Rivers LaBarge
Silverton Or. 97381

Dear Chair Salinas and members of the House Committee on Health Care, For the Record My name is Rivers LaBarge. I live in the rural area of Drakes Crossing, and a climbing arborist of 25+ years for I am grateful this opportunity to speak before the esteemed members of this committee. I am here to describe to you what happened to my son after exposure to chlorpyrifos drift.

On July 17, 2019 a pesticide applicator made a helicopter aerial application of Lorsban Advance, a chlorpyrifos insecticide, to a Christmas tree farm across the road from and adjacent to our family's property and our home in the early morning. We had all the windows and doors open because it was a hot summer evening the night before. We witnessed in the aftermath of this spraying terrible harm to the flora and fauna that live on our property. The spray killed all of the insects on our property, including the bees and butterflies that pollinate our vegetable garden. Our son, Honor LaBarge, began feeling lethargic and sick that evening. Three days after the spraying, Honor developed a fever. His condition rapidly spiraled downwards from there until he was diagnosed on August 22nd, 2019, with Burkett's Lymphoma & Leukemia. This disease is currently known the fastest growing human tumor. A double strand break in his DNA @ 8-14 Chromosomes

We were so consumed with shock and fear about our son's illness, we were unaware that we could file a pesticide drift complaint with the Oregon Department of Agriculture until much later. On November 17, a Department of Agriculture investigator came to my property to interview me and also spoke to the pesticide applicator. He returned on November 22 and December 3 to take pesticide residue samples. It is important to note that ODA tested more than four months after the spray.

The official agency investigation concluded that the pesticide applicator, Industrial Aviation, made an application of a chlorpyrifos product, Lorsban Advanced, and that the insecticide drifted onto my property and was detected in lab samples more than four months after the application. The pilot claimed he left a 20 ft. no-spray buffer zone between his application and my property line, however the concentration of chlorpyrifos in samples taken on my property were well over the permissible limits.

In our research, we have further investigated the chemical history of chlorpyrifos, and the results are conclusive. Medical sources and our experience all point to the same conclusion: exposure to chlorpyrifos drift and residues can result in the chromosomal double-strand break, just like the cancer that Honor developed. He developed a number of tumors focused in his liver and kidneys. He has had to go through five months of hospitalization and intense chemotherapy treatments for leukemia and lymphoma. As of February 6, only 5 days ago Honor is in remission, 3 years without occurrence is considered cured.

We ask that the State pass HB 4109 to phase-out chlorpyrifos as a promise to the future safety of our children and their children. No child should have to go through what Honor has been through. Oregon must end the use of chlorpyrifos.

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Linking Pesticide Exposure with Pediatric Leukemia: Potential Underlying Mechanisms

Describes the HOW

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Abstract

Leukemia is the most common cancer in children, representing 30% of all childhood cancers. The disease arises from recurrent genetic insults that block differentiation of hematopoietic stem and/or progenitor cells (HSPCs) and drives uncontrolled proliferation and survival of the differentiation-blocked clone. Pediatric leukemia is phenotypically and genetically heterogeneous with an obscure etiology. The interaction between genetic factors and environmental agents represents a potential etiological driver. Although information is limited, the principal toxic mechanisms of potential leukemogenic agents (e.g., etoposide, benzene metabolites, bioflavonoids and some pesticides) include topoisomerase II inhibition and/or excessive generation of free radicals, which may induce DNA single- and double-strand breaks (DNA-DSBs) in early HSPCs. Chromosomal rearrangements (duplications, deletions and translocations) may occur if these lesions are not properly repaired. The initiating hit usually occurs *in utero* and commonly leads to the expression of oncogenic fusion proteins. Subsequent cooperating hits define the disease latency and occur after birth and may be of a genetic, epigenetic or immune nature (*i.e.*, delayed infection-mediated immune deregulation). Here, we review the available experimental and epidemiological evidence linking pesticide exposure to infant and childhood leukemia and provide a mechanistic basis to support the association, focusing on early initiating molecular events.

Keywords: infant and childhood leukemia, hematopoietic stem/progenitor cells, chromosomal rearrangements, topoisomerase II, pesticides, DNA double-strand break, oxidative stress

1. Introduction

Leukemia is the most common childhood cancer, accounting for 30% of all cancers diagnosed in children under 15 years of age, with an annual incidence of up to 40 cases per million children in developed countries and an incidence peak between three and five years of age [1,2]. Pediatric acute leukemia is a phenotypically- and genetically-heterogeneous disease of immature hematopoietic stem and progenitor cells (HSPCs). Phenotypically, it can target B-cell progenitors (B-cell acute lymphoblastic leukemia (B-ALL)), T-cell progenitors (T-ALL) or myeloid progenitors (acute myeloid leukemia (AML)). Acute leukemia can be further stratified according to the differentiation stage at which HSPCs are blocked; for example, B-ALL can have a pro-B (proB-ALL) or pre-B phenotype (preB-ALL) [3]. Similarly, AML can affect both immature (subtype M0 of the French-American-British classification of AML) and mature lineage-committed types, such as erythroblastic or megakaryoblastic leukemia (subtypes M6 and M7, respectively). Seventy percent of pediatric acute leukemias are ALL and 30% are AML. Genetically, ALL and AML can be further stratified according to molecular cytogenetics [4,5], which represents a prognostic factor.

Fetal hematopoiesis begins in the aorta gonad-mesonephros region to subsequently colonize the fetal liver (FL) and ultimately, just before birth, the bone marrow [6]. FL hematopoiesis entails an active proliferation of progenitors, rendering fetal HSPCs susceptible to oncogenic transformation through DNA damage mediated by chemical exposure during pregnancy [7]. Although the etiology of ALL remains elusive, ionizing radiation, congenital genetic syndromes and *in utero* exposure to specific genotoxic chemicals, including household pesticides, represent prime etiological suspects [8]. Importantly, altered patterns of infection during early childhood might also contribute to acute leukemia in children [9,10,11].

We here review the available experimental and epidemiological evidence linking pesticide exposure with infant and childhood leukemia and provide a mechanistic basis to support the association, focusing on early molecular events. However, the paucity of mechanistic data is a major obstacle to fully understanding the toxicological pathways involved. Causation pathways are likely to be multifactorial, and it is possible that the risk of pediatric leukemia from environmental exposure is influenced by genetic susceptibility.

2. Evidence Linking Pesticide Exposure with Pediatric Leukemia

2.1. Epidemiological Studies Supporting the Association

There is a growing concern about whether chronic low-level pesticide exposure during pregnancy or childhood increases the risk of childhood leukemia. Epidemiological studies suggest that pesticide exposure may have a greater impact on children than adults [12,13]. Almost all of the available evidence has focused on pediatric leukemia without making a distinction between infant and childhood leukemia, which are etiologically and pathologically different entities. However, most epidemiological studies are limited because no specific pesticides have been directly associated with the risk of leukemia, but rather the broad term "pesticide exposure" [13,14]. Such associations are mainly based on subjects' recall of the pesticide exposure, which hampers the drawing of conclusions because of recall/information bias.

In contrast to childhood leukemia, very few studies have examined the risk of infant leukemia and pesticide exposure. An international collaborative study on transplacental chemical exposure and risk of infant leukemia found an increased risk after *in utero* exposure to household pesticides (propoxur and other methylcarbamate insecticides), the therapeutic analgesic dipyrone and hormonal intake (estrogens). In these cases, infant leukemia was associated with the mixed lineage leukemia (*MLL*) gene fusion, likely as a result of topoisomerase II inhibition [15,16]. Although the aforementioned study was based on a rather small sample size, an increased risk (Odds Ratio—OR: 2.18) of infant leukemia was shown in mothers exposed to domestic insecticides during pregnancy. Since estrogens can be metabolized to catechol estrogen-3,4-quinones [17], the association found for infant leukemia might be due to topoisomerase II inhibition caused by quinone metabolites generated during estrogen metabolism [7]. A further Brazilian study found that over use of pesticides during pregnancy was associated with ALL and AML (OR: 2.10 and 5.01, respectively) in children <1 year of age [18]. Moreover, maternal exposure to the insecticide permethrin (assessed by self-reporting)

was associated with a higher risk of leukemia in children <1 year of age, with an OR of 2.47 for ALL and 7.28 for AML. This finding was also supported by a case-control study in China where the use of pyrethroids (assessed by urine levels of major metabolites) was associated with a greater risk of ALL [19].

The presence of the herbicide chlorthal in household dust samples was also associated with an increased risk of ALL in children <8 years, with a significant dose-response trend [20]. The association was greater with the herbicide mixture chlorthal plus alachlor. Other studies, however, report no significant associations. For example, no significant risk of childhood leukemia was found with exposure to some agricultural and residential herbicides, such as metolachlor, bromoxynil, cyanazine and 2,4-dichlorophenoxyacetic acid [20,21]. Furthermore, a case-control study on leukemia in children <1 year old from the American Children's Oncology Group failed to find a significant association between household exposure to insecticides or rodenticides and ALL or AML [22].

Different meta-analyses have consistently shown an increased risk of childhood leukemia associated with pesticide exposure [13,23]. However, this review will focus on the latest quantitative synthesis of evidence from studies. A recent meta-analysis has shown that maternal occupational pesticide exposure during pregnancy and/or paternal occupational pesticide exposure near-to-conception increases the risk of leukemia in offspring [24]. The authors pooled data from 13 case-control studies participating in the Childhood Leukemia International Consortium (CLIC) and found an almost two-fold increased risk of AML in mothers exposed to pesticides during pregnancy, whereas no significant risk was found for paternal exposure around conception. In relation to ALL, the same study observed a 20% increased risk with paternal exposure around conception, which appeared to be more evident for pediatric T-cell ALL. By contrast, no significant association was found between maternal exposure during pregnancy and risk of B or T-cell ALL. In a separate study investigating residential pesticide exposure, Bailey *et al.* [25] pooled data from 12 case-control studies in the CLIC and found a significant increased risk of ALL associated with exposure to any pesticide shortly before conception, during pregnancy and after birth (OR: 1.39, 1.43 and 1.36, respectively). Little variation was observed with the type of pesticide. Regarding AML, an increased risk was found for exposure to any pesticide in the few months prior to conception and during pregnancy (OR: 1.49 and 1.55, respectively); however, exposure after birth failed to demonstrate an increased leukemogenic risk. A recent meta-analysis conducted by Chen *et al.* [12] pooled 16 case-control studies and found that childhood exposure to indoor, but not outdoor, residential insecticides was associated with an increased risk of pediatric leukemia (OR: 1.47). A slightly weaker association was found for herbicide exposure (OR: 1.26). Notwithstanding these positive associations, observational studies on pesticide exposure and pediatric leukemia have a number of weaknesses to claim causal relationships. The consistency of findings across meta-analyses may be due to the considerable overlap in the studies included in the different meta-analyses undertaken. Many epidemiological analyses have not been performed using methodologically-rigorous association studies. Limitations include the lack of an accurate exposure estimate (from both a qualitative and quantitative standpoint), lack of temporal concordance (most studies were case-control in design) and little information on the dose-response relationship. In addition, the available epidemiological evidence may be challenged by endogenous or exogenous factors, such as genetic susceptibility, lifestyle and co-exposure to other environmental agents.

2.2. In Vitro Studies

The few *in vitro* studies available so far have shown that captan and captafol (two related chloroalkylthiocarboximide fungicides) decrease the activity of topoisomerase II by 50% and 20%, respectively, at a concentration of 1 μM [26]. Similarly, thiram (a dithiocarbamate fungicide) inhibits topoisomerase II at 10 μM [27]. However, genotoxic potential (*i.e.*, genetic abnormalities, mutations) of these fungicides occurred only at very high doses (10–100 mM) *in vivo* using common fruit flies [26]. More

recently, the organophosphate (OP) insecticide chlorpyrifos has been reported to induce DNA double-strand breaks (DSBs) and *MLL* gene rearrangements in human fetal liver $CD34^+$ HSPCs as a consequence of topoisomerase II inhibition [14].

Other OP pesticides have been implicated in leukemogenesis, particularly isofenphos, diazinon and fenitrothion. An *in vitro* study using the human leukemic cell line K562 demonstrated metabolic changes consistent with a leukemogenic potential of isofenphos [28]. In addition, human peripheral blood lymphocytes exposed to isofenphos exhibited dose-dependent damage to chromosomal DNA, as well as disruption of the cholinergic nuclear signaling pathway, which collectively could lead to genomic instability and leukemogenesis [29]. In an *in vitro* study using diazinon, a concentration of 0.1 μM induced hypermethylation of several genes involved in cell cycle arrest, such as cyclin-dependent kinase inhibitor 1A (*CDKN1A*) and 1C (*CDKN1C*), and tumor suppressor genes, such as *p53* and *PTEN* [30]. Fenitrothion at low concentrations (1 μM) also induced chromosomal damage in the B-cell leukemia/lymphoma-2 cell line BCL-2 [31].

3. Gene-Environment Interactions

For most pediatric leukemias, multiple genetic polymorphisms of xenobiotic metabolizing enzymes may interact with environmental, dietary and maternal factors to modulate the development of the disease. For example, quinones, which are capable of inhibiting topoisomerase II and can cleave the *MLL* gene at topoisomerase II cleavage sites, may be poorly detoxified depending on the activity of NAD(P)H:quinone oxidoreductase 1 (NQO1), an enzyme that detoxifies chemicals with quinone rings, such as bioflavonoids and benzene metabolites. Thus, genetic polymorphisms of *NQO1* resulting in low-activity variants might be associated with an increased risk of infant leukemia. By contrast, in childhood ALL without *MLL* rearrangements, deficiency of the *NQO1* gene is not associated with the etiology of the disease [32].

Global DNA hypomethylation is associated with activation of oncogenes and neoplastic processes [33], whereas the hypermethylation of 5′ cytosine-phospho-guanine (CpG) islands in promoter regions of some tumor suppressor genes prevents their transcription and promotes the development of tumors [34]. The genetic regulation of folate metabolism may have an influence on the preleukemic clone origin via DNA hypomethylation of key regulatory genes, rendering the genome vulnerable to genomic instability [35]. The presence of some polymorphisms in genes involved in folate metabolism reduces enzyme activity, leading to inadequate folate levels and DNA hypomethylation, ultimately contributing to the neoplastic process [35,36]. The insufficient input of folate increases the plasma concentration of homocysteine and *S*-adenosylhomocysteine, with the latter being a general inhibitor of adenosylmethionine-dependent methyltransferases [37]. Inhibition of these enzymes may alter both DNA methylation and transcriptional regulation [36,38]. The 677C>T gene polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) has been linked to a decreased risk of childhood ALL, likely as a result of higher production of 5,10-MTHF and thymidine, which improve the fidelity of DNA synthesis and repair [39]. On the other hand, inactivating polymorphisms of detoxifying enzymes involved in carcinogen metabolism, such as glutathione *S*-transferases (GST), in parents have been associated with the development of ALL in their children <1 year old. The deletion of both the *GSTT1* and *GSTM1* genes in either parent might affect the risk of infant leukemia [40]. Furthermore, genetic polymorphisms of xenobiotic transport and metabolism pathways are associated with the risk of childhood ALL. In particular, polymorphisms of the *ABCB1* gene, which encodes a membrane transporter of lipophilic compounds, may interact with household insecticide exposures to increase the risk of disease [41]. Genetic variability in DNA repair pathways and cell cycle checkpoints might also interact with environmental, dietary, maternal and other external factors affecting the development of ALL. In summary, the limited data available suggest that dietary and environmental exposure to substances targeting topoisomerases together with the reduced ability of fetuses or their mothers to detoxify such compounds because of polymorphic variants of given genes could contribute to the development of pediatric leukemia [8,42].

The International Childhood Acute Lymphoblastic Leukemia Genetics Consortium revealed limitations in current studies on genetic susceptibility and the risk of ALL because of difficulties in conducting statistically- and methodologically-rigorous investigations [43]. Genome-wide association studies of childhood ALL have provided robust evidence for four low-penetrance susceptibility variants, which confer only a modest increase in risk. Moreover, the well-recognized ethnic differences in the risk of ALL represent a weakness in assessing the interplay between inherited and non-genetic risk factors. Given the small frequency of many ALL subgroups, the identification of differential effects will realistically be possible only through multi-center pooled analyses [43].

4. Early Molecular Events Involved in Pesticide-Associated Pediatric Leukemogenesis

Despite the rather comprehensive epidemiologic evidence linking pesticide exposure during different reproductive stages (pre-conception, pregnancy and early postnatal life) and pediatric leukemia, robust underlying pathological mechanisms remain unknown. The initiating event at the molecular level might be the induction of chromosomal rearrangements as a result of pesticide exposure and subsequent topoisomerase II inhibition or generation of oxidative stress, leading directly or indirectly to DNA damage. A mechanistic explanation follows.

4.1. DNA Double-Strand Breaks (DSBs)

Under some circumstances, oxidative lesions can lead to DNA DSBs formation in HSPCs. Environmental exposures to numerous chemicals, including many pesticides, have been shown *in vivo* and *in vitro* to generate oxidative species that can ultimately induce DNA base or sugar oxidative damage, leading to single-strand breaks (SSBs) and DSBs formation in the DNA [44]. For example, OP insecticides (chlorpyrifos, methyl-parathion, malathion), methyl-carbamates (methomyl) and the herbicide paraquat all cause oxidative DNA damage followed by DNA SSBs and DSBs [45,46,47,48]. There is also evidence of pesticide-induced oxidative stress and DNA damage in agricultural workers [47]. Additionally, oxidative species may interact with biological molecules to disrupt normal DNA synthesis and repair, and so, inhibition/inactivation of antioxidant proteins or DNA repair enzymes may also be an underlying molecular mechanism [49]. Along this line, pesticides can disrupt a number of antioxidant enzymes, including superoxide dismutase and catalase [50], rendering oxidative stress [51].

DSBs can arise under different circumstances: (i) when two SSBs form close to each other on opposite strands; (ii) upon enzymatic DNA cleavage next to an SSB on the opposite strand; or (iii) when either DNA replication or transcription takes place at sites of misrepaired DNA. DSBs constitute the first molecular event in the generation of chromosomal aberrations [52]. For instance, chlorpyrifos is reported to cause DNA DSBs and further chromosomal rearrangements (*i.e.*, *MLL*) through oxidative stress in human FL HSPCs [53]. However, chlorpyrifos can also induce DNA DSBs as a result of topoisomerase II inhibition in FL HSPCs in a manner similar to that produced by etoposide [14]. Analogously, blood lymphocytes from pesticide sprayers have greater fragile site breakage than normal individuals following treatment with aphidicolin, an inhibitor of DNA polymerases [54]. Chromosomal fragile sites are regions of the genome prone to breakage following exposure to many chemicals, including environmental and chemotherapeutic agents. During DNA replication, fragile site-inducing conditions can uncouple the helicase complex from the DNA polymerase, resulting in long stretches of single-stranded DNA and further DNA breakage [55]. Aphidicolin can also induce fragile site breakage through a topoisomerase II-mediated mechanism [56].

