**Chlorpyrifos Doses to Women of the Columbia University Cohort and Neurodevelopmental Impairment—A Bayesian-Inspired Uncertainty Analysis and Risk Projection Reflecting Inputs from Different Sources of Information**

**Supplemental Material**

**Detailed material on individual sources of information and inferences for the uncertainty distribution for the dose/blood level ratio at low doses**

### 1. Constructing the Initial “Prior” From Measured Air Exposures and Metabolite Excretion in Relation to Observed Blood Levels

The Columbia University researchers have conducted extensive measurements of air levels of chlorpyrifos for individual women over the various years of their study, using both 48-hour personal monitoring, and longer duration measurements of indoor air. The Columbia cohort women likely received inhalation, dermal and hand-to-mouth oral exposures from chlorpyrifos residues in their dwellings. It is also possible that some airborne chlorpyrifos may have become effective oral exposures by dissolution in fat-containing foods. Therefore, the observed relationship of the women’s blood levels to air exposures provides a set of minimum estimates of chlorpyrifos dose per unit of blood level (Table S1—corresponds to Table II in the final technical report). Because many blood levels in later years are non-detects, there is some merit in using ratios from the earlier years, when non-detects were not a major problem for the analysis. We therefore have bolded one specific entry in Table S1--0.56 ng/kg-day per pg/g maternal blood as providing a minimal estimate. Later PBPK modeling, however requires a revision of this floor estimate. Even with zero chlorpyrifos metabolism rates, both the ingestion and inhalation models with baseline partition coefficients yielded minimum dose/blood level ratios of at least 1.92 ng/kg-day/pg/g blood. With tissue/blood partition coefficients corresponding to 2 standard deviations below the inferred central estimate calculated from Lowe et al, (that is, a fat/blood partition coefficient of 94), a zero metabolism rate would yield a dose/blood level ratio of 1.59 ng/kg-day/pg/g blood. Because of this we chose this value as a modified lower bound estimate for both our inhalation and ingestion dose/blood level ratios

An upper bound estimate of the inhalation dose/blood level ratio is constructed from similar Columbia cohort data on the relationship between excretion of the chlorpyrifos metabolite TCPy (3,5,6-trichloro-2-pyridinol) and chlorpyrifos blood levels. Such ratios (Table S2—corresponds to Table III of the final technical report) represent an upper bound because there are are several contributions to the urinary excretion of TCPy in addition to breackdown from absorbed chlorpyrifos, most notably preformed TCPy in the diet resulting from agricultural uses of chlorpyrifos([1](#_ENREF_1)).

**Table S1—Corresponds to Table II of the Final Technical Report.** Ratios of Columbia University Measured Air Exposures (Projected to the Birth Dates of the Babies) to Observed Maternal Blood Levels (ng/kg-day per pg/g of blood)

|  |  |
| --- | --- |
|  |  |
| Dates | air ng/kg-day per pg/g maternal blood  |
| 3/15/98-4/27/99 | 0.69 |
| 4/28/99-4/27/00 | 0.43 |
| 4/28/00-4/27/01 | 0.78 |
| 4/28/01-4/27/02 | 2.85 |
| 4/28/02-4/27/03 | 4.45 |
| 4/28/03-6/27/04 | 10.13 |
| 1998-4/27/2001 Average | **0.56a** |
| All Years Average | 0.83 |

**a**This bolded value, for years when non-detects did not appreciably complicate estimation of the blood levels, was chosen as an initial lower bound for the inhalation dose/blood level ratio. However later PBPK modeling established that dose/blood level ratios could not be as low as this. Even with zero chlorpyrifos metabolism rates, both the ingestion and inhalation models with baseline partition coefficients yielded minimum dose/blood level ratios of at least 1.92 ng/kg-day/pg/g blood. With tissue/blood partition coefficients corresponding to 2 standard deviations below the inferred central estimate calculated from Lowe et al, (that is, a fat/blood partition coefficient of 94), a zero metabolism rate would yield an inhalation dose/blood level ratio of 1.59 ng/kg-day/pg/g blood. Because of this, we chose to revise the lower bound of the inhalation dose/blood level ratio for our “bounding” calculation to 1.59 ng/kg-day/(pg/g blood).

**Table S2—Corresponds to Table III of the final technical report.** Upper Bound Estimates of the Chlorpyrifos Dose/Blood Level Based on Urinary TCPy Excretion

|  |  |  |  |
| --- | --- | --- | --- |
| Time Period | ng/kg-day per pg/g maternal blood from volume-adjusted TCPy excretion b | ng/kg-day per pg/g maternal blood from creatinine-adjusted TCPy excretion b | ng/kg-day per pg/g maternal blood from average of volume- and creatinine-adjusted TCPy excretion |
|  1998 a |  |  |  |
| 1999 |  |  |  |
| 2000 |  |  |  |
| 2001 | 71.2 | 61.9 | 66.6 |
| 2002 | 41.2 | 37.0 | 40.0 |
| 2003 | 134.3 | 112.2 | 112.2 |
| 2004 | 135.4 | 160.5 | 147.9 |
| 2001-2 | 57.8 | 50.8 | **54.3** c |
| All years | 63.3 | 56.7 | 59.8 |

a No measurements of urinary TCPy were made before 2001.

b Volume- and creatinine-correction formulae were based on data from Knuppel et al. (1979) ([2](#_ENREF_2)).

c This value, after rounding up to 55, was chosen to represent the upper bound of the oral dose/blood level distribution in the “prior” bounding calculation for the ingestion route of exposure. However, for the inhalation route, the PBPK modeling revealed that even with an infinite rate of liver metabolism of chlorpyrifos (implemented in the model by reducing the release of chlorpyrifos from the liver to the venous circulation to zero) and baseline partition coefficients, the maximum inhalation chlorpyrifos dose/blood level was not expected to exceed 32.53. With tissue/blood partition coefficients corresponding to 2 standard deviations above the inferred central estimate calculated from Lowe et al, (that is, a fat/blood partition coefficient of about 148), the inhalation chlorpyrifos dose/blood level was not expected to exceed 32.98. In the light of this constraint, we choose to revise the upper bound of the inhalation dose/blood ratio for our “bounding” calculation to 32.98 ng/kg-day/(pg/g blood) and we represent the uncertainty in the inhalation dose/blood level ratio as a normal distribution, with the 1%-99% confidence limits set at the lower and upper bounds as specified here and in Table II, with a truncation limits of 1.0 and 35. PBPK-based metabolism rates derived from 100 fractiles of the inhalation model were then first input into the version of the PBPK model with absorption via the oral route. This yielded unrestricted ratios of ingestion dose/blood level ranging from 2 to over 1120 for values of the inhalation dose/blood ratio ranging from 1.8 to 32.5 with central estimate tissue/blood partition coefficients. The full unrestricted model equation was:



Where

base = 1.89

coeff = 175.7

liv met rate = liver metabolism rate (per minute) (inferred for each modeled value of the distribution of the bounded inhalation dose/blood level relationship)

fitted exponent = 1.07

liv bl partco = liver blood partition coefficient (with a central estimate value of 6.12).

