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Sharlene R. Matten
Designated Federal Official
Office of Science Coordination and Policy (7201M)
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460-0001

Re: FIFRA Scientific Advisory Panel; Review of the U.S. EPA's Evaluation of the Toxicity Profile of Chlorpyrifos, Docket Identification Number EPA–HQ–OPP–2008– 0274

Dear Ms. Matten:

Thank you for the opportunity to respond to the Agency's request for comments on the hazard identification and hazard characterization for chlorpyrifos.

Chlorpyrifos remains one of the most widely used organophosphate pesticides, despite a long history of documented human and ecological risks from exposure through food, drinking water, and residential and occupational applications. In 2001, residential uses of chlorpyrifos were restricted, although numerous studies illustrate that indoor exposure to this persistent organophosphate continue. In 2004, Dow Chemical (the manufacturer of chlorpyrifos) paid a $2 million fine to settle a complaint by the State of New York that alleged Dow had violated a 1994 agreement to stop advertising claims of chlorpyrifos' safety. In 2006, the EPA's Office of Inspector General highlighted numerous deficiencies in the Agency's testing protocol and data requirements for risk assessments for food-use pesticides, including a particular focus on chlorpyrifos (U.S. EPA, 2006). Millions of pounds of chlorpyrifos are still applied annually in agricultural and non-agricultural settings, despite continued and growing concerns among scientists about substantial data gaps regarding the exposure risk of chlorpyrifos, particularly for infants and children.

I welcome the Agency's efforts to review recent data pertaining to this developmental toxicant. Briefly, my comments that follow illustrate that 1) non-symptomatic exposures of humans to chlorpyrifos lead to adverse neurological and development endpoints in humans that are predicted by animal studies; 2) non-cholinergic mechanisms are important for developmental neurotoxicity outcomes and are critical to evaluating hazard and risk to infants and children; and 3) despite industry claims to the contrary, animal studies using different routes of administration (e.g., oral gavage and subcutaneous injection) have resulted in virtually identical outcomes.

I would be happy to provide the FIFRA Scientific Advisory Panel any additional data or information regarding my comments. Please feel free to contact me.

Sincerely,

Theodore Slotkin, Ph.D.
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Professor in Psychiatry & Behavioral Sciences
Professor in Neurobiology
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Comments of
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Re: FIFRA Scientific Advisory Panel; Review of the U.S. EPA's Evaluation of the Toxicity Profile of Chlorpyrifos, Docket Identification Number EPA–HQ–OPP–2008– 0274

The Agency requested specific comment on the following areas:

1. Interpretation of recent epidemiological studies associating in utero and/or post-natal chlorpyrifos exposure with health outcomes;

2. Aspects of chlorpyrifos metabolism, such as differences in paraoxonase 1 (PON 1) expression and activity, which affects population variability with respect to the effects of chlorpyrifos and its oxon metabolite; and

3. Cholinergic and non-cholinergic modes/mechanisms of toxicity which are relevant to evaluating hazard and risk to infants and children.

4. The relevance of animal studies conducted by different routes of administration (e.g., gavage or subcutaneous injection), to different age groups and by different exposure pathways, to human health risk assessment.

My comments specifically address recent epidemiological studies (point #1), mechanisms of toxicity (point #3), and the relevance of different routes of exposure in animal studies (point #4).

Briefly, my comments that follow illustrate that 1) non-symptomatic exposures of humans to chlorpyrifos lead to adverse neurological and development endpoints in humans that are predicted by animal studies; 2) non-cholinergic mechanisms are important for developmental neurotoxicity outcomes and are critical to evaluating hazard and risk to infants and children; and 3) despite industry claims to the contrary, animal studies using different routes of administration (e.g., oral gavage and subcutaneous injection) have resulted in virtually identical outcomes.

1. Interpretation of recent epidemiological studies associating in utero and/or postnatal chlorpyrifos exposure with health outcomes.

Numerous recent studies confirm that non-symptomatic exposures of humans to chlorpyrifos lead to adverse health endpoints that are predicted from animal studies. Table 1 summarizes four relevant studies that I commend to the Panel for review. For convenience, these studies are appended as Appendix A.