Topoisomerase II has critical functions in both DNA replication and transcription processes, and the so-called "topoisomerase II poisons" disrupt the DNA-induced topoisomerase II cleavage-religation equilibrium through the stabilization of ternary (drug-DNA-enzyme) complexes, termed cleavage complexes [57]. Chemical-induced breakpoints are strongly associated with predicted topoisomerase II cleavage sites (*i.e.*, *MLL*), thus supporting a role for topoisomerase II-mediated breakage upon exposure to environmental agents. The high

frequency of topoisomerase II recognition sites in specific DNA regions and the high expression of this enzyme in human $CD34^+$ HSPCs represent favorable conditions for breakage following exposure to agents targeting topoisomerase II activity (*i.e.*, bioflavonoids and quinones). Because $CD34^+$ HSPCs appear to be more sensitive to DNA damage than committed progenitor cells, exposure to low levels of different chemicals may induce DNA breakage at certain sites in HSPCs, increasing the risk of chromosomal rearrangements. If affected cells survive, they continue growing and dividing, thus perpetuating DNA lesions and starting the chain of events that will eventually lead to leukemogenesis [55].

4.2. Chromosomal Translocations

Key molecular events leading to pediatric leukemia pathogenesis are chromosomal translocations. These generally result from the exchange of chromosomal arms between heterologous chromosomes, and DNA DSBs are prerequisites for their occurrence. Chromosomal translocations ultimately result in the deregulation of key cellular proteins, especially those encoded by proto-oncogenes and tumor suppressor genes, which are critical functional regulators of the cell [58]. Two functional classes of translocations are known. The first one relocates a proto-oncogene (or genes encoding for non-antigen receptors or transcription factors) into regulatory regions of actively-transcribed genes (such as those encoding for immunoglobulin chains or T-cell receptors), causing dysregulated expression of an intact protein. The second class of translocations juxtaposes two genes to encode a chimeric protein, which is functionally distinct from the wild-type proteins [1].

Although the mechanistic generation of chromosomal translocations is not well understood, they may arise from improper DNA repair or erroneous recombination of variable (V), diversity (D) and joining (J) gene segments (a process known as V(D)J recombination). As for improper DNA repair, reactive oxygen species (ROS)-induced DSBs in human FL $CD34^+$ HSPCs following maternal exposure to chemicals triggers recombination/repair pathways by non-homologous end-joining (NHEJ) [14]. The majority of damaged HSPCs may either successfully repair the DNA DSBs or fail to do so and undergo apoptotic cell death. In a fraction of cells, the repair of the DNA DSBs within particular breakpoint cluster regions (bcr) is not completed correctly, giving rise to chromosomal translocations or deletions [59]. For fusion genes to be leukemogenic, DSBs must occur simultaneously in two chromosomes and must also involve the coding region of the genes to generate an exon-exon in-frame functional chimeric gene product. Importantly, this has to occur in an HSPC that has managed to bypass cell death and displays a sustainable lifespan and clonal potential to propagate the chimeric gene product [60].

Erroneous V(D)J recombination usually occurs in developing lymphocytes during cell maturation, where V(D)J gene segments of immunoglobulin chains or T-cell receptors are rearranged to yield a wide range of immunoglobulins and T-cell receptors. The process entails the cleavage of the V(D)J gene at the flanking recombination signal sequences (RSS) by lymphocyte-specific recombination-activating gene (RAG) endonucleases and subsequent ligation of the segments via the classical NHEJ pathway [61]. In pediatric leukemia, chromosomal translocations and deletions often arise as a result of mistakes in V(D)J rearrangements because RAG enzymes can erroneously recognize and target RSS-like sequences. V(D)J-recombinase-mediated rearrangements may occur at both immune RSS and non-immune cryptic RSS (cRSS), which are widely distributed throughout the genome [62]. There is growing evidence that *in vivo* exposure to DNA-damaging agents, including pesticides, can increase the frequency and alter the recombination site distribution of V(D)J rearrangements at cRSS [63,64]. An increase in V(D)J-recombinase-mediated events at either immune or non-immune RSS following exposure to DNA-damaging agents could play an important role in environmentally-induced genetic alterations associated with leukemia development. Nonetheless, the mechanism by which exposure to DNA-damaging agents could increase the frequency of V(D)J-recombinase-mediated genomic rearrangements remains unclear [64].

5. Pathobiology of Pediatric Leukemias

Given the distinct natural history and pathogenesis of infant and childhood leukemia, both entities will be addressed separately, although a chromosomal translocation is frequently the common initiating oncogenic event in both entities.

5.1. Infant Leukemia

Infant acute leukemia shows unique clinical and biological features and is commonly associated with rearrangements in the *MLL* gene (*MLL*-r), a master gene located on chromosome 11q23 that regulates normal human hematopoietic development and differentiation [65]. The *MLL* gene encodes a methyltransferase with activity for lysine 4 of histone H3 (H3K4), which mediates changes in chromatin associated with epigenetic transcriptional activation that plays an essential role in regulating gene expression during early development and hematopoiesis [66]. Rearrangements involving the *MLL* gene have been reported to occur only in mice with defects in DNA damage response and not in wild-type animals [67]. *MLL*-r functions as the initiating, and perhaps the sole driving, oncogenic event by dysregulating epigenetic and/or transcriptional programs [33] (Figure 1). Epidemiological and genetic studies have suggested that *MLL*-r may result from transplacental exposure to DNA topoisomerase-II inhibitors during gestation, such as chemotherapeutic agents, benzene metabolites (*i.e.*, benzoquinone), quinolone antibiotics, bioflavonoids present in some fruits and vegetables and some pesticides [7,33,68]. However, exposure to topoisomerase-II inhibitors is not sufficient *per se* for rearrangement of *MLL*, and the genetic background, such as mutations in the DNA damage response pathway, may influence the likelihood of *MLL*-r [67].

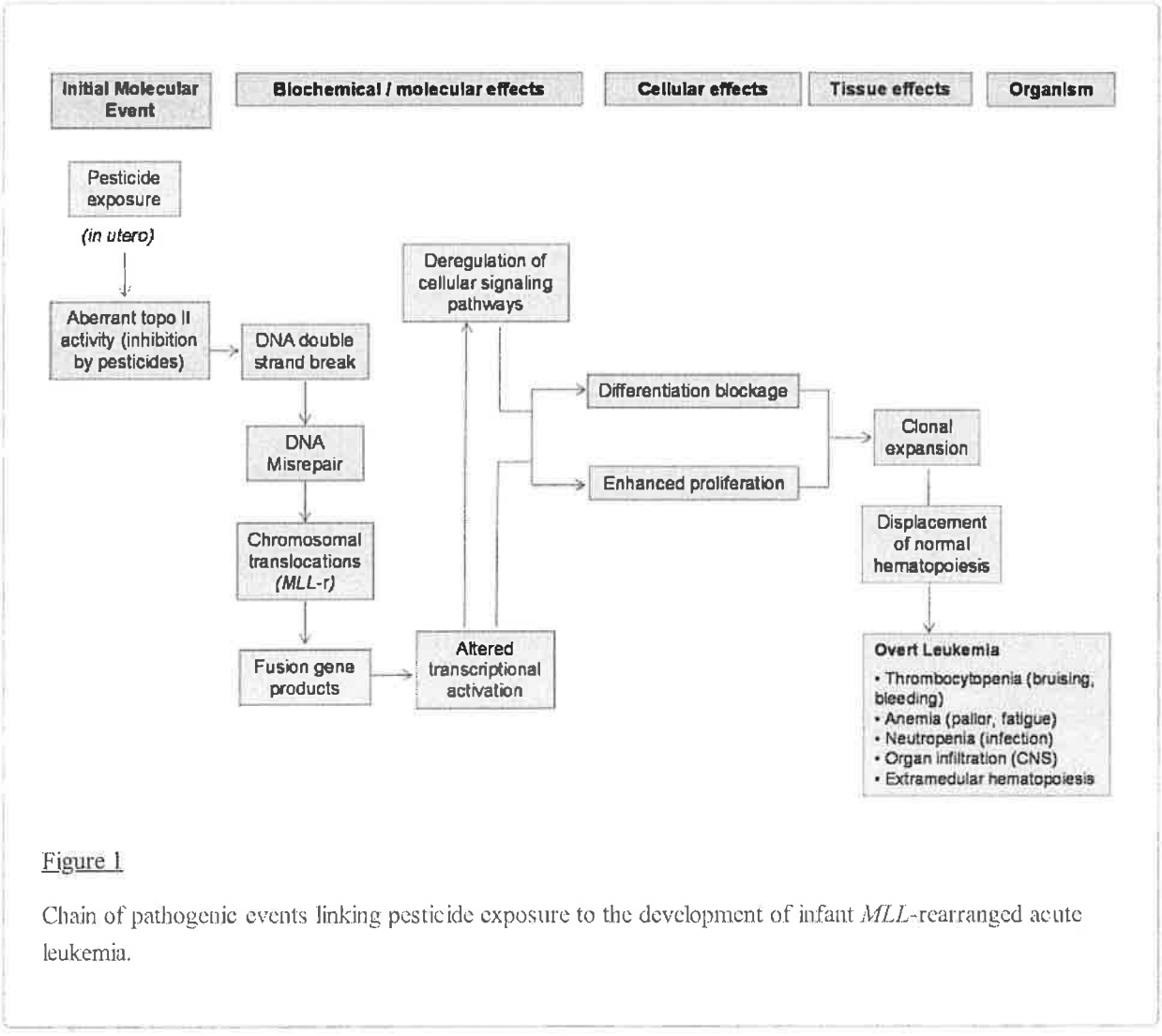

Figure 1

Chain of pathogenic events linking pesticide exposure to the development of infant *MLL*-rearranged acute leukemia.

The existence of recombination-prone sequences in the *MLL* bcr region supports the contention that *MLL*-r results from DNA breakage and recombination events. The genomic instability within *MLL* bcr may be the consequence of increased ROS generation [69]. The *MLL* fusion gene renders HSPCs more vulnerable to DNA repair and cell-cycle deregulation, facilitating the rapid acquisition of additional, secondary genetic changes, particularly upon continued exposure to genotoxic chemicals *in utero* [7,70]. These chemicals target early mesodermal precursors or HSPCs residing mainly in the FL where they inhibit topoisomerase-II activity and produce DNA DSBs within the *MLL* bcr, which are not properly repaired by homologous recombination or NHEJ. Because those mesodermal precursors or HSPCs are rapidly dividing and have high topoisomerase II content, they may be particularly sensitive to damage by topoisomerase II-targeting chemicals during a critical developmental window of vulnerability [33,71,72,73,74,75]. However, because of the very short latency of infant leukemia, it remains obscure whether the fusion gene generated from chromosomal translocations requires additional cooperating oncogenic hits for leukemogenesis. Although recurrent activating mutations of genes associated with cellular proliferation, such as components of the RAS signaling pathway, have been reported [76,77,78,79], functional studies revealed that these mutations are important for tumor maintenance rather than initiation in human HSPCs [80]. *MLL* breakage itself is not sufficient for the development of full-blown infant leukemia, even if the DNA damage response is defective. Activation of cellular proliferation by mutation of other genes might be necessary for overt leukemia [67]. The transformation mediated by the aberrant proteins encoded by fusion genes might depend on alternative (epi)-genetic cooperating lesions at a critical developmentally-earlier window of stem cell vulnerability to develop overt leukemia [33].

Intriguingly, and in contrast to the global dogma of cancer biology, *MLL*-r infant leukemia has been shown to have abnormal hypermethylation in non-enhancer, non-promoter regions, perhaps contributing to genomic stability and a silenced mutational landscape [76,81,82]. Extensive hypermethylation of tumor suppressor genes resulting in gene silencing has been observed in some cases of *MLL*-r infant leukemia [83].

5.2. Childhood Leukemia

Childhood leukemia has a prevalence peak at ~3–5 years of age, suggesting that environmental exposures *in utero* or during early childhood might be risk factors [25]. Under the current paradigm, the first initiating oncogenic mutation usually involves structural or numerical chromosomal alterations, impairing normal cell differentiation, while secondary hits more commonly comprise mutations affecting developmentally-regulated master transcription factors or membrane-proximal signaling pathways conferring proliferation and survival advantages to the differentiation-blocked clone [1,7,8,84,85]. The development of leukemia requires the activation of cell proliferation in addition to differentiation blockage [67]. Numerical aberrations (*i.e.*, hyperdiploidy) are also common hallmarks in childhood B-cell ALL.

The most common chromosomal aberrations are *E2A-PBX1*, *TEL-AML1* and *MLL*-r for B-ALL and *AML1-ETO* and *MLL*-r for AML. Similar to *MLL* rearrangements, the resulting aberrant chimeric proteins alter the normal transcriptional program and block normal B-cell and/or myeloid differentiation [8,86,87,88] (Figure 2). Although the *AML1* gene has been linked to anti-topoisomerase II agents, similar to the *MLL* gene, *TEL-AML1* is not sufficient to cause the disease by itself. As this fusion gene is observed in cord blood from about 1% of normal newborns, a significant proportion of the population carries self-limiting preleukemic clones, and the majority of them do not result in disease [3]. The longer latency observed in childhood leukemia unequivocally indicates that the initiating chromosomal translocation itself is unlikely to convert a preleukemic clone into an overt disease, thus suggesting the need for secondary cooperating (epi)-genetic events.

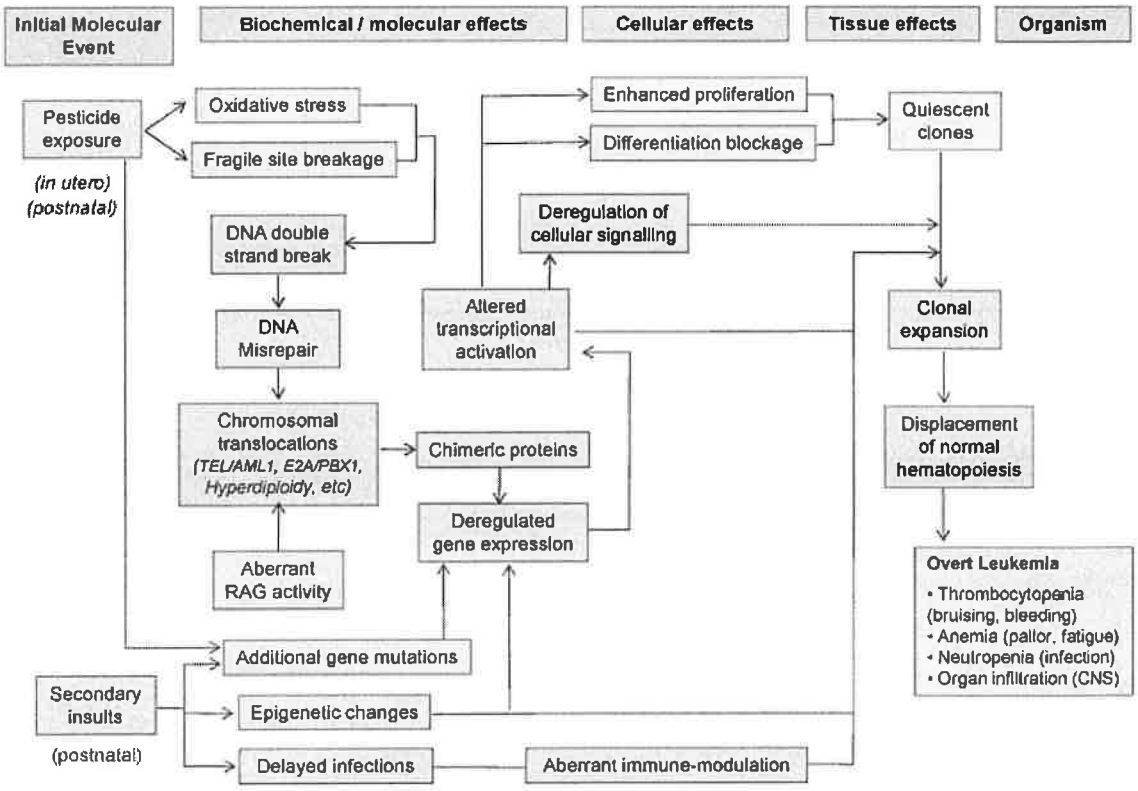

Figure 2

Chain of pathogenic events linking pesticide exposure to the development of childhood leukemia. Blue arrows indicate events related to the "first hit" and red arrows events related to the "second hit" (for more details, see Section 5.2).

Dysfunction of the immune system and delayed infections have been linked to childhood leukemia [9,89]. Two distinct underlying mechanisms might explain this association: (i) a lower repertoire of infections during early immune development; and (ii) an altered congenital responder status to infection resulting in functionally-aberrant clinical presentation of occasional infections. Thus, an untimely and excessive inflammatory response abolishes normal hematopoiesis, promoting selective expansion of a preleukemic clone (Figure 2) because of proliferative advantage and increased likelihood for a second mutation required for the development of the disease to occur [33]. In turn, early childhood infections or vaccination may reduce the likelihood of leukemia [90]. Importantly, the major histocompatibility genes might play a role in the linkage between patterns of infection and leukemia risk, as several HLA haplotypes have been associated with childhood leukemia [3]. However, other studies have suggested that major histocompatibility complex-defined variation in immune-mediated response is unlikely to be a major risk factor [91].

Aberrant RAG activity resulting in genomic rearrangements may be a crucial secondary mechanism leading to B-cell ALL. Aberrant RAG activities can result in various oligoclonal V(D)J recombination events and the inactivation of genes required for B-lineage differentiation [87]. A clear link between RAG and childhood leukemia through inflammatory mechanisms has been recently reported [89], further connecting immune system-RAG-childhood leukemia.

6. Role of Acetylcholinesterase in Leukemogenesis

Moderate acetylcholine (ACh) levels are crucial for controlling immune and inflammatory functions in peripheral tissues. An increase in ACh above a certain threshold can suppress the production of pro-inflammatory cytokines. Acetylcholinesterase (AChE) contributes to regulating ACh levels and, thus, modulates inflammation [92]. In particular, ACh produced by the vagus nerve and/or by peripheral leukocytes [93] can potently modulate several classical immune reactions by activating the α7-nicotinic ACh receptor on the leukocyte membrane, which in turn blocks the nuclear factor kappa B (NF-κB)-mediated production of pro-inflammatory cytokines, such as IL1β and tumor necrosis factor alpha [92]. Because mesenchymal stromal/stem cells carry both nicotinic and muscarinic ACh receptors [94], niche-derived cholinergic signals may play a role in hematopoiesis by regulating proliferation and apoptosis of HSPCs undergoing erythroid and myeloid differentiation [95].

The *ACHE* gene includes multiple putative binding sites for hematopoietic transcription factors. Alternative splicing gives rise to "synaptic" (AChE-S) multimers, which control ACh levels in the brain and muscles, "erythrocyte" (AChE-E) dimers and stress-induced "read-through" (AChE-R) monomers [96]. AChE-R is involved in cell proliferation, whereas AChE-S can be induced during apoptosis [97]. Under stress responses, blood AChE-R undergoes C-terminal cleavage rendering a C-terminal peptide (ARP) of 55 kDa, which promotes the myeloproliferation and thrombopoiesis characteristics of cellular stress [98]. Because ARP functions as a hemopoietic growth factor promoting proliferation of $CD34^+$ HSPCs, circulating AChE-R and/or ARP might be involved in directing $CD34^+$ HSPCs towards prolonged granulocytosis [96]. Furthermore, *ACHE* has been reported to play a role in hematopoiesis by regulating proliferation, differentiation and apoptosis of erythroid and myeloid progenitors. This might explain, at least in part, the association of perturbations in *ACHE* gene expression with myeloid leukemia [99], particularly after exposure to anticholinesterase insecticides, such as OPs.

