removed}

{1'-oxoestragole by individual human liver microsomes}
        {Monte Carlo simulation}
        VmaxLOEcc = init(exp(normal(1.1,0.3)))          ;nmol/min/mg microsomal protein
        VmaxLOEc  = IF VmaxLOEcc > 1.22 AND VmaxLOEcc < 7.39 THEN  VmaxLOEcc  ELSE 100001

;metabolites of 1'-hydroxyestragole, scaled maximum rate of metabolism (umol hr-1)
VMaxLHEG = VmaxLHEGc/1000*60*MPL*L*BW
VMaxLOE = VmaxLOEc/1000*60*S9PL*L*BW
VMaxLHES = VmaxLHESc/1000*60*S9PL*L*BW

;metabolites of 1'-hydroxyestragole, affinity constants (umol/L)
KmLHEG = 708
{1'-sulfoxxyestragole by individual human liver microsomes}
        {Monte Carlo simulation}
        KmLHESc = init(exp(normal(5.4,0.4)))            ;umol/L
        KmLHES  = IF KmLHESc > 66.36 AND KmLHESc < 738.75 THEN  KmLHESc  ELSE 100001
```

```
{1'-oxoestragole by individual human liver microsomes}
        {Monte Carlo simulation}
        KmLOEc  = init(exp(normal(6.2,0.3)))              ;umol/L
        KmLOE  = IF KmLOEc > 200.34 AND KmLOEc < 1211.87 THEN  KmLOEc  ELSE 100001

;==============================================================================
;Run settings
;==============================================================================

;Molecular weight
MWE = 148.2;                    Molecular weight estragole
MWHE=164.2;                     Molecular weight 1'-hydroxyestragole

;Given dose (mg/ kg bw) and oral dose umol/ kg bw}
GDOSE = 0.07                            ;GDOSE = given dose mg/ kg bw
ODOSE = (GDOSE*1E-3)/MWE*1E6      ; ODOSE = given dose recalculated to umol/kg bw
DOSE=ODOSE*BW;                                          ; DOSE = umol

;Time
Starttime = 0;                  in hrs
Stoptime = 24;                  in hrs

;==============================================================================
;Dynamics
;==============================================================================
;slowly perfused tissue compartment

;AS = Amount estragole in slowly perfused tissue, umol
    AS' = QS*(CA-CVS)
    Init AS = 0
    CS = AS/VS
    CVS = CS/PSE
;------------------------------------------------------------------------------
;richly perfused tissue compartment

;AR = Amount estragole in richly perfused tissue, umol
    AR' = QR*(CA-CVR)
    Init AR = 0
    CR = AR/VR
    CVR = CR/PRE
;------------------------------------------------------------------------------
;fat compartment

;AF = Amount estragole in fat tissue, umol
    AF' = QF*(CA-CVF)
    Init AF = 0
    CF = AF/VF
    CVF = CF/PFE

;------------------------------------------------------------------------------
;uptake estragole from GI tract

;AGI = Amount estragole remaining in GI tract (umol)
AGI' =-Ka*AGI
Init AGI = DOSE
```

```
;-----------------------------------------------------------------------------------------------------------------------------------------------------
;liver compartment

;estragole
;AL = Amount Estragole in liver tissue, umol
      AL' =  QL*(CA -CVL)+ Ka*AGI - AMLHE' - AMLAP' - AMLEE' -AMLHA'
      Init AL = 0
      CL = AL/VL
      CVL = CL/PLE

;AMLHE=Amount estragole metabolized in liver to 1'-hydroxyestragole (HE)
      AMLHE' = VmaxLHE*CVL/(KmLHE + CVL)
      init AMLHE = 0
      CMLHE =AMLHE/VL

;AMLAP=Amount estragole metabolized in liver to 4-allylphenol (AP)
      AMLAP' = VmaxLAP*CVL/(KmLAP + CVL)
      init AMLAP = 0
      CMLAP =AMLAP/VL

;AMLEE=Amount estragole metabolized in liver to estragole-2',3'-oxide (EE)
      AMLEE' = VmaxLEE*CVL/(KmLEE + CVL)
      init AMLEE = 0
      CMLEE =AMLEE/VL

 ;AMLHA=Amount estragole metabolized in liver to 3'-hydroxyanethole (HA)
      AMLHA' = VmaxLHA*CVL/(KmLHA + CVL)
      init AMLHA = 0
      CMLHA =AMLHA/VL


;1'-hydroxyestragole
;ALHE = amount 1'-hydroxyestragole in liver tissue, umol
      ALHE' = AMLHE' - AMLHEG' - AMLHES' - AMLOE'
      Init ALHE = 0
      CLHE = ALHE/VL
      CVLHE = CLHE/PLHE

