**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**

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Neurodevelopmental effects of chlorpyrifos have been observed in human studies that relate indices of function at age 7 to fetal cord blood levels at birth. Consideration of these findings in setting regulatory guidelines for exposure requires a way to translate between the fetal and maternal blood levels and external exposures. This work integrates information from four sources and associated parameter uncertainties to develop the needed low-dose dosimetry translation factors. There is more qualitative treatment of model uncertainties. The results illustrate the narrowing of parameter uncertainties with the progressive integration of different types of information, although even with all data inputs considered, 95% confidence ranges on the potency of chlorpyrifos for inducing neurodevelopmental effects still span over 10-fold ranges, depending on the route of exposure and the specific index of function used. Extensive sensitivity analyses show the effects of including vs excluding specific information sources, and other analytical choices. Despite the residual uncertainty, the human observations suggest greater sensitivity to these effects than is provided for by standard inferences of protective exposure levels from observed inhibition of cholinesterase in adult rats, combined with traditional uncertainty factors. Ultimate recommendations for risk management standards (RfDs and RfCs) based in part on these results will depend on the development of guidance that takes into account both human variability in susceptibility and the remaining uncertainty—that is, what percentile of the population is to be protected from how much impairment with what degree of confidence?

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# 1. Background

The preliminary EPA risk assessment for chlorpyrifos ([1](#_ENREF_1)) derives proposed reference doses (RfDs) and reference concentrations (RfCs) entirely from observations of cholinesterase inhibition in animals. However,recent epidemiological data from Columbia University researchers([2](#_ENREF_2)) ([3](#_ENREF_3), [4](#_ENREF_4)) ([5](#_ENREF_5)) and others([6](#_ENREF_6)) ([7](#_ENREF_7)) ([8](#_ENREF_8)) indicate long term neurodevelopmental effects from exposures of pregnant women. Furthermore, there is some mechanistic support for the idea that chlorpyrifos can influence neurodevelopmental processes in at least some cases at concentrations below those needed to produce appreciable inhibition of acetylcholinesterase enzyme activity ([9](#_ENREF_9)) and pathways that do not necessarily involve chlorpyrifos oxon formation. For example inhibition of axon growth in vitro occurred to the same extent with chlorpyrifos or its oxon, and was observed at concentrations below those where cholinesterase inhibition was detected.([10](#_ENREF_10)) The human neurodevelopmental data cited above do not assess cholinesterase but rather focus upon functional endpoints in neonates that are relevant to learning and behavior and thus are relevant to human risk assessment. The present paper evaluates the dose response for two of these endpoints from the Columbia cohort (reduction of working memory and IQ at age 7) in an effort to bring the epidemiological findings into a risk context and inform the selection of RfDs and RfCs.

An important feature of the Columbia University observations is that the effects observed are related to an internal biomarker of exposure—blood levels of chlorpyrifos observed in both newborns and their mothers shortly after birth. In order to quantitatively use observations of this type in consideration of “Reference Doses” or Acceptable Daily Intakes, it is necessary to translate these blood levels to estimates of long term steady-state external doses. Some of the women in this cohort received exposures related to the insecticidal spraying of their apartment on a repeated basis during the 1990s, a time when chlorpyrifos was common in household sprays. Measurements of indoor air in a limited subset of the cohort confirmed that chlorpyrifos was a major indoor contaminant during gestation in these women. This paper develops blood-level-to-absorbed dose translation factors with explicit treatment of the uncertainties in combining information from different sources, and then provides a preliminary discussion of implications for risk management choices.