ACHE is located on chromosome 7q22 within a critical region subject to non-random chromosomal abnormalities. The remarkable abundance of SINEs (short interspersed elements), in particular Alu repeats, in the *ACHE* locus implies exceptional susceptibility to retrotransposition events, which are assisted by the existence of chromosomal breakages. Alu repeats also facilitate unequal crossing-over, altogether contributing to the instability of this region. Chromosomal rearrangements could result in the loss of upstream transcription factor binding sites and, thus, may affect *ACHE* gene expression under stress or exposure to anti-AChE agents. This explains the reported chromosomal aberrations involving 7q22 in leukemic patients [100]. The proximal promoter of the *ACHE* gene contains consensus motifs for the leukemia-associated factor AML1/Runx1 and c-fos, a transcription factor known to regulate *ACHE* gene expression under stress [101]. Hence, the loss of DNA on chromosome 7 may play a significant role in AML [95,96,97,98,99,100,101,102]. Furthermore, a study of 1880 children with ALL reported that 4% of them had DNA losses involving chromosome 7 [103].

A pivotal role of AChE has been suggested in apoptosis. While the 55-kDa AChE protein is selectively induced during apoptosis, its suppression inhibits apoptosome formation and rescues cells from apoptosis [104]. The 55-kDa AChE protein is negatively regulated by the activation of the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) pathway [104,105]. This signaling cascade is crucial to cell cycle progression, transcription, translation, differentiation, apoptosis, motility and metabolism [106]. The decrease in AChE activity and the consequent increased level of ACh could cause cholinergic overstimulation and enhance cell proliferation in lung cancer [97]; however, whether a similar effect can occur in leukemogenesis is unknown. On the other hand, AChE can hydrolyze lipid peroxides, raising the possibility that a reduction in enzyme activity increases oxidative stress and cellular damage [97].

7. Conclusions

Overall, there is sustained epidemiological evidence to suggest a risk of pediatric leukemia upon exposure (*in utero* and/or after birth) to some classes of pesticides, but scientific/mechanistic studies to definitively support this association are lacking. Pesticides may induce topoisomerase II inhibition or generation of oxidative stress,

consistently leading to misrepaired DNA cleavage and further chromosomal aberrations in HSPCs. This early molecular event might be sufficient for triggering infant leukemia, but not childhood leukemia, which requires further postnatal events for overt disease. The combination of epidemiological and case-based genomic studies together with cell biology analyses would be useful to elucidate the etiology of pediatric leukemia. In particular, this approach would help to better understand the biological and genetic evidence that is pertinent to the mechanisms by which pesticides might impact on the risk of pediatric leukemia.

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Author Contributions

Antonio F. Hernández and Pablo Menéndez conceived of and wrote the review.

Conflicts of Interest

The authors declare no conflict of interest.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: November 3, 2016

SUBJECT: Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review

PC Code: 059101
Decision No.: 522687
Petition No.: NA
Risk Assessment Type: Single Chemical Aggregate
TXR No.: NA
MRID No.: NA

DP Barcode: D436317
Registration No.: NA
Regulatory Action: Registration Review
Case No.: NA
CAS No.: 2921-88-2
40 CFR: 40 CFR§180.342

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1.0 Executive Summary

This document presents the revised human health risk assessment for the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Registration Review of the organophosphate (OP) insecticide chlorpyrifos.

Background
A preliminary human health risk assessment (HHRA) for chlorpyrifos was completed on June 30, 2011 (D. Drew *et. al*, D388070, 06/30/2011) as part of the FIFRA Section 3(g) Registration Review program. A revised HHRA was completed in 2014 (D. Drew *et. al*, D424485, 12/29/2014) to address comments received on the preliminary HHRA and to incorporate new information and new approaches that had become available since the June 2011 risk assessment. Most notably, the 2014 revised HHRA incorporated the following: (1) a physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model for deriving toxicological points of departure (PoDs) based on 10% red blood cell (RBC) acetyl cholinesterase (AChE) inhibition; and (2) evidence on neurodevelopmental effects in fetuses and children resulting from chlorpyrifos exposure as reported in epidemiological studies, particularly the results from the Columbia Center for Children's Environmental Health (CCCEH) study on pregnant women which reported an association between fetal cord blood levels of chlorpyrifos and neurodevelopmental outcomes. The 2014 revised HHRA retained the 10X Food Quality Protection Act (FQPA) Safety Factor (SF) because of the uncertainties that neurodevelopmental effects may be occurring at doses lower than those that cause 10% RBC AChE inhibition and used for the PoD.

Based on the aggregate risks identified in 2014 (D. Drew *et. al*, D424485, 12/29/2014), a proposed rule (PR) for revoking all tolerances of chlorpyrifos was published in the Federal Register on November 6, 2015 (80 FR 69079). At that time, the EPA had not completed a refined drinking water assessment or additional analysis of the hazard from chlorpyrifos that was suggested by several commenters to the EPA's 2014 registration review revised HHRA. Those commenters raised the concern that the use of 10% RBC AChE inhibition for deriving PoDs for chlorpyrifos may not provide a sufficiently health protective human health risk assessment given the potential for neurodevelopmental outcomes. Accordingly, following the issuance of the proposed rule, the EPA conducted additional hazard analyses using data on chlorpyrifos levels in fetal cord blood (reported by the CCCEH study investigators) as the source for new PoDs for risk assessment.

The EPA consulted the FIFRA Scientific Advisory Panel (SAP) for scientific advice on the proposed approach of using the CCCEH cord blood data at a meeting on April 19 – 21, 2016. The 2016 SAP did not support using the cord blood data quantitatively for deriving PoDs. However, the Panel concluded that epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% RBC AChE inhibition, which was used as the PoD in the EPA's 2014 RHHRA and for the 2015 proposed revocation rule. The SAP therefore appears to have rejected both the approach the EPA put forward in its proposed rule derived from the 2014 risk assessment as well as the EPA's initial efforts to address the results of the CCCEH study quantitatively.

The SAP report, however, did present the EPA with a path forward for a third approach to setting the PoDs. First, as a foundation, it is important to note that the SAP was supportive of the EPA's use of the PBPK model as a tool for assessing internal dosimetry from typical Office of Pesticide Programs (OPP) exposure scenarios using peer reviewed exposure assessment approaches (e.g., food, water, residential, occupational). Use of the PBPK model coupled with typical exposure scenarios provides the strongest scientific foundation for chlorpyrifos human health risk assessment and is the approach used in this 2016 assessment. Given that the window(s) of susceptibility are currently not known for the observed neurodevelopmental effects, and the uncertainties associated with quantitatively interpreting the CCCEH cord blood data, the SAP recommended that the agency use a time weighted average (TWA) blood concentration of chlorpyrifos for the CCCEH study cohort as the PoD for risk assessment. The EPA has chosen to follow that advice in this assessment. Thus, for this assessment, the PBPK model was used to determine the TWA blood level expected from post-application exposures from the chlorpyrifos indoor crack and crevice use scenario. This scenario was selected as it represents the most appropriate exposure for the women in the CCCEH cohort (i.e., crack and crevice was the predominant application type during the time of the CCCEH study and is considered protective of other possible exposures for the women in the cohort). In order to derive a TWA of chlorpyrifos concentrations in blood for a predicted risk assessment endpoint, the dose reconstruction analysis assumed exposures for 2 hours per day with a daily shower, for a total of 30 days. Additionally, chlorpyrifos residues were assumed to dissipate 10% daily; that is, the total amount of residue available for transfer from the treated floor is assumed to reduce by 10% for each subsequent day of exposure until the end of the 30th day prior to the next application.

The TWA blood level was used as the internal dose for determining separate PoDs for infants, children, and adults exposed to chlorpyrifos. These separate PoDs have been calculated by PBPK modeling for dietary (food, drinking water), residential, and occupational exposures. With the exception of the acute (single day) exposure assessment for non-occupational bystander post-application inhalation exposures, only steady state[1] (repeat) exposure durations are considered in this assessment as assessing the steady state exposure duration most closely matches the TWAs calculated for the PoDs. The PoDs derived from the TWA blood level are protective of any additional acute exposures to chlorpyrifos.

The TWA blood level resulting from chlorpyrifos exposure from the crack and crevice scenario is considered a lowest-observed-adverse-effect-level (LOAEL) rather than a no-observed-adverse-effect-level (NOAEL), since this is the exposure level likely to be associated with neurodevelopmental effects reported in the CCCEH study. In situations where the agency selects a PoD from a study where a NOAEL has not been identified, the EPA generally will retain the FQPA SF of 10X to account for the uncertainty in using a LOAEL. Therefore, the 10X FQPA SF has been retained in this revised risk assessment for chlorpyrifos. The revised risk assessment also applies a 10X uncertainty factor for intraspecies variability because of the lack

[1] Organophosphates (OPs), including chlorpyrifos, exhibit a phenomenon known as steady state AChE inhibition. After repeated dosing at the same level, the degree of inhibition comes into equilibrium with the production of new, uninhibited enzyme. At this point, the amount of AChEI at a given dose remains relatively consistent across duration. In general, OPs reach steady state within 2-3 weeks. Therefore, for OPs it is appropriate to assess steady state exposure durations (up to 21 days) instead of longer term exposures. The steady state point of departure is protective of any longer exposure duration, including chronic exposure.

of sufficient information to reduce or remove this factor. Typically, the agency uses animal studies for selection of PoDs and, as such, retains a 10X interspecies factor for extrapolation of the animal data to assess human health. However, with use of the PBPK-PD model which accounts for the pharmacokinetic and pharmacodynamic differences between animals and humans to derive PoDs, it is appropriate to reduce the interspecies factor to 1X. Therefore, the total uncertainty factor for chlorpyrifos in this 2016 risk assessment is 100X.

For the dietary assessment, PoDs are divided by the total uncertainty factor (100) to derive a population adjusted dose (PAD). The chlorpyrifos exposure values resulting from dietary modeling are compared to the PAD. There are potential risks of concern when estimated dietary risk exceeds 100% of the PAD.

For the residential and occupational assessments, margins of exposure (MOEs) are calculated by comparing the PoDs to the calculated exposures for each scenario. The resulting MOEs are then compared to the level of concern (LOC) of 100 (the total uncertainty factor is the LOC). If calculated MOEs are less than 100 then a risk of concern is identified for that exposure scenario.

This 2016 human health risk assessment only provides limited summary information and substantially relies on the following previous documents developed for chlorpyrifos, and the updated drink water assessment, which contain more detailed evaluations of the risk assessment approach, scientific literature, and the PBPK model:

- D. Drew *et al.,* Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review, December 29, 2014, D424485;
- U.S. Environmental Protection Agency, Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides, September 15, 2015, D331251;
- R. Bohaty and J. Hetrick. Chlorpyrifos Registration Review Drinking Water Assessment, April 14, 2016, D432921
- U.S. Environmental Protection Agency, Chlorpyrifos Issue Paper: Evaluation of Biomonitoring Data from Epidemiology Studies, March 11, 2016 and supporting analyses presented to the FIFRA Scientific Advisory Panel's (SAP) meeting on April 19-21, 2016, (EPA-HQ-OPP-2016-0062).

Use Profile

Chlorpyrifos is a broad-spectrum, chlorinated OP insecticide. Registered use sites include a large variety of food crops, and non-food use settings. Public health uses include aerial and ground-based fogger adulticide treatments to control mosquitoes. There is a wide range of registered formulations, application rates, and application methods. Registered labels generally require that handlers use normal work clothing (i.e., long sleeved shirt and pants, shoes and socks) and coveralls, chemical resistant gloves, and dust/mist respirators. Also, some products are marketed in engineering controls such as water soluble packets. The restricted entry intervals (REIs) on the registered chlorpyrifos labels range from 24 hours to 5 days. The pre-harvest intervals (PHIs) range from 0 days (Christmas trees) to 365 days (ginseng).

Dietary Risk Assessment

This assessment indicates that steady state dietary exposure analysis is highly refined. The large

majority of food residues used were based upon U. S. Department of Agriculture's Pesticide Data Program (PDP) monitoring data. Percent crop treated information and food processing factors were included, where available. All commodities with U.S. tolerances for residues of chlorpyrifos are included in the assessment.

The steady state dietary (food only) exposures for chlorpyrifos are of risk concern (> 100% steady state PAD for food ($ssPAD_{food}$)) at the 99.9th percentile of exposure for all population subgroups analyzed. Children (1-2 years old) is the population subgroup with the highest risk estimate at 14,000% of the $ssPAD_{food}$.

For chlorpyrifos, a drinking water level of comparison (DWLOC) approach is used to calculate the amount of exposure available in the dietary 'risk cup' for chlorpyrifos in drinking water after accounting for chloropyrifos exposure from food. This DWLOC is then compared to the estimated drinking water concentration (EDWC) to determine if there is a risk of concern for drinking water exposures. However, because this assessment indicates that dietary risks from food alone are of concern it is not possible to calculate a DWLOC; essentially the steady state DWLOC is '0' after accounting for food exposures.

Hypothetically, if there were no exposure to chlorpyrifos from food and the entire dietary 'risk cup' was available for drinking water, the resulting steady state DWLOC for infants (the most highly exposed population subgroup for water) would be 0.014 ppb. An EDWC at or exceeding this concentration would be considered a risk of concern for exposures to chlorpyrifos in drinking water. The refined chlorpyrifos EDWCs are presented in the revised drinking water assessment (DWA) (Bohaty, R., 4/14/2016, D432921, Chlorpyrifos Revised Drinking Water Assessment for Registration Review).

Residential (Non-occupational) Risk Assessment
Residential post-application exposures can occur for adults and children golfing on chlorpyrifos-treated courses. The residential post-application assessment considered and incorporated all relevant populations and chemical-specific turf transferable residue (TTR) data. This assessment indicates that all residential post-application exposures are of concern (i.e., MOEs are < 100) on the day of application (Day 0); all MOEs < 1 (LOC = 100). Further, all residential post-application exposure scenarios assessed following aerial and ground Ultra Low Volume (ULV) mosquitocide applications result in risks of concern; MOEs ranged from < 1 to 68 (LOC = 100).

Non-Occupational Spray Drift Exposure and Risk Assessment
A quantitative non-occupational spray drift (from treatment of agricultural fields) assessment was conducted for this assessment. Adult dermal and children's (1 < 2 year old) dermal and incidental oral risk estimates from indirect exposure to chlorpyrifos from spray drift result in risk estimates of concern at the field edge. All scenarios require buffer distances of > 300 feet to be below the level of concern.

Non-Occupational Bystander Post-Application Inhalation Exposure and Risk Assessment
In the 2014 risk assessment, the agency did not include a quantitative assessment of post-application inhalation exposure to bystanders. This assessment was not included since two vapor-phase AChE inhibition inhalation toxicity studies were submitted and reviewed which

demonstrated that no inhibition of AChE occurred even at the saturation concentration. Therefore, it was assumed that there were no anticipated risks of concern from exposure to the volatilization of either chlorpyrifos or chlorpyrifos oxon. However, in the current assessment, the points of departure for risk assessment have been chosen to be protective of potential neurological effects that occur below levels where AChE inhibition could occur. For that reason, a quantitative bystander/volatilization assessment has been included in this update.

The EPA has assessed residential bystander exposure from field volatilization of applied chlorpyrifos based on available *ambient* (five studies/11 locations) and *application site* (one study/2 locations) air monitoring data. Of the 11 acute *ambient* air concentrations assessed, six resulted in risk estimates that are of concern (i.e., MOEs < 100). Only one steady-state *ambient* air concentration resulted in a risk estimate not of concern (i.e., MOEs > 100). For the *application site* air concentrations assessed, all resulted in risk estimates of concern (i.e., MOEs < 100).

Aggregate Risk Assessment
For the chlorpyrifos aggregate assessment, the EPA has traditionally used a DWLOC approach to calculate the amount of exposure available in the total 'risk cup' for chlorpyrifos in drinking water after accounting for any chloropyrifos exposures from food and residential use. This DWLOC is then compared to the EDWC to determine if there is an aggregate risk of concern. However, because the dietary risks from food exposure alone and from residential exposure alone are of concern, it is not possible to calculate a DWLOC; essentially, the steady state aggregate DWLOC is '0' after accounting for food and residential exposures. Quantitatively aggregating (combining) residential, food, and drinking water exposures would result in risks of concern.

Occupational Risk Assessment
Steady state occupational handler and post-application exposure analyses were previously completed for the registered uses of chlorpyrifos. However, occupational exposures and risk estimates have been updated to incorporate the revised PBPK-derived PoDs. The scenarios, assumptions, and exposure inputs have not changed since the previous assessment.

Using the updated PBPK-derived steady state PoDs and uncertainty factors (dermal and inhalation LOC = 100), all agricultural occupational handler scenarios, all primary seed treatment handler scenarios, and all secondary seed treatment (planter) scenarios are of concern with label-specified and maximum levels of personal protective equipment (PPE) or engineering controls (MOEs < 100).

Using the updated PBPK-derived steady state PoDs and uncertainty factors (dermal LOC = 100), all occupational dermal post-application scenarios were of concern on Day 0. The REIs on the registered chlorpyrifos labels range from 24 hours to 5 days. On average, scenarios were not of concern ≥ 18 days after treatment.

2.0 Use Profile

Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum,

chlorinated OP insecticide. Registered use sites include a large variety of food crops (including fruit and nut trees, many types of fruits and vegetables, and grain crops), and non-food use settings (e.g., golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products). Public health uses include aerial and ground-based fogger adulticide treatments to control mosquitoes. There are also residential uses of roach bait products and ant mound treatments. Permanent tolerances are established (40 CFR§180.342) for the residues of chlorpyrifos in/on a variety of agricultural commodities, including meat, milk, poultry and eggs. There are also tolerances for use in food handling/service establishments (FHE or FSE). Chlorpyrifos is manufactured as granular, microencapsulated liquid, soluble concentrate liquid, water dispersible granular in water soluble packets (WSP), wettable powders in WSPs, impregnated paints, cattle ear tags, insect bait stations and total release foggers. There is a wide range of application rates and methods. The residues of concern for risk assessment purposes are chlorpyrifos and chlorpyrifos oxon under some circumstances.

3.0 Tolerance Considerations

See Section 2.0 and Appendix 8 of D22485 (D. Drew *et al.,* 12/29/2014) for details regarding the analytical enforcement method, U.S. tolerances and international residue levels for chlorpyrifos.

4.0 Chemical Identity and Physical/Chemical Properties

See Sections 3.1 and 3.2 and Appendix 7 of D22485 (D. Drew *et al.,* 12/29/2014) for details regarding the chemical identity and physical/chemical characteristics of chlorpyrifos.

5.0 Hazard Characterization and Dose-Response Assessment

5.1 Introduction & Background

Historically, the EPA has used AChE inhibition as the critical effect for deriving risk assessment PoDs for OP pesticides, including chlorpyrifos. However, there is a breadth of information available on the potential adverse neurodevelopmental effects in infants and children as a result of prenatal exposure to chlorpyrifos. Over the last several years, the agency has taken a stepwise, objective, and transparent approach to evaluate, interpret, and characterize the strengths and uncertainties associated with the available neurodevelopmental information. This effort has involved extensive collaboration across the EPA and also within the Federal government.