Using these unlimited ingestion dose ratio results as inputs, the upper limit of 55 was imposed via a Michaelis-Menten-like saturation equation form:

Limited oral dose/blood ratio = 

Where

max ratio = 55

half-sat value = 35

Choice of these values allowed the predicted oral dose/blood level ratios to maintain agreement throughout the entire range of fractiles with the dual constraints that (1) the oral dose/blood ratio should be less than 55 and (2) greater than the inhalation dose/blood level ratio that was used to derive the liver metabolism rate.

However later PBPK modeling required a modification of this upper bound, as well for the inhalation route of exposure. Model variants constructed to provide essentially infinite rates of liver metabolism were constructed by setting the output from the liver to the venous blood compartment to zero. On this basis we find that maximum inhalation dose/blood level ratios at 400 hours of continuous exposure cannot be expected to exceed 32.53 for central estimates of tissue/blood partition coefficients. With tissue/blood partition coefficients corresponding to 2 standard deviations above the inferred central estimate calculated from Lowe et al, (that is, a fat/blood partition coefficient of about 148), the inhalation chlorpyrifos dose/blood level was not expected to exceed 32.98. In the light of this constraint, we choose to revise the upper bound for our “bounding” calculation to 32.98 ng/kg-day/(pg/g blood) Recognizing that the extreme “bounds” derived from these data are not absolute limits, but represent some percentiles of the uncertainty distribution ([3](#_ENREF_3)) ([4](#_ENREF_4)), we choose to allow for a 2% chance that the true value of the blood to dose conversion factor is outside of our extreme bounds and we represent the uncertainty in the inhalation dose/blood level ratio as a normal distribution, with the 1%-99% confidence limits set at the lower and upper “bounds” derived above. A truncated normal, rather than a lognormal distribution is selected in this case because of the natural limitations imposed by the zero-infinite metabolism rate cases. To further recognize this, we impose absolute truncation limits of 1-35 for the inhalation dose/blood level ratio.

The footnote to Table S2 describes the procedure for relating the bounding distribution estimates for the inhalation dose/blood ratios first to metabolism rates and then to oral dose/blood ratios associated with those metabolism rates.

### 2. Input from with New Human Liver Microsome Metabolism Data Interpreted Using the PBPK Model Initially Developed by Timchalk and Coworkers

A physiologically-based pharmacokinetic model developed by Timchalk and coworkers ([5](#_ENREF_5)), was adapted to pregnant women by Lowe et al. ([6](#_ENREF_6)). Lowe et al. associate essentially continuous (3 hours per day over a prolonged period—400 hours) and a hearing presentation by Timchalk([7](#_ENREF_7)), associate essentially continuous (3 times per day over a prolonged period—400 hours) oral exposures of 153 ng/kg body weight-day (0.436 nmole/kg-day) with blood levels of about 10.6 pmol/liter. After unit conversion (based on a molecular weight for chlorpyrifos of 350.6), this implies a low dose ratio of about 39.7 ng oral chlorpyrifos/kg body weight/day per pg/liter of blood. Timchalk (2008b) also report that because a large fraction of oral chlorpyrifos is expected to be metabolized on its “first pass” through the liver, about 26 times more blood chlorpyrifos is expected per unit of chronic inhalation administration than is expected for oral exposure. Therefore for inhalation exposure the reported expectation under the Timchalk model is about 39.7/26 = 1.53 ng/kg-day per pg/g of chlorpyrifos in blood.

The alert reader will notice that the projected inhalation dosimetry ratio of 1.53 ng/kg-day per pg/g of chlorpyrifos in blood from the Timchalk report is smaller than the revised lower limit from our bounding calculation, suggesting that there must either be some modeling error or a reporting error in either our PBPK or Timchalk’s model implementation. We therefore ran a zero-metabolism check on the overall mass balance and compartmental contents of our model for pregnant women after 400 hours of inhalation dosing, using the Lowe et al fat/blood partition coefficient from late pregnancy and other tissue/blood partition coefficients scaled in proportion from the original Timchalk([5](#_ENREF_5)) values. Over this period, the total chlorpyrifos absorbed would be expected to be 400 hours \* (0.436 nmole/kg-day)\*83.9 kg body weight/24 hrs/day= 610 nmoles = 0.61 µmoles. It can be seen in Table S3 that with zero metabolism, all of the absorbed chlorpyrifos is accounted for, and is distributed among the compartments in reasonable proportion to the partition coefficients. The one exception is the fat compartment which, at 400 hours, has somewhat less than its expected full steady state concentration. In the light of these results we reluctantly conclude that we cannot reconcile the low inhalation dose/blood level ratio implied by the Timchalk presentation with the results of our own modeling.

Unfortunately neither the original Timchalk et al paper and hearing presentation nor the Lowe et al. adaptation for pregnancy provide estimates of the uncertainty of their modeling results for an average person, or the expected person-to-person variability of the blood levels expected in their model for a given dose. Also absent are confidence limits for the estimates of parameters making up the human model. The basic calibration of the original human model was based on in vitro observations of activating and detoxifying reactions in microsome preparations from rats.

And although Timchalk mentions several updates to the model to accommodate more recent information, none of these apparently includes a recalibration of the human metabolism parameters([8](#_ENREF_8)) based on in vivo or in vitro measurements of metabolism in humans. The consequences of uncertainty in metabolic rates are limited in this case by a common convention in PBPK modeling, which places all metabolism in the liver. If nearly all metabolism is in fact in the liver, and if metabolism is expected to be rapid enough that a large fraction of chlorpyrifos entering the liver is metabolized before exiting to the venous circulation, then the whole-body elimination rate of primarily determined by the liver blood flow rate and the fraction of the whole body burden of chlorpyrifos that is contained in that blood (vs storage compartments such as fat). One alteration in the Lowe et al. paper from the original Timchalk (2002) model assumptions is a reduction in the fat blood partition cofficients for nonpregnant people from the 435 originally calculated by Timchalk to 178, based on new measurements. A further reduction to 121 is indicated for the third trimester of pregnancy because of the increased fat content of maternal plasma in late gestation. Other modifications of compartment sizes and partition coefficients with pregnancy are documented in Table S4. These changes are not expected to alter the expected rate of loss of chlorpyrifos from blood passing through the liver, but the reduced tissue/blood partition coefficients increase the faction of the whole body burden delivered to the liver per unit time so that the expected whole body half-life is reduced by several fold with the increase in blood fat content (data not shown).