;AMLHEG= amount 1'-hydroxyestragole metabolized in liver to 1'-hydroxyestragole glucuronide (HEG)
      AMLHEG' = VmaxLHEG*CVLHE/(KmLHEG + CVLHE)
      init AMLHEG = 0
      CLHEG = AMLHEG/VL

;AMLOE= amount 1'-hydroxyestragole metabolized in liver to 1'-oxoestragole (OE)
      AMLOE' = VmaxLOE*CVLHE/(KmLOE + CVLHE)
      init AMLOE = 0
      CLOE = AMLOE/VL

;AMLHES= amount 1'-hydroxyestragole metabolized in liver to 1'-sulfooxyestragole (HES)
      AMLHES' = VmaxLHES*CVLHE/(KmLHES + CVLHE)
      init AMLHES = 0
      CLHES = AMLHES/VL
```

```
;-----------------------------------------------------------------------------------------------------------------------------------------------
;lung compartment

;ALu = amount estragole in lung tissue, umol
      ALu' = QC*(CV-CALu)
      Init ALu = 0
      CLu = ALu/VLu
      CALu = CLu/PLuE
;-----------------------------------------------------------------------------------------------------------------------------------------------
; arterial blood compartment

;CA = Concentration arterial blood estragole
     AA' = QC*(CALu-CA);
     Init AA = 0
     CA =  AA/VA
;-----------------------------------------------------------------------------------------------------------------------------------------------
; venous blood compartment

;CV = Concentration venous blood estragole (umol/L)
     AV' = QF*CVF + QR*CVR + QS*CVS + QL*CVL  - QC*CV
     Init AV = 0
     CV = AV/VV

;==============================================================================
;Mass balance calculations
;==============================================================================
{Mass Balance}
Total = DOSE
Calculated = AF + AS + AR + AL +  ALu + AV+ AA + AGI + AMLEE + AMLHA+ AMLHE + AMLAP