The stepwise evaluation began with the September 2008 FIFRA SAP. The SAP evaluated the agency's preliminary review of available literature and research on chlorpyrifos, with a particular focus on effects seen in women and children following chlorpyrifos exposures (USEPA, 2008). Subsequently, the agency has developed approaches for risk assessment of semi-volatile pesticides (USEPA, 2009), and developed the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" to better integrate epidemiology data with other types of experimental data in pesticide risk assessments (USEPA, 2010; FIFRA SAP 2010a,b). In early 2011, the FIFRA SAP reviewed the chlorpyrifos physiologically based pharmacokinetic – pharmacodynamic (PBPK-PD) model to conduct quantitative risk assessment.

The model estimates AChE inhibition in humans following exposure to chlorpyrifos and/or the oxon from a variety of exposure pathways (FIFRA SAP 2011).

In 2012, the agency convened another FIFRA SAP to review the latest experimental data related to AChE inhibition, cholinergic and non-cholinergic adverse outcomes, including neurodevelopmental studies on behavior and cognition effects (FIFRA SAP 2012[2]). Similarly, the agency also performed an in-depth analysis of the available chlorpyrifos biomonitoring data and of the available epidemiologic studies from three major children's health cohort studies in the U.S., including those from the Columbia University. The agency also explored plausible hypotheses on mode of actions/adverse outcome pathways (MOAs/AOPs) leading to neurodevelopmental outcomes seen in the biomonitoring and epidemiology studies.

Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological cohorts.[3] Also, the agency evaluated the potential for chlorpyrifos exposure to lead to the neurobehavioral outcomes seen in the cohorts, and the ability of other environmental exposures to affect the interpretation of the results from the Columbia University studies.

In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" (herein called "HHRA", D. Drew *et al.,* D424485, 12/29/2014). The 2014 assessment used a PBPK-PD model (Appendix 2) to derive human PoDs based on 10% RBC AChE inhibition; for more information see Appendix 2 of D424485 (D. Drew *et al.,* 12/29/2014). In accordance with the recommendation of the FIFRA SAP (2012), the agency conducted a dose reconstruction analysis based on registered uses available for use in indoor residential areas prior to the year 2000. The highest exposures resulted from the registered broadcast use in residential homes. Based on the output from the PBPK-PD model, for the highest exposure considered (i.e., contact with hard floors following indoor broadcast use of a 1% chlorpyrifos formulation), <10% RBC AChE inhibition in pregnant women and young children would be expected from residential uses. It is noteworthy that all estimates of exposure based on conservative assumptions lead to predicted AChE inhibition levels < 10%. The chlorpyrifos 2014 revised HHRA included retention of the 10X FQPA SF for all populations assessed; including infants, children, youths, and women of childbearing age. The 10X FQPA safety factor was retained based on the conclusion that, given the totality of evidence, chlorpyrifos likely played a role in the neurodevelopmental outcomes reported by the Columbia University investigators but uncertainties, such as the lack of an established MOA/AOP for neurodevelopmental effects and the exposure to multiple AChE-inhibiting pesticides, precluded definitive causal inferences. As a result, there is sufficient uncertainty in the human dose-response relationship for neurodevelopmental effects which prevents the agency from reducing or removing the statutory 10X FQPA SF (D. Drew *et al.,* D424485, 12/29/2014).

In 2013, the EPA sought to obtain the original raw data used to support certain epidemiological analyses of *in* utero exposure to chlorpyrifos and subsequent adverse neurodevelopmental health outcomes in children generated by the CCCEH. While the researchers did not agree to provide

[2] https://www.regulations.gov/docket?D=EPA-HQ-OPP-2012-0040
[3] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170

these data to the EPA, agency staff gained valuable insight into the conduct of the study and the data that were collected in a visit to Columbia University in April 2013. The agency wrote a summary of the 2013 meeting with researchers from Columbia University which can be found in "Appendix 6 Columbia Center for Children's Environmental Health (CCCEH) Epidemiology Data Acquisition "Raw Data Request" of Drew *et. al*, D424485, 12/29/2014. In the summer of 2015, Dr. Dana Barr of Emory University (formerly of CDC) provided the EPA with limited raw urine and blood data in her possession from the three cohorts. However, the files provided from Dr. Barr are not useful for the EPA's current purpose of assessing risk to chlorpyrifos (D. Vogel, Record of Correspondence, 10/2016). The EPA does not have any of the other measurements of the children in the cohort (e.g., chlorpyrifos blood data, interviews, test or IQ scores).

In a 2016 white paper, the agency proposed using data on cord blood reported from the investigators at the Columbia Center for Children's Environmental Health (CCCEH) as the source for new PoDs for risk assessment. This 2016 white paper was reviewed by the FIFRA SAP in April, 2016[4]. The 2016 Panel did not support using the CCCEH chlorpyrifos concentrations in cord blood quantitatively to derive PoDs for risk assessment. The Panel noted a number of uncertainties, including: the use of results from a single longitudinal study without replication from another cohort; the lack of verification and replication of the analytical chemistry results that reported very low levels of chlorpyrifos (pg/g); and the lack of raw data available for independent evaluation. Importantly, however, the Panel agreed that "both epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% red blood cell (RBC) acetylcholinesterase (AChE) inhibition (i.e., toxicity at lower doses)." Moreover, the Panel did support the use of the PBPK model to assess internal dosimetry from various exposure scenarios. The SAP specifically stated that PBPK modelling "is a valuable tool to interpret the biomonitoring data in circumstances where multiple routes of exposure occur and when based on best available information as inputs."

Therefore, based on the evidence collected from 2014 to date, as summarized above, the agency has updated its HHRA for the existing uses of chlorpyrifos. This 2016 human health risk assessment provides limited, summary information and substantially relies on previous documents developed for chlorpyrifos which contain more detailed evaluations of scientific literature and the PBPK model:

- D. Drew *et al.,* Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review, December 29, 2014, D424485; and
- U.S. Environmental Protection Agency, Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides, September 15, 2015, D331251.

5.2 Summary of the Literature Review on Neurodevelopmental Effects

Detailed summaries of the epidemiological studies used in this literature review can be found either in the 2014 chlorpyrifos HHRA (D. Drew *et al.,* D424485, 12/29/2014), the 2015 literature review for other organophosphates (OPP/USEPA, D331251, 09/15/2015), and reviews of newer studies (E. Holman, D432184, 03/25/2016). Only brief summaries of the literature reviews are

[4] https://www.regulations.gov/docket?D=EPA-HQ-OPP-2016-0062

provided below.

Newer lines of research on OPs have raised some uncertainty about the agency's risk assessment approach of using AChE inhibition for deriving PoDs. These uncertainties are in the areas of potential AOPs; *in vivo* animal studies; and notably results seen in epidemiological studies in mothers and children, with regard to the potential for neurodevelopmental effects in fetuses and children. Many of these studies have been the subject of review by the agency over the last several years as part of the development of the 2014 chlorpyrifos HHRA (D. Drew *et al.,* D424485, 12/29/2014).

A review of the scientific literature on potential MOAs/AOPs[5] leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (USEPA, 2012) and updated for the December 2014 chlorpyrifos HHRA (D. Drew *et al.,* D424485, 12/29/2014). In short, multiple biologically plausible hypotheses and pathways are being pursued by researchers that include targets other than AChE inhibition, including cholinergic and non-cholinergic systems, signaling pathways, proteins, and others. However, no one pathway has sufficient data to be considered more credible than the others. Published and submitted guideline developmental neurotoxicity (DNT) laboratory animal studies have been reviewed for OPs (D. Drew *et al.,* D424485, 12/29/2014 and USEPA, D331251, 09/15/2015). Neurobehavioral alterations in laboratory animals were often reported; however, at AChE inhibiting doses. Moreover, there was generally a lack of consistency in pattern, timing, and dose-response for these effects; and a number of studies were of low quality. However, the information on neurobehavioral effects as a whole provides evidence of long-lasting neurodevelopmental disorders in rats and mice following gestational exposure to OPs.

Initially, the agency focused on epidemiological studies from three US cohorts: 1) The Mothers and Newborn Study of North Manhattan and South Bronx performed by the CCCEH at Columbia University; 2) the Mt. Sinai Inner-City Toxicants, Child Growth and Development Study or the "Mt. Sinai Child Growth and Development Study;" and 3) the Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by researchers at University of California Berkeley. The agency has evaluated these studies and sought external peer review (FIFRA SAP reviews in 2008 and 2012; federal panel, 2013[6]) and concludes they are of high quality. In the three US epidemiology cohort studies, mother-infant pairs were recruited for the purpose of studying the potential health effects of environmental exposures during pregnancy on subsequent child development. Each of these cohorts has evaluated the association between prenatal chlorpyrifos and/or OP exposure with adverse neurodevelopmental outcomes in children through age 7-11 years. For the 2014 chlorpyrifos HHRA (D. Drew *et al.,* D424485, 12/29/2014), the EPA included epidemiologic research results from these three US prospective birth cohort studies but primarily focused on the results of CCCEH since this cohort has published studies on the association between cord blood levels of chlorpyrifos and neurodevelopmental outcomes. The agency retained the FQPA 10X SF in the 2014 chlorpyrifos revised risk assessment, in large part, based on the findings of these studies.

[5] Mode of action (MOA) and adverse outcome pathways (AOPs) describe a set of measureable key events that make up the biological processes leading to an adverse outcome and the causal linkages between such events.
[6] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170

In the 2015 updated literature review (USEPA, D331251, 09/15/2015), the agency conducted a systematic review expanding the 2012/2014 review which was focused only on US cohort studies with particular emphasis on chlorpyrifos. The expanded 2015 review includes consideration of the epidemiological data on any OP pesticide, study designs beyond prospective cohort studies, and non-U.S. based studies. The updated literature review identified seven studies which were relevant (Bouchard *et al.,* 2010; Fortenberry *et al.,* 2014; Furlong *et al.,* 2014; Guodong *et al.,* 2012; Oulhote and Bouchard, 2013; Zhang *et al.,* 2014; Shelton *et al.,* 2014). These seven studies have been evaluated in context with studies from the 2012/2014 review (D. Drew *et al.,* D424485, 12/29/2014). In addition, the agency has also reviewed more recent studies from CCCEH (Rauh *et al.*, 2015) and a pooled analysis of U.S. cohort studies (Engel *et al.*, 2015) (E. Holman, D432184, 03/25/2016). As discussed below, Rauh *et al.* (2015) provides further evidence of neurodevelopmental outcomes in the CCCEH study. The Engel *et al.* (2015) study shows relatively consistent results compared to previous studies conducted at 24 months (Engel *et al.*, 2011; Rauh *et al.*, 2006). Only a brief summary of this review is provided below. The agency continues to conclude that the 3 U.S. cohort studies (CCCEH, CHAMACOS, and Mt. Sinai) provide the most robust available epidemiological evidence.

The agency acknowledges the lack of established MOA/AOP pathway, the inability to make strong causal linkages, and the unknown window(s) of susceptibility. These uncertainties do not undermine or reduce the confidence in the findings of the epidemiology studies. The epidemiology studies reviewed in the 2012/2014 and 2015 literature reviews represent different investigators, locations, points in time, exposure assessment procedures, and outcome measurements. Despite differences in study design, with the exception of two negative studies in the 2015 literature review (Guodong *et al.,* 2012; Oulhote and Bouchard, 2013) and the results from the more recent Engel *et al.* (2015) study[7], all other study authors have identified associations with neurodevelopmental outcomes associated with OP exposure; these conclusions were across four cohorts and twelve study citations. Specifically, there is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures.

The CCCEH study primarily tested for the presence of chlorpyrifos in cord blood, and therefore remains the most relevant for the purposes of chlorpyrifos risk assessment. As summarized above, when comparing high to low exposure groups at 3 years of age in the CCCEH study (Rauh *et al.*, 2006), there were increased odds of:

- Mental delay (odds ratio; OR=2.4; 95% Confidence interval (CI): 1.1–5.1);
- Psychomotor delay (OR=4.9; 95% CI: 1.8–13.7);
- Attention disorders (OR=11.26; 95% CI: 1.79–70.99);
- Attention deficit hyperactivity disorder (ADHD) (OR=6.50; 95% CI: 1.09–38.69); and
- Pervasive Developmental Disorders (PDD) (OR=5.39; 95% CI: 1.21–24.11).

In a follow-up study at age 11, CCCEH study authors observed increased odds of mild to

[7] It is noted that the CCCEH study participants included in the Engel et al (2015) study are women enrolled from 2000-2001, i.e. after the cancellation of the residential uses of chlorpyrifos.

moderate tremor when comparing high to low exposure groups (Rauh *et al.*, 2015). Rauh *et al.*, (2011) evaluated relationship between prenatal chlorpyrifos exposure and neurodevelopment in 265 of the CCCEH cohort participants at age 7 years. They described the log of Working Memory Index (WMI) of children as linearly associated with concentration of chlorpyrifos (CPF) in cord blood: Slope = -0.006 (95% CI = -0.01, -0.002). For each standard deviation increase in exposure (4.61 pg/g), they observed a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.

In summary, the EPA's assessment is that the CCCEH study, with supporting results from the other 2 U.S. cohort studies and the seven additional epidemiological studies reviewed in 2015, provides sufficient evidence that there are neurodevelopmental effects occurring at chlorpyrifos exposure levels below that required for AChE inhibition.

5.3 Dose-Response Assessment

5.3.1 Conceptual Approach

As noted above, the agency has historically used 10% inhibition of RBC AChE as the critical effect for deriving PoDs for chlorpyrifos and other OPs. For example, the 2014 HHRA on chlorpyrifos used the PBPK-PD model to derive PoDs that could result in 10% RBC AChE inhibition for multiple exposure scenarios (e.g., worker, dietary, residential). While significant uncertainties remain about the actual exposure levels experienced by mothers and infant participants in the children's health cohorts, it is unlikely that these exposures resulted in RBC AChE inhibition at or above the 10% AChE inhibition response level. For example, as part of the CHAMACOS study, Eskenazi *et al.*, (2004) measured AChE activity and showed that no inhibition in AChE activity were observed. Additionally, following the recommendation of the FIFRA SAP in 2012, the agency conducted a dose reconstruction analysis for pregnant women and young children based on registered residential chlorpyrifos uses available prior to 2000 inside the home (D. Drew *et al.*, D424485, 12/29/2014). The PBPK-PD model using this dose reconstruction analysis indicates that for the highest exposure considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <1% RBC AChE inhibition was produced in pregnant women. While uncertainty exists as to actual chlorpyrifos exposure at (unknown) critical windows of exposure, the agency believes it is unlikely individuals in the epidemiology studies experienced RBC AChE inhibition from their exposure to chlorpyrifos. The 2016 SAP concluded that "epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% RBC AChE inhibition (i.e., toxicity at lower doses)." As such, the use of 10% RBC AChE inhibition for deriving PoDs for chlorpyrifos may not provide a sufficiently protective human health risk assessment. Therefore, the agency has endeavored to derive PoDs and uncertainty/safety factors for risk assessment that are protective of both the AChE inhibition and any adverse effects that could occur at lower doses.

As noted, however, the 2016 SAP did not support using the CCCEH cord blood quantitatively in deriving revised PoDs. In their verbal comments, multiple panelists suggested a 'hybrid' approach. In the written report, the SAP did not provide a suggested approach for how the EPA might continue to use the epidemiology data results in a quantitative risk assessment without

attempting to derive the PoD from cord blood data. Specifically, the SAP stated that, given the absence of a particular key window of exposure for the effects shown in the CCCEH study, the EPA should use estimated peak blood concentrations or TWA blood concentrations within the prenatal period as the PoD rather than blood concentrations at delivery. The Panel was also positive and supportive of the agency's use of the PBPK model as a tool for assessing internal dosimetry from the typical OPP exposure scenarios using peer reviewed exposure assessment approaches (e.g., food, water, residential, worker). As such, use of the PBPK model coupled with the typical OPP exposure scenarios to derive PoDs based on TWA blood concentrations, as recommended by the SAP, provide the strongest scientific foundation for moving forward in human health risk assessment for chlorpyrifos. This approach:

- incorporates peer reviewed and accepted inputs for both chlorpyrifos and standard pesticide risk assessment, including: the Residential SOPs[8], the EPA Exposure Factors Handbook 2011 Edition, chlorpyrifos-specific residential exposure modeling inputs and others;
- does not directly rely on quantitative measures of chlorpyrifos in cord blood obtained from the CCCEH, which were the source of uncertainty identified by the 2016 SAP, while still accepting the qualitative findings that chlorpyrifos contributed to the outcomes reported by the CCCEH, which were supported by the 2008 and 2012 SAPs; and
- does not directly rely on quantitative measures of chlorpyrifos in cord blood obtained from the CCCEH, and thus, the lack of access to the raw data from the CCCEH is less of an uncertainty.

The following sections describe the use of the PBPK model to 1) predict TWA of blood concentrations from an exposure scenario likely to be experienced by women in the CCCEH study (indoor use of chlorpyrifos-containing products), and 2) determine the external doses (PoDs for risk assessment) for infants, children, youths, and adults using current exposure assumptions and methodologies (i.e., The 2012 Residential SOPs, and chemical-specific exposure data, etc.) that result in the predicted TWA of blood concentration. The likely indoor use scenario which was experienced by the women in the CCCEH study was derived from the indoor crack and crevice uses of chlorpyrifos; reasoning for selecting this specific scenario is detailed below.

5.3.2 Deriving Internal Concentrations of Chlorpyrifos from Indoor, Crack & Crevice Use

In order to derive a protective PoD for risk assessment from the internal concentrations of chlorpyrifos, the agency reviewed the chlorpyrifos registered uses that would have been available to the CCCEH cohort. The following two risk mitigation actions were the basis for the agency's conclusion that the crack and crevice uses of chlorpyrifos was the most appropriate scenario to assess exposure to the women in the CCCEH cohort in the approximate 1998-2000 timeframe:

- In January 1997, the technical registrants agreed to cancel all broadcast and total release/aerosol foggers containing chlorpyrifos in order to reduce indoor exposures, especially to children and other sensitive groups. The following chlorpyrifos uses were

[8] https://www.epa.gov/sites/production/files/2015-08/documents/usepa-opp-hed_residential_sops_oct2012.pdf

also cancelled: all direct application of pet products including sprays, shampoos, and dips (pet collars not included); and all insecticidal paint additives. Further, all concentrates which required mixing were eliminated, limiting the household consumer's access to only ready-to-use products. Although the above uses were cancelled in 1997, existing stocks could be phased out, or applied until depleted. Indoor crack and crevice (perimeter) and spot treatment as a termiticide uses of chlorpyrifos continued to be registered.

- In June 2000, the technical registrants of chlorpyrifos, agreed to eliminate or phase out nearly all remaining uses that resulted in residential exposure, including: home lawn, crack and crevice, and other indoor uses. Non-residential uses where children could be exposed, such as schools and parks, were also cancelled, with the exception of roach and ant baits in child resistant packaging, and mosquito and fire ant control. For uses that were cancelled, retailers had a stop sale date of December 31, 2001. A phase out of existing stocks was allowed following the 2001 stop sale.