**Table S3.** 400 Hour Compartmental Contents of the Inhalation Model Variant Exposed to 153 ng/kg Body Weight per Day with No Metabolism

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Tissue volumes (liters) | Nmoles CPF at 400 hours | CPF Concentration pg/g | Dosimetry ratio (ng/kg-day)/(pg/g conc. In blood) | Tissue/venous blood conc | Input tissue/blood partition coefficient |
| Arterial blood | 2.1 | 0.47 | 80 | 1.90 |  | 1.0 |
| Venous blood | 3.8 | 0.86 | 79 | 1.93 |  | 1.0 |
| Liver | 1.7 | 2.36 | 492 |  | 6.22 | 6.1 |
| Brain | 1.1 | 2.36 | 738 |  | 9.33 | 9.2 |
| diaphram | 0.017 | 0.01 | 134 |  | 1.70 | 1.7 |
| raidly perfused | 1.9 | 1.23 | 224 |  | 2.83 | 2.8 |
| mammary | 1.1 | 0.71 | 224 |  | 2.83 | 2.8 |
| uterus | 2.6 | 1.66 | 222 |  | 2.81 | 2.8 |
| placenta and fetus | 4.0 | 2.57 | 224 |  | 2.83 | 2.8 |
| slowly perfused | 30.3 | 11.57 | 134 |  | 1.69 | 1.7 |
| fat | 29.4 | 584.16 | 6967 |  | 88.10 | 121 |
| skin | 5.9 | 2.24 | 134 |  | 1.70 | 1.7 |
| Total | 83.9 | 610.19 |  |  |  |  |

**Table S4.** Consequences of Partition Coefficient and Compartment Size Changes Between the Original Timchalk Model and a Model Patterned After the Lowe et al. for the Third Trimester of Pregnancy

|  |  |  |  |
| --- | --- | --- | --- |
|  | Patition Coefficients Relative to Blood | Compartment Volumes (l) | Blood Volume Equivalents (liters) |
| Compartment | Orig. Timchalk Model | Lowe nonpregnant Model | Lowe CPF 37 Week Model | Orig. Timchalk | Lowe Nonpregnant | Lowe 37 week pregnant | Orig Timchalk | Lowe Nonpregnant | Lowe 37 week pregnant |
| Blood | 1 | 1 | 1 | 4.0 | 4.0 | 5.9 | 4.0 | 4.0 | 5.9 |
| Brain | 33 | 13.5 | 9.2 | 1.2 | 1.2 | 1.1 | 38.2 | 15.6 | 10.3 |
| diaphram | 6 | 2.5 | 1.7 | 0.02 | 0.02 | 0.02 | 0.1 | 0.0 | 0.0 |
| Fat | 435 | 178 | 121 | 23.4 | 23.4 | 29.4 | 10184 | 4167 | 3557 |
| Liver | 22 | 9.0 | 6.1 | 1.7 | 1.7 | 1.7 | 38.2 | 15.6 | 10.3 |
| rapidly perfused | 10 | 4.1 | 2.8 | 2.3 | 2.3 | 1.9 | 23.1 | 9.5 | 5.4 |
| slowly perfused | 6 | 2.5 | 1.7 | 31.2 | 31.2 | 30.3 | 187.3 | 76.6 | 50.5 |
| Skin | 6 | 2.5 | 1.7 | 5.2 | 5.2 | 5.9 | 31.2 | 12.8 | 9.8 |
| Mammary |  |  | 2.8 |  |  | 1.1 |  |  | 3.1 |
| Uterus  |  |  | 2.8 |  |  | 2.6 |  |  | 7.3 |
| Placenta |  |  | 2.8 |  |  | 0.6 |  |  | 1.8 |
| Fetus |  |  | 2.8 |  |  | 3.4 |  |  | 9.5 |
| Total |  |  |  | 69.1 | 69.1 | 83.9 | 10506 | 4301 | 3671 |

Given this, it is important to try to quantify the uncertainty in these expected partition coefficients. The human plasma used by Lowe et al. for determination of the third trimester fat/blood partition coefficient was a pool from 10 women. Therefore the uncertainty in the partition coefficient derived from this pool depends in part on the variability in fat content of late pregnant women’s plasma, and any errors resulting from analytical uncertainty. Three sources report means and standard deviations for cholesterol and triglycerides for a total of 161 women in late pregnancy ([9](#_ENREF_9)) ([10](#_ENREF_10)) ([11](#_ENREF_11)). Based on these data we derive a coefficient of variation for the sum of the variances of the cholesterol and triglyceride levels of about 35%. From this, the mean fat content of 10 plasma samples should have a sampling uncertainty standard error of about 0.35/(100.5) = 11.1% of the mean. Therefore the estimated fat/blood partition coefficient of 121 for late pregnant women should be considered to have a ± 1 standard error range at least as broad as about 107.5-134.5. Corresponding uncertainties in tissue/blood partition coefficients for other tissues were inferred from this range (for example, the liver/blood partition coefficient was assumed to have a central value of 6.1 with a a ± 1 standard error range of 5.4-6.8. This uncertainty is used below in our PBPK modeling analysis of the uncertainty in the external dose/blood translation factor.

Despite the expected insensitivity of these results to modest differences in human metabolism rates for chlorpyrifos, one can still ask whether more recent data in humans should lead to any revision in the metabolism rate assumptions derived by Timchalk et al. from rat data. Recently, Smith et al. (2011) have published a fairly extensive set of chlorpyrifos metabolism measurements from liver microsome preparations from deceased people of a variety of ages ([12](#_ENREF_12)) (Table S5—corresponds to Table IV of the final technical report). These data provide the basis to reevaluate the original Timchalk et al. assumptions, and further test for differences by age and gender. There are trends toward some differences by gender, gender, with women having modestly lower rates of intrinsic clearance, but these differences are not large enough to reach statistical significance by usual criteria (P = 0.22 considering all females vs all males; P = 0.13 considering only data for five adult females vs five adult males).

Conversion of these *in vitro* results to estimates of *in vivo* clearance requires an assumption about the amount of microsomal protein present in human liver. Based on a compilation of earlier observations([13](#_ENREF_13)) ([14](#_ENREF_14)) by Cubitt ([15](#_ENREF_15)) an average liver microsome content is about 39.8 mg of microsomal protein per gram of liver (coefficient of variation = 27%) and an average value for the size of the human liver is about 1.59 liters (coefficient of variation = 12%.