Table 1. Summary of Recent Epidemiological Studies

Citation	Findings
Farahat, TM, GM Abdelrasoul, MM Amr, MM Shebl, FM Farahat, WK Anger, 2003. Neurobehavorial effects among workers occupationally exposed to organophosphorous pesticides. *Occup. Environ. Med.* **60**:279-286.	Occupational exposure to OP pesticides was associated with deficits in a wider array of neurobehavioral functions than previously reported, perhaps because of higher exposure in this population. Moderate chronic OP exposure may not only affect visuomotor speed as reported previously, but also verbal abstraction, attention, and memory.
Perera FP, Rauh V, Whyatt RM, Tang D, Tsai WY, Bernert JT, Tu YH, Andrews H, Barr DB, Camann DE, Diaz D, Dietrich J, Reyes A and Kinney PL (2005). A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures. *Neurotoxicology* **26**:573-587.	Cord chlorpyrifos, and a combined measure of cord chlorpyrifos, diazinon, and propoxur-metabolite, were inversely associated with birth weight and/or length ($P < 0.05$).
Rauh VA, Garfinkel R, Perera R, Andrews H, Hoepner L, Barr D, Whitehead D, Tang D and Whyatt RM (2006) Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics* **118**:1845-1859.	The adjusted mean 36-month Psychomotor Development Index and Mental Development Index scores of the highly and lower exposed groups differed by only 7.1 and 3.0 points, respectively, but the proportion of delayed children in the high-exposure group, compared with the low-exposure group, was 5 times greater for the Psychomotor Development Index and 2.4 times greater for the Mental Development Index, increasing the number of children possibly needing early intervention services.
Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J and McCauley L (2006) Organophosphate pesticide exposure and neurobehavioral performance in agricultural and nonagricultural Hispanic workers. *Environ. Health Perspect.* **114**:691-696.	The neurobehavioral performance of Hispanic immigrant farm workers was lower than that observed in a nonagricultural Hispanic immigrant population, and within the sample of agricultural workers there was a positive correlation between urinary organophosphate metabolite levels and poorer performance on some neurobehavioral tests. These findings add to an increasing body of evidence of the association between low levels of pesticide exposure and deficits in neurobehavioral performance.

2. Cholinergic and non-cholinergic modes/mechanisms of toxicity which are relevant to evaluating hazard and risk to infants and children

There is widespread acceptance among the scientific community that non-cholinergic mechanisms are important for developmental neurotoxicity outcomes following exposure to chlorpyrifos. The Israel Ministry of Health has issued a succinct review of the scientific studies that considered whether acetylcholinesterase (AChE) inhibition is the only valid point of departure for the assessment of risk from exposure to OP insecticides (Winston, 2006).

The Israel review concludes that:

> "When all of the facts are reviewed open-mindedly, with an eye for scientific objectivity and truth, it becomes apparent that AChE as a point of departure for risk assessment is one of historical convenience and fails to account for a myriad of recent scientific findings."

The findings of numerous animal studies consistently report neurotoxic behavior of chlorpyrifos at concentrations and doses that are below those that inhibit AChE activity. The following review articles summarize the much larger primary research literature on non-cholinergic mechanisms, and are recommended to the Panel for review at their convenience, appended as Appendix B.

Casida JE and Quistad GB (2004) Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. *Chem. Res. Toxicol.* **17**:983-998.

Colborn T (2006) A case for revisiting the safety of pesticides: a closer look at neurodevelopment. *Environ. Health Perspect.* **114**:10-17.

Costa LG (2006) Current issues in organophosphate toxicology. *Clin. Chim. Acta* **366**:1-13.

Gupta RC (2004) Brain regional heterogeneity and toxicological mechanisms of organophosphates and carbamates. *Toxicol. Mech. Meth.* **14**:103-143.

Jamal GA, Hansen S and Julu PO (2002) Low level exposures to organophosphorus esters may cause neurotoxicity. *Toxicology* **181-182**:23-33.

Mauro RE and Zhang L (2007) Unique insights into the actions of CNS agents: lessons from studies of chlorpyrifos and other common pesticides. *CNS Agents Med. Chem.* **7**:183-199.