The standard approach to developing such dosimetry translations is to use a physiologically based pharmacokinetic model, ideally calibrated with the aid of human observations of the levels of the in vivo biomarkers over time following known external exposures to the toxicant. However in this case there are unusual complications. Because of perceived deficiencies in the procedures used to obtain informed consent, EPA has concluded that it is barred from using the more recent (late 1990s) Kisicki ([11](#_ENREF_11)) observations of blood levels in relation to known oral doses of chlorpyrifos (J.Carley memo dated 5/29/09; <http://www.epa.gov/hsrb/files/1d6-ethics-rvw-kisicki-etal-060109.pdf>). Therefore it is desirable to develop estimates of the blood to external dose translation factors with and without dependence on this source of information. The difficulty is compounded because there is an appreciable but unexplained quantitative inconsistency between the blood levels observed in these Kisicki data in relation to chlorpyrifos dosing and an older set of observations made by Nolan and coworkers in the early 1980s. ([12](#_ENREF_12)) Thus the use of the Kisicki data requires making choices for resolving that inconsistency, in addition to the usual needs to make many small choices to address incompleteness in data sets and the translation of information developed in different settings.

A Bayesian-inspired analytical framework is well suited to the task of developing uncertainty distributions based on inputs of different sets of information. (See, for example, Bedford and Cooke ([13](#_ENREF_13)). Also, with differing perspectives, Gelman et al., ([14](#_ENREF_14)), and Robert ([15](#_ENREF_15))). Our analysis of the uncertainty distribution for the low dose blood/external air translation factor draws on:

* A “prior” lognormal uncertainty distribution constructed by using measured air exposures and measured urinary metabolite excretion for the Columbia cohort women in relation to observed blood levels to bound the range of plausible blood/external air translation factors.
* Results of runs of a physiologically based pharmacokinetic model derived by Timchalk, ([16](#_ENREF_16)) recalibrated using recently published in vitro human metabolism data ([17](#_ENREF_17)) .
* Two mutually contradictory sets of results of direct human oral exposure studies, done in the early 1980s by Nolan et al. ([12](#_ENREF_12)) and the late 1990s by Kisicki et al., respectively. ([11](#_ENREF_11))

We do not attempt a complete Bayesian analysis, based on a “full probability model”([14](#_ENREF_14)). Our more limited goal is to make an informative description of what can reasonably be inferred from those various data sources interpreted as probabilistic predictions. For the myriad small issues that arise in interpreting each particular data set, our approach is to make reasonable choices and to be transparent about those choices and their justification. For considering the influence of different data sets we combine them in a sequence of steps and present results at each step. The results after combining information from three sources show what can inferred while ignoring the Kisicki data. Considering the Kisicki data raises additional challenges because they appear to contradict the Nolan data. The appearance of contradiction raise the question of whether one (or both) of the data sets might have unknown sources of error. These possibilities can be elucidated by considering the findings that incorporate both, one or the other, or neither of the Nolan and Kisicki data sets.

The discussion of methods and intermediate results below is kept brief to stay within space limitations. More details of the application of the methods and intermediate results are available as supplementary material in a more extended version of this paper (http://www2.clarku.edu/faculty/dhattis/).

# 2. Analytical Methods

## 2.1 PBPK Modeling for Dosimetry Translation Among Different Routes of Exposure

The object of our model predictions is the low dose ratio of external absorbed CPF dose (by either inhalation or ingestion) to the blood CPF level in units of ng CPF/kg Body Weight-day absorbed external dose/(pg CPF/g blood). In many cases, primary data for one route of exposure (e.g. ingestion for the Nolan and Kisicki data sets) are interpreted for the other route of exposure using PBPK modeling. To do this, metabolism rates and associated uncertainties are transferred between models incorporating the different exposure routes. PBPK model calculations of this ratio are not taken to infinite time but are done to a standard time (400 hours, or somewhat over 2 weeks) that is long enough for most compartments except fat to closely approach steady state, but not long enough to be unrealistic in representing the changing compartment sizes and blood flows of late pregnancy.

In doing this it is important to recognize the effects of some common conventions in PBPK modeling that create non-obvious differences in the mathematical form of the relationships between metabolism rates and the external dose needed to produce a given long term blood level. These conventions are (1) all metabolism occurs in the liver, and (2) following ingestion, all blood carrying absorbed material flows to the liver before reaching the systemic circulation.