**Table S5—Corresponds to Table IV of the Final Technical Report**. Summary Statistical Analysis of Data from Smith et al (2011) on Metabolic Activation and Inactivation Rates Observed in Liver Microsome Preparations from Recently Deceased Humans of Different Ages

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Subject | Dearylation Ci µl/(min-mg microsomal protein) | Desulfuration Ci µl/(min-mg microsomal protein) | Fraction activated (desulf/(dear+desulf) | Total Intrinsic Clearance µl/(min-mg microsomal protein) |
| Age 0-5 Females |  |  |  |
| 354 | 2.1 | 1.98 | 0.485 | 4.08 |
| 57 | 25.02 | 25.8 | 0.508 | 50.82 |
| 776 | 19.63 | 6.03 | 0.235 | 25.66 |
| 689 | 6.2 | 3.79 | 0.379 | 9.99 |
| Gmean | 8.94 | 5.85 | 0.385 | 15.18 |
| Age 25-48 Females |  |  |  |
| 459 | 6.61 | 1.5 | 0.185 | 8.11 |
| 25 | 7 | 4.15 | 0.372 | 11.15 |
| 434 | 2.92 | 0.68 | 0.189 | 3.60 |
| 251 | 18.25 | 3.48 | 0.160 | 21.73 |
| 115 | 4.56 | 1.51 | 0.249 | 6.07 |
| Gmean | 6.46 | 1.86 | 0.220 | 8.44 |
|  |  | Approx lognormal 5%-95% confidence range for Gmean | 5.15-13.85 |
| Age 0-10 Males |  |  |  |
| 845 | 9.91 | 5.88 | 0.372 | 15.79 |
| 282 | 13.39 | 3.54 | 0.209 | 16.93 |
| 671 | 7.35 | 3.06 | 0.294 | 10.41 |
| 268 | 17.15 | 3.89 | 0.185 | 21.04 |
| 270 | 5.05 | 2.54 | 0.335 | 7.59 |
| 395 | 21.25 | 3.99 | 0.158 | 25.24 |
| 825 | 30.37 | 16.28 | 0.349 | 46.65 |
| 322 | 39.03 | 3.3 | 0.078 | 42.33 |
| 346 | 43.41 | 25.75 | 0.372 | 69.16 |
| 551 | 23.55 | 5.07 | 0.177 | 28.62 |
| 852 | 43.67 | 9.91 | 0.185 | 53.58 |
| 792 | 38.63 | 5.29 | 0.120 | 43.92 |
| 675 | 1.42 | 1.6 | 0.530 | 3.02 |
| 215 | 9.44 | 5.6 | 0.372 | 15.04 |
| 59 | 5.02 | 0.87 | 0.148 | 5.89 |
| 485 | 2.28 | 0.1 | 0.042 | 2.38 |
| Gmean | 12.86 | 3.58 | 0.207 | 17.32 |
| Age 17-75 Males |  |  |  |
| 133 | 21.99 | 12.16 | 0.356 | 34.15 |
| 743 | 12.33 | 1.95 | 0.137 | 14.28 |
| 752 | 4.22 | 2.68 | 0.388 | 6.90 |
| 201 | 22.48 | 6.42 | 0.222 | 28.90 |
| 203 | 10.63 | 4.1 | 0.278 | 14.73 |
| Gmean | 12.23 | 4.41 | 0.259 | 17.03 |
|  |  |  | Approx lognormal 5%-95% confidence range for Gmean | 10.6-27.2 |

There are two principal reasons for concern about the use of liver microsomes from recently deceased people to predict in vivo clearance rates for living people. First, the in vitro measurements might not perfectly replicate the actual in vivo conditions under which the metabolic enzymes are operating because of possible differences in cofactor concentrations, pH, and other aspects of the incubation mixtures relative to the normal environment within liver cells. Second, it is difficult to know whether there might have been changes in the activity of liver metabolizing enzymes in the interval between the humans’ deaths and the harvesting of the livers for metabolic enzyme extraction. Ito and Houston (2005) ([16](#_ENREF_16)) have published an extensive set of comparisons of in vivo intrinsic metabolic clearance rates predicted from liver microsome measurements in comparison with those observed in in vivo studies for 52 drugs. The estimates of in vivo intrinsic clearance were defined by the equation:

Clint,h,*in vivo* = 

where Qh represents hepatic blood flow (20.7 ml/min), fu is the unbound fraction and RB is the blood-to-plasma concentration ratio. The units of internal clearance are the effective volume of blood “cleared” of chlorpyrifos per unit of time. These data indicate that microsomal data tend to underpredict in vivo clearance by a geometric mean ratio of 7.9 fold with a GSD for differences among chemicals of 2.98 (Figure S1). This mean and log variance are used to represent the likely systematic bias and random error uncertainty in the microsome-based metabolism data.

The microsome metabolism data in Table S5 were of course collected from liver samples from people not known to be pregnant. We therefore need to ask whether we should expect systematic changes in liver metabolism rates for women during pregnancy. Loebstein([17](#_ENREF_17)) have compiled a helpful set of comparisons of classical pharmacokinetic parameters in pregnant and nonpregnant women (Table S6, which corresponds to Table VII in the final technical report). Unfortunately the exact times during pregnancy when these data were collected are not documented in their review paper. Overall, however, these data indicate that half-lives of the studied drugs tend to be somewhat less, and clearance rates 1.93 fold as great in pregnant women, although considerable differences among chemicals/drugs are apparent in the geometric standard deviations shown in the table. For purposes of our late stage pregnancy models, we assume that chlorpyrifos follows a similar pattern to this limited sample of drugs. We therefore have adjusted the central estimate liver metabolism rates for chlorpyrifos upward by 1.93 fold from the rates derived from the in vitro observations for adult women in Table S5. For uncertainty analysis, we assess differences that would be produced by assuming this adjustment is larger or smaller than the central estimate by an amount corresponding to ± 1 standard deviation of the data in Table S6—approximately 0.85 to 4.39 times the liver clearance values derived directly from the calibration for nonpregnant women.



Regression fit:

Log(Observed Clearance) = 0.952 (SE = .090) predicted clearance + 0.942 (SE = .069)

R2 = 0.788

.952 coefficient of predicted clearance is close to the theoretically expected 1.0

Root mean square error (standard deviation of the points from the line) = 0.476

Geometric mean ratio: 7.94 (used for gmean bias correction); GSD for diversity of ratios among chemicals: 2.98 (used for uncertainty in gmean bias correction)

**Fig. 2.** Lognormal Plot of Observed vs Expected Clearance Rates from Human Microsome Measurements (ml Blood/Kg Body Weight-min) Based on a Physiologically-Based Scaling Factor (856 mg microsomal protein/kg body weight) After Application of a Geometric Mean Bias correction of 7.94, Based on Data of Ito and Houston (2005) ([16](#_ENREF_16)). N = 52.