Ray DE and Richards PG (2001) The potential for toxic effects of chronic, low-dose exposure to organophosphates. *Toxicol. Lett.* **120**:343-351.

Slotkin TA (2005) Developmental neurotoxicity of organophosphates: a case study of chlorpyrifos, in *Toxicity of Organophosphate and Carbamate Pesticides* (Gupta RC ed) pp 293-314, Elsevier Academic Press, San Diego.

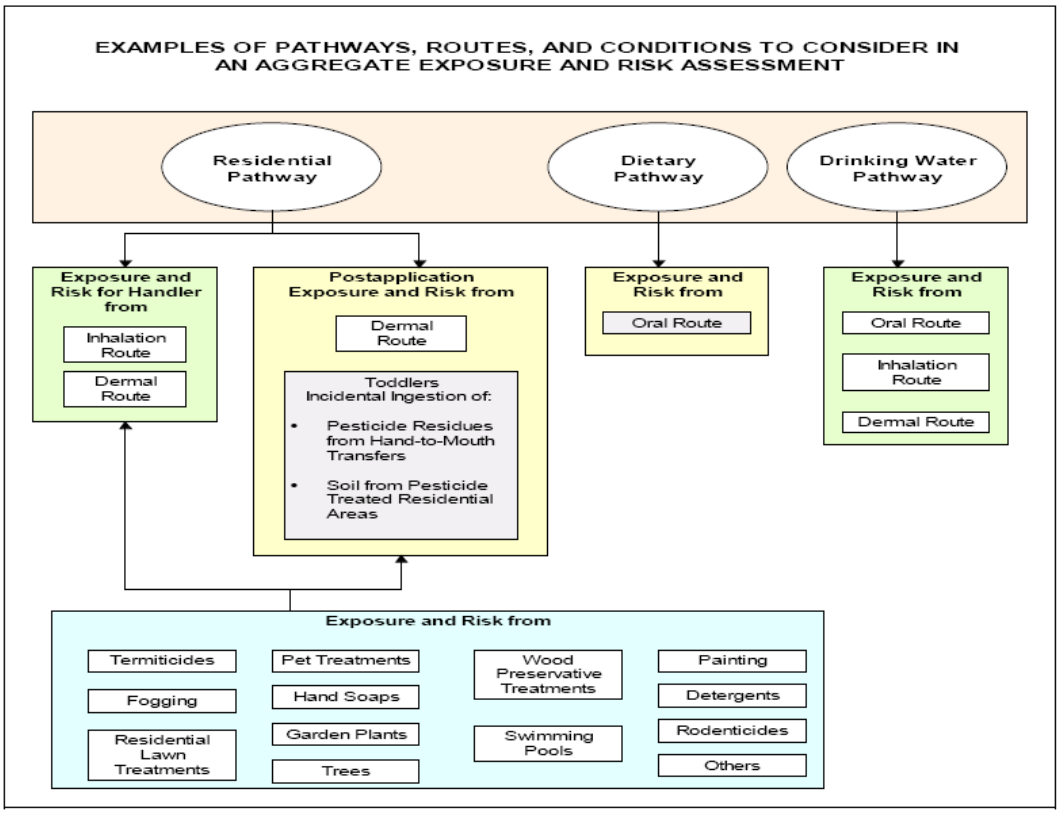
3. The relevance of animal studies conducted by different routes of administration (e.g., gavage or subcutaneous injection) for conducting human health risk assessment to different age groups and by different exposure pathways.

3.1 Influence of Route of Administration on Exposure Concentration and Duration

As shown in Figure 1, there are numerous pesticide exposure pathways that must be considered in an aggregate exposure and risk assessment. Regardless of the route by which chlorpyrifos gains entry into the body, the key parameters are (a) the concentration of chlorpyrifos reaching the target tissue (i.e., the developing brain), (b) how long it stays there, and (c) the critical developmental period in which exposure occurs. While the route of

administration influences these parameters, the selection of the route is not necessarily "automatic," nor, as it turns out, do studies with differing routes of administration lead to conflicting conclusions. For example, in considering toxicity in the fetus, it is important to keep in mind that fetal exposure occurs via the maternal bloodstream, not via oral ingestion.