It turns out that for the ingestion route of exposure, these typical assumptions lead to a very straightforward relationship between metabolism rates and the dosimetry translation factor, even where the metabolism rate is such that a large fraction of the chlorpyrifos is expected to be removed on the “first pass” through the liver. However, with the inhalation route, only about a quarter of the chloropyrifos absorbed into the arterial blood in the lungs is subjected to liver metabolism before reaching the venous blood. The effect of this is that even for very high rates of metabolism, the external dose rate/blood level ratio approaches a maximal value (Figure 1).

This maximum can be determined directly by making model variants where the release of chlorpyrifos from the liver to the venous blood pool is set to zero. For central estimates of tissue/blood partition coefficient, as depicted in Figure 1, that maximum is about 32.53 ng/kg-day per pg/g of blood. This maximum changes only slightly for ± 1 standard error estimates of the tissue/blood partition coefficients (32.30-32.76).

The saturation-type form in Figure 1 suggests a Michaelis-Menten type relationship between the liver metabolism rate and the inhalation dose/blood level ratio. For the central estimate partition coefficients, the points are well fit (average root mean square error for 18 data points = 0.5% of the inhalation dose ratio values) by



where the liver metabolism rate is in units of 1/min, and “base”, “max ratio”, and “half-saturation rate” are fitted constants. For the central estimate tissue/blood partition coefficients (liver/blood = 6.1) these fitted values are:

base = 0.325

max ratio = 32.6

half-saturation rate = 0.189

Trials with a range of values for the liver/blood and other related tissue/blood partition coefficients reveal that the “max ratio” and “half-saturation rate” components of this equation are

**Fig. 1.** Contrasting relationships between liver metabolism rate and dose/blood level ratios for PBPK-modeled ingestion vs inhalation routes of absorption for central estimates of tissue/blood partition coefficients. The non-linearity in the inhalation curve results from the facts all metabolism is placed in the liver and that only about a quarter of the chlorpyrifos absorbed via inhalation passes through the liver before it can reach and be measured in the venous blood. Thus at high metabolism rates an upper limit is reached in the dose/blood ratio that corresponds to nearly complete elimination of chlorpyrifos from the blood going to the liver, but little or no elimination from blood that reaches the venous circulation after first passing through other organs. By contrast all blood absorbed via ingestion is expected to first pass through the liver before reaching the venous blood—leading to a near linear relationship between the liver metabolism rate and the absorbed dose needed to produce a given level of chlorpyrifos in venous blood.

influenced by the liver/blood partition coefficient. A revised equation including this dependency is



where minimizing the squares of the deviations of observed from model fitted values yields:

base = 0.335

max ratio = 32.6

half-saturation rate = 0.189

fitted exponent = 0.0606

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

With these values, the root mean square error of the model fit to 41 data points is 0.37% of the mean inhalation dose/blood level. This equation is used below to model the combined effects of uncertainties in metabolism rates and tissue/blood partition coefficients.

The oral dose/blood level relationship is similar, but with a modified saturable element created to accommodate the upper limit dose/blood level relationship derived in the bounding analysis (see Table III below, footnote c).

## 2.2. Combining Uncertainty Distributions from Various Sources

For each of our four sources of information (j = 1-4) we derive a probability distribution Pj(i) that gives the probability that a median pregnant woman would have the value of that ratio in the small incremental range i based only on that source of data. We then combine the probability density functions into “posterior” distributions that represent inferences drawn from more than one data source. The formula for doing so is the standard form for conditioning (Bedford and Cooke ([13](#_ENREF_13)) pp. 65-66) where the products include the “prior” (j =1) and whatever other sources of information are being combined.



The denominator of this function essentially normalizes the individual probability estimates to 1 by summing the combined probability products in the numerator over all the i intervals that are considered remotely possible.

## 2.3 Deriving Probability Distributions for Each Source of Information

Limitations on the length of the manuscript preclude a detailed discussion of the derivation of uncertainty distributions from each of the four types of information inputs. This is provided in supplemental information available via our website (http://www2.clarku.edu/faculty/dhattis). Instead, we describe here the kinds of uncertainties included in interpreting information from each source, some specific inputs to the uncertainties that may possibly be useful in other analyses, and the general way we derived probability density functions from each type of input.

Where non-detect values were present in primary data, imputations of mean values for the non-detects were done by fitting lognormal distributions to the data above the non-detect limits. This was needed only in the case of the Kisicki et al. data.