**Table S6 (Corresponds to Table VII in the Final Technical Report)**. Comparisons of Classical Pharmacokinetic Parameters for Selected Drugs Between Pregnant and Nonpregnant Women

 Elimination Half-Life (minutes) Volume of Distribution (Liters) Total Clearance (ml/minute)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Drug | Pregnant | Nonpregnant | Preg/nonpreg ratio | Pregnant | Nonpregnant | Preg/nonpreg ratio | Pregnant | Nonpregnant | Preg/nonpreg ratio |
| Ampicillin | 52.4 | 69.6 | 0.753 | 32.8 | 34.5 | 0.951 | 450 | 370 | 1.216 |
| Cefuroxime | 44 | 58 | 0.759 | 17.8 | 16.3 | 1.092 | 282 | 198 | 1.424 |
| Imipenem | 36 | 41 | 0.878 | 47.1 | 18.9 | 2.492 | 973 | 338 | 2.879 |
| Piperacillin | 46.5 | 53.7 | 0.866 | 67.6 | 41.9 | 1.613 | 1538 | 540 | 2.848 |
| Azlocillin | 65.4 | 72 | 0.908 | 15.4 | 24.7 | 0.623 | 126.1 | 195.7 | 0.644 |
| Nifedipine | 81 | 360 | 0.225 |  |  |  | 266 | 27 | 9.852 |
| Labetolol | 102 | 320 | 0.319 |  |  |  | 1704 | 1430 | 1.192 |
| Sotalol | 396 | 558 | 0.710 | 106.4 | 87.3 | 1.219 | 196 | 109 | 1.798 |
|  |  | Gmean | 0.614 |  |  | 1.212 |  |  | 1.931 |
|  |  | GSD | 1.694 |  |  | 1.603 |  |  | 2.272 |
|  |  | Gstd error | 1.205 |  |  | 1.212 |  |  | 1.337 |

Data source: Loebstein et al., 1997

The implication of these data for the central estimate of the liver tissue metabolic clearance rate for women in late pregnancy is



The combined uncertainties in these multiplicative factors are assessed via the addition of lognormal variances (using base 10 logs)

Variance in log(loss rate) = 0.085 (microsome-based clearance) + 0.127 (pregnant/non-pregnant metabolism ratio) + 0.225 (in vivo/in vitro microsome bias correction) = 0.438

This variance translates into a geometric standard deviation for these sources of uncertainty of about



This yields a ± 1 standard error range for the liver metabolic loss rate of 0.186 – 3.91/min. (Of course, because of the dependency on liver/blood partition coefficient in the equation above, these metabolism ranges differ for different partition coefficient values).

One further step is required to convert these clearance and partition coefficient data into expectations for the ratio of exposure to blood levels. This is to incorporate them into variants of the Timchalk et al. PBPK model for chlorpyrifos. For this purpose, we created a simplified version of the model, implemented in Microsoft Excel for transparency and ease of use. As observed by Mosquin et al.,([18](#_ENREF_18)) ordinary conditions of human exposure are not expected to approach those needed to come near to the Km’s of the various enzymes involved in CPF and CPF-oxon metabolism. Therefore the saturable forms for the metabolism equations were replaced by

Loss of CPF from liver (µmoles/hr) = time step (hr)\*CPF µmoles in liver\*K

Where K is a simple rate constant (in units of 1/hr) representing the sum of conversion of CPF to TCP and CPF-Oxon.

The results of running this model for 400 hours of continuous dosing (at 153 ng/kg-day) with ± 1 log standard deviation variations in metabolism rates and tissue/blood partition coefficients are shown in Table S7. It can be seen that in this case the effect of uncertainty in partition coefficients is quite small relative to the metabolism rate uncertainty. This is because changes in

**Table S7.** Expected Consequences of ± 1 Standard Error Variations in Pregnancy-Related Metabolism Rates and Tissue/Blood Partition Coefficients for the Low Dose CPF Blood Level to External Dose Conversion Factor for Ingestion vs Inhalation Routes of Exposure, Inferred from Microsome Measurements of Liver Metabolism and Pregnancy-Related Changes in Metabolism

|  |  |  |  |
| --- | --- | --- | --- |
|  | cent est metab preg | "-1 SD metab preg" | "+1 SD metab preg" |
| **Baseline partition coefficients** |  |  |  |
| Ingestion | 155.5 | 34.7 | 709.8 |
| Inhalation | 27.0 | 17.1 | 31.1 |
| ingestion/inhalation ratio | 5.7 | 2.0 | 22.8 |
|  |  |  |  |
| **1 std error low tissue/blood partition coefficients** |  |  |  |
| ingestion | 154.4 | 34.3 | 705.4 |
| inhalation | 26.8 | 16.9 | 30.9 |
| ingestion/inhalation ratio | 5.7 | 2.0 | 22.8 |
|  |  |  |  |
| **1 std error high tissue/blood partition coefficients** |  |  |  |
| ingestion | 156.6 | 35.1 | 714.2 |
| inhalation | 27.3 | 17.3 | 31.4 |
| ingestion/inhalation ratio | 5.7 | 2.0 | 22.8 |

the liver/blood partition coefficient give rise to mostly offsetting changes in the liver metabolism rate inferred from the in vitro data. This is different than will be observed for the Nolan and Kisicki observations, where the data give rise to expectations for the in vivo metabolism rates that are not directly affected by partition coefficient uncertainties. The results summarized in Table S7 were in turn used to create overall distributions of the expected inhalation and ingestion dose/blood level ratios produced by the metabolism rate and partition coefficient uncertainties. In the case of the ingestion blood level to dose ratio, this was a lognormal distribution with gmean = 156.5 and gsd = 4.501. The relatively large uncertainty in these results for this source of information derives from the strong dependence of the ingestion dose/blood factor on the metabolism rate which in this case is not bounded on the upside by observations such as those in Table S2 from the Columbia researchers’ observations. The limitation of these results to values for this factor of no more than 55 will be seen when the microsome-based information is combined with the bounding “prior”.

### 3. Inferences Based on the Nolan et al. (1984) ([19](#_ENREF_19)) Experimental Human Dosing Study

Nolan et al. administered 0.5 mg/kg of chlorpyrifos orally to a total of 6 male subjects. (One of these served as a pilot study and no detected blood chlorpyrifos levels are available for him). Blood chlorpyrifos observations for the remaining men, based on an EPA summary are shown in Table S8 (which corresponds to Table V in the final technical report). At the bottom of the table are calculations of the “Area Under the Curve” (AUC) of chlorpyrifos blood levels X time over the 12 hours of observation with and without use of an imputed value of 3.1 ng/ml in place of the “nondetect” observations. Overall these AUC estimates with the imputed values have a geometric mean of 107.8 ng-hr/ml and a geometric standard error of 1.147 (the ± 1 standard error range is 94.0-123.6).