Figure 1. Aggregate Exposure Pathways and Risk Assessment (U.S. EPA, Office of Inspector General, 2006)



Thus, oral dosing of newborn rats makes no sense if the objective is to mimic human fetal exposure, given that neonatal rats are developmentally equivalent to a late 2nd-3rd trimester human fetus. Further, the oral route introduces a "first-pass" effect, where the toxicant passes through the hepatic portal circulation before delivery to the systemic circulation. The first-pass effect allows the liver to metabolize a portion or even the majority of a compound; this is especially important for chlorpyrifos, where the liver provides both activation (formation of chlorpyrifos oxon) as well as inactivation (cleavage to trichloropyridinol). It is critically important to note, then, that the first-pass effect changes drastically with development, so that net delivery to the systemic circulation after ingestion will be inconsistent between species that have differing developmental profiles, and also in comparing exposures at different ages within species. With the subcutaneous route, the dose is delivered in a consistent manner because it avoids the first-pass through the liver.

Another cogent reason to prefer subcutaneous administration over oral gavage is that it is easier to do and less stressful for the animal. Stress is a potential confound in all developmental neurotoxicity studies, and a quick subcutaneous injection requires far less manipulation than oral gavage (even when gavage is done correctly, without injury to the animal).

For these reasons, administration by other routes (e.g., subcutaneous injection) often gives more reliable and consistent results than oral gavage for comparing animal models to humans. Finally, for children, as illustrated by Figure 1, exposure involves a combination of dermal, inhalation, and ingestion pathways, not just one or the other; so again, what really matters is the total concentration of chlorpyrifos reaching the brain and the duration over which the brain is exposed.

3.2 Consistency of Results Among Studies with Different Routes of Administration

Despite the inherent differences that might arise for effects of chlorpyrifos modeled with different routes of administration, it is important to note that virtually all studies in the extant literature, whether conducted with oral gavage or injection routes, have resulted in virtually identical outcomes. Despite claims that different routes of administration lead to different results, the scientific evidence leads to the conclusion that the route of exposure doesn't seem to be critical in terms of outcome, so long as the exposures remain within comparable bioeffective parameters, namely a range from those that are below the threshold for any measurable cholinesterase inhibition, up to those that cause significant inhibition but without overt symptoms of intoxication.

Table 2 summarizes specific comparisons between our results (Slotkin Laboratory), which use subcutaneous chlorpyrifos administration over a short period (four days), and those of another leading research in the field, Dr. Janice Chambers of Mississippi State University, whose group gives chlorpyrifos in higher doses by oral gavage, producing significant cholinesterase inhibition, but without overt symptoms of exposure.

Table 2. Comparison of Results between Studies Using Oral Gavage and Subcutaneous Injection as Routes of Administration

Chambers Group	Slotkin Group
Atterberry TT, Burnett WT, Chambers JE. 1997. Age-related differences in parathion and chlorpyrifos toxicity in male rats: target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. Toxicol Appl Pharmacol 147:411-418.	*Song X, Seidler FJ, Saleh JL, Zhang J, Padilla S, Slotkin TA. 1997. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signaling cascade. Toxicol Appl Pharmacol 145:158-174*
Experimental Design: Evaluated the developmental changes in cholinesterase (ChE) activity in the normal brain in males only.	Experimental Design: Evaluated ChE in developing brain regions.
Findings: ChE increases with age in the cerebral cortex but not the medulla oblongata.	Findings: 1. Age-related increases in the forebrain (a region that contains the cerebral cortex and is thus comparable to the measures in Atterberry et al., (1997)). We also found an increase in the brainstem (which contains the medulla oblongata studied by Dr. Chambers, but also some additional brain areas. (See Carr et al., (2001), described below for study where the Chambers Group also reports the same increases in this region.) 2. Significant reductions in muscarinic receptors (comparable with Tang et al. ,(1999)).
Tang J, Carr RL, Chambers JE. 1999. Changes in rat brain cholinesterase activity and muscarinic receptor density during and after repeated oral exposure to chlorpyrifos in early postnatal development. Toxicol Sci 51:265-272.	*Dam K, Garcia SJ, Seidler FJ, Slotkin TA. 1999. Neonatal chlorpyrifos exposure alters synaptic development and neuronal activity in cholinergic and catecholaminergic pathways. Dev Brain Res 116:9-20.*
Experimental Design: 1. Did not assess sex effects (i.e., males and females combined). 2. Chlorpyrifos was given by subcutaneous injection in oil to pregnant rats at a dose of 100, 150 or 200 mg/kg on postnatal (PN) day 1. 3. Treatment of pups over a long duration (PN1-21) giving the doses by oral gavage in oil vehicle in three dose groups designated "low," "medium" and "high," treatments that were all designed to cause "continuous ChE inhibition during the period of the brain growth spurt.	Experimental Design: 1. Treat with much lower doses of chlorpyrifos(compared to Tang, et al., (1999) for a much shorter period of time using subcutaneous route of administration which causes less systemic toxicity and no persistent ChE inhibition, (as demonstrated in Song et al.).

