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CHLORPYRIFOS - A SCIENTIFIC PERSPECTIVE ON THE RELIABILITY AND UTILITY OF INFORMATION FROM EPIDEMIOLOGY STUDIES

Dear Dr. Bradbury,

It is our understanding that the Office of Pesticide Programs (OPP) is preparing a revised human health risk assessment for chlorpyrifos and that this risk assessment could be available for public comment in 2014. As OPP health scientists work toward completion of this task, existing epidemiology data must be considered in addition to many other complex, technical toxicology datasets that have been developed for chlorpyrifos over a number of years.

In considering epidemiology data, it is important that the data be critically evaluated to determine whether they are sufficient to inform risk assessment. Though robust epidemiologic studies have the potential to be useful, preliminary and incomplete studies are often used to fuel sensationalized media messages that may lead to unwarranted public fear and confusion. A recent example of how certain epidemiology studies have been misrepresented and pushed beyond what can reasonably be concluded for chlorpyrifos is the September 3, 2013 article by the Pesticide Action Network in Mother Earth News, entitled, "*Pesticides in Food are Keeping Children From Learning.*"

EPA's Scientific Advisory Panel noted in 2010 when commenting on the Office of Pesticide Programs (OPP) proposed framework to incorporate human studies in its health risk assessments: "*like all information considered in risk assessments, the quality and reliability of the information provided by epidemiologic studies needs to be closely scrutinized.*" Thus, Dow AgroSciences (DAS) would like to take this opportunity to provide you with a scientific perspective on the reliability and utility of the information provided within the epidemiology studies available for chlorpyrifos. We have engaged scientists within DAS as well as qualified external scientific and regulatory consultants to prepare the attached white paper. Within this paper, a number of citations are provided to support the points, which we hope will be useful to the assessment team. We would be happy to answer questions on the document or provide any additional clarifications or information you or your scientific staff may need.

Respectfully,

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Epidemiology Studies Pertaining to Chlorpyrifos Exposures: Considerations of Reliability and Utility

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Summary
The utility and application of epidemiology data in risk assessment and regulatory decision-making has received considerable attention in recent years and continues to be vetted by the U.S. Environmental Protection Agency (USEPA) when evaluating chemical risk to human populations (*e.g.*, SAP 2010). For the insecticide chlorpyrifos, there exists a growing number of studies that may inform the risk assessment for this chemical, although one cohort investigated by Columbia University is being considered by the USEPA as providing evidence for the relationship between chlorpyrifos exposure and children's development and cognitive function (*i.e.*, Rauh *et al.*, 2006, Rauh *et al.*, 2011; Whyatt *et al.*, 2004). A critical analysis of published information from varying scientific disciplines and perspectives reveals that findings from this singular cohort have limitations, including reliability of reported results, exposure to other risk factors, lack of reproducibility of findings in other studies, and incompatibility with the voluminous toxicology database for chlorpyrifos (Eaton *et al.*, 2008; Prueitt *et al.*, 2011; Li *et al*., 2012; Burns *et al*., 2013). In fact, the European Food Safety Authority (EFSA) recently concluded in its review on epidemiological studies linking exposure to pesticides and health effects that there is "no evidence" to suggest an association between pesticide exposure and neurodevelopmental related outcomes, due to a number of deficiencies in the available data (Ntzani *et al.*, 2013). This EFSA review included neurodevelopment/IQ studies on chlorpyrifos that were published in 2006 and later. The totality of problems relating to the reliability of the reported findings on the Columbia cohort renders the study inappropriate for risk assessment. The study is not useful for informing the question of whether neurodevelopmental effects occur at exposure levels lower than those associated with acetylcholinesterase inhibition or for "bounding" dose-response estimates from animal studies.

The following are key reasons for our position:

1. The analytical method used in the Columbia study has not been validated at the low concentrations reported in maternal/cord blood from Columbia study subjects. Also, the exposure assessment picture is incomplete for chlorpyrifos (and other chemical exposures), given that the reported effects on neurodevelopment (which is a continuous process throughout pregnancy and during early infant and childhood years) are predicated upon a single exposure measurement (snapshot in time).

2. There is a lack of credible scientific evidence to support the biological plausibility of the Columbia cohort findings at the exposures reported. The animal database for chlorpyrifos is one of the most robust across all chemistries and it is not possible to explain adverse neurodevelopmental effects occurring below the threshold point of departure for acetylcholinesterase without significant speculation and conjecture.

3. Chlorpyrifos cannot reliably be deemed as a causal factor for the reported neurodevelopmental effects. There are credible alternative explanations for the observed effects. In particular, influences of other chemical and nonchemical stressors could contribute to or account for the reported associations of impaired neurodevelopment in the Columbia study. Also many of the outcomes reported may be chance occurrences, given the methods used to assess cognitive function.

4. When considering the specificity of biomarkers for chlorpyrifos exposure and lack of concordance across reported outcomes, the results reported in the Columbia study have not, in effect, been replicated or confirmed in other independent studies.

5. Although sufficient information is available to identify the serious limitations with the Columbia study, nonetheless the data on which the Columbia study findings are based have not been shared in a public format for further independent evaluation by either government agencies or other interested investigators. This is not only counter to the principles of transparency in federally funded research, but precludes further scientific analysis which could ultimately assist in rendering a more informed and objective analysis of the data.

1. The analytical method used in the Columbia study has not been validated at the low concentrations reported in blood:

Because a majority of samples from the Columbia cohort are below the validated limit of detection (LOD) for chlorpyrifos analysis in plasma/serum, any conclusions regarding outcomes and associated exposure based on chlorpyrifos levels < 15 picograms (pg)/gram (g) are not reliable. This impacts the reliability of the correlation analyses between exposures and most outcomes since the study dichotomized exposure as "low" and "high" using 6.17 pg/g as its cut point (Whyatt *et al*., 2004; Rauh et al., 2006). When all values are used in linear analyses, such as when evaluating IQ (Rauh *et al*., 2011), there may be substantial misclassification of exposure.