We can use these AUC observations over the first 12 hours after oral dosing to re-calibrate our version of the Timchalk et al (2002) PBPK model for men of the average weight reported for the Nolan experiments (85.6 kg). The only other adaptation needed for the model is to specify the fraction of chlorpyrifos absorbed following oral administration. From observations of TCPy excretion over the 5 days following dosing, the preliminary indicated fraction of TCPy absorbed appears to be about 72.2% with a standard error of about 6%.

At least two types of corrections are indicated for these primary observations:

* Not all of the TCPy excreted by these men probably originated from the oral dose of chlropyrifos administered. Some undoubtedly resulted from background dietary levels of TCPy and CPF resulting from the use of CPF in agriculture.
* Some fraction of the TCPy that did result from administered CPF remained in the body following the end of the observation period at 108 hours after dosing.

**Table S8 (corresponds to Table V in the final technical report.** Observations of Blood Chlorpyrifos Levels in Five Subjects Given 0.5 mg/kg Orally in the Early 1980s Set of Human Exposure Experiments, and Calculated Integral “Area Under the Concentration X Time Curve” (AUC) Values

 **Individual Subjects**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hours After Dosing** | **B**  | **C**  | **D**  | **E**  | **F**  |
| **Blood Chlorpyrifos Levels (ng/ml)**  |
| 0  | ND a | ND  | ND  | ND  | 9 |
| 1  | ND  | ND  | 6 | 5 | 9 |
| 2  | ND  | 19 | ? (blank in table) | 11 | 15 |
| 4  | ND  | 9 | 21 | 8 | 9 |
| 6  | 12 | 12 | 21 | 8 | 18 |
| 8  | 7 | 30 | ND  | ND  | ND  |
| 10  | 8 | 6 | ND  | ND  | ND  |
| 12  | 28 | 15 | ND  | ND  | ND  |
| Calculated AUC 0-12 hours (ng-hr/ml), assuming imputed values of 3.1 ng/ml for the ND values | 97.5 | 162.1 | 123.5 | 70.5 | 105.5 |
| Calculated AUC 0-12 hours (ng-hr/ml assuming 0 for the ND values | 82.0 | 157.5 | 106.5 | 53.5 | 90.0 |
|  |  |  |  |  |  |

a ND = Not Detected—limit of detection was reportedly 5 ng/ml.

Source: EPA summary (Anonymous, 2008) of data of Nolan (1982) reported in MRID 00124144. ([20](#_ENREF_20))

For the first point, we use a set of nationally representative data from a later period([21](#_ENREF_21))—900 men and women from the NHANES III study ages 20-59 in 1988-94. From a lognormal fit to the creatinine-corrected data, the geometric mean and geometric standard deviation for urinary excretion of TCPy in this group were 2.18 µg/gram of creatinine and 2.297 respectively (Figure S2). The arithmetic mean for this distribution is expected to be about 3.1 µg/gram of creatinine. Multiplying by a male/female average creatinine excretion rate of 1.7 g/day [calculated from distributional data of Garde et al. (2004) ([22](#_ENREF_22))], the expectation is for background TCPy excretion of about 5.2 µg/day, or about 26 µg over the 5 days of the Nolan et al. observations. As it happens this is a fairly small fraction of the average reported excretion of TCPy in the Nolan experiment of 17,800 µg. Relative to the average administered dose of 23.5 mg of TCPy-equivalents (23,500 µg) the background represents about 0.11%). After subtracting this amount from the reported excretion observations, we can now estimate the fraction of generated TCPy expected to remain in the men’s bodies from the terminal rate of exponential decline in the TCPy excretion observed in the Nolan et al. data (Figure S3) ([20](#_ENREF_20)).

Considering the rate of exponential decline from 18 hours on, the indicated half life of TCPy is 30.9 hours. If TCPy generation is essentially complete by 6 hours after dosing, this half life implies that about 7.8% of TCPy generated from chlorpyrifos would be expected to remain in the body after 5 days. This amount therefore needs to be added to the estimate of the total oral absorption of chlorpyrifos, giving us 72.2% (from the original ratio of excretion/dose) – 0.11% (from background TCPy excretion) + 7.8% (from TCP remaining in the body) = 84.8% total mean ingested CPF absorbed. The mean AUC per mg/kg absorbed dose for the five men studied by Nolan is therefore 111.8/(.848\*.5 mg/kg) = 264 (ng-hr/ml)/(mg/kg absorbed). We take the standard error of the mean absorption fraction to be proportionate to the mean of the total percentage excretion, giving us a standard error for uncertainty calculations of about 6%\*84.8/72.2 = 6.6%.

Combining both absorption rate and observed AUCs as sources uncertainty, the indicated liver metabolism rate for the 5 males observed by Nolan et al. is describable as a lognormal distribution with a geometric mean of 0.2565/min and a geometric standard deviation of 1.1789.

Of course the central estimate and uncertainty derived in this way pertains to measurements in the Nolan et al. males. This must be corrected for

* Male/female differences
* Pregnant/non-pregnant differences (as derived before in section 2 above, including both metabolic rate and partition coefficient uncertainties).

The first of these can be inferred from the liver microsome metabolism rate information in Table X. The differences between the metabolism rates for adult men vs adult women was almost twofold, based on the geometric means, but this difference is not statistically significant by usual criteria (P = 0.13). Nevertheless the comparative rate data can be described by an uncertainty/confidence distribution. The central estimate of this female/male metabolism rate

.



**Fig S2.** Lognormal plot of the distribution of TCPY excretion (µg) per gram of creatinine in 900 NHANES III spot samples from adults. Antilog geometric mean = 2.18 µg/g; geometric standard deviation = 2.297

Data Source: Hill et al. ([30](#_ENREF_30))



**Fig. S3.** Mean decline of TCP excretion following a 0.5 mg/kg dose of CPF for 5 subjects studied by Nolan. Plot of ln(TCP in µg/hr) vs time. The slope of the line for the 18+ hours after dosing is -0.0224 with a standard error of 0.0009.

Data Source: Nolan et al. (1982), summarized in ([20](#_ENREF_20)).

distribution is just the ratio of the female/male geometric mean metabolism rates, or about 0.496. Because the variance of a sum or difference of two uncertain quantities is the sum of the component variances, and because dividing the female metabolism rate by the male metabolism rate is equivalent to subtracting their logarithms, the lognormal uncertainty variance for the ratio of adult female/adult male CPF metabolism rates is expected to be:

[Log(Geom std error for female metabolism)]2 + [Log(Geom std error for male metabolism)]2

= [Log(1.351)]2+ [Log(1.331)] 2 = 0.03224

The combined log(geometric standard error) is just the square root of this variance, or 0.1801, yielding an antilog geometric standard error for the female/male metabolic rate ratio of 1.514. Therefore for nonpregnant women we derive a central estimate the CPF liver metabolism rate from the Nolan observations of 0.256 \* 0.496 = 0.127/min.