The very different functional forms of the relationship between liver metabolism rate and dose/blood level ratios for ingestion vs inhalation routes of absorption led to appreciable differences in our approaches for quantifying the P(i)s based on different input information. In the case of ingestion, where the uncertainty distributions derived from sources other than the truncated prior are well described as lognormal (See Figure 2), P(i)s are simply calculated in log increments from the fraction of a lognormal distribution falling between the bounds of each interval. By contrast, for the inhalation case, where the saturation-type relationship to metabolic rates yields uncertainty distributions that are not well described as either lognormal or normal (See Figure 3), we use empirical distributions derived from 100 X 100 combinations of pharmacokinetic model outputs representing probabilistically equal fractiles in the uncertainties in both metabolic rates inferred from different information sources and uncertainties in partition coefficients.

Table I (with details in Tables II-VII) lists the uncertainties that are involved in deriving probability density functions from each of the four sources of information. It can be seen that some of these are shared between the analyses of different sets of primary data. Therefore, although each of the primary data sources is independent of the others, the shared analytical elements mean that the derived density functions are not completely independent.

# 3. Pharmacokinetic Results

Table VIII summarizes the effects of the progressive addition of information from various sources to obtain the final results. It can be seen that with each addition of data, the assessed uncertainty distribution tends to narrow, even if the new data have appreciable differences from the previously analyzed data. However it can be seen in the comparison between the last line and the second to the last line under the “inhalation dose/blood” section that this is not always the case. The final combination of all four sources of information has a slightly larger GSD than the combination of three sources excluding the Nolan information. The final assessments indicate that the uncertainties are modest enough for the results to be useable—with 2.5-97.5 fractile ranges spanning about 2.6 fold for oral absorption and 1.5 fold for inhalation absorption.



**Fig. 2.** Lognormal plot of the cumulative probability density function for the ingestion dose/blood level ratio resulting from all combinations of 100 equal-probability weighted values of metabolism rate (derived from the Kisicki observations) and 100 equal probability weighted values of the liver and other tissue/blood partition coefficients derived based on lipophilicity information for pregnant women from Lowe et al. (2009) and other sources. The line represents a fitted lognormal distribution, which reasonably describes the plotted points over the central ± 3 standard deviation range of Z-Scores.



**Fig. 3.** Lognormal plot of the cumulative probability density function for the inhalation dose/blood level ratio resulting from all combinations of 100 equal-probability weighted values of metabolism rate (derived from the Kiskicki observations) and 100 equal probability weighted values of the liver and other tissue/blood partition coefficients derived based on blood lipophilicity information for pregnant women from Lowe et al. (2009) and other sources. It can be seen that the lognormal hypothesis fits the distribution poorly, necessitating use of the distribution in empirical form. The discrepancy is smaller, but still pronounced, for a similar plot constructed based on the uncertainty in liver metabolic rate derived from the Nolan observations.

**Table I.** Shared Uncertainties in Analyses of Each of the Four Sources of Primary Information on CPF External Dose/Blood Level Ratios