It is a basic foundation of the scientific process that researchers must show that a quantitative exposure measurement is accurate, precise, and reproducible across the range of values determined within a study. For example, the USEPA method validation guidelines call for replicate determinations of analyte recovery from a given matrix (substrate) down to the stated LOD (USEPA 1998). However, this has not occurred within the Columbia study. There were no data generated during validation of the plasma/serum analysis method (Barr *et al.*, 2002) or during the subsequent analysis of the Columbia cohort samples to show that chlorpyrifos levels could be accurately measured in plasma/serum matrix down to the stated LOD of 0.5-1 pg/g (note: authors

use LOD term when discussing limit of quantitation). The lowest concentration for which analyte recovery in plasma/serum was determined using this method was 15 pg/g. This is a critical point, as more than 80% of the Columbia subjects had levels below this validation level. Further, there was no evaluation of possible sample contamination during blood collection in the hospital, processing to plasma, or during shipment to the CDC. Analysis of sample integrity is a critical parameter of all biomonitoring studies, especially those at the trace levels reported for this cohort. Note that Barr *et al.* (2002) also reported background chlorpyrifos levels of 9 pg/g in control serum samples, 50% higher than the "high" exposure Columbia cohort criteria, the source of which was never determined. Finally, the CDC has stated that chlorpyrifos blood measurements from the 2003-2004 NHANES survey will not be released because the CDC lab was not able to meet its own QC/QA (Quality Control/Quality Assurance) criteria for the assay (personal communication, CDC). This is the same method used in the Columbia study, so any decisions based on the use of such methodology should be highly scrutinized.

2. There is not a supportable basis for biological plausibility:

Based on a consideration of all available data, acetylcholinesterase inhibition (AChEI) data remain the most sensitive and robust source of dose-response data for deriving points of departure for chlorpyrifos (EPA SAP 2012, p. 28). Currently, there is no established biological mode of action to explain the potential neurodevelopmental effects reported in the Columbia study (EPA SAP 2012, p.30). Based on reliable animal experimentation, neurodevelopmental and/or behavioral effects have been reported at higher exposures, *i.e.*, at or above 1.0 mg/kg body weight per day which appears to be a threshold below which neurodevelopmental effects have not been reported (Li *et al*., 2012; Maurissen *et al*., 2000; EPA SAP 2012, p. 36). As noted by the SAP in 2012 (p.36), *" . . . effects of CPF at 1 mg/kg are difficult to interpret because of methodological limitations, inconsistencies, and variation in study design, sometimes lack of control for litter effects, oversampling issues, behavioral methods used, and lack of dose-response findings. At doses exceeding 1 mg/kg, the data show somewhat more consistency, but even here, dose response experiments are the exception."*

The threshold of 1 mg/kg/day is 30-fold higher than the threshold of 0.03 mg/kg/day for the most sensitive red blood cell (RBC) AChEI metric (USEPA 2011 p. 25). The quantitative dose response data for AChEI are especially robust and comprehensive, and include data at the time of peak effect from different ages including rat fetus, young pups, and pregnant and non-pregnant adult rats (Mattsson *et al.,* 2000; Maurissen *et al.,* 2000, Marty and Andrus, 2010).

This threshold of 1 mg/kg/day is also at least 5,000 times higher than estimated exposures from the Columbia study. Lowe *et al*. (2009) estimated prenatal chlorpyrifos exposure levels in the Columbia study to be 0.15 ug/kg/day based on mean maternal and cord blood concentrations reported by Whyatt *et al.,* 2005. These dose levels do not produce RBC AChEI in humans (Garabrant *et al*., 2009; Farahat *et al*., 2011). Using a biomonitoring equivalent approach, all blood concentrations for the Columbia study subjects, as well as those of other epidemiology cohorts, were predicted to be well below the level of RBC AChE inhibition (see Attachment A).

One hypothesis that has been put forth is that the neurodevelopmental effects at these low dose levels are a result of hypothetical non-cholinergic modes of action reported in the animal literature. However, as discussed previously, the animal literature indicates that dose levels that cause adverse neurodevelopmental outcomes only occur at exposures that inhibit AChE in pregnant rats or offspring (Li *et al.*, 2012; Prueitt *et al.*, 2011). Thus, the scientific data support that neurodevelopmental effects attributed to chlorpyrifos in the rodent occur at doses at or above 1 mg/kg/day. Based on the animal model, it would take significant conjecture and speculation to conclude that altered neurodevelopment in humans resulting from non-cholinergic pathway perturbations would occur at doses lower than those associated with AChE inhibition.

3. There are credible alternative explanations for the neurodevelopmental effects observed in the Columbia study:

Chlorpyrifos cannot reliably be deemed a causal factor for the neurodevelopmental effects reported by Columbia University. Alternative explanations are credible and present themselves logically when considering exposure measurement error (as discussed under point #1 and below), the incompatibility with the rodent model (as discussed under point #2), methodological issues with analysis of data (as discussed below and in Attachment B), exposure to other toxic chemicals, including neurotoxicants, and the published literature documenting that children who grow up in poverty or low income households have difficulties with neurocognitive function. In commenting on the Columbia University study findings, an independent group of experts acknowledged, "*The authors attempted to control for confounding factors, including other known neurodevelopmental risk factors in this inner-city cohort, such as maternal perinatal smoking and alcohol; nevertheless, it is difficult to dismiss the contribution of these and perhaps other confounding factors*" (Eaton *et al.*, 2008).