Additionally, in the summer of 2016, OPP contacted several professional pesticide applicators working in New York City apartment buildings around the time of the CCCEH cohort. These professional pesticide applicators recalled that the crack and crevice[9] use was the predominant use around 1998-2000 (D. Friedman, Record of Correspondence, 10/2016). Based on this input, and the mitigation rationale outlined above, the agency has focused on crack and crevice exposures for the 2016 risk assessment.

The 2012 FIFRA SAP (2012) recommended that the EPA conduct a "dose reconstruction" analysis of indoor residential uses to assess potential for RBC AChE inhibition. The dose reconstruction analysis was conducted and presented in the 2014 HHRA[10]. The goal of the dose reconstruction exercise was to estimate upper limit, bounding level exposures, to test the hypothesis of whether RBC AChE at or above the 10% inhibition level used by the agency for typical AChE PoDs may have occurred in the CCCEH cohort. For example, in the dose reconstruction analysis, exposure to the women was assumed to occur 24 hours a day without adjustments for bathing, showering, or leaving the residence for 14 consecutive days. For the 2014 HHRA, residential handler and post-application exposures from indoor broadcast applications resulted in the highest risk estimates and, therefore, were the only exposure estimates presented. The purpose of 2016 analysis for this risk assessment is to predict typical product usage and behaviors thereby deriving more accurate and realistic estimates of exposure compared to the 2014 analysis.

For the 2016 risk assessment, the agency has assessed chlorpyrifos exposures resulting from post-application exposures only. Whyatt *et al.* (2002) reported that many women applied pesticide products themselves, and that majority who reported using pesticide products used them at least once per month. However, as the agency has shown in the 2014 dose reconstruction analysis, post-application exposures are greater in magnitude than exposures which occur during an application. Therefore, the assessment of post-application exposure ensures that the highest potential exposures are evaluated. Specifically, the 2016 risk assessment

[9]Per the 2012 Residential SOPs, a crack and crevice application is defined as application of pesticides with the use of a pin stream nozzle, into cracks and crevices in which pests hide or through which they may enter a building. Such openings commonly occur at expansion joints, between different elements of construction, and between equipment and floors.

[10] The methods, algorithms, and exposure data used to conduct the dose reconstruction analysis can be referenced in Appendix 10 of the 2014 HHRA.

focuses on the post-application exposures from the chlorpyrifos in crack and crevice use since this was the predominant application type during the time of the CCCEH cohort.

The dose reconstruction in the 2016 risk assessment is based on the methods outlined in the 2012 Residential SOPs[11] which describe specific algorithms and inputs, on a scenario-specific basis.[12] Appendix 10 of the 2014 HHRA (D. Drew *et al.,* D424485, 12/29/2014) can be referenced for a description of the methods, algorithms, and inputs used. Specifically, the 2012 Residential SOPs[13] have been used to predict the range of potential exposures which could have occurred to individuals in the cohort for crack and crevice hard surface and carpet treatments. The present analysis uses the same chemical-specific exposure data inputs recommended in the 2012 Residential SOPS (*i.e.*, the fraction of chlorpyrifos residues transferred from treated carpet and hard surfaces to the exposed individual; and exposure data used to derive the liquid formulation transfer coefficient (TC)). Additionally, chemical-specific exposure data were used to define the concentrations of chlorpyrifos present in air following indoor applications. The differences between the previous dose reconstruction and the present analysis are: (1) the exposure duration was 24 h/day for the 2014 dose reconstruction analysis, and 2 h/day for the present analysis; (2) predicted endpoint for the dose reconstruction analysis was the peak RBC AChE inhibition level during the 14 days post-application, and the predicted endpoint for the present analysis was time-weighted average of chlorpyrifos concentrations in blood; (3) no shower was assumed to occur over the 14-day exposure period for the dose reconstruction analysis, whereas a daily shower is assumed to occur for the present analysis; (4) the total exposure duration was 14 days in the dose reconstruction analysis, and 30 days in the present analysis. The assumption that women followed in the CCCEH cohort showered immediately after exposure leads to significantly more conservative estimates of risk assessment PoDs (i.e., neurodevelopmental effects may have occurred at lower exposure levels when assuming that the women showered after daily exposure vs. when it is assumed that the women did not shower after daily exposure); however, since other inputs (e.g., 50% of the body exposed) lead to less conservative PoD estimates, the combination of inputs used to estimate exposures is expected to reasonably approximate exposures to these women resulting in reasonable risk assessment PODs.

For the 2016 risk assessment, the agency assumed a once daily shower occurred immediately following exposure activities. The PBPK model simulation were conducted for a 30-day post-application in the crack & crevice scenario. Daily exposure durations for post-application dermal contact with carpets and hard surfaces were selected based on the recommendation in the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment[14] (herein referred to as the 2012 Residential SOPs). Specifically, for adults, the recommended exposure durations for post-application dermal contact are 8 and 2 hours daily for carpets and hard surfaces, respectively. These values are based on the EPA Exposure Factors Handbook 2011[15] Edition that provides information on the total time spent in a residence and time spent in various rooms within a residence. The hard surface exposure scenario resulted the highest estimated exposures and, therefore, was selected for PBPK model PoD derivation. Additionally, chlorpyrifos residues were assumed to dissipate 10% daily; that is, the total amount of residue

[11] https://www.epa.gov/sites/production/files/2015-08/documents/usepa-opp-hed_residential_sops_oct2012.pdf
[12] The 2012 Residential SOPs were subjected to peer review by FIFRA SAP in October 2009. http://www.regulations.gov/#!docketBrowser;rpp=50;po=0;D=EPA-HQ-OPP-2009-0516
[13] https://www.epa.gov/sites/production/files/2015-08/documents/usepa-opp-hed_residential_sops_oct2012.pdf
[14] http://www.epa.gov/pesticides/science/residential-exposure-sop.html
[15] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

available for transfer from the treated floor is assumed to reduce by 10% for each subsequent day of exposure until the end of the 30th day prior to the next application. The 10% value was based on an evaluation of all available chlorpyrifos-specific floor residue data. For all post-application exposure scenarios a female bodyweight reflective of all trimesters of pregnancy, 75 kg, was assumed to reflect the population of interest from the CCCEH cohort. This value was derived from the EPA Exposure Factors Handbook 2011 Edition (adult female: Tables 8-3 through 8-5; body weight of pregnant women: Table 8-29).

The results of the 2016 dose reconstruction assessment of the post-application exposures following contact with hard surfaces following indoor chlorpyrifos crack and crevice treatment is presented in Table 5.3.2.

Table 5.3.2. Residential Post-application Exposures to Women in the CCCEH Cohort Following Indoor Chlorpyrifos Crack and Crevice Treatment.

Exposure Scenario	Formulation	Deposited Residue[1] ($\mu g/cm^2$)	Fraction Transferred[2]	Transferable Residue[3] ($\mu g/cm^2$)	Transfer Coefficient (cm^2/hr)	Exposure Time (hr/day)	Dermal Dose[4] (mg/kg/day)	Airborne Concentration of Chlorpyrifos[5] (mg/m^3) - Day of Application
Crack and Crevice (Hard Surfaces)	1% PCO Crack and Crevice Application	0.30	0.13	0.039	6,800	2	0.00707	0.00089

1 Estimated based on the recommendations of the 2012 Residential SOPs: Indoor Environments SOP.
2 Chlorpyrifos-specific fraction transfer as recommended in the 2012 Residential SOPs: Indoor Environments SOP (Table 7-9; Arithmetic Mean).
3 Transferable Residue ($\mu g/cm^2$) = Deposited Residue ($\mu g/cm^2$) * Fraction Transferred (unitless)
4 Dermal Dose (mg/kg/day) = Transferable Residue ($\mu g/cm^2$) * Transfer Coefficient (cm^2/hr) * Exposure Time (hr/day) * Conversion Factor (0.001 mg/μg)
5 Average airborne concentration of chlorpyrifos from crack and crevice on the day of product application as determined from 3 literature studies and 1 registrant submitted study.

The PBPK model-predicted time course of chlorpyrifos concentrations in blood based on the crack and crevice scenario is provided in Figure 1. The predicted TWA of chlorpyrifos concentration in blood from this scenario was 0.004 µg/L, shown as the solid horizontal line in Figure 1.

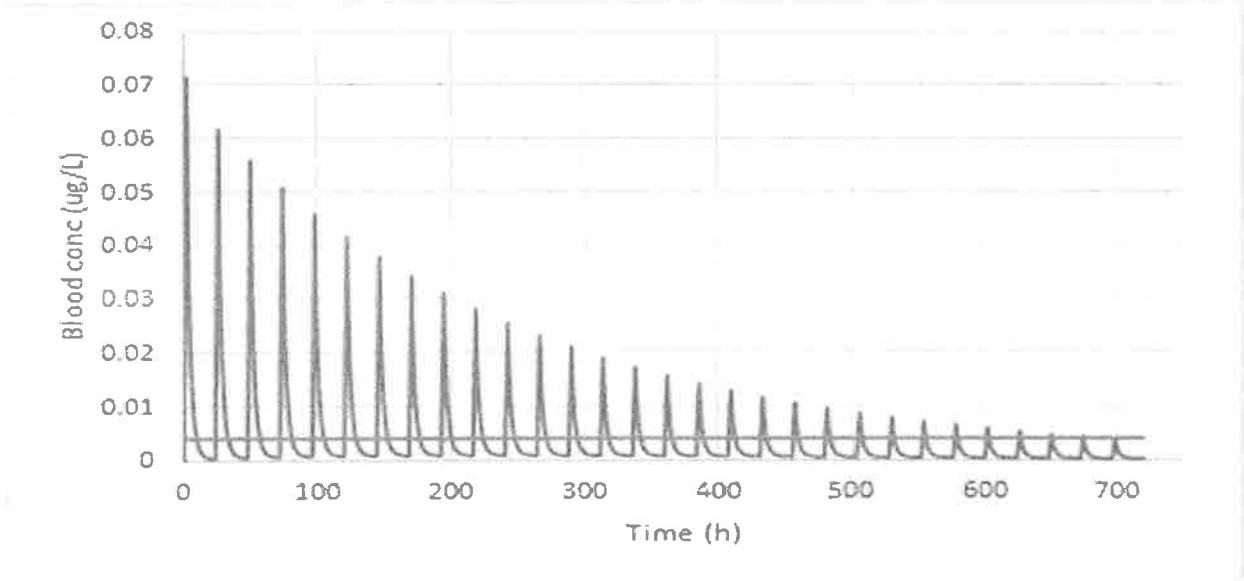

Figure 1: The PBPK model-predicted time course of chlorpyrifos concentrations in blood based on the crack and crevice scenario. The predicted TWA of chlorpyrifos concentration in blood (0.004 µg/L) is shown by the solid line.

5.3.3 Determining PoDs

In typical risk assessments, PoDs are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by use of 10X factors. In the case of chlorpyrifos, the PBPK model for chlorpyrifos was used as a data-derived extrapolation approach to estimate individual PoDs for pregnant women and children. As noted above, the PBPK model was first used to predict, from the crack and crevice post-application scenario, the TWA of chlorpyrifos concentration in blood as the internal dose metric for deriving PoDs in the subsequent analyses.

For the 2014 HHRA (D. Drew *et. al*, D424485, 12/29/2014), the EPA developed PoDs based on AChE inhibition to protect against cholinergic toxicity; such cholinergic toxicity could occur to any lifestage if exposure is sufficiently high. As such, in 2014, the EPA evaluated the spectrum of lifestages from the fetus through adulthood. Fetuses may be exposed to chlorpyrifos through the mother while infants and children may be exposed directly. Studies in laboratory animals do not suggest any specific critical period or lifestage, but instead suggest pre- and post-natal periods of susceptibility. The EPA acknowledges that the epidemiology literature regarding associations between post-natal (infancy, childhood) biomarker metrics and neurodevelopmental outcomes is limited to the Bouchard *et al.,* (2010) study, a cross-sectional study that observed positive association between attention and behavior problems and total dialkyl phosphate metabolites (DAPs) and dimethyl alkylphosphate metabolites (DMAPs), using urinary National Health and Nutrition Examination Survey (NHANES) data in children 8–15 years old. The other studies which evaluated postnatal biomarker metrics and neurodevelopment outcomes have found no statistically significant associations. Specifically, postnatal exposure to OPs (measured as DAPs) has been assessed in the CHAMACOS cohort (Eskenazi *et al.*, 2007; Young *et al.*,

2005; Bouchard *et al.*, 2011), two other cross-sectional studies (Guodong *et al.*, 2012; Oulhote and Bouchard, 2013) and Engel *et al.,* (2016). Despite the limited epidemiological evidence from postnatal exposure, the EPA is proposing to use the TWA as the most relevant source of information for deriving a PoD specific for chlorpyrifos for fetuses, infants, and children. Consistent with the advice from the 2016 SAP, the EPA believes that the CCCEH results are directly relevant to fetal exposure and newborns; however, the EPA acknowledges they may be less relevant to older infants, toddlers, and children. The EPA has conducted exposure assessments for all typical age groups for completeness and acknowledges that the exposure and risk assessment results for females 13-49 years old are the most relevant to the CCCEH data.

The PBPK model accounts for pharmacokinetic characteristics to derive age, duration, and route specific PoDs (Table 5.3.3.3). Separate PoDs have been calculated for dietary (food, drinking water), residential, and occupational exposures by varying inputs on types of exposures and populations exposed to obtain a predicted time-weighted average of 0.004 µg/L chlorpyrifos in blood using inputs specific to each scenario (i.e., duration exposed, amount consumed, etc). Specifically, the following characteristics have been evaluated: route (dermal, oral, inhalation); body weights which vary by life-stage; exposure duration (hours per day, days per week); and exposure frequency [events per day (eating, drinking)].

To derive a PoD for each non-dietary and dietary exposure scenario and subpopulation, the appropriate body weight for each age group or sex was taken from the Exposure Factors Handbook (USEPA, 2011) (for occupational exposures) or from the NHANES/What We Eat in America (WWEIA) Survey[16] (for dietary exposures). All body weights used are consistent with those assumed for typical pesticide dietary, occupational, and residential exposure assessments and shown in Table 5.3.3.1.

Table 5.3.3.1. Body Weight Assumptions Incorporated into PBPK Model for Chlorpyrifos.

Exposure Scenario	Exposure Pathway	Population & Body Weight (kg)				
		Infants (< 1 yr old)	Young Children (1 - 2 years old)	Children (Residential:6-11 years old; Dietary:6-12 years old)	Youths (Residential:11-16 years old; Dietary:13-19 years old)	Females (13 – 49 years old)
Dietary	Food and Drinking Water	4.8[1]	12.6[2]	37.1[2]	67.3[2]	72.9[2]
Residential (Golfers)	Dermal			32[5]	57[6]	69[4]
Residential (Mosquitocide Application)	Dermal, Oral, Inhalation		11[3]			
Residential (Bystander/ Volatilization Assessment)	Inhalation		11[3]			
Occupational	Dermal, Inhalation					

1 For infants from birth to < 1 year old, the agency has selected the body weight for the youngest age group, birth to < 1 month old, 4.8 kg (Exposure Factors Handbook, Table 8-3, mean body weight for the birth to < 1 month age group).
2 NHANES/WWEIA
3 Exposure Factors Handbook, Table 8-3, mean body weight for the 1 to < 2 year old age group.

[16] http://www.ars.usda.gov/Services/docs.htm?docid=13793

4 Exposure Factors Handbook, Table 8-5, mean body weight for females 13 to < 49 years old.
5 Exposure Factors Handbook, Table 8-3, mean body weight for the 6 to < 11 year old age group.
6 (Exposure Factors Handbook, Table 8-3, mean body weight for the 11 to < 16 year old age group).

Table 5.3.3.2 shows the durations (days) of exposure included in the PBPK model to derive PoDs.

Table 5.3.3.2. Days of Exposure Assumptions Incorporated into PBPK Model for Chlorpyrifos.

Exposure Scenario	Exposure Pathway	Population & Days of Exposure				
		Infants (< 1 yr old)	Young Children (1 - 2 years old)	Children (Residential:6-11 years old; Dietary:6-12 years old)	Youths (Residential:11-16 years old; Dietary:13-19 years old)	Females (13 – 49 years old)
Dietary	Food and Drinking Water	21	21	21	21	21
Residential (Golfers)	Dermal			21	21	21
Residential (Mosquitocide Application)	Dermal, Oral, Inhalation		21			
Residential (Bystander/ Volatilization Assessment)	Inhalation		1 & 21			
Occupational	Dermal, Inhalation					

To derive the dietary exposure PoDs, dietary exposure was estimated daily for 21 days. For drinking water exposures, the daily water consumption volume was set to 0.688557 L for infants, children between 1-2 year old, and children 6-12 years old; 1.71062 L for youths 13-19 years old and female adults. Infants and children were assumed to consume water six times a day; youths and female adults were assumed to consume water four times a day. For food exposures, the eating event was set to one meal per day. The daily volumes consumed and number of daily consumption events for all populations are mean values by age group based on USDA's WWEIA. The mean daily water consumption amounts for children 1- 2 years old (0.35 L) and children 6-12 years old (0.58 L), were less than that for infants (0.688557 L); the infant daily water consumption volume was selected for all child sub-populations to be protective. For youths 13-19 years old, the mean daily water consumption amount (0.93 L) was less than that for the female adults (1.71062 L); therefore, the adult daily water consumption was selected for both subpopulations to be protective.

For all residential dermal exposures to chlorpyrifos, the fraction of skin in contact with chlorpyrifos was set to 50% to reflect uncovered skin areas for adults and children wearing shorts and a tee shirt. A daily shower (i.e., washing off the chlorpyrifos) was assumed immediately following chlorpyrifos exposure. All residential exposures were set to be continuous for 21 days. For residential exposures via golfing on treated turf, the daily exposure time is assumed to be 4 hours/day; for residential exposures via contact with turf following public health mosquitocide application, the daily exposure duration is assumed to be 1.5 hours for ground applications and 1 hour for aerial applications. For residential inhalation exposures following public health mosquitocide application, the exposure duration was set to 1 hour per

day. These exposure times selected were based on those recommended in the 2012 Residential SOPs. For residential bystander exposures from volatilization following treatment of nearby fields, the inhalation exposure time was set to 24 hours per day. For inhalation exposures following mosquitocide application and from volatilization, the inhalation rates were set to 0.33 m^3/hour for children 1 to < 2 years old and 0.64 m^3/hour for adults.

In addition to dietary and residential exposures, the PBPK model was also used to estimate PoDs resulting in a time-weighted average of 0.004 µg/L chlorpyrifos in blood following occupational exposures (Table 5.3.3.3). Dermal exposures for workers assumed even distribution across the entire body surface area. A daily shower (i.e., washing off the chlorpyrifos) was assumed following chlorpyrifos exposure. The worker was assumed to be a female adult between the ages of 13 to 49, and had a body weight of 69 kg. This worker is exposed to chlorpyrifos either via inhalation or skin for 8 hours/day, 5 days/week, for a total of 21 days.

Table 5.3.3.3. PBPK Model-Predicted Chlorpyrifos Point of Departures (PoDs) Corresponding to a Time-Weighted Average of 0.004 µg/L Chlorpyrifos in Plasma*.