ERROR=((Total-Calculated)/Total+1E-30)*100
MASSBBAL=Total-Calculated + 1

;==============================================================================
;Calculation with model
;==============================================================================;
DNAdGUO=629.8*CLHES
```

Figure S1: DNA adduct formation reported for rats exposed to methyleugenol

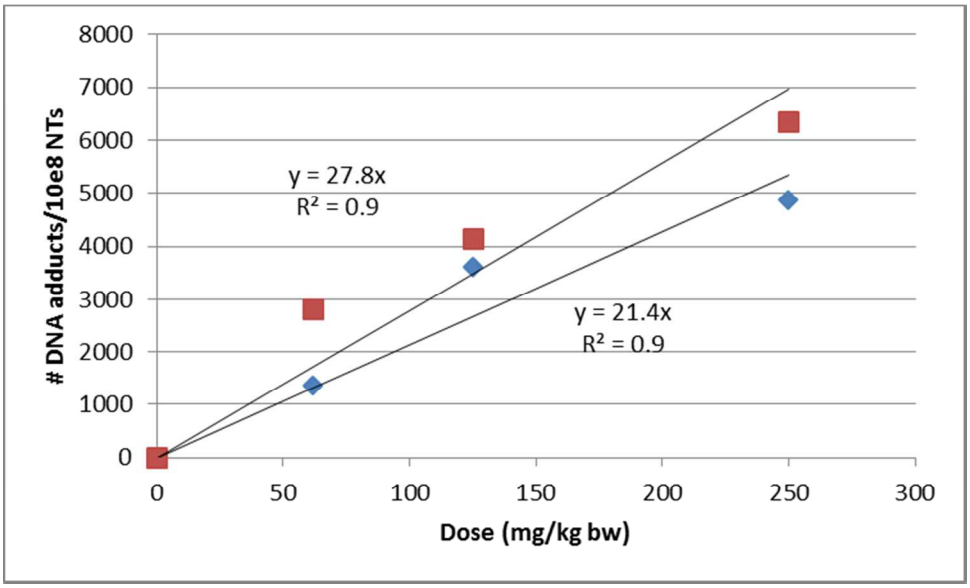

Figure S1. DNA adduct formation reported by Williams et al[1] in liver of rats exposed 3 days a week for 8 weeks (bleu) or 16 weeks (red) to different oral doses of methyleuenol. On average a linear increase in DNA adduct formation of 25 adducts in 10^8 nts per mg/kg bw was assumed.

Figure S2: DNA adduct formation reported for mice exposed to methyleugenol

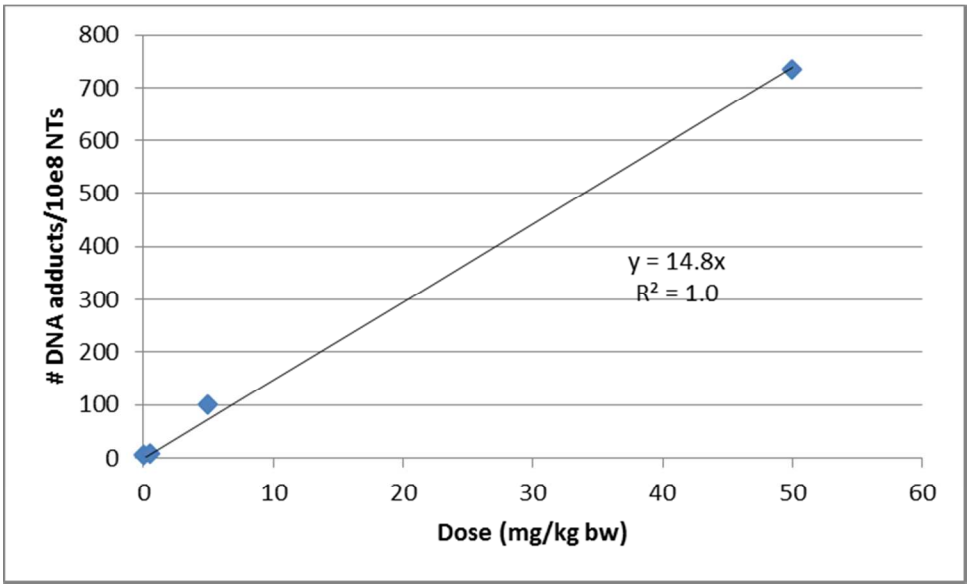

Figure S2. DNA adduct formation reported by Herrmann et al[2] in liver of mice exposed to single oral doses of methyleugenol. A linear increase in DNA adduct formation of 15 adducts in 10^8 nts per mg/kg bw was assumed.

Figure S3: DNA adduct formation reported for rats exposed to estragole

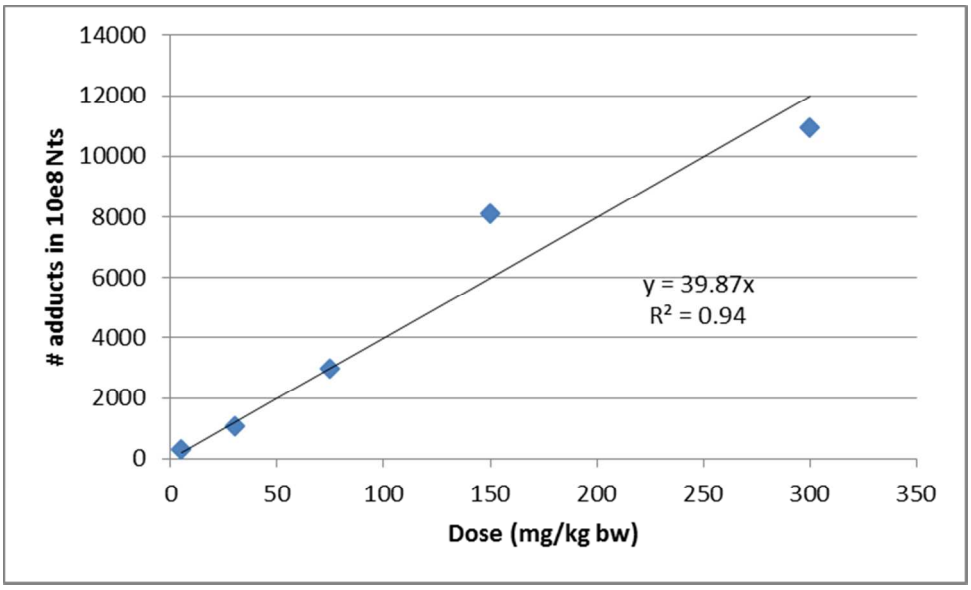

Figure S3. DNA adduct formation reported by Paini et al[3] in liver of rats exposed to single oral doses of estragole. A linear increase in DNA adduct formation of 40 adducts in 10^8 nts per mg/kg bw was assumed.

References

1. Williams, G. M., Iatropoulos, M. J., Jeffrey, A. M., & Duan, J.-D. (2013) Methyleugenol hepatocellular cancer initiating effects in rat liver. *Food Chem. Toxicol. 53*, 187–196.
2. Herrmann, K., Engst, W., Meinl, W., Florian, S., Cartus, A. T., Schrenk, D., Appel, K. E., Nolden, T., Himmelbauer, H., Glatt, H. (2014) Formation of hepatic DNA adducts by methyleugenol in mouse models: drastic decrease by Sult1a1 knockout and strong increase by transgenic human SULT1A1/2. *Carcinogenesis 35*, 935–941.
3. Paini, A., Punt, A., Scholz, G., Gremaud, E., Spenkelink, B., Alink, G., Schilter, B., van Bladeren, P. J., Rietjens, I. M. C. M. (2012) In vivo validation of DNA adduct formation by estragole in rats predicted by physiologically based biodynamic modelling. *Mutagenesis 27*, 653–663.