The mothers and children within the Columbia study who had measurable exposures to chlorpyrifos were also exposed to other chemicals that have the potential to subtly or profoundly affect child neurodevelopment. For example, the Columbia cohort was exposed to polycyclic aromatic hydrocarbons (PAHs), which were reported to be associated with neurodevelopmental effects in these same children (Perera *et al.*, 2006). Also**,** lead levels are an important variable in the Columbia study, especially for low-income families living at or near the poverty level. Blood lead levels have consistently been correlated with IQ loss (Healey *et al.*, 2010), as well as achievement and behavioral deficits (Chandramouli *et al.*, 2009). Lead levels were not properly controlled in the Columbia study (Rauh *et al.*, 2006; 2011) for the entire sample, and it is plausible that associations between chlorpyrifos and neurodevelopmental effects could be partially or wholly attributable to lead (see Attachment B).

Poverty and pesticide exposure are highly correlated. Mothers and children who live in crowded, substandard housing are more likely to encounter exposure to multiple and heavily applied pesticides, both legal and illegal (*e.g.*, Morbidity and Mortality Weekly,1997). Indeed, a survey of the Columbia cohort indicated that pesticide use was frequent (Whyatt *et al.*, 2002). It has been proposed that insecticide exposure (regardless of the chemical) may be a marker for insect infestation (and other related factors) and may not itself be the causal agent driving the neurodevelopmental results (Burns *et al.*, 2013). In fact, the Columbia authors reported an

association with the Bayley Scales of Infant Development (BSID) and piperonyl butoxide, a synergist used with pyrethrins and synthetic pyrethroid insecticides, which replaced the use of chlorpyrifos in residential settings after 2001.

It is well documented in the literature that social hardships related to poverty and maternal depression can affect scores on intelligence tests and other measures of cognitive ability (*e.g.,* Luby *et al.,* 2013; Duncan and Brooks-Gunn, 1997; Feinstein, 2003; Canadian Paediatric Society, 2004; center on the Developing Child at Harvard University, 2009). In the Columbia study (Rauh *et al.,* 2006), neurodevelopmental effects were only observed at 3 years of age, not before. As the children age from birth to 3 years, there are a number of other well-known nonchemical risk factors that affect brain development. Efforts were made by the Columbia study investigators to account for certain risk factors, including the influence of race/ethnicity, gestational age, maternal education and maternal IQ (albeit, there were missing IQ data for several dozen women in the study). Observational data on the quality of the home care-taking environment were also considered, but it is unclear to what extent key risk factors were addressed. For example, information was collected on mothers' feelings and state of mind but there is no indication that these potential risk factors were explicitly addressed. Interestingly, maternal depression was a significant factor for influencing childhood behavior, as modeled by the UC Berkeley investigators (Eskenazi *et al.*, 2007; Marks *et al*., 2010).

Despite efforts made by the Columbia study investigators to account for other risk factors, the influences of other chemical and nonchemical stressors which could contribute to or account for the observed associations of impaired neurodevelopment cannot easily be attributed to the independent effects of a single chemical (*i.e.,* chlorpyrifos) in the multi-chemical exposure scenario experienced by the Columbia cohort, particularly spanning a multi-year period that encompasses an important period of sequential neurodevelopment (*e.g.*, SAP, 2012; Eaton *et al*., 2008). Any inferences based on the Columbia study regarding the degree to which chlorpyrifos contributes to the measured outcomes cannot be separated easily from other risk factors, and thus the study cannot be used to reliably address the question of whether chlorpyrifos can cause neurodevelopmental effects at the exposure levels reported. Although it is legitimate for academic scientists to propose and investigate hypotheses, the Columbia study cannot serve as a reliable basis for addressing key questions regarding a single chemical in a regulatory risk assessment.

Another significant weakness of the Columbia study relates to the exposure data, *i.e.,* measurements of chlorpyrifos do not reflect exposure over time. Evaluations of neurodevelopmental scores/function on the cohort continued into childhood and early adolescence, which is well beyond the single snapshot in time of chlorpyrifos measurement. This is especially pertinent since neurodevelopment occurs both prenatally and postnatally, essentially a continuous process throughout early infant and childhood years (Selevan *et al.,* 2000). The Columbia University study focused exclusively on prenatal exposure as measured by cord/maternal blood measures of chlorpyrifos within two days of birth. Furthermore, the maternal and cord blood measurements represent a single sample, or snapshot (*i.e.,* only one point in time), collected for convenience (at birth) and with no information regarding the chlorpyrifos home application. Given the rapid metabolism of chlorpyrifos in humans and subsequent short residence time in the body, a single sample obtained at the time of delivery or shortly after would have little relationship or meaning to exposure levels that were present during

most of the pregnancy (or thereafter). Also, as discussed earlier in this paper (Point #1), the chlorpyrifos blood measurements cannot be deemed accurate. The inadequate investigation on exposures to either chlorpyrifos or other pesticides and chemicals (*e.g.,* polycyclic aromatic hydrocarbons, lead, *etc*.) results in an incomplete exposure picture.

Lastly, there are issues regarding how cognitive testing was assessed in the Columbia cohort (Rauh *et al.,* 2006) raising the question of whether the reported associations with chlorpyrifos are real or not. Briefly, the cohort was inappropriately dichotomized into two exposure groups and use of a cut-off of a standard score of "85" for BSID scores to denote children as "High Risk" is an arbitrary decision. Also, the "multiple simultaneous" comparisons in Rauh *et al.* 2006 and 2011 can lead to chance errors (see Attachment B for more details).