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primary Information Source | "Prior" Bounding Analysisa of Inhaled CPF Dose and Metabolite Excretion/Blood Levels in Columbia University Cohort ([2](#_ENREF_2)) Women | Metabolism Rates of CPF in Vitro by Liver Microsomes ([18](#_ENREF_18)) | Blood Levels (Interpreted as 12 Hour AUCs) and Metabolite Excretion After a Single CPF Oral Dose (Nolan et al. 1984) ([12](#_ENREF_12)) ,([19](#_ENREF_19))(Table V) | Blood Levels (Interpreted as 12 Hour AUCs) and Metabolite Excretion After a Single CPF Oral Dose (Kisicki et al. 1999) ([11](#_ENREF_11)) ([20](#_ENREF_20)) (Table VI) |
| Population Studied | Pregnant women exposed in their residences via air and likely other routes (85-453 women with different types of measurements over 3-6 years) | 30 Recently deceased people with no known special exposure to CPF, including 5 adult women used for final analysis (Table IV) | 5 male subjects given 0.5 mg/kg CPF orally | 3 groups of 6 male and 6 female subjects each given single doses of 0.5, 1.0, and 2.0 mg/kg orally  |
| **Types of Uncertainties Included in Analysis:** |
| Imputation of values for non-detects |  |  |  | Only 8 of 24 subjects at the two higher dose levels had measured CPF in blood over detection limits, requiring imputation for nondetects |
| In Vitro/In Vivo Projection |  | yes (systematic and random error in using liver microsome data from recently deceased people to project human in vivo clearanceb) |  |  |
| Male/Female Projection of Metabolism Rates |  |  | yes--from comparison of male and female in vitro microsomal metabolism rates (Table IV); with uncertainty quantified by addition of variances in log space |  |
| Pregnant/Nonpregnant Metabolic Rate Projection |  | yes [represented as a random draw of clearance ratios from 7 drugs studied by Loebstein ([21](#_ENREF_21)) (Table VII), treated as lognormal) | yes, from Loebstein data (Table VII) | yes, from Loebstien data (Table VII) |
| PBPK Model Assumptions Other than Metabolism | Yesa | yes (all metabolism in the liver; pregnancy-related partition coefficient changes estimated by Lowe; ([22](#_ENREF_22)) with uncertainties for non-fat tissue patterned after those from fat; pregnancy-related compartment size changes) | yes  | yes  |

a See Tables II and III. An initial lower bound was inferred from ratio of measured air exposures/blood levels for relatively early years where blood levels had few non-detect values (Table II). Later PBPK modeling established that even with a zero liver metabolism rate, and a 2 standard deviation low estimate of tissue/blood partition coefficients, the model could not produce a ratio as low as that indicated by the lower bound. An initial upper bound was inferred from the ratio of excretion of the CPF metabolite TCP to the blood levels (Table III). This was an upper bound because of other sources of TCP in urine, prominently including preformed TCP in diet from use of CPF in agriculture.([23](#_ENREF_23)) These bounds were treated as subjective 1%-99% confidence limits on the inhalation/blood ratio. PBPK-model based metabolism rates from the inhalation bounding distribution were used to infer corresponding percentiles of the liver metabolism rate distribution.

b Data of Ito and Houston (2005) ([24](#_ENREF_24)) for 52 drugs 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. This mean and log variance is used to represent the likely systematic bias and random error uncertainty in the microsome-based metabolism data.

**Table II.** 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 III.** 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) ([25](#_ENREF_25)).

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.

**Table IV**. 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 |

**Table V.** Observations by Nolan et al. (1984) 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. ([19](#_ENREF_19))

**Table VI.** Observations of Blood Chlorpyrifos Levels in 8/24 Kisicki et al. (1999) 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) ([20](#_ENREF_20)) and Kisicki et al. (1999) ([11](#_ENREF_11)) as summarized by Doherty and Taylor (1999). ([26](#_ENREF_26))

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.