Findings: 1. Part 1: Greater ChE inhibition in the brains of dams than in the pups. 2. Part 2: Assays used the whole brain except the cerebellum finding signs of acute intoxication: tremors, mortality. 3. Chlorpyrifos exposure inhibits ChE and causes a decrease in muscarinic acetylcholine receptor binding consequent to the ChE effect, that is, the decrease was directly related to the degree of ChE inhibition. 4. Effects of anticholinesterases during this critical time may delay or disrupt nervous system development and cause permanent effects.	Findings: 1. CPF exposure during the postnatal period of synaptogenesis elicits widespread disruption of cholinergic and catecholaminergic pathways. As this is the period in which patterns of synaptic responsiveness is programmed by neural input, the period of developmental vulnerability to CPF is likely to extend into childhood. 2. Consonant results with Atterberry et al., (1997) and Tang et al., (1999) despite differences in dose, route and duration of chlorpyrifos administration.
Carr RL, Chambers HW, Guarisco JA, Richardson JR, Tang J, Chambers JE. 2001. Effects of repeated oral postnatal exposure to chlorpyrifos on open-field behavior in juvenile rats. Toxicol Sci 59:260-267.	*Dam K, Seidler FJ, Slotkin TA. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. Dev Brain Res 121:179-187.*
Experimental Design: Neonatal rats received the same treatment regimens described in Tang et al and just as presented in that paper, there was significant ChE inhibition.	Experimental Design: Focused on chlorpyrifos exposures well below those used by Carr et al., (2001), both in terms of absolute dose and duration of treatment, and in terms of biologic activity as monitored by ChE inhibition and signs of systemic toxicity.
Findings: 1. Significant ChE inhibition. Although there was no effect on locomotor activity up to PN20, a deficit emerged after the termination of chlorpyrifos exposure and was significant by PN25 and PN30. 2. Did not find a statistically significant sex difference in the overall effects on locomotor activity 3. At the lower dose (similar to Dam et al., 2000) the adverse effects emerge earlier in the males (first trend seen on PN20 and significant by PN25), whereas it is never significant in females (consistent with Dam et al., 2000). 4. Sex differences in locomotor activity, such as those observed following a single subcutaneous administration were not observed in this study, which utilized an oral exposure route. (Note that this conclusion was revised in Johnson, et al., (2006) – see below.)	Findings: 1. Chlorpyrifos exposure produced developmental behavioral delays in both males and females, with emergence of many of the effects days or weeks after the termination of chlorpyrifos treatment and after the restoration of ChE activity and muscarinic receptor levels 2. For late-emerging effects on locomotor activity, we found preferential deficits in males when chlorpyrifos was given on PN1-4, with the locomotor assessments done 3-4 weeks later (PN21, PN30).