4. Adverse results reported in the Colombia study are not found in other populations:

An important aspect of determining the validity of an epidemiology study is whether findings can be reproduced; that is, associations between similar outcomes and exposures to the chemical of interest should be found in different populations. Chlorpyrifos is measured in different ways across studies, with some measuring chlorpyrifos itself, and others measuring other biomarkers that represent exposure to chlorpyrifos and other chemicals, such that exposure to chlorpyrifos itself cannot be teased out. The order of reliability of biomarkers is as follows: chlorpyrifos > 3,5,6-trichloro-2-pyridinol (TCPy) > diethylphosphates (DEPs). The metabolite DEP can reflect exposure to pesticides other than chlorpyrifos. TCPy and DEPs in urine can also result from exposure to these OP metabolites in food or the environment rather than to chlorpyrifos or other OPs.

When considering the order of reliability of biomarkers, the results are not consistent across the existing epidemiology studies. (See Attachment C of this document for details). Specifically, studies at Mount Sinai Hospital and the University of California (UC) at Berkeley do not confirm the results reported by the Columbia University researchers (Eaton *et al.,* 2008; Prueitt *et al.*, 2011; Li *et al.*, 2012; Burns *et al.* 2013). The Mount Sinai (Berkowitz *et al*., 2004; Engel *et al.,* 2011) and UC Berkeley (Eskenazi *et al.,* 2004: Eskenazi e*t al*., 2007; Marks *et al*., 2010; Bouchard *et al*., 2011) studies report some neurodevelopmental effects associated only with DEP, a less specific biomarker. Outcomes associated with the more specific biomarker, TCPy, are either not tested or not analyzed. Further, there are two new epidemiology studies that have not observed consistent associations with birth weight or developmental outcomes. Two of these studies measured chlorpyrifos or TCPy with reported exposure levels higher than (China cohort; Wickerham *et al.,* 2012) or comparable to (Mexico City cohort; Fortenberry *et al.,* 2013) the Columbia or UC Berkeley studies. Although these studies did not investigate all of the outcomes measured in the Columbia study, findings for endpoints that were evaluated do not confirm findings from the Columbia study (Rauh *et al.*, 2006 and 2011; Whyatt *et al.*, 2004). Based on epidemiological data published through 2007, Eaton *et al.* (2008) also concluded that there were no consistent associations observed when neurodevelopmental outcomes of the Columbia, Mount Sinai, and Berkley studies were compared.

5. Data access has not been provided:

Transparency and documentation of the decision process are at the core of a credible risk assessment. EPA's Office of Pesticide Programs has a long history of transparency as well as data disclosure in risk assessments to ensure the credibility of its registration and reevaluation decisions. OPP's transparency in its risk assessments and decision-making adheres to President Barack Obama's January 21, 2009 Memorandum to Heads of Executive Departments and Agencies on "Transparency and Open Government" (Obama, 2009). Given the concerns about the reliability of exposure assessment in the Columbia study, there would be value in accessing the data for the purposes of exposure (dose) reconstruction and review of the health effect analyses. Similarly, the UC Berkeley and Mount Sinai studies (Eskenazi *et al*., 2007; Marks *et al*., 2010; Bouchard *et al.,* 2011; Engel *et al.,* 2011) conducted no health analyses using chlorpyrifos in cord blood or with urinary TCPy after age two. Access to and independent analyses of these data would also be informative to determine the reliability of the Columbia results.

OPP has indicated publicly, "*The studies that are the most relevant and informative to risk assessment are those that clearly and fully describe study design, conduct and methods, as well as providing access to the underlying data*" (http://www.epa.gov/pesticides/science/literature-studies.html). OPP has considered the Columbia study, which is a federally funded study, as a source of data intended for consideration in its chlorpyrifos risk assessment (see SAP 2008; 2012). However, though DowAgroSciences has made repeated requests through the Freedom of Information Act (FOIA) to the Agency to obtain the Columbia data, there is a restriction placed on data access by the authors. Thus, independent verification of the analyses (including an evaluation of different cutoff points for exposure and BSID outcomes) and the ability to answer specific questions regarding the Columbia study (*e.g.*, other risk factors) are not possible by DowAgroSciences, EPA scientists, or the public. This lack of access is counter to the recent February 22, 2013 John P. Holdren Memorandum to Heads of Executive Departments and Agencies on "Increasing Access to the Results of Federally Funded Scientific Research" (Holdren, 2013). Thus, any significant cited line of evidence in OPP's chlorpyrifos risk assessment should be based on accessible data sets that allow for independent analysis and verification of conclusions. We acknowledge the importance of protecting the privacy of the subjects in epidemiology studies, but there are well-recognized and accepted ways to provide data on cohort subjects while protecting the privacy of the subjects. Given the problems and complex issues involved with the chlorpyrifos cohort data, including the type of cognitive assessment used, a more thorough and multidisciplinary scientific review is needed, which provides some access to the data and includes pediatricians, epidemiologists, clinicians and neuropsychologists experienced in evaluation of pediatric cognitive function.