Exposure Scenario	Exposure Pathway	Infants (< 1 year old)	Young Children (1 - 2 years old)	Children (Residential:6-11 years old; Dietary:6-12 years old)	Youths (Residential:11-16 years old; Dietary:13-19 years old)	Females (13 – 49 years old)
Dietary	Drinking Water (µg/kg/day)	1.4	3.2	7.1	4.8	5.1
	Food (µg/kg/day)	0.2	0.17	0.13	0.12	0.12
Residential (Golfers)	Dermal (µg/kg/day)			2.2	1.4	1.3
Residential (Mosquitocide Application)	Dermal (µg/kg/day)		14.9			3.4
	Oral (µg/kg/day)		0.17			
	Inhalation (concn. in air mg/m^3)[1]		*Aerial:* 0.00165 *Ground:* 0.0011			*Aerial:* 0.0051 *Ground:* 0.0034
Residential (Bystander/ Volatilization Assessment)	Inhalation (concn. in air mg/m^3)		*Steady State:* 0.00068 *Acute:* 0.0013			*Steady State:* 0.00021 *Acute:* 0.004
Occupational	Dermal (µg/kg/day)					0.47
	Inhalation (concn. in air mg/m^3)					0.0011

*PoDs and exposure and risk estimates for females 13-49 yrs covers all youths >13 yrs.

1. PBPK model inputs for inhalation mosquitocide scenarios differ based on the exposure scenario being assessed. Since the AgDISP (v8.26) model predicts the 1 hour average air concentration following aerial applications, the PBPK-PD was model was run assuming 1 hr of inhalation exposure/day, 7 days/week, and 21 days of exposure. For ground based ULV applications, risks are estimated based on the inhalation exposure duration for time spent outdoors (1.5 hours/day) and, therefore, the PBPK-PD model was run assuming 1.5 hours of inhalation exposure/day, 7 days/week, 21 days of exposure.

5.3.4 Uncertainty, Extrapolation, & FQPA Safety Factors

The TWA blood level resulting from chlorpyrifos exposure from the crack and crevice scenario

is considered a LOAEL rather than a NOAEL, since this is the exposure level likely to be associated with neurodevelopmental effects reported in the CCCEH study. In situations where the agency selects a PoD from a study where a NOAEL has not been identified, the EPA generally will retain the FQPA SF of 10X to account for the uncertainty in using a LOAEL. In the 2016 revised risk assessment this is being done for chlorpyrifos. The 2016 revised risk assessment also applies a 10X uncertainty factor for intraspecies variability because of the lack of sufficient information to reduce or remove this factor. Typically, the agency uses animal studies for selection of PoDs and, as such, retains a 10X interspecies factor for extrapolation of the animal data to assess human health. However, with use of the PBPK-PD model which accounts for the pharmacokinetic and pharmacodynamic differences between animals and humans to derive PoDs, it is appropriate to reduce the interspecies factor to 1X. Therefore, the total uncertainty factors for chlorpyrifos in this 2016 risk assessment are 100X (10x for intra-species extrapolation and 10x for the FQPA 10 safety factor).

6.0 Dietary Exposure and Risk Assessment

HED had previously conducted both acute and steady state dietary (food only) exposure analyses for chlorpyrifos using DEEM and Calendex software with the Food Commodity Intake Database (FCID) (D. Drew *et al.,* D424486, 11/18/2014), respectively.

For the current assessment, the steady state exposure values resulting from the 2014 dietary assessment are compared to the updated PBPK-derived steady state Population Adjusted Dose (ssPAD). When the dietary exposure exceeds 100% of the ssPAD there is a potential risk concern.

Since the steady state dietary assessment is protective of any acute food exposures, only the results of the steady state assessment are discussed herein. The steady state analysis calculated exposures for the sentinel populations of infants <1 year old, children 1-2 years old, youth 6-12 years old, and females 13-49 years old.

All details pertaining to the assumptions, data inputs, and exposure outputs for the dietary analysis may be found in the 2014 dietary assessment memorandum (D. Drew *et al.,*D425586, 11/18/2014).

6.1 Food Residue Profile

The residue of concern for tolerance expression and risk assessment in plants (food and feed) and livestock commodities is the parent compound chlorpyrifos. Based on the available crop field trials, metabolism studies, and PDP monitoring, the cholinesterase inhibiting metabolite, chlorpyrifos oxon, would be not be present in edible portions of the crops, or in livestock tissue or milk and, therefore, is not included in the food assessment.

The steady state dietary exposure analysis is highly refined. The large majority of food residues used were based upon USDA's PDP monitoring data except in a few instances where no appropriate PDP data were available. In those cases, field trial residues or tolerance level residues were assumed. The Biological & Economic Analysis Division (BEAD) provided

percent crop treated information in the Screening Level Usage Analysis (SLUA; May 1, 2014). Food processing factors from submitted studies were used as appropriate. All commodities with current U.S. tolerances for residues of chlorpyrifos are included in this assessment (40 CFR§180.342).

6.2 Steady State Dietary (Food Only) Exposure and Risk Estimates

The steady state dietary (food only) exposures for chlorpyrifos are of concern at the 99.9th percentile of exposure for all population subgroups analyzed. Children (1-2 years old) is the population subgroup with the highest risk estimate at 14,000% of the ssPAD$_{food}$.

Table 6.2. Steady State Dietary (Food Only) Exposure and Risk Estimates for Chlorpyrifos.

Population Subgroup	ss PoD$_{food}$[1] (µg/kg/day)	ssPAD$_{food}$[2] (µg/kg/day)	Food Exposure[3] (µg/kg/day)	% of ssPAD$_{food}$
Infants (< 1 yr)	0.20	0.002	0.186	9,300
Children (1-2 yrs)	0.17	0.0017	0.242	14,000
Youths (6-12 yrs)	0.12	0.0012	0.128	11,000
Adults (Females 13-49 yrs)	0.12	0.0012	0.075	6,200

1 Steady state point of departure; daily dose predicted by PBPK-PD for steady state (21 day) dietary (food) exposures (see Table 5.3.3.3 for PoDs).
2 ssPAD= Steady state population adjusted dose = PoD (Dose predicted by PBPK model ÷ total UF; Total uncertainty factor =100X (10X intraspecies factor and 10X LOAEL to NOAEL extrapolation factor).
3 Steady state (21 day) food-only exposure estimates from Calendex (at 99.9th percentile).

6.3 Steady State Dietary (Food Service/Food Handling Establishments) Exposure and Risk Estimate

There are chlorpyrifos uses in food handling establishments (FHE) where food and food products are held, processed, prepared or served. These may include areas such as boxcars, shipping containers, and warehouses. FHE uses in restaurants, or similar service areas where food is prepared and served, may also be referred to as *food service establishment* (FSE) uses. There are no tolerances for the chlorpyrifos uses in FHEs except for the specific use of chlorpyrifos in FSEs as stated in the 40 CFR§180.342 (a) (3):

A tolerance of 0.1 part per million is established for residues of chlorpyrifos, per se, in or on food commodities (other than those already covered by a higher tolerance as a result of use on growing crops) in food service establishments where food and food products are prepared and served, as a result of the application of chlorpyrifos in microencapsulated form.

Typically, where there are established tolerances for FSE (or FHE) uses, anticipated residues for *all* foods would be included in the dietary assessment along with the residues on the foods with crop tolerances. The food only exposures in Section 6.2do not incorporate potential exposure from residues that may result on foods from FSE uses and, therefore, may underestimate actual exposures. A previous dietary risk assessment included a chronic analysis for FSE uses (D. Soderberg, D388166, 6/11/2011). This analysis was based on a BEAD estimate of < 2% of

establishments treated with chlorpyrifos and half the analytical limit of detection (½ LOD; 0.01 ppm) based on all nondetectable residues in a chlorpyrifos FHE study. That analysis resulted in a chronic dietary exposure of 0.009 µg/kg for children ages 1-2 years old (highest exposed population subgroup). HED has used this exposure value to compare to the ssPAD for children ages 1-2 years old. For the FSE uses alone, the children ages 1-2 years old steady state dietary (food only) exposures for chlorpyrifos are of concern, with an estimated risk of 530% of the ssPAD.

6.4 Dietary Drinking Water Risk Assessment

The total dietary exposure to chlorpyrifos is through both food and drinking water. EFED has provided a revised drinking water assessment (DWA) for chlorpyrifos (R. Bohaty, D432921, 04/14/2016) which includes the updated EDWCs for dietary risk assessment. A DWLOC approach is used to calculate the amount of exposure available in the total dietary 'risk cup' for chlorpyrifos in drinking water after accounting for chloropyrifos exposure from food. This DWLOC is then compared to the EDWC to determine if there is a risk of concern for drinking water exposures (See D. Drew, D424485, 12/29/2014 for details on the DWLOC approach and calculations). However, because the dietary risks from food alone are of concern (exceed the ssPAD), it is not possible to calculate a DWLOC; essentially the steady state DWLOC is '0' after accounting for food exposures.

Hypothetically, if there were no exposure to chlorpyrifos from food, and the entire dietary 'risk cup' was available for drinking water, the resulting steady state DWLOC for infants (the most highly exposed population subgroup for water) would be 0.014 ppb. An EDWC at or exceeding this concentration would be considered a risk of concern for exposures to chlorpyrifos in drinking water.

7.0 Residential (Non-Occupational) Exposure/Risk Characterization

Residential exposures to chlorpyrifos are currently expected from homeowner use. Formulations/use sites registered for homeowner use include a granular ant mound use and roach bait in child-resistant packaging. Additionally, chlorpyrifos is labeled for public health aerial and ground-based fogger ULV mosquito adulticide applications and for golf course turf applications. All residential exposures and risks were previously assessed in support of the 2014 HHRA (W. Britton, D424484, 12/29/2014). The previous assessment included evaluation of residential post-application risks from playing golf on chlorpyrifos-treated courses and from exposures which can occur following aerial and ground-based ULV mosquito adulticide usage. The potential for residential exposures from the roach bait product was determined to be negligible. Further, residential exposures from the ant mound use were also determined to be negligible since these products can only be applied professionally and direct exposure with treated ant mounds is not anticipated.

In addition to the assessment of residential exposure, the potential for post-application exposures to residential bystanders who live on, work in, or frequent areas adjacent to treated fields from spray drift and volatilization were also evaluated and presented in the 2014 HHRA.

The previously assessed residential post-application, residential bystander/volatilization, and non-occupational spray drift risk estimates have been updated to incorporate the approach applied for PBPK-derivation of PoDs for infants, children, and adults based on the exposures estimated from the indoor crack and crevice uses of chlorpyrifos during the time of the CCCEH cohort.

7.1 Residential Handler Exposure/Risk Estimates

HED uses the term "handlers" to describe those individuals who are involved in the pesticide application process. HED believes that there are distinct tasks related to applications and that exposures can vary depending on the specifics of each task. Residential handlers are addressed somewhat differently by HED as homeowners are assumed to complete all elements of an application without use of any protective equipment.

Based upon review of all chlorpyrifos registered uses, only the roach bait products can be applied by a homeowner in a residential setting but the application of roach bait products has not quantitatively assessed because these exposures are negligible. The roach bait product is designed such that the active ingredient is contained within a bait station which eliminates the potential for contact with the chlorpyrifos containing bait material. Therefore, updated residential handler risks are not required for these uses.

7.2 Residential Post-application Exposure/Risk Estimates

Residential post-application exposures are likely from being in an environment that has been previously treated with chlorpyrifos. Chlorpyrifos can be used in areas frequented by the general population including golf courses and as an aerial and ground-based ULV mosquito adulticide applications made directly in residential areas. Post-application exposure from residential ant mound treatment was assessed qualitatively as addressed above because negligible exposures are anticipated.

All of the residential post-application exposure scenarios, data and assumptions, and algorithms used to assess exposures and risks from activities on golf course turf following chlorpyrifos application are the same as those used in the 2014 HHRA and ORE assessment. Additionally, this updated assessment makes use of the same chemical-specific turf transferable residue (TTR) data used previously to assess exposures and risks from golfing. Only the PoDs and LOCs have changed.

The residential post-application exposures and risks resulting from aerial and ground-based ULV mosquito adulticide applications have also been updated to reflect the updated PoDs and LOCs. However, the risks from the exposure scenarios have also been updated to reflect 1) the current default deposition fraction recommended for ground applied ULV mosquitocides (i.e., 8.7 percent of the application rate vs the previous 5 percent) and 2) several iterations of aerial applications modeled assuming differing winds speeds and release heights allowed by chlorpyrifos mosquitocide ULV labels. All other inputs and algorithms used for assessment of these exposure scenarios in 2014 remain the same, including the use of the chemical-specific TTR data. The AgDISP (v8.2.6) model input parameters, outputs, and the algorithms used to estimate residential post-application exposures following aerial and ground-based ULV

mosquitocide application can be found in Appendix A.

Default deposition fraction for ground applied ULV mosquitocides: Previously, an off-target deposition rate of 5 percent of the application rate was used by HED to evaluate ground-based ULV applications (i.e., 5 percent of the target application rate deposits on turf). This recommendation was based on data from Tietze *et al.,* and Moore *et al.* In a 2013 analysis (C. Peck, D407817, 3/28/2013), the Environmental Fate and Effects Division (EFED) reviewed eight published studies on ground ULV application in which deposition was measured. The studies varied in collection media (i.e., grass clippings and coupons), distance from application or spray head (ranging from 8 meters to 500 meters), and chemical measured (i.e., fenthion, malathion, naled, and permethrin). The analysis included the Moore *et al.,* and Tietze *et al.*, studies cited above. After considering the available data, HED has determined that an off-target deposition rate of 8.7 percent of the application rate may be used by HED to evaluate ground-based ULV applications (i.e., 8.7 percent of the target application rate deposits on turf). This value is the 90 percent upper confidence limit on the mean and is slightly higher than the mean values from all the data points observed in the studies (7.1%, n= 94). The adjusted application rate was then used to define TTR levels by scaling the available TTR data as appropriate.

Aerial application wind speed, volume median diameter, and release height: Previously, HED used the AgDISP (v8.2.6) model to assess deposition and air concentrations from aerial ULV applications assuming a 1 mph wind speed, volume median diameter is less than 60 µm (Dv 0.5 < 60 µm), and 300 foot release height. For this updated assessment, bounding risks have been estimated using the model based on a range of labeled application parameters. Lower spray height and lower wind speeds, and a greater Dv 0.5, results in the worst case potential exposures, or reduced potential for spray drift and, as a result, a greater deposition fraction and 1 hour average concentration. Therefore, estimated dermal and inhalation risks would be greater under these application conditions. The reverse is true for the best-case modeling scenario.

- Worst-case - 1 mph wind speed, Dv 0.5 = 60 µm, and 75 foot release height; and
- Best-case - 10 mph wind speed, Dv 0.5 = 40 µm, and 300 foot release height.

The following inputs were used for AgDISP (v8.26) modeling of chlorpyrifos ULV aerial applications.

Table 7.2.1. AGDISP Inputs (v8.26): Chlorpyrifos Mosquitocide ULV Aerial Application.

Input Parameters	Inputs to include in the AgDISP model	Notes/Comments
Application Method	Aerial	Default
Aircraft	Air Tractor AT-401	Default
Release Height	75, 300 Feet minimum release	Label allows a release height ranging from 75 to 300 feet.
Spray Lines	20 Reps	Default
Application Technique	Liquid	Default
Application Technique *Nozzles*	3; Extent 76.3%; Spacing 18.7 ft	Default
Application Technique *Drop Size Distribution*	User defined Parametric; $D_{V0.5}$: 40, 60 µm; and relative span: 1.4.	A $D_{v0.5}$ value of < 60 µm is allowable on the label. A $D_{v0.5}$ value of < 40 µm was modeled to estimate a lower droplet size

Table 7.2.1. AGDISP Inputs (v8.26): Chlorpyrifos Mosquitocide ULV Aerial Application.		
Input Parameters	**Inputs to include in the AgDISP model**	**Notes/Comments**
	no conversion to Malvern Drop Size Distribution	as is typically used for ULV aerial application.
Swath Width	500 feet	Default
Swath Displacement	Worst case application parameters: -130 feet Best case application parameters: 3,729 feet	The modeled spray deposition shows the peak deposition to be at a distance other than 0 feet. Therefore, the swath displacement w changed to the horizontal distance from the y axis where the peak deposition occurred and then the air concentration value was selected at this distance.
Meteorology	Wind type: single height Wind speed: 1, 10 mph Wind direction: -90 deg Temperature: 85 F° Relative humidity: 50%	No wind speed was identified on the label. The wind speeds of 1 and 10 mph were modeled to represent a reasonable range of wind speeds typical of ULV aerial applications.
Spray Material	Name: Oil Spray Material Evaporates: Yes Spray volume rate: 1.5 (gal/A) Active Fraction: 0.1936 Nonvol Fraction: 1	Spray material criteria as defined by the product label.
Atmospheric Stability	Overcast	Default
Surface	Upslope angle: 0 deg Sideslope angle: 0 deg Canopy: None	Default
Transport	Distance: 0 feet	Default
Advanced	Default Swatch offset: 0 Swath Specific Gravity carrier: Oil Specific Gravity active and additive= 0.929 Evaporation Rate: 84.76	Inputs based on criteria as defined by the product label.

Summary of Residential Post-application Non-Cancer Exposure and Risk Estimates

A summary of risk estimates is presented in Tables 7.2.2 through 7.2.8 below.

All residential post-application exposure scenarios assessed for playing golf on chlorpyrifos-treated courses, including all relevant populations and in consideration of all TTR data state sites, result in risks of concern (i.e., MOEs are < 100). Further, all residential post-application exposure scenarios assessed following aerial and ground ULV mosquitocide application result in risks of concern. All risk estimates are provided in Appendix B.

Table 7.2.2. Residential Post-application Non-cancer Exposure and Risk Estimates from Playing Golf on Chlorpyrifos-Treated Courses.						
Lifestage	**Post-application Exposure Scenario**		**Application Rate[1]**	**State (TTR Data)**	**Dose (mg/kg/day)[2]**	**MOEs[3]**
	Use Site	**Route of Exposure**				
Adult	Golf Course	Dermal	1.0	CA	0.010	0.13

Table 7.2.2. Residential Post-application Non-cancer Exposure and Risk Estimates from Playing Golf on Chlorpyrifos-Treated Courses.

Lifestage	Post-application Exposure Scenario		Application Rate[1]	State (TTR Data)	Dose (mg/kg/day)[2]	MOEs[3]
	Use Site	Route of Exposure				
(Females)	Turf		(Emulsifiable Concentrate)	IN	0.0069	0.19
				MS	0.012	0.11
				Mean	0.0095	0.14
Youths 11 to < 16 years old				CA	0.010	0.14
				IN	0.0070	0.20
				MS	0.012	0.12
				Mean	0.0096	0.15
Children 6 to < 11 years old				CA	0.012	0.19
				IN	0.0082	0.27
				MS	0.014	0.16
				Mean	0.011	0.20
Adult (Females)			1.0 (Granular)	CA	0.0088	0.15
Youths 11 to < 16 years old					0.0088	0.16
Children 6 to < 11 years old					0.010	0.21

1 Based on the maximum application rates registered for golf course turf use.
2 Dose (mg/kg/day) equations for golfing are provided in Appendix B of the 2014 HHRA. For dose estimation from exposures to golfing on treated turf TTR data was used. Doses have been presented for all State sites, including the mean of all State sites.
3 MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day). See Table 5.3.3.3 for PODs.