**Table VII**. 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

Table VIII. Effects of Progressive Addition of Information from Different Sources on the Central Estimates and Uncertainties of Inhalation and Ingestion Dose/Blood Level Ratios [ng CPF absorbed/kg body weight-day]/pg CPF/g maternal blood plasma)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Inhalation Dose/Blood Level Ratios**  | Gmean | GSD | 2.5th percentile | 97.5th percentile |
| "Prior" Bounding Analysis (Tables II and III) | 15.3 | 1.734 | 2.93 | 30.5 |
| In Vitro Microsome Metaoblism([18](#_ENREF_18)) ([24](#_ENREF_24)) | 23.3 | 1.476 | 7.44 | 32.3 |
| **2-way Comb.--Prior and In Vitro Microsome Metab** | **20.2** | **1.402** | **8.78** | **31.5** |
|  |  |  |  |  |
| Nolan Oral Human Dosing Experiments | 17.7 | 1.460 | 6.98 | 29.3 |
| **3-way Comb. Excluding Kisicki** | **20.5** | **1.293** | **11.2** | **29.9** |
|  |  |  |  |  |
| Kisicki Oral Human dosing Experiments | 29.9 | 1.078 | 24.2 | 32.3 |
| **3-way Comb excluding Nolan** | **28.5** | **1.091** | **22.3** | **31.6** |
|  |  |  |  |  |
| **4-way Comb. including all sources of information** | **27.6** | **1.103** | **21.1** | **31.0** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ingestion Dose/Blood Level Ratios** | Gmean | GSD | 2.5th percentile | 97.5th percentile |
| "Prior" Bounding Analysis | 24.4 | 1.719 | 4.58 | 48.7 |
| In Vitro Microsome Metaoblism ([18](#_ENREF_18)) | 156 | 4.501 | 8.06 | 3040 |
| **2-way Comb.--Prior and In Vitro Microsome Metab** | **24.5** | **1.638** | **7.38** | **48.0** |
|  |  |  |  |  |
| Nolan Oral Human Dosing Experiments | 45.9 | 2.485 | 7.61 | 276 |
| **3-way Comb. Excluding Kisicki** | **25.1** | **1.469** | **10.4** | **46.6** |
|  |  |  |  |  |
| Kisicki Oral Human dosing Experiments | 479 | 2.295 | 93.1 | 2460 |
| **3-way Comb excluding Nolan** | **35.3** | **1.289** | **19.8** | **52.1** |
|  |  |  |  |  |
| **4-way Comb. including all sources of information** | **33.7** | **1.288** | **19.0** | **49.9** |

Overall, it can be seen that chlorpyrifos absorbed via the oral route is expected to have a slightly smaller and more uncertain impact in raising blood levels than chlorpyrifos absorbed via inhalation. Based on all four sources of information, final geometric mean estimates of the dose/blood ratio differ by a little more than 20% between the two routes (33.7/27.6 = 1.22). This difference is solely due to “first pass” metabolism in the liver, and does not reflect any assumption about the fraction absorbed via oral vs inhalation exposures. If oral absorption is less than complete, that should be reflected in an additional factor added to assessments of absorbed dose in relation to external exposures.

As indicated earlier, the current EPA position is that it is barred from using the Kisicki data because of deficiencies in the informed consent procedures used by the investigators. The effect of this is to lower the central estimate dose/blood level ratios by about a quarter for both routes of absorption. This would correspondingly increase the expected potency of chlorpyrifos for inducing developmental effects. Additionally excluding the Kiskicki data increases the estimated uncertainty in the dose/blood level ratios with 2.5-97.5 fractile ranges increasing to 2.7 fold for inhalation absorption and 4.5 fold for oral absorption.

A final feature of methodological interest is that despite substantial uncertainty, the exercise of developing the “prior” bounding estimates does turn out to have an appreciable influence on the final results. In particular the upper limit on the potential absorbed dose/blood level ratios developed by comparing the observed ratios of mean chlorpyrifos metabolite excretion to mean chlorpyrifos blood levels in the Columbia University data appreciably narrowed the range of oral dose/blood level ratios that would otherwise have been inferred from the statistically strong Kisicki data.

# 4. Preliminary Implications for the Potency of Chlorpyrifos for Inducing Modest Impairments in Neurological Development

Both IQ and the measure of working memory found to be significantly affected at age 7 in the Columbia work ([2](#_ENREF_2)) are indexes of function defined as having a mean of 100 and a standard deviation of 15 in a normal population. Regression analyses controlling for confounders (home observation for measurement of the environment; sex of the child; completed years of maternal education, maternal IQ, race/ethnicity, annual income less than $20,000/year, second-hand smoke exposure in pregnancy, polycyclic aromatic hydrocarbons measured by personal air sampling, and exact child age at testing) yielded the following estimates of effect sizes:

Ln(Working memory)—0.006 decrease per pg CPF/g cord blood plasma (95% CL = 0.002 –
 0.01) p = 0.003

Ln(Full scale IQ)—0.003 decrease per pg CPF/g cord blood plasma (95% CL = 0.000 – 0.006)
 p = 0.05.