<u>Conclusions</u>:

Despite not having access to the data published by Columbia University, there is sufficient information available to conclude that there are serious limitations that impact their utility and reliability in risk assessment. The Columbia study is not suitable as a basis to "bound" dose-response estimates from animal studies or to inform whether neurodevelopmental effects occur at exposure levels lower than those associated with AChEI. This is because of the difficulty of

disentangling the potential of other chemical and nonchemical stressors to account for or contribute to the observed associations. Further, the analytical method used in collecting plasma biomonitoring data to address the issue of whether any health outcome could potentially occur below exposure levels resulting in AChEI has not been adequately validated. The incompatibility with the rodent model and the lack of biological plausibility for chlorpyrifos causing neurodevelopmental outcomes in children at estimated exposure levels, as well as the lack of consistency with other populations, indicate that any reported statistical associations within the Columbia study are likely due to factors other than chlorpyrifos exposure. The data are not accessible for public viewing and independent analysis, which is counter to the basic tenets of transparency in government-funded research. Confidence in the reliability of the conclusions for risk assessment-based decisions is inseparably tied to transparency and transparency cannot be achieved when data access is denied. Collectively, these represent very compelling and independent bases for precluding the use of the Columbia study data in an evaluation of exposure and effect for chlorpyrifos or for calling into question the robust and comprehensive animal database on pre- and postnatal toxicity and its adequacy to characterize the dose-response curve at lower dose levels for the young.

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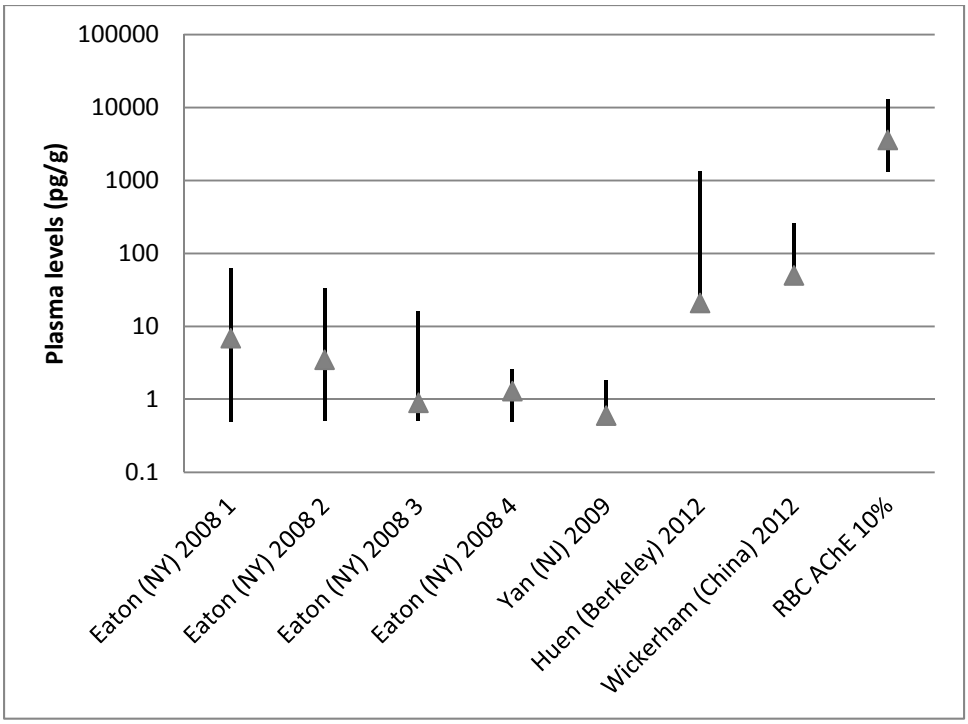
Attachment A: Consideration of AChE depression in Columbia study subjects

The purpose of this attachment is to provide additional explanation supporting the prediction, based on the available biomonitoring data, that all blood concentrations for the Columbia study subjects, as well as those of other epidemiology cohorts, were well below the level of RBC AChE inhibition.

It is cumbersome to compare chronic dose levels administered to animals with concentration levels observed in spot samples collected in humans. Biomonitoring equivalents (BE) address this problem. For chlorpyrifos, BE values were developed for blood CPF that are associated with a predicted maximum of 10% inhibition of red blood cell acetylcholinesterase (RBC AChE) using a physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) model (Arnold *et al*., 2013). Inhibition of RBC AChE, while not part of the cholinergic toxicity pathway in the central nervous system, is a conservative marker of inhibition of brain AChE, since inhibition of these blood enzymes occurs pre-systemically in the liver during CPF metabolism, and is the USEPA regulated endpoint.

There are four human studies in which chlorpyrifos was measured in cord blood and or maternal serum (Whyatt *et al*., 2004; Yan *et al*., 2009; Huen *et al*., 2012; Wickerham *et al*., 2012). The cord blood levels are shown for each study in the graph below. Since the levels declined appreciably over time in the Columbia study, the levels are shown by year of birth (as reported in Whyatt *et al*., 2004 and Eaton *et al.,* 2009). Also shown is the range of plasma chlorpyrifos concentrations predicted to cause 10% inhibition of RBC AChE (RBC AChE 10%). A few subjects in the UC Berkeley study (Huen *et al*., 2012) may have had concentrations near the lowest estimate for 10% RBC AChE inhibition. All other study subjects were well below all estimates for RBC AChE inhibition.



AChE: Acetylcholinesterase; Eaton 2008 1 – 4 (birth years 1999, 2000, 2001, 2002)

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Attachment B: Scientific perspective on specific Columbia study parameters and analyses

The purpose of this attachment is to provide additional points contributed by Dr. Alan S. Kaufman (Clinical Professor of Psychology at the Yale Child Study Center at the Yale University School of Medicine) related to the Columbia study findings (Rauh et al., 2006 and Rauh et al., 2011) and reported interpretation within the broader context of developmental/cognitive testing and assessment. Personal Communication, November 6, 2013.

Columbia Cohort and Lead Exposure

Please comment on exposure of the Columbia cohort to lead and its impact on the exposure-response relationship to chlorpyrifos in the Columbia study?