For pregnant women, as before, we need to multiply the central estimate by the pregnant/non-pregnant ratio from Table S6 (1.931) giving us an overall central estimate metabolism rate for pregnant women of 0.246/min. The uncertainty around the central estimate derived from the Nolan et al. observations is the result of combining the lognormal variances in liver metabolism rate from the Nolan estimates themselves (GSD 1.1789), with variances from the male/female comparison (GSD 1.514) and the variance from the standard deviation of the pregnant/nonpregnant ratio comparison in Table S6 (GSD 2.272) (the standard deviation is used here rather than the standard error because CPF is modeled as a random draw from the specific chemicals for which we have data). The combined lognormal uncertainty in the expected metabolism rate from the Nolan observations is therefore a lognormal distribution with a combined GSD of

2.545

Given this, a ± 1 standard error range for the Nolan-calibrated metabolism rate is 0.096 – 0.625/min. This in turn provides input for an analysis of the distribution for the expected dosimetry ratio (external dose/blood level for either inhalation or ingestion modes of absorption) for the combined uncertainties in metabolic rate and pregnancy-related tissue/blood partition coefficients. The results of this analysis are shown in Table S9. It can be seen that, as expected, the reduced metabolism rates derived from the Nolan observations lead to smaller estimates of the external dose/blood level ratio, and also smaller differences related to the route of exposure (ingestion vs inhalation). This is because, for the central estimate Nolan metabolism rate, the fraction of chlorpyrifos removed from the blood flowing to the liver has been reduced to about 62% (± 1 standard error range =39%-80% assuming central estimate tissue/blood partition coefficients).

**Table S9.** Expected Consequences of ± 1 Standard Error Variations in Pregnancy-Related Metabolism Rates Inferred from the Nolan et al. (1984) Observations and Tissue/Blood Partition Coefficients for the Low Dose CPF Blood Level to External Dose Conversion Factors for Ingestion vs Inhalation Routes of Exposure

|  |  |  |  |
| --- | --- | --- | --- |
|  | cent est metab preg | "-1 SD metab preg" | "+1 SD metab preg" |
| **Baseline partition coefficients** |  |  |  |
| ingestion | 45.5 | 18.6 | 114.2 |
| inhalation | 19.2 | 12.1 | 25.5 |
| ingestion/inhalation ratio | 2.36 | 1.53 | 4.48 |
|  |  |  |  |
| **1 std error low tissue/blood partition coefficients** |  |  |  |
| ingestion | 40.1 | 16.4 | 100.8 |
| inhalation | 18.1 | 11.1 | 24.6 |
| ingestion/inhalation ratio | 2.21 | 1.47 | 4.10 |
|  |  |  |  |
| **1 std error high tissue/blood partition coefficients** |  |  |  |
| ingestion | 50.9 | 20.8 | 127.7 |
| inhalation | 20.2 | 13.1 | 26.3 |
| ingestion/inhalation ratio | 2.51 | 1.59 | 4.86 |

## 4. Inferences Based on the Kisicki et al.([23](#_ENREF_23)) Experimental Human Dosing Study

The human dosing experiments of Kisicki et al. were considerably more extensive than those carried out by Nolan et al. Where Nolan exposed only 6 men to a 0.5 mg/kg and detected significant blood levels of at least 5 ng/ml in a minimum of 5, Kisicki et al. gave chlorpyrifos orally to 6 men and 6 women each in doses of either 0.5, 1.0, or 2 mg/kg body weight and used an analytical method with a reported detection limit of 1 ng/ml. Despite the greater doses and the smaller detection limit, only 8 of the 24 Kisicki et al. subjects exposed at the two higher doses reportedly had blood levels above the reduced detection limit of 1 ng/ml. The data for these 8 subjects are reproduced in Table S10 (which corresponds to Table VI in the final technical report).

As before we have chosen to consolidate these observations into the overall integral of blood CPF X time [that is, the Area Under the Curve (AUC)] over the period from 0 to 12 hours after dosing. Then, to help normalize by dosing level and differential absorption among subjects and experiments, we have divided each individual subject’s AUC by the mg/kg absorbed dose for that subject, as calculated by the Dow researchers from the ultimate amount of TCP excreted in urine by 168 hours.

The results of these calculations are shown in Table S11. It can be seen that even without taking into account the fact that 2/3 of the subjects in the more recent experiments had reported blood levels below the detection limit, the indicated blood CPF/absorbed dose is markedly reduced compared to the earlier Nolan et al. (1982, 1984) findings. The averages for the men and women are 34.4 and 58.5 ng-hr/ml/(mg/kg absorbed) respectively. This compares with the figure from the Nolan et al. study of 264 ng-hr/ml/(mg/kg absorbed). Thus the Kisicki et al. observations must lead to considerably higher expected metabolism rates than those of Nolan et al., and similarly higher estimates of the dose/blood level conversion factors.

After fitting the individual detected values in Table S10 to lognormal distributions that factor in the presence of the nondetects (where the “nondetect” value was considered to be just below the lowest detected value for each sex), the mean values for men and women are reduced to 11.4 and 42.6 ng-hr/ml/(mg/kg absorbed) respectively. Assessment of the uncertainties in these values after imputation of the mean values in the ranges below the male and female detection limits is not straightforward. One approach is to do a parametric bootstrap analysis—doing 24 draws (one for each subject) per trial from the lognormal distribution fit to the data in the detected region; and then re-fitting the randomly drawn values in the detected region to new lognormal distributions to estimate average values for the whole distribution. Using this approach through 20 iterations for the distribution of female log(AUC)/absorbed dose distribution yields an approximately lognormal distribution with a geometric mean of 41.8 (ng-hr/ml)/(mg/kg absorbed) and a geometric standard deviation of 1.1267. We choose to use these estimates for calibration of the metabolism rate of the PBPK model for non-pregnant women. This results in a central estimate liver metabolism rate of 1.36/min with ± 1 standard error confidence limits of

**Table S10 (corresponds to Table VI in the final technical report).** Observations of Blood Chlorpyrifos Levels in 8/24 Subjects Given 1-2 mg/kg in the More Recent Set of Human Exposure Experiments and Reported with at Least One CPF Blood Level Above The Reported Detection Limit of 1 ng/ml