For modest levels of CPF in cord blood (1-10 pg/g) the effects on population mean IQ expected from these relationships are very nearly linear—thus for full scale IQ the central estimate is for a change in the population mean IQ of -0.300 points at 1 pg/g and -2.95 IQ points for a concentration of 10 pg/g.

Bringing these relationships together with our earlier estimates of dose/maternal blood plasma CPF and associated pharmacokinetic uncertainty requires a conversion between maternal and cord blood plasma. Based on 338 paired observations from the Columbia University data in maternal and cord blood mean CPF levels ± standard errors were 2.88 ± 0.26 pg/g for maternal blood plasma compared to 3.26 ± 0.40 pg/g for fetal cord blood plasma. Based on a total of 15,000 trials of these distributions, a mean estimate of maternal/cord concentration ratio is about 0.90 with a 95% confidence range of 0.66 to 1.22.

The final distributional implications of the combined pharmacokinetic and pharmacodynamic uncertainties are derived in simulations of 5000 trials (i) each of



The numerator of this expression derives the expected index change/maternal blood concentration for each trial, and the denominator converts to units of absorbed daily dose via either inhalation or ingestion routes. Because the distributions for the absorbed dose/maternal blood ratio in were not simply describable as either normal or lognormal, a table-lookup function was used to select derived values for this. The Excel spreadsheets used for these calculations are included in the supplementary material made available on our website.

Table IX shows the arithmetic mean results of three 5000-trial runs of this expression for the arithmetic mean and confidence/uncertainty fractiles of the index change/ng/kg-day of absorbed dose based on independent selections from the three sets of uncertainty distributions derived earlier. The arithmetic mean IQ change is included here in order to facilitate economic “expected value” juxtapositions of possible costs and benefits from changes in population exposures. On the other hand, upper fractiles of the uncertainty distributions may well be considered relevant under various risk management scenarios. The eight lines of the table represent all combinations of (1) the index of neurodevelopmental function (visual memory index vs full-scale IQ), (2) the inhalation vs ingestion route of absorption, and (3) exclusion vs inclusion of the Kisicki et al. source of data related to the ratio of absorbed daily dose/maternal blood CPF concentration.

It should be stressed that not all sources of uncertainty are included in this quantitative assessment. We have not attempted to quantitatively assess model uncertainties related to the form of the dose response relationship used in the primary analysis of the Columbia data, uncertainties related to the control of confounders in that work, and assumptions related to the distributional forms of both human interindividual variability and the uncertainties related to each source of information.

# 5. Discussion

The possible implications of these results for risk management choices (e.g., RfDs, RfCs, ADIs) must necessarily be left for decision-makers operating under their different legislative mandates. There presently is no unambiguous risk management guidance on this in the U.S. However it is not unreasonable to make some comparisons with the existing suggested RfD for chlorpyrifos based on a range of some not-clearly-incorrect choices of the uncertainty and variability criteria for risk management.

Based on the most recent EPA analysis (which is now under consideration for revision) the chronic point of departure benchmark dose based on the lower confidence limit of the dose causing 10% inhibition of cholinesterase levels in pregnant rats, is 0.03 mg/kg-day. After application of a traditional 100x adjustment to account for possible animal to human, and sensitive human to average human sensitivity differences, they arrive at a candidate oral reference dose of 0.0003 mg/kg-day, which is equivalent to 0.3 µg/kg-day or 300 ng/kg-day.

When basing risk assessment on a small shift at the individual level that is within the clinically normal range, one mus choose and effect size that can be considered biologically meaningful. For the chlorpyrifos-induced decrement of population mean IQ, the most directly applicable precedent for a target level response to neurodevelopmental toxicant can be found in a recent paper by researchers at the California EPA’s Office of Environmental Health Hazard Assessment ([27](#_ENREF_27)). These authors derive a target change in blood level to be prevented that is associated with an expected change in population mean IQ of 1 point (1/15th of the standard deviation of IQ values in a normal population). Such a criterion would lead to definition of an oral chlorpyrifos RfD somewhere in the range of 24-100 ng/kg-day assuming 100% absorption, mean-.975 fractile choices in the uncertainty distribution, and inclusion vs exclusion choices for the use of the Kisicki et al. data. Thus even using this relatively permissive choice of a 1 full IQ point criterion for population change in full scale IQ, the Columbia observations would seem to indicate a need for a several fold more stringent RfD than would ordinarily be derived from the available animal data.