Table 7.2.3. Residential Post-application Inhalation Steady State Exposure Estimates from Chlorpyrifos ULV Aerial Mosquitocide Application - AgDISP Model.

Application Parameters	Population	Air Concentration Estimate (mg/m^3)[1]	MOE[2]
1 mph Wind Speed Dv 0.5 = 60 µm 75 Foot Release Height	Adults	0.0047	**1.1**
	Children 1 to <2 years old		**0.35**
10 mph Wind Speed Dv 0.5 = 40 µm 300 Foot Release Height	Adults	0.00070	**7.3**
	Children 1 to <2 years old		**2.4**

1 Air concentration estimate modeled using AGDISP v8.2.6 at breathing height of adults and children.
2 MOE = PoD (mg/m^3) ÷ Dose (mg/m^3). See Table 5.3.3.3 for PODs.

Table 7.2.4. Residential Post-application Inhalation Steady State Exposure Estimates from Chlorpyrifos ULV Ground Mosquitocide Application - WMB Model.

Population	Air Concentration Estimate (mg/m^3)[1]	MOE[2]
Adults	0.0013	**0.66**
Children 1 to <2 years old		**0.21**

1 Air concentration estimate modeled using the well mixed box model. The inputs and algorithms used are presented in Appendix C of the 2014 HHRA.
2 MOE = PoD (mg/m^3) ÷ Dose (mg/m^3). See Table 5.3.3.3 for PODs.

Table 7.2.5. Residential Post-application Dermal Steady State Exposure Estimates Resulting from Chlorpyrifos Aerial ULV Mosquitocide Application.						
Application Parameters	**Lifestage**	**Application Rate (lb ai/A)**	**AgDISP Deposition Fraction[1]**	**Adjusted TTR[2] ($\mu g/cm^2$)**	**Dermal Dose[3] (mg/kg/day)**	**MOE[4]**
1 mph Wind Speed Dv 0.5 = 60 µm 75 Foot Release Height	Adults	0.010	1.0	0.00038	0.0015	**2**
	Children 1 to < 2 Years Old				0.0026	**6**
10 mph Wind Speed Dv 0.5 = 40 µm 300 Foot Release Height	Adults	0.010	0.086	0.000033	0.00013	**27**
	Children 1 to < 2 Years Old				0.00022	**68**

1 Aerial fraction of mosquitocide application rate deposited on turf as determined using AgDISP model v8.2.6.
2 TTR_t ($\mu g/cm^2$) = [(Day 0 Residue from MS TTR study ($\mu g/cm^2$) x Application Rate (0.010 lb ai/A)) / Application Rate of MS TTR Study (3.83 lb ai/A))] * AgDISP Deposition Fraction
3 Dermal Dose (mg/kg/day) = [(TTR_t ($\mu g/cm^2$) * CF1 (0.001 mg/µg) * Transfer Coefficient (180,000 cm^2/hr, adults; 49,000 cm^2/hr, children) * ET (1.5 hrs))] ÷ BW (kg)
4 MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day). See Table 5.3.3.3 for PODs.

Table 7.2.6. Residential Post-application Dermal Steady State Exposure Estimates Resulting from Chlorpyrifos ULV Ground Mosquitocide Application.					
Lifestage	**Application Rate (lb ai/A)**	**Deposition Fraction[1]**	**Adjusted TTR[2] ($\mu g/cm^2$)**	**Dermal Dose[3] (mg/kg/day)**	**MOE[4]**
Adults	0.010	1.0	0.00038	0.0015	**26**
Children 1 to < 2 Years Old				0.0026	**67**

1. Ground fraction of mosquitocide application rate deposited on turf as determined using eight published studies on ground ULV application in which deposition was measured.
2. TTR_t ($\mu g/cm^2$) = [(Day 0 Residue from MS TTR study ($\mu g/cm^2$) x Application Rate (0.010 lb ai/A)) / Application Rate of MS TTR Study (3.83 lb ai/A))] * AgDISP Deposition Fraction
3. Dermal Dose (mg/kg/day) = [(TTR_t ($\mu g/cm^2$) * CF1 (0.001 mg/µg) * Transfer Coefficient (cm^2/hr - 180,000, adults; 49,000, children) * ET (1.5 hrs))] ÷ BW (kg)
4. MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day). See Table 5.3.3.3 for PODs.

Table 7.2.7. Residential Post-application Steady State Incidental Oral Exposure Estimates Resulting from Chlorpyrifos ULV Aerial Mosquitocide Application.					
Application Parameters	**Lifestage**	**Application Rate (mg ai)**	**Dermal Exposure (mg/day)[1]**	**Incidental Oral Dose (mg/kg/day)[2]**	**MOE[3]**
1 mph Wind Speed Dv 0.5 = 60 µm 75 Foot Release Height	Children 1 to < 2 Years Old	0.010	0.028	$5.2x10^{-5}$	**3**
10 mph Wind Speed			0.0022	$4.5x10^{-6}$	**38**

Dv 0.5 = 40 µm 300 Foot Release Height					

1 Dermal exposure (mg/day) as calculated for children's aerial based ULV applications using the algorithms described in Table 6.2.4 above, and as described in Appendix C of the 2014 HHRA.
2 Incidental Oral Dose estimated using the algorithms as described below in Appendix C of the 2014 HHRA.
3 MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day). See Table 5.3.3.3 for PODs.

Table 7.2.8. Residential Post-application Steady State Incidental Oral Exposure Estimates Resulting from Chlorpyrifos ULV Ground Mosquitocide Application.

Lifestage	Application Rate (mg ai)	Dermal Exposure (mg/day)[1]	Incidental Oral Dose (mg/kg/day)[2]	MOE[3]
Children 1 to < 2 Years Old	0.010	0.0024	$4.5x10^{-6}$	**37**

1 Dermal exposure (mg/day) as calculated for children's ground based ULV applications using the algorithms described in Table 6.2.5 above, and as described below in Appendix C of the 2014 HHRA.
2 Incidental Oral Dose estimated using the algorithms as described in Appendix C of the 2014 HHRA.
3 MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day). See Table 5.3.3.3 for PODs.

7.3 Residential Risk Estimates for Use in Aggregate Assessment

All residential risks assessed with the updated PBPK-derived PODs are of concern (i.e., all MOEs are < the LOC of 100). Therefore, quantitatively aggregating residential exposures with food and drinking water exposures would also result in risks of concern.

8.0 Non-Occupational Spray Drift Exposure and Risk Estimates

Spray drift is a potential source of exposure to those nearby pesticide applications. This is particularly the case with aerial application, but, to a lesser extent, spray drift can also be a potential source of exposure from the ground application methods (e.g., groundboom and airblast) employed for chlorpyrifos. Sprays that are released and do not deposit in the application area end up off-target and can lead to exposures to those it may directly contact. They can also deposit on surfaces where contact with residues can eventually lead to indirect exposures (*e.g.*, children playing on lawns where residues have deposited next to treated fields). The potential risk estimates from these residues can be calculated using drift modeling coupled with methods employed for residential risk assessments for turf products.

In the 2011 occupational and residential exposure assessment, the potential risks to bystanders from spray drift and exposure from volatilization were identified as possible concerns. Spray drift is the movement of aerosols and volatile components away from the treated area during the application process. The potential risks from spray drift and the impact of potential risk reduction measures were assessed in July 2012 (J. Dawson *et al.,* D399483, 07/13/2012). This evaluation supplemented the 2011 assessment where limited monitoring data indicate risks to bystanders. To increase protection for children and other bystanders, chlorpyrifos technical registrants voluntarily agreed to lower application rates and to other spray drift mitigation measures (R. Keigwin, 2012). As of December 2012, spray drift mitigation measures and use restrictions appear on all chlorpyrifos agricultural product labels. For the 2014 HHRA, spray drift risks were updated due to the use of the PBPK-PD model which impacted the PoDs, and

thus spray drift risk estimates. This assessment updates chlorpyrifos risks once more to incorporate the approach applied for PBPK-derivation of PoDs for infants, children, and adults based on the exposures estimated from the indoor crack and crevice uses of chlorpyrifos during the time of the CCCEH cohort.

With a dermal and incidental oral LOC of 100, all non-occupational spray drift risk estimates are of concern at the field edge with the use of certain application rates, nozzle droplet sizes, and application methods. Buffer distances > 300 feet are needed for MOEs to be not of concern. The estimated buffer distances are in excess of those agreed to by the technical registrants in July 2012. All drift risk estimates are presented in Appendix C.

9.0 Non-Occupational Bystander Post-Application Inhalation Exposure and Risk Estimates

In January 2013, a preliminary assessment of the potential risks from volatilization was conducted (R. Bohaty *et al.,* D399484 and D400781, 01/31/2013). The assessment evaluated the potential risks to bystanders, or those who live and/or work in proximity to treated fields, from inhalation exposure to vapor phase chlorpyrifos and chlorpyrifos-oxon emitted from fields following application of chlorpyrifos. The results of the January 2013 assessment indicated that offsite concentrations of chlorpyrifos and chlorpyrifos-oxon may exceed the target concentration based on the toxicological endpoints used at that time (J. Hotchkiss *et al.,* EPA MRID 48139303).

In June 2014, a re-evaluation of the 2013 preliminary volatilization assessment was conducted since the Registrant had conducted and submitted two, high quality nose-only vapor phase AChE inhibition inhalation studies for both chlorpyrifos and chlorpyrifos-oxon (W. Irwin, D411959, 06/25/2014) to address the uncertainty surrounding exposure to aerosol versus vapor phase chlorpyrifos. In the vapor studies, female rats were administered a saturated vapor, meaning that the test subjects received the highest possible concentration of chlorpyrifos or chlorpyrifos-oxon which can saturate the air in a closed system. At these saturated concentrations, no statistically significant inhibition of AChE activity was measured in RBC, plasma, lung, or brain at any time after the six-hour exposure period in either study. Under actual field conditions, indications are that exposures to vapor phase chlorpyrifos and its oxon would be much lower as discussed in the January 2013 preliminary volatilization assessment. Since the studies demonstrated that no toxicity occurred even at the saturation concentration, the agency concluded that there was no risk potential, as risk is a function of both exposure and hazard.

However, in the current risk assessment for chlorpyrifos, the PoDs for risk assessment have been chosen to be protective of potential neurological effects below levels where AChE inhibition could occur. For that reason, a quantitative bystander/volatilization assessment has been included in this update. This assessment is an update to the 2013 assessment and has been updated to reflect air monitoring data collected since 2006, and the updated PoDs for chlorpyrifos.

There are six available chlorpyrifos air monitoring studies that were conducted since 2006 (brief study summaries available in W. Britton, D388165, 06/27/2011). These include:

- One application site study conducted in North Central and Yakima Valley, OR by the University of Washington Department of Environmental and Occupational Health Sciences, and
- Five ambient air studies
    - one conducted in North Central and Yakima Valley, by the University of Washington Department of Environmental and Occupational Health Sciences;
    - two conducted by Pesticide Action Network North America (PANNA) in Washington and Minnesota; and
    - two conducted by CalDPR.

Application site air monitoring refers to the collection of air samples around the edges of a treated field during and after a pesticide application. Samples are generally collected for short intervals (e.g., < 8 hours), for at least the first day or two after application with subsequent samples increasing in duration. In this type of study, it is typically known when an application occurred, the equipment used for the application, and the application rate. Application site monitoring data represents an exposure to vapors at or near the field edge resulting from an application.

Ambient air monitoring typically is focused on characterizing the airborne pesticide levels within a localized airshed or community structure of some definition (e.g., city, township, or municipality). This type of monitoring effort also can be focused on capturing chronic background levels or other temporal characteristics of interest such as focusing on seasonal pesticide use patterns. Typically, samples are taken for 24 consecutive hours and collected at the same site over an extended period of time (e.g., several weeks or months). In contrast to application site air monitoring, information on the precise timing and location of pesticide applications are rarely collected in ambient air monitoring studies. However, this does not mean that an application did not occur near an ambient sampler during the monitoring period

The EPA has assessed residential bystander exposure to chlorpyrifos based on the available ambient and application site air monitoring data (Tables 9.1 and 9.2). The chlorpyrifos bystander volatilization inhalation exposure assessment includes acute and steady state exposure scenarios. The acute scenario compares the maximum air concentration detected in the monitoring studies to the acute PoD. The steady state scenario compares the arithmetic mean chlorpyrifos air concentration from several monitoring studies to the steady state PoD.

The EPA has assessed residential bystander exposure from field volatilization of applied chlorpyrifos based on available *ambient* (five studies/11 locations) and *application site* (one study/2 locations) air monitoring data. For adults, of the 11 acute *ambient* air concentrations assessed, six resulted in risk estimates that are of concern (i.e., MOEs < 100). Only one steady state *ambient* air concentration resulted in a risk estimate not of concern (i.e., MOEs > 100). For the *application site* air concentrations assessed, all resulted in risk estimates of concern (i.e., MOEs < 100). For children 1 to <2 years old, of the 11 acute *ambient* air concentrations assessed, all resulted in risk estimates that are of concern (i.e., MOEs < 100). Only four steady state *ambient* air concentration resulted in a risk estimate not of concern (i.e., MOEs > 100). For the *application site* air concentrations assessed, all resulted in risk estimates of concern (i.e.,

MOEs < 100). All bystander risk estimates are presented in Appendix D.

Table 9.1. Chlorpyrifos Preliminary Volatilization Risk Analysis for Residential Adult Bystanders.					
Study, Year	**Sampler/ Site Location**	**Maximum Air Concentration (ng/m³)**	**Arithmetic Mean Air Concentration (ng/m³)**	**Acute MOEs[1] (LOC = 100)**	**Steady State MOEs[2] (LOC = 100)**
Application Site Data					
WA DOH, 2008	North Central District Perimeter Site	1145	153	**3.5**	**1.4**
	Yakima Valley Perimeter Site	1002	294	**4**	**0.71**
Ambient Air Data					
WA DOH, 2008	North Central District Ambient	21	7	190	**31**
	North Central District Receptor	606.8	33	**6.6**	**6.4**
	Yakima Valley Ambient	30	9	130	**23**
	Yakima Valley Receptor	243	30	**16**	**6.9**
Parlier, CA (CalDPR) 2009		150	96	**27**	**2.2**
Cowiche PANNA 2006		462	155	**8.7**	**1.4**
PANNA MN Drift Study (2006-2009)	Browerville Site B	15	2.7	270	**79**
	Perham Site C	47	1.9	**85**	110
CDPR 2014 Air Monitoring Network	Salinas, CA	14.1	5.4	280	**39**
	Shafter, CA	337.9	92.1	**12**	**2.3**
	Ripon, CA	14.1	14.1	280	**15**

1 Acute MOE = Acute PoD (4,000 ng/m³) / Study maximum air concentration (ng/m³).
2 Steady State MOE = Steady State PoD (210 ng/m³) / Study arithmetic mean air concentration (ng/m³).

Table 9.2. Chlorpyrifos Preliminary Volatilization Risk Analysis for Residential Children (1 to <2 Years Old) Bystanders.					
Study, Year	**Sampler/ Site Location**	**Maximum Air Concentration (ng/m³)**	**Arithmetic Mean Air Concentration (ng/m³)**	**Acute MOEs[1] (LOC = 100)**	**Steady State MOEs[2] (LOC = 100)**
Application Site Data					
WA DOH, 2008	North Central District Perimeter Site	1145	153	**1.1**	**4.4**
	Yakima Valley Perimeter Site	1002	294	**1.3**	**2.3**
Ambient Air Data					
WA DOH, 2008	North Central District Ambient	21	7	**62**	100
	North Central District Receptor	606.8	33	**2.1**	**21**
	Yakima Valley Ambient	30	9	**43**	**73**
	Yakima Valley Receptor	243	30	**5.3**	**22**

Table 9.2. Chlorpyrifos Preliminary Volatilization Risk Analysis for Residential Children (1 to <2 Years Old) Bystanders.					
Study, Year	**Sampler/ Site Location**	**Maximum Air Concentration (ng/m^3)**	**Arithmetic Mean Air Concentration (ng/m^3)**	**Acute MOEs[1] (LOC = 100)**	**Steady State MOEs[2] (LOC = 100)**
Parlier, CA (CalDPR) 2009		150	96	**8.7**	**7.1**
Cowiche PANNA 2006		462	155	**2.8**	**4.4**
PANNA MN Drift Study (2006-2009)	Browerville Site B	15	2.7	**87**	260
	Perham Site C	47	1.9	**28**	350
CDPR 2014 Air Monitoring Network	Salinas, CA	14.1	5.4	**92**	130
	Shafter, CA	337.9	92.1	**3.8**	**7.4**
	Ripon, CA	14.1	14.1	**92**	**48**

1 Acute MOE = Acute PoD (1,300 ng/m^3) / Study maximum air concentration (ng/m^3).
2 Steady State MOE = Steady State PoD (680 ng/m^3) / Study arithmetic mean air concentration (ng/m^3).

Characterization of Bystander Risk Assessment/Uncertainties

Some of the limitations and considerations that have been identified that should be considered in the interpretation of these results include:

- Most of the data utilized in this preliminary assessment are 24-hour air samples. When these data are used, an assumption is made that an individual is exposed to the same air concentration for 24-hours every day. However, this is not always the case as real world time-activity data indicate that many parts of the population move from site to site on a daily basis (e.g., go to work and back).

- This assessment is only representative of outdoor concentrations (i.e., the exposure and risk estimates assume an individual is outdoors all the time). It does not take into account potential effects of air conditioning systems and similar air filtration systems which could potentially reduce air concentrations indoors. The agency believes that indoor concentrations will be at worst equivalent to outdoor concentrations and may potentially be lower.

- All of the data used for this analysis have been generated in California and Washington; however, chlorpyrifos is used in many regions throughout the country. Therefore, the results based on the limited available air monitoring data were used to represent the rest of the country due to a lack of adequate information for any other region. It is unclear what potential impacts this extrapolation might have on the risk assessment. Factors such as meteorology and cultural practices may impact the overall amounts of chlorpyrifos that volatilize from a treated field as well as the rate at which it volatilizes.

- As part of the December 2009 SAP, the agency presented their analysis of several models that could be used as screening tools to predict the air concentration and volatilization flux based on intrinsic properties and transport behaviors of pesticides. These models would allow the agency to better represent the potential volatilization of semi-volatile

pesticides across various regions of the country and thus would provide refinement to this assessment over using straight air monitoring data. The SAP provided a number of comments regarding the agency's model analysis, including the recommendation to evaluate some additional models. The agency is currently in the process of evaluating the SAP's comments. As appropriate, the agency will revise the modeling approach presented to the SAP for determining the rate of volatilization (flux) for semi-volatile pesticides and for estimating air concentrations of applied pesticides in the atmosphere under varying environmental conditions. After any policies or procedures are put into place, the agency may revisit the residential bystander exposure and risk assessment.