Individual Subjects (number, sex, paroxonase genotype, dose)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hours After Dosing | 47 M QQ 2 mg/kg | 49 F QQ 2 mg/kg | 56 F QQ 2 mg/kg | 59 F QQ 2 mg/kg | 11 M QQ 1 mg/kg | 14 M QQ 1 mg/kg | 21 F QQ 1 mg/kg | 30 F QR 1 mg/kg |
| 0 | ND a | ND | ND | ND | Ns b | ND | ND | ND |
| 2 | **3.1** | **3.1** | ND | **2.2** | **1** | ND | **5.6** | ND |
| 4 | **1.3** | **xx** | ND | **4.1** | ND | **2.7** | **2.9** | ND |
| 8 | **3.4** | **1.7** | **18** | **4.1** | ND | **1.5** | ND | **1.1** |
| 12 | **1.8** | ND | **2.5** | **1.5** | ND | ND | ND | ND |
| AUC with Imputation of 0.671 for ND (ng-hr/ml) | 28.0 | 22.9 | 81.0 | 36.8 | 9.0 | 17.5 | 24.6 | 9.8 |
| AUC assuming ND = 0 | 27.3 | 20.9 | 77.0 | 36.1 | 3.0 | 14.1 | 19.9 | 4.4 |
| Fraction Absorbedc | 0.59 | 0.20 | 0.86 | 0.30 | 0.27 | 0.38 | 0.36 | 0.16 |
| AUC/Absorbed dose (ng-hr/ml)/mg/kg) | 23.6 | 57.0 | 47.0 | 60.9 | 33.4 | 46.2 | 67.6 | 60.1 |

a ND = Not Detected

b Ns is presumably “no sample”.

Source: Brzak (1999) ([24](#_ENREF_24)) and Kisicki et al. (1999) ([23](#_ENREF_23)) as summarized by Doherty and Taylor (1999). ([25](#_ENREF_25))

cFrom calculations by Dow authors from total TCP metabolite excretion up to 168 hours after dosing, adjusted by us for the individual amounts of TCP likely to remain in the body at 168 hours.

1.21 to 1.54. These findings therefore indicate a definitely greater metabolism rate than the than the 0.256/min central estimate (with ± 1 standard error confidence limits of 0.222-0.296) that was inferred from the Nolan et al. observation for men.

As with the in vitro microsome derived estimates of adult female metabolism rates, we need to add adjustments related to pregnant/nonpregnant differences in metabolism rates and tissue blood partition coefficients. The adjustment for pregnancy and its uncertainty leads to a central estimate metabolism rate of 2.63/min with a combined ± 1 standard error confidence range of 1.15-6.03/min. Nearly all (98%) of the uncertainty variance contributing to this range comes from the pregnant/nonpregnant adjustment rather than from the uncertainty derived from the Kisicki observations themselves. Relative to the Nolan-derived estimates, the expected metabolism rate using the Kisicki observations is a bit more than 10-fold larger than those derived from the Nolan observations. The ± 1 standard deviation range derived from the Kisicki data (5.25 fold) is slightly narrower than the 6.3 fold derived from the Nolan data. This slightly narrower uncertainty range probably in part reflects the greater number of subjects studied by Kiskicki et al. and the absence of the need for the projection between males and non-pregnant females for the Nolan-calibrated case.

Parallel to the Nolan-derived observations, Table S11 shows the consequences of our assessed uncertainties in both metabolism rate and tissue/blood partition coefficients for expected low dose CPF blood level to dose conversion factors for continuous 400-hour ingestion and inhalation exposures for pregnant women. Again we see the much greater sensitivity of the dose conversion factor for ingestion to changes in metabolism rate. Whereas the central estimate of the ingestion factor is increased by ten fold for the Kisicki-based calibration relative to the Nolan calibration, the indicated central estimate ratio for the inhalation route is increased by less than two-fold.

From these data, an overall model of the uncertainty distribution for the inhalation dosimetry factor results in a geometric mean of 30.1 and 2.5th – 97.5th percentile confidence limits of 26.9 – 33.7. This can be compared with the inhalation dose factor uncertainty distribution derived from the Nolan observations of a geometric mean 18.1 and 2.5 percentile – 97.5th percentile confidence limits of 9.0-36.6.

It may well be asked why the expected range of the inhalation dosimetry factor is so much smaller for the Kisicki et al. calibration than for the Nolan et al. calibration. The GSD for the uncertainty in underlying metabolism rates is not that much different (Nolan 1.455 vs Kisicki 1.298). The answer is that for the higher range of Kisicki metabolism rates, the model is essentially flow limited. For the ± 1 standard error range of the Kisicki-calibrated metabolism rates, the fractional clearance of CPF from blood flowing through the liver is between 88.3-97.5%. In contrast, for the ± 1 standard error range of the Nolan-calibrated metabolism rates, the fraction clearance ranges from 38.8-80.4%--a much more consequential difference for the rates of overall elimination of CPF from the body.

A similarly derived lognormal distribution for the ingestion dose projection factor indicates a geometric mean of 478 and 2.5th – 97.5th percentile confidence limits of 93.9-2,438. This can be compared to the similar statistics of geometric mean 99.4 and 2.5th – 97.5th percentile confidence

**Table S11.** Expected Consequences of ± 1 Standard Error Variations in Pregnancy-Related Metabolism Rates Inferred from the Kisicki et al. (1984) Observations and Tissue/Blood Partition Coefficients for the Low Dose CPF Blood Level to External Dose Conversion Factors for Ingestion vs Inhalation Routes of Exposure

|  |  |  |  |
| --- | --- | --- | --- |
|  | cent est metab preg | "-1 SD metab preg" | "+1 SD metab preg" |
| **Baseline partition coefficients** |  |  |  |
| Ingestion | 478 | 209 | 1095 |
| Inhalation | 30.5 | 28.3 | 31.6 |
| ingestion/inhalation ratio | 15.7 | 7.4 | 34.6 |
|  |  |  |  |
| **1 std error low tissue/blood partition coefficients** |  |  |  |
| Ingestion | 423 | 185 | 968 |
| Inhalation | 30.1 | 27.6 | 31.3 |
| ingestion/inhalation ratio | 14.1 | 6.7 | 30.9 |
|  |  |  |  |
| **1 std error high tissue/blood partition coefficients** |  |  |  |
| Ingestion | 535 | 234 | 1224 |
| Inhalation | 30.9 | 28.9 | 31.9 |
| ingestion/inhalation ratio | 17.3 | 8.1 | 38.3 |

limits of 30-329 derived from the Nolan et al. data. Therefore we must conclude that although there is some overlap in the 95% confidence bounds, there is likely to have been some unsuspected source of systematic measurement error that has caused the ingestion dosimetry implications of the Kisicki observations to diverge by about 5 fold from those of Nolan et al. The model structure assumption involved with first pass metabolism amplifies this difference compared with the much more modest 30.1/18.1 = 1.66 fold difference at the geometric means implied by the two sets of data for the inhalation dosimetry factor.

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