Table IX. Expected Arithmentic Means and Confidence Distributions for Changes in Indices of Neurodevelopmental Function Per Unit of Absorption of Chlorpyrifos Per Day During Pregnancy Based on Dosimetry and Effect Observations in People [Index Change/(ng/kg body weight-day)]

 --Fractile of Confidence/Uncertainty Distribution--

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Index of Function | Route of Absorption | Include vs Exclude Kisicki | Arithmetic Mean | 2.5 | 10 | 50 | 90 | 97.5 |
| Working Memory | Inhalation | Exclude | 0.035 | 0.010 | 0.017 | 0.032 | 0.055 | 0.076 |
| Working Memory | Inhalation | Include | 0.025 | 0.0080 | 0.013 | 0.024 | 0.037 | 0.046 |
| Working Memory | Ingestion | Exclude | 0.030 | 0.0075 | 0.012 | 0.026 | 0.051 | 0.076 |
| Working Memory | Ingestion | Include | 0.021 | 0.0063 | 0.010 | 0.019 | 0.034 | 0.045 |
| Full Scale IQ | Inhalation | Exclude | 0.017 | -0.0001 | 0.0052 | 0.016 | 0.031 | 0.043 |
| Full Scale IQ | Inhalation | Include | 0.012 | 0.0001 | 0.0041 | 0.012 | 0.021 | 0.027 |
| Full Scale IQ | Ingestion | Exclude | 0.015 | -0.0001 | 0.0040 | 0.013 | 0.027 | 0.042 |
| Full Scale IQ | Ingestion | Include | 0.010 | -0.0001 | 0.0032 | 0.010 | 0.019 | 0.026 |

The California OEHHA IQ target is not the only approach for establishing risk criteria for neurodevelopmental outcomes. Another possible criterion would be to allow no more than a 10% change in the risk of being below the 1st percentile of normal function (that is, and extra 1/1000 population risk of profound impairment). For IQ and working memory, which are defined as having population means of 100 with a standard deviation of 15, the first percentile of a normal population is expected to correspond to an index value of 65.1. Assuming no change in the population standard deviation, an extra 10% chance of being below an IQ or working memory index value of 65.1 would be produced by a population mean shift of about 0.54 index points. Adopting this criterion, and including as an option, change in working memory index would lead to candidate RfDs in the range of 7 – 50 ng/kg-day.

Consideration of the potential economic consequences provides additional perspective on the acceptable effect size for neurodevelopmental outcome. In the light of the association between IQ and worker productivity, Grosse et al. ([28](#_ENREF_28)) estimate that each loss of one IQ point for a 2-year old is expected to produce about a 2% reduction in the present value of lifetime income (with confidence limits of 1.8 – 2.4 %). Based on an average 2-year old’s estimated lifetime income of $723,000, each IQ point change is therefore expected to lead to economic damage of about $14,500 in 2002 dollars (with confidence limits of about $13,000 to $17,000). Based on this, a population mean shift in IQ of even as much as 0.5 index points, as implicitly assumed in the previous paragraph, may not be considered “de minimis”.

# Summary

In carefully controlled and extensively reported epidemiology investigations, prenatal chlorpyrifos exposure has been associated with neurodevelopmental deficits in IQ and working memory in the Columbia cohort. These findings are generally supported by similar investigations by other groups examining prenatal effects of chlorpyrifos exposure. To make the Columbia findings useful for dose-response assessment, we have applied a Bayesian inspired PBPK model approach to convert biomarker data to intake dose. While the uncertainty in this conversion is substantial, the range of chlorpyrifos potency estimates for changes in IQ and working memory of consequential effect size yield a greater potency than the current RfD/RfC approach on IRIS based upon cholinesterase inhibition in rodents. Thus, we provide a way to quantitatively inform future chlorpyrifos dose response assessments with epidemiological endpoints of direct relevance to human risk.

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