Lead levels are an important variable in the Columbia study, especially for low-income families living at or near the poverty level. Blood lead levels have consistently been correlated with IQ loss (Healey *et al.,* 2010), as well as achievement and behavioral deficits (Chandramouli *et al.,* 2009). In the Columbia cohort, it was reported "*Lead levels were available, however, for only a subset of children (n = 89). Within this subset, there was no significant relationship between prenatal lead levels and chlorpyrifos levels (r = -0.08; P = .49).*" (Rauh *et al.,* 2006). However, an important question regarding lead that is not addressed in the Columbia study is how did high prenatal lead levels correlate with changes in the Bayley mental and motor development scores? Lead levels were not properly controlled in the Columbia study for the entire sample, and it is plausible that significant relationships could have emerged between lead levels and high chlorpyrifos levels and also between lead levels and Bayley scores. Thus, it is feasible that lead - uncontrolled in this study - played an unknown role in the subjects with "high" chlorpyrifos levels.

Although Rauh *et al.* in the 2006 study neglected to examine the relationship between cord lead and Bayley scores, in their 2011 study, Rauh *et al.* did determine whether cord lead was related to both chlorpyrifos and the WISC-IV scores. Nonetheless, there was cord blood information on too few mothers to be able to control this important variable and blood lead levels of the children in the study were lacking, which should have been done when the children were 1-3 years old.

Columbia Cohort and Cognitive Testing Assessment

Please comment on the use of dichotomized scores on the Bayley Scales of Infant Development (BSID) outcomes.

Regarding the dichotomizing of the Columbia cohort into two portions: Rauh *et al.* (2006) say the issue concerns the dichotomizing of the subjects into two portions. Rauh et al. (2006) indicate: "*The most highly exposed group and the undetectable group had lower mean MDI and PDI scores than did the 2 middle levels. On the basis of these preliminary analyses, and consistent with our previous reports, a dichotomized exposure variable was used, classifying subjects into high exposure (>6.17 pg/g) or lower exposure (≤6.17 pg/g).*" The four groups should have been analyzed separately. There was a "U-shaped" relationship between the Bayley scores and level of chlorpyrifos exposure. The "undetectable" group and the "high exposure"

group both scored lowest on the mental and motor scales of the Bayley. There is no scientific justification for combining the two middle groups with the "undetectable" group. Further, it is inappropriate to examine the Bayley scores for the four groups *before* making the decision of how to combine the data. The Bayley scores are the dependent variable for the study (*i.e.,* the outcome variables). It is not good scientific practice to examine data on the outcome variables before deciding how to analyze the data.

Columbia Cohort and Mental/Psychomotor Performance Across Exposure Groups

Please comment on the mental and psychomotor delay at age 3 when comparing high to low exposure groups within the Columbia cohort.

Regarding the loss of mental function at age 3 years, Rauh *et al.,* (2006) report that "*Highly exposed children (chlorpyrifos levels of ≥ 6.17 pg/g plasma) scored, on average, 6.5 points lower on the Bayley Psychomotor Development Index and 3.3 points lower on the Bayley Mental Development Index at 3 years of age compared with those with lower levels of exposure. Children exposed to higher, compared with lower, chlorpyrifos levels were also significantly more likely to experience Psychomotor Development Index and Mental Development Index delays, attention problems, attention-deficit/hyperactivity disorder problems, and pervasive developmental disorder problems at 3 years of age.*" Thus, the motor and mental results were treated as if they are the same, which they are not. A 3.3 point discrepancy on the mental index is not a meaningful difference, and that difference did not even approach statistical significance at the 0.05 level ($p = 0.155$ in Table 2; Rauh *et al.,* 2006). It is inappropriate to speak of significant mental "delays" as in the Rauh *et al.,* 2006 paper. First, the significance level = 0.048 (Table 2; Rauh *et al.,* 2006) is barely under the $p < 0.05$ guideline. Nonetheless, that probability has no meaning because of the "multiple simultaneous" comparisons in Table 2 (12 comparisons, to be exact - four contrasts at each of three ages). Whenever more than one comparison is made at a time, it is incumbent on the researcher to exercise some type of control over the chance error that inevitably occurs when many comparisons are made at once. Rauh *et al*. made no such control (*e.g.,* demanding that each separate probability must be $p < 0.02$ or $p < 0.01$ to achieve a "family-wise" error rate of 0.05). In short, the value of 0.048 is "not" significant, but likely a result of making so many comparisons such that a few will be "significant" just by chance occurrence. Secondly, the use of a cut-off of a standard score of "85" to denote children as "High Risk" is an arbitrary decision. Scoring below 85 is not a diagnostic category. It is not even a common "cut" score for determining who is at high risk; values below 85 are much more common. Harrison (1990, pp. 53-56), for example, uses cut-off scores of 70, 75, and 80 (but not 85) to illustrate the use of the *Early Screening Profiles* for identifying high risk children between the ages of 2 to 6 years. Changing categories, in any event, is not meaningful. IQ and motor development tests have a built-in standard error of measurement of 3 or 4 points--and that error is even higher when testing very young children tested on tests developed for infants and toddlers. For example, Black and Matula (2000) point out that for the second edition of the Bayley Scales (normed for ages 1-42 months): *"The average standard error of measurement is 5.21 for the Mental Scale and 6.01 for the Motor Scale"* (pp. 68-69); those values are far lower than the values of about 3.00 for Wechsler's IQ scales at ages 3-7 years (Pearson, 2012, Table 4.3; Psychological Corporation, 2003, Table 4.3). Categories such as "High Risk" will change dramatically from Test 1 to Test 2 when the same child is tested twice simply due to errors of

measurement. Whether a child scores 82 or 83 or 84 or 85 or 86 or 87 is just pure chance due to measurement error. A single arbitrary cut-off is inadequate to identify children as normal or with "delays." Such an arbitrary approach takes unfair advantage of the known errors of measurement that characterize even the best measures of mental and motor ability; 85 is just an arbitrary number that may or may not mean delay or high risk.