Richardson J, Chambers J. 2003. Effects of gestational exposure to chlorpyrifos on postnatal central and peripheral cholinergic neurochemistry. J Toxicol Environ Health 66:275-289.	See *Dam et al., 1999; and Slotkin T, Cousins MM, Tate CA, Seidler FJ. 2001. Persistent cholinergic presynaptic deficits after neonatal chlorpyrifos exposure. Brain Research 902(2):229-243.*
Experimental Design: Pregnant rats were given 3, 5, or 7 mg/kg chlorpyrifos daily by corn oil gavage, from gestational days 6-20. Pups were examined on PN1,3,6,9,12. Sexes were not examined separately.	
Findings: 1. Reductions in forebrain ("brain, without cerebellum and medulla pons") ChE persisting to PN6 in the low dose group and through PN9 at medium or high dose. 2. Choline acetyltransferase (ChAT), an enzyme biomarker for presynaptic acetylcholine nerve terminals was reduced after ChE returned to normal 3. Reduction in ChAT activity observed here may be the result of insufficient availability of choline because of a decrease in HACU	Despite differences in dose, route of administration, duration of treatment and vehicle, Richardson and Chambers, 2003 report the same findings and same conclusions as Dam et al., (1999); and Slotkin et al., (2001). Same endpoints targeted, with the same outcomes: ChAT and choline transporter.
Richardson JR, Chambers JE. 2004. Neurochemical effects of repeated gestational exposure to chlorpyrifos in developing rats. Toxicol Sci 77:83-90.	See *Dam et al., 1999; and Slotkin T, Cousins MM, Tate CA, Seidler FJ. 2001. Persistent cholinergic presynaptic deficits after neonatal chlorpyrifos exposure. Brain Research 902(2):229-243.*
Experimental Design: Same treatments were used as in Richardson and Chambers, 2003, without the 5 mg/kg dose group; determinations were conducted out to PN30. Sex effects not assessed.	
Findings: 1. The decrease in ChAT was persistent and other presynaptic cholinergic neuronal markers, the vesicular acetylcholine transporter (VAChT) and HC3 binding to characterize the HACU process. 2. Data suggest that gestational exposure to 7 mg/kg/day CPS results in long-term alterations of presynaptic cholinergic neurochemistry. 3. Results confirm previous observations of the effects of gestational exposure to CPS on brain ChE and ChAT activity, and extend them by demonstrating that exposure to CPS during gestation results in transient reductions of total mAChR and mAChR subtypes, but more persistent reductions of HACU and VAChT levels. 4. Starting on PND 9 there was a progressive decline in HACU levels	Despite differences in dose, route of administration, duration of treatment and vehicle, Richardson and Chambers, (2004) report the same findings and same conclusions as Dam et al., (1999); and Slotkin et al., (2001). Again, the same variables were measured (ChAT, HC3 binding), with precisely the same outcomes as found by the Chambers group.

in the high-dosage group, which reached a maximum reduction of 21% on PND 30, the last day examined. A similar long-term reduction of HACU levels has also been observed by Qiao *et al*. (2003) in the offspring of dams subcutaneously exposed to 1 or 5 mg/kg/day CPS in DMSO from GD 17–20. 5. The reductions observed in ChAT activity, along with reductions in VAChT and HACU levels, suggest that presynaptic cholinergic neurons are especially sensitive to the effects of gestational exposure to CPS at the doses used in this study.	
Richardson JR, Chambers JE. 2005. Effects of repeated oral postnatal exposure to chlorpyrifos on cholinergic neurochemistry in developing rats. Toxicol Sci 84:352-359.	See *Dam et al., 1999; and Slotkin T, Cousins MM, Tate CA, Seidler FJ. 2001. Persistent cholinergic presynaptic deficits after neonatal chlorpyrifos exposure. Brain Research 902(2):229-243.*
Experimental Design: Single chlorpyrifos treatment regimen by corn oil gavage, starting at 1.5 mg/kg, then increasing the dose to 3 mg/kg and then 6 mg/kg as the animals got older, with the treatment continued from PN1-21. Sex differences not assessed.	
Findings: 1. ChE inhibition lasted through PN30, and as in previous studies, decreased ChAT and VAChT, as well as decreased HC3 binding that exceeded the effect on ChAT, all persisted to the end of study at PN30. 2. Repeated postnatal exposures to CPS result in transient reductions of mAChR and more persistent alterations of presynaptic cholinergic neurons. 3. In addition to identical findings reported in Richardson and Chambers, 2003, 2004 (see above); vesicular acetylcholine transporter (VAChT) levels were persistently decreased as well, suggesting that presynaptic cholinergic neurons may be especially vulnerable to gestational CPS exposure.	Richardson and Chambers (2005) findings are identical to those in our studies, which typically carry out the measurements even further, to adulthood. Again, both groups assess the same measures and reach the same conclusions.