10.0 Aggregate Exposure/Risk Characterization

In accordance with the FQPA, HED must consider and aggregate (add) pesticide exposures and risks from three major sources: food, drinking water, and residential exposures. In an aggregate assessment, exposures from relevant sources are added together and compared to quantitative estimates of hazard, or the risks themselves can be aggregated. The steady state aggregate assessment includes food, drinking water, and residential exposures.

For chlorpyrifos aggregate assessment, a DWLOC approach is used to calculate the amount of exposure available in the total 'risk cup' for chlorpyrifos in drinking water after accounting for any chloropyrifos exposures from food and residential uses. This DWLOC is then compared to the EDWC to determine if there is an aggregate risk of concern. However, because the dietary risks from food exposure alone and from residential exposure alone are of concern, it is not possible to calculate a DWLOC; essentially, the steady state aggregate DWLOC is '0' after accounting for food and residential exposures.

[See the December 2014 chlorpyrifos HHRA for details of the DWLOC approach and calculations. See the April 2016 DWA for the EDWCs.]

11.0 Occupational Exposure and Risk Estimates

HED had previously conducted both steady state occupational handler and post-application exposure analyses for chlorpyrifos (W. Britton, D424484, 12/29/2014). However, occupational exposures and risks have been updated to incorporate the approach applied for PBPK-derivation of PoDs for infants, children, and adults based on the exposures estimated from the indoor crack and crevice uses of chlorpyrifos during the time of the CCCEH cohort. The scenarios, assumptions, and exposure inputs have not changed since the previous assessment; the assessment below estimates occupational handler exposures using the updated PBPK-derived steady state PoDs. Details on the exposure inputs, scenarios, and assumptions can be found in the 2014 ORE assessment (W. Britton, D424484, 12/29/2014).

It is agency policy to use the best available data to assess exposure. The same chemical-specific dislodgeable foliar residue (DFR) studies were used for the 2014 assessment of occupational post-application exposure to chlorpyrifos have been used for this update, including: emulsifiable concentrate formulations on sugarbeets, pecans, citrus, sweet corn, cotton, and turf; wettable powder formulations on almonds, apples, pecans, cauliflower, tomato and turf; granular

formulations on sweet corn and turf; a total release aerosol formulation on ornamentals; and a microencapsulated liquid formulation on ornamentals.

Several sources of generic data were used in this assessment as surrogate data including: Pesticide Handlers Exposure Database Version 1.1 (PHED 1.1); the Agricultural Handler Exposure Task Force (AHETF) database; the Outdoor Residential Exposure Task Force (ORETF) database; the Agricultural Reentry Task Force (ARTF) database; ExpoSAC Policy 14 [Standard Operating Procedures (SOPs) for Seed Treatment]; HED's 2012 Residential SOPs for Residential Pesticide Exposure Assessment: Lawns/Turf, Outdoor Fogging/Misting Systems, registrant-submitted exposure monitoring studies MRIDs 44180401, 44301301, 44793301, 44829601, 42974501, 43062701, 44748101, 44748102, 46722701, and 46722702, and published literature studies. Some of these data are proprietary, and subject to the data protection provisions of the *Federal Insecticide, Fungicide, and Rodenticide Act (*FIFRA).

In the 2011 HHRA (D. Drew *et al.,* D388070, 06/30/2011), additional studies were recommended to address uncertainties regarding the formation of chlorpyrifos oxon and its decay following applications in greenhouses. To date, no additional data have been submitted.

11.1 Steady State Occupational Handler Risk

The term handlers is used to describe those individuals who are involved in the pesticide application process. There are distinct job functions or tasks related to applications and exposures can vary depending on the specifics of each task. Job requirements (amount of a chemical used in each application), the kinds of equipment used, the target being treated, and the level of protection used by a handler can cause exposure levels to differ in a manner specific to each application event. Based on the anticipated use patterns and current labeling, types of equipment and techniques that can potentially be used, occupational handler exposure is expected from chlorpyrifos use. For purpose of occupational handler assessment, the parent chlorpyrifos is the relevant compound.

Current labels generally require that handlers use normal work clothing (i.e., long sleeved shirt and pants, shoes and socks) and coveralls, chemical resistant gloves, and dust/mist respirators. Also, some products are marketed in engineering controls such as water soluble packets. In order to determine what level of personal protection is required to alleviate risk concerns and to ascertain if label modifications are needed, steady state exposure and risk estimates were updated for occupational handlers of chlorpyrifos for a variety of scenarios at differing levels of personal protection including engineering controls.

The occupational handler scenarios, assumptions, and exposure inputs have not changed since the previous assessment.

Summary of Occupational Handler Non-Cancer Exposures and Risk Estimates

Using the updated PBPK-derived steady state PODs and uncertainty factors (dermal and inhalation LOC = 100), all agricultural occupational handler scenarios, all primary seed treatment handler scenarios, and all secondary seed treatment (planter) scenarios are of concern with label-specified and maximum levels of personal protective equipment (PPE) or engineering

controls (MOEs < 100). Detailed result tables are provided in Appendix E.

11.2 Steady State Occupational Post-Application Risk Estimates

HED uses the term, post-application, to describe exposures that occur when individuals are present in an environment that has been previously treated with a pesticide (also referred to as reentry exposure). Such exposures may occur when workers enter previously treated areas to perform job functions, including activities related to crop production, such as scouting for pests or harvesting. Post-application exposure levels vary over time and depend on such things as the type of activity, the nature of the crop or target that was treated, the type of pesticide application, and the chemical's degradation properties. In addition, the timing of pesticide applications, relative to harvest activities, can greatly reduce the potential for post-application exposure. Chlorpyrifos parent compound is the residue of concern for occupational post-application dermal exposures; however, it may be possible that the formation of the oxon is greater and its deactivation slower in greenhouses when compared to the outdoor environment and that an assessment may be needed for exposure to the oxon in greenhouse settings.

11.2.1 Occupational Post-application Inhalation Exposure/Risk Estimates

There are multiple potential sources of post-application inhalation exposure to individuals performing post-application activities in previously treated fields. These potential sources include volatilization of pesticides and resuspension of dusts and/or particulates that contain pesticides. Previously, a quantitative post-application inhalation risk assessment was not conducted for chlorpyrifos or chlorpyrifos oxon due to the lack of toxicity seen in the available nose-only vapor phase AChE inhibition inhalation studies (W. Britton, D424484, 12/29/2014). The studies did not demonstrate inhalation toxicity, or inhibition of AChE activity measured in RBC, plasma, the lungs, and the brain following exposure to chlorpyrifos or chlorpyrifos oxon vapor, even at the saturation concentration. However, since the previous assessment, the PODs have been updated to reflect the PBPK-derived steady state PoD based on a TWA of blood concentrations corresponding to levels likely to have occurred in the CCCEH cohort, as discussed in Section 5.3.3. Therefore, the agency will be assessing occupational post-application inhalation from the registered uses of chlorpyrifos.

The agency has sought expert advice and input on issues related to volatilization of pesticides from its Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) in December 2009, and received the SAP's final report on March 2, 2010 (https://www.regulations.gov/document?D=EPA-HQ-OPP-2009-0687-0037). The agency has evaluated the SAP report and has developed a Volatilization Screening Tool and a subsequent Volatilization Screening Analysis (https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0219-0001). During Registration Review, the agency will utilize this analysis, and take into consideration the risks identified from the residential bystander assessment, to determine if data (i.e., flux studies) or further analysis is required for chlorpyrifos.

In addition, the agency is continuing to evaluate the available post-application inhalation exposure data generated by the Agricultural Reentry Task Force. Given these two efforts, the agency will continue to identify the need for and, subsequently, the way to incorporate

occupational post-application inhalation exposure into the agency's risk assessments.

The Worker Protection Standard for Agricultural Pesticides contains requirements for protecting workers from inhalation exposures during and after greenhouse applications through the use of ventilation requirements.[40 CFR 170.110, (3) (Restrictions associated with pesticide applications)].

11.2.2 Occupational Post-application Dermal Exposure/Risk Estimates

Occupational post-application assessments were previously performed for: 1) exposures to the parent compound chlorpyrifos in outdoor environments (uses other than greenhouse), 2) exposures to the parent chlorpyrifos (only) in greenhouses and 3) exposures to both the parent and the oxon metabolite in greenhouses; and incorporated: 1) a PBPK modeled dermal PoD specific for occupational assessment 2) the updated master use summary document, 3) the updated adult (female) default body weight, and 4) the changes relating to agricultural transfer coefficients (TC) as described in the *Science Advisory Council for Exposure (ExpoSAC) Policy 3 – Revised March 2013*[17] (W. Britton, D424484, 12/29/2014).

However, the steady state PODs and uncertainty factors have changed since the previous assessment. Therefore, the occupational post-application exposure assessment has been revised. The scenarios, assumptions, and exposure inputs have not changed since the previous assessment; the assessment below estimates occupational post-application dermal exposures using the updated PBPK-derived steady state PODs. Details on the exposure inputs, scenarios, and assumptions can be found in W. Britton, D424484, 12/29/2014. Detailed result tables are provided in Appendix F.

Summary of Occupational Post-application Non-Cancer Exposures and Risk Estimates

263 total occupational post-application scenarios were evaluated. The restricted entry intervals (REIs) on the registered chlorpyrifos labels range from 24 hours to 5 days. All scenarios were of concern on Day 0 with a dermal LOC of 100. On average, scenarios were not of concern $\geq$ 18 days after treatment.

[17] http://www.epa.gov/opp00001/science/exposac-policy-3-march2013.pdf

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13.0 List of Appendices

Appendix A: Non-occupational exposure estimates following mosquitocide applications
Appendix B: Residential (golfing) post-application exposure estimates
Appendix C: Non-occupational spray drift exposure and risk estimates
Appendix D: Non-occupational bystander post-application inhalation exposure and risk estimates
Appendix E: Occupational handler exposure and risk estimates
Appendix F: Occupational post-application dermal exposure and risk estimates

LC 265
2020 Regular Session
12/20/19 (CDT/am/ps)

DRAFT

SUMMARY

Prohibits aerial pesticide applicator from spraying or otherwise applying by aircraft pesticide product containing chlorpyrifos.

Prohibits person from applying pesticide product containing chlorpyrifos within 300 feet of campus of school.

Requires person that employs worker to take steps to ensure that worker does not enter into area in which pesticide product containing chlorpyrifos was applied within eight preceding calendar days.

Effective January 1, 2022, prohibits sale, purchase or use of pesticide product containing chlorpyrifos.

Requires State Department of Agriculture to revoke, on January 1, 2022, pesticide registrations for pesticide products containing chlorpyrifos.

Declares emergency, effective on passage.

A BILL FOR AN ACT

Relating to prevention of health impacts from exposure to chlorpyrifos; and declaring an emergency.

Be It Enacted by the People of the State of Oregon:

SECTION 1. Sections 2 to 11 of this 2020 Act are added to and made a part of ORS chapter 634.

SECTION 2. The purpose of this 2020 Act is to prevent health impacts from chlorpyrifos, which is known to be harmful to human health, by reducing exposure to this pesticide.

SECTION 3. (1) As used in this section, "aerial pesticide applicator" means an individual engaging in activities that require a certificate under ORS 634.128.

(2) An aerial pesticide applicator may not spray or otherwise apply by aircraft a pesticide product containing chlorpyrifos.

NOTE: Matter in **boldfaced** type in an amended section is new; matter [*italic and bracketed*] is existing law to be omitted. New sections are in **boldfaced** type.

SECTION 4. (1) As used in this section, "campus" and "school" have the meanings given those terms in ORS 634.700.

(2) A person may not apply a pesticide product containing chlorpyrifos within 300 feet of the campus of a school.

SECTION 5. A person that employs a worker shall take steps to ensure that the worker does not enter into an area in which a pesticide product containing chlorpyrifos was applied within eight preceding calendar days.

SECTION 6. Notwithstanding ORS 634.900 (1), a violation of sections 3 to 5 of this 2020 Act constitutes a violation subject to imposition of civil penalties under ORS 634.900 to 634.915.

SECTION 7. Sections 3 to 6 of this 2020 Act are repealed on January 2, 2023.

SECTION 8. (1) A person may not sell, purchase or use a pesticide product containing chlorpyrifos.

(2) Notwithstanding ORS 634.900 (1), a violation of this section constitutes a violation subject to imposition of civil penalties under ORS 634.900 to 634.915.

SECTION 9. Section 8 of this 2020 Act becomes operative on January 1, 2022.

SECTION 10. On January 1, 2022, the State Department of Agriculture shall immediately revoke any registration issued under ORS 634.016 prior to January 1, 2022, for a pesticide product containing chlorpyrifos.

SECTION 11. Section 10 of this 2020 Act is repealed on January 2, 2023.

SECTION 12. This 2020 Act being necessary for the immediate preservation of the public peace, health and safety, an emergency is declared to exist, and this 2020 Act takes effect on its passage.

Proc Natl Acad Sci U S A. 2012 May 15;109(20):7871-6. doi: 10.1073/pnas.1203396109. Epub 2012 Apr 30.

Brain anomalies in children exposed prenatally to a common organophosphate pesticide.

Rauh VA[1], Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS.

Author information

Abstract

Prenatal exposure to chlorpyrifos (CPF), an organophosphate insecticide, is associated with neurobehavioral deficits in humans and animal models. We investigated associations between CPF exposure and brain morphology using magnetic resonance imaging in 40 children, 5.9-11.2 y, selected from a nonclinical, representative community-based cohort. Twenty high-exposure children (upper tertile of CPF concentrations in umbilical cord blood) were compared with 20 low-exposure children on cortical surface features; all participants had minimal prenatal exposure to environmental tobacco smoke and polycyclic aromatic hydrocarbons. High CPF exposure was associated with enlargement of superior temporal, posterior middle temporal, and inferior postcentral gyri bilaterally, and enlarged superior frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere. Group differences were derived from exposure effects on underlying white matter. A significant exposure × IQ interaction was derived from CPF disruption of normal IQ associations with surface measures in low-exposure children. In preliminary analyses, high-exposure children did not show expected sex differences in the right inferior parietal lobule and superior marginal gyrus, and displayed reversal of sex differences in the right mesial superior frontal gyrus, consistent with disruption by CPF of normal behavioral sexual dimorphisms reported in animal models. High-exposure children also showed frontal and parietal cortical thinning, and an inverse dose-response relationship between CPF and cortical thickness. This study reports significant associations of prenatal exposure to a widely used environmental neurotoxicant, at standard use levels, with structural changes in the developing human brain.

Comment in

Differentiating experimental animal doses from human exposures to chlorpyrifos. [Proc Natl Acad Sci U S A. 2012]

US environment agency cuts funding for kids' health studies. [Nature. 2019]

PMID: 22547821 PMCID: PMC3356641 DOI: 10.1073/pnas.1203396109

[Indexed for MEDLINE] **Free PMC Article**

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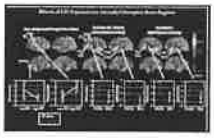

Publication types, MeSH terms, Substances, Grant support

LinkOut - more resources

Format: Abstract

Pediatrics. 2015 Oct;136(4):719-29. doi: 10.1542/peds.2015-0006. Epub 2015 Sep 14.

Residential Exposure to Pesticide During Childhood and Childhood Cancers: A Meta-Analysis.

Chen M[1], Chang CH[1], Tao L[1], Lu C[2].

Author information

Abstract

CONTEXT: There is an increasing concern about chronic low-level pesticide exposure during childhood and its influence on childhood cancers.

OBJECTIVE: In this meta-analysis, we aimed to examine associations between residential childhood pesticide exposures and childhood cancers.

DATA SOURCES: We searched all observational studies published in PubMed before February 2014 and reviewed reference sections of articles derived from searches.

STUDY SELECTION: The literature search yielded 277 studies that met inclusion criteria.

DATA EXTRACTION: Sixteen studies were included in the meta-analysis. We calculated effect sizes and 95% confidence intervals (CIs) by using a random effect model with inverse variance weights.

RESULTS: We found that childhood exposure to indoor but not outdoor residential insecticides was associated with a significant increase in risk of childhood leukemia (odds ratio [OR] = 1.47; 95% CI, 1.26-1.72; I(2) = 30%) and childhood lymphomas (OR = 1.43; 95% CI, 1.15-1.78; I(2) = 0%). A significant increase in risk of leukemia was also associated with herbicide exposure (OR = 1.26; 95% CI, 1.10-1.44; I(2) = 0%). Also observed was a positive but not statistically significant association between childhood home pesticide or herbicide exposure and childhood brain tumors.

LIMITATIONS: The small number of studies included in the analysis represents a major limitation of the current analysis.

CONCLUSIONS: Results from this meta-analysis indicated that children exposed to indoor insecticides would have a higher risk of childhood hematopoietic cancers. Additional research is needed to confirm the association between residential indoor pesticide exposures and childhood cancers. Meanwhile, preventive measures should be considered to reduce children's exposure to pesticides at home.

PMID: 26371195 DOI: 10.1542/peds.2015-0006

[Indexed for MEDLINE] **Free full text**

Publication types, MeSH terms, Substance

LinkOut - more resources

very detailed, but difficult to understand

Format: Abstract

Toxicol Sci. 2015 Oct;147(2):588-606. doi: 10.1093/toxsci/kfv153. Epub 2015 Jul 20.

Chlorpyrifos Induces MLL Translocations Through Caspase 3-Dependent Genomic Instability and Topoisomerase II Inhibition in Human Fetal Liver Hematopoietic Stem Cells.

Lu C[1], Liu X[1], Liu C[1], Wang J[1], Li C[1], Liu Q[1], Li Y[1], Li S[1], Sun S[2], Yan J[3], Shao J[4].

Author information

Abstract

Household pesticide exposure during pregnancy has been associated with a more than 2-fold increased risk in infant leukemia, and chlorpyrifos (CPF) is among the most frequently applied insecticides. During early fetal development, liver is a hematopoietic organ with majority of cells being CD34(+) hematopoietic stem cells (CD34(+)HSC). The in utero injury to CD34(+)HSC has been known to underlie the pathogenesis of several blood disorders, often involving rearrangements of the mixed-lineage leukemia (MLL) gene on 11q23. In this study, we evaluated the leukemogenic potential of CPF in human fetal liver-derived CD34(+)HSC. Specifically, exposure to 10 μM CPF led to decrease in viability, inhibition in proliferation and induction of DNA double-strand breaks (DSBs) and occurrence of MLL(+) rearrangements. In particular, we observed CPF-mediated cell cycle disturbance as shown by G0/G1 arrest, in contrast to etoposide (VP-16), an anticancer drug used as a positive control and known to induce G2/M arrest. Further study on mechanisms underlying DNA DSBs and MLL(+) rearrangements revealed that CPF might act as topoisomerase II poison, a mechanism of action similar to VP-16. On the other hand, CPF was also shown to induce early apoptosis through active caspase-3 activation, a pathway known to underlie DNA DSBs and MLL(+) translocations. Our data indicate that in utero injury of CD34(+)HSC by CPF may contribute to the increased risk of infant leukemia. Future work will elucidate the mechanism and the type of CPF-induced MLL(+) translocations in HSC.

KEYWORDS: DNA double strand break; MLL rearrangement; apoptosis; chlorpyrifos; hematopoietic stem cells; topoisomerase II

PMID: 26198043 DOI: 10.1093/toxsci/kfv153

[Indexed for MEDLINE]

Publication type, MeSH terms, Substances

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