In the 2011 study, the Rauh *et al.* analysis of chlorpyrifos and WISC-IV scores appears sound. However, the multiple comparisons (as mentioned above) are still an issue. In Table 2, there are five adjusted value comparisons; the authors made no attempt to control for errors that occur when several comparisons are made at once. Consequently, the $p = 0.048$ value for Full Scale IQ is suspect and most likely a chance finding. Nonetheless, the significant finding for working memory is robust and not likely due to chance. However, whenever only one of four mental indexes is found to be significant, such a finding should be replicated with independent samples to verify that it is a "real" relationship between chlorpyrifos and intelligence, not a chance finding.

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Attachment C: Epidemiology Data for Chlorpyrifos – Considerations of Reproducibility

The purpose of this attachment is to explain the importance of type and specificity of exposure when evaluating evidence for and against causality, namely using chlorpyrifos and/or urinary TCPy compared to less specific metabolites. Demonstration of reproducibility between studies (not within) is critical for risk assessment decisions.

To support causality, associations between exposures to the chemical of interest and health outcomes should be found in different populations. The summary Table 1 presented below, which uses the format of EPA's Table 10 (USEPA 2012, page 59), includes recent data from two new cohorts (China cohort; Wickerham et al., 2012; Mexico City cohort; Fortenberry et al., 2013) that have exposure levels higher than or comparable to the Columbia and University of California (UC) Berkeley studies. Another study conducted in New Jersey (Yan et al., 2009; Barr et al., 2010) did not observe any significant associations for fetal growth; however, in this study, chlorpyrifos was near the limit of detection in most maternal and cord blood samples.
A major challenge in determining reproducibility of the Columbia study is a lack of consistency of exposure metrics. Some other cohort studies measured urinary biomarkers of organophosphate (OP) exposure that have different levels of specificity as a biomarker for chlorpyrifos exposure. The most specific urinary biomarker of chlorpyrifos exposure is 3,5,6-trichloro-2-pyridinol (TCPy), but this also can be a metabolite of chlorpyrifos-methyl. Dialkylphosphates (DAPs) are non-specific biomarkers of OPs that are comprised of diethylphosphates (DEPs) and dimethyl phosphates (DMPs). The DEPs include two metabolites of chlorpyrifos, diazinon and other diethyl-OPs (Table 2). In contrast, the DMPs are not biomarkers for chlorpyrifos but can be biomarkers of many dimethyl-OPs (Table 2). These urinary metabolites of OPs can also be a result of exposure to these metabolites in food or the environment rather than to chlorpyrifos or other OPs. Thus, in evaluating consistency across studies it is necessary to consider the studies in terms of the level of information they provide about chlorpyrifos specifically, as opposed to information about OPs generally, as well as what is meant by "consistent findings" in the context of these different biomarkers of exposure (Li *et al*., 2012; Mink *et al*., 2012). The summary Table 1 in this attachment differs from EPA's Table 10 in that associations with DEPs are tabulated instead of those for DAPs.

<u>Points to Note in Reviewing the Table 1:</u>

1. EPA's 2012 SAP clearly stated that total DAPs "are not selective enough to be a useful biomarker for chlorpyrifos" (EPA SAP, P. 58). Chlorpyrifos is the biomarker deemed to be the highest priority because of its specificity (EPA SAP, P. 58). EPA SAP also concluded that "the next biomarker of choice is TCPy, then DETP/DEP in urine," although both are present in the environment as degradates of the active ingredient

(USEPA SAP, p. 58). Thus, this table omits DAPs but includes DEPs. The columns are shaded to reflect the order of priority based on specificity of the biomarker as described above.

2. We define null findings as those with a p value >0.1, non-significant findings as those with a p value>0.5, and present the direction (either as positive for score increased or inverse for score decreased) for findings with a p value<0.1. Values were calculated from the confidence interval when p values were not provided (Altman and Bland, 2011). The table is only a brief synopsis; for more in depth analysis including tables of the magnitude and direction of effects and all statistical testing conducted, we direct the reader to one of several published reviews (Prueitt *et al*, 2011; Li *et al*., 2012; Mink *et al*., 2012, Burns *et al*., 2013).

3. Table 1 only includes associations reported for prenatal exposures (*i.e*. maternal DEPs and TCPy) to be comparable to the Columbia cohort study, which only measured cord/maternal blood at birth.

4. Few findings of the Columbia cohort have been tested by independent investigators using TCPy and/or chlorpyrifos in blood, although these data are available (Huen *et al*. 2012). For example, the UC Berkeley study has not evaluated any neurodevelopmental outcomes using available cord blood chlorpyrifos levels or reported any results using TCPy in children over 2 years of age. It is unknown why the UC Berkeley investigators have not published any health results using these data that would contribute more informed data for use in decision-making relevant to chlorpyrifos.

5. Pervasive Development Disorder (PDD) and Attention-Deficit Hyperactivity Disorder-like behaviors (ADHA) were not clinically diagnosed in the Table 1 studies. Rather, they were based on checklists completed by the mother. Furthermore, maternal depression, a potential confounder of maternal reporting of behavioral problems in children, was not controlled by the Columbia or Mexico City investigators, although it was a significant factor in the UC Berkeley study.

<u>Analysis of summary table</u>

Table 1 is a high level summary of many analyses and purely looks at statistical associations. Even without discussing methodological differences, most of the findings of the Columbia study are not replicated. After age 2 years, it might appear that the UC Berkeley cohort shows limited consistency with the Columbia cohort because of the association with mothers' report of Attention-Deficit Hyperactivity Disorder-like behaviors (ADHD) and DEPs. Unfortunately, the UC Berkeley study did not report any testing for TCPy or chlorpyrifos in older children. Notably, there was no significant overall association found with ADHD and other attention

problems and TCPy in the Mexico cohort. Both the UC Berkeley and Mexico investigators conducted multiple tests for attention and behavioral problems without control for multiple testing. For example, in Table 5 of the Mexico cohort, 27 tests for trend were presented for which two were considered borderline statistically significant ($p<0.1$) while none was statistically significant at the standard p-level (i.e., ($p<0.05$). Overall, since positive results were only reported for DEP and only at age 5 in the UC Berkeley study, and not for TCPy, there is little support for a consistent exposure and ADHD association and less support for any effects attributable to chlorpyrifos.

Both the UC Berkeley and Mount Sinai cohorts show some consistency with Full Scale IQ with DEPs ($0.05<p<0.1$), which are not specific to chlorpyrifos. Again, no testing was reported for TCPy or chlorpyrifos in the other cohorts. Thus, although one could selectively focus on the statistically significant DEPs association from the UC Berkeley study and the findings in the Columbia study as evidence of consistency across cohorts, the null findings with the more specific biomarker TCPy significantly weakens the weight of evidence. The observations in the Columbia study have not been sufficiently tested with chlorpyrifos exposure in other studies to confirm if these are true or false observations. Without robust replication, the Columbia data should not be used for risk assessment.

Table 1. Summary of findings from key epidemiology studies for chlorpyrifos.

	Columbia	Mount Sinai		UC Berkeley			Mexico City	China
Markers of exposure	**CPF**	**TCPy**	**DEPs**	**CPF**	**TCPy**	**DEPs**	**TCPy**	**CPF**
Birth Length	Inverse (Null post 2000)	Null	Null	Collected, analysis not available	Null	Null	N.I.	N.I.
Birth Weight	Inverse (Null post 2000)	Null	Inverse	Collected, analysis not available	Null	Null	N.I.	Null
Bayley Scores 12 months (MDI/PDI)	Null/Null	Collected, analysis not available	Null/null	Collected, analysis not available	Null/Null	Null/Null	N.I.	N.I.
Bayley Scores 24 months (MDI/PDI)	Null/Null	Collected, analysis not available	Null/Null	Collected, analysis not available	Null/Null	Null/Null	N.I.	N.I.
Bayley Scores 36 months (MDI/PDI)	Inverse/ Inverse	Not tested	Not tested	Not tested	Not tested	Not tested	N.I.	N.I.
Pervasive Development Disorder (PDD)	Positive (36 mo)	Not tested	Not tested	Collected, analysis not available	Null (24 mo)	Null (24 mo)	N.I.	N.I.
ADHD/ attention and behavior problems ages 2 - 7 years	Positive (36 mo)	Not tested	Not tested	Collected, analysis not available	Null (24 mo)	Null (3.5 yr) Positive (5 yr)[1]	Null[2] (6-11 yr)	N.I.
Mental Development (WISC-IV, age 7 – 9 years)	Inverse (Full-scale IQ and Working Memory);Null (Others)	Collected, analysis not available	Inverse (NS) FSIQ, Working memory	Collected, analysis not available	Collected, analysis not available	Inverse (FSIQ) Null (working memory)	N.I.	N.I.

1. P< 0.1 for a single test at 3.5 years. All testing for other attention and behavioral problems at age 5 years were not statistically significant (Marks et al., 2010).
2. P < 0.1 for a single ADHD index in boys and Hit RT block change in all subjects. All testing for other models for changes in psychometric assessment scores were not statistically significant (Fortenberry, et al., 2013).
NS=Not statistically significant, [0.1>p>0.05]. p values calculated from confidence interval using following equation: SE = (u - l)/(2 x 1.96); z = Est/SE; P = exp(-0.717 x z - 0.416 x z^2) (Altman and Bland, 2011)):
Inverse= higher levels of exposure associated with adverse health outcomes (score decreased)
Positive= higher levels of exposure associated with adverse health outcome (score increased)
Null = No association observed, p> 0.1
MDI = Mental development index
PDI = Psychomotor development index
Not tested = study did not measure the outcome at the age listed
N.I. = no information available
Collected, analysis not available = biomarker and outcome measured but associations never publicly released

Table 2. Urinary biomarkers of pesticide exposure.

Pesticide	Dimethyl-phosphate	Dimethylthio-phosphate	Dimethyldithio-phosphate	Diethyl-phosphate	Diethylthio-phosphate	Diethyldithio-Phosphate
Azinphos methyl	X	X	X			
Chlorethoxyphos				X	X	
Chlorpyrifos				X	X	
Chlorpyrifos methyl	X	X				
Coumaphos				X	X	
Dichlorvos	X					
Diazinon				X	X	
Dicrotophos	X					
Dimethoate	X		X			
Disulfoton				X	X	X
Ehtion				X	X	X
Fenitrothion	X	X				
Fenthion	X	X				
Isazaphos-methyl	X	X				
Malathion	X	X	X			
Methidation	X	X	X			
Methyl parathion	X	X				
Naled	X					
Oxydemeton-methyl	X	X				
Parathion				X	X	
Phorate				X	X	X
Phosmet	X	X	X			
Pirimiphos-methyl	X	X				
Sulfotepp				X	X	
Temephos	X	X				
Terbufos				X	X	X
Tetrachlorviphos	X					
Trichlorfon	X					

The table shows the six urinary metabolites and the parent organophosphate insecticides responsible for these metabolites. DAPs measures all six of these metabolites, only two of which are associated with chlorpyrifos. (CDC Fourth National Report on Human Exposure to Environmental Chemical, 2009)

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