Johnson FO, Chambers JE, Carr RL. 2006. Effects of repeated postnatal exposure to chlorpyrifos or methyl parathion in the elevated plus-maze model of anxiety. Soc Toxicol Abstract no. 1445.	*Aldridge JE, Levin ED, Seidler FJ, Slotkin TA. 2005. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. Environ Health Perspect 113:527-53.*
Experimental Design: Administered chlorpyrifos in oil by gavage at doses causing no signs of overt toxicity (doses not specified) but sufficient to still have reduced hippocampal ChE on PN35 in both sexes.	See *Dam et al., 1999; and Slotkin et al., 2001*
Findings: 1. On PN35, there was an increased open-arm time in the plus-maze for both males and females, but by PN70 the effect on males was much greater than the "limited effect" shown in females. 2. Passive avoidance retention was affected only in males at the lower doses but also in females at the high dose. 3. Postnatal exposure to OP insecticides can modulate fearless behavior and impair avoidance learning in a gender-related manner that persists well beyond cessation of exposure.	These findings are identical to our conclusions as presented in our behavioral papers.

3.3 Dow Chemical Conclusions are Inconsistent with Experimental Data

In addressing the pharmacokinetic issues engendered by different routes of administration, I would like to call the Panel's attention to inconsistencies in Dow's representation of supposed "problems" with the injection route. Originally, Dow-supported publications proposed that the injection route would produce enormous "spikes" of chlorpyrifos concentrations, that is, very rapid absorption resulting in inappropriately high concentrations in the bloodstream and in the brain (Zhao et al., 2006). In a subsequent paper, they argued the opposite, namely that absorption after subcutaneous injection was slower and incomplete (Marty et al., 2007). Indeed, it is worth examining this latter paper because their data actually support the view that the oral and injection routes produce quite similar results, notwithstanding their statements to the contrary in the Abstract and Discussion.

Marty et al., (2007) begin with the assumption that oral administration of chlorpyrifos in milk is the only valid route of administration for neonatal rats. As already discussed, this underlying assumption is incorrect, given that the neonatal rat is functionally equivalent to a human fetus, which gets exposed via the bloodstream. Nevertheless, let us for the moment grant them this assumption. If the Panel will examine Marty et al., (2007), Table 2 (page 364), it is evident that the pharmacokinetics of a single dose given via oral milk administration are virtually identical to that of subcutaneous injection in DMSO vehicle, in terms of Tmax, Cmax, AUC, t1/2, metabolite kinetics, etc. Nonetheless, the Abstract opines that "A bolus dose of 1 mg/kg CPF in DMSO administered s[ub]c[utaneously] appeared to have substantially altered pharmacokinetics from orally administered CPF"! Table 2 indicates clearly that this is not the case.

The only differences that are pointed out are an apparently longer Tmax with the injection model for the chlorpyrifos metabolite, trichloropyridinol (TCP), and a supposed "depot" from the injection. These are both incorrect as shown by their experimental data. The variability of the TCP measurements is high (coefficient of variation is 60%), so that a 4 vs. 6 hr Tmax isn't a significant difference (notably, neither statistics nor variability measures are shown for this claimed difference in Marty et al., 2007). As to the supposed "depot," this conclusion is also unsupported by the data because if two different administration routes result in the same Tmax, Cmax, AUC and t1/2, then their absorption rate constants and bioavailabilities **must** be identical (unless one is willing to forego the first law of thermodynamics). The data shown in Figure 2 (page 366) that purport to show a depot were apparently not conducted for any other route of administration, so a claim of "unique" properties is unsubstantiated. Moreover, the variability of the subcutaneous results is such that the 95% confidence limit covers a 2-3 fold range of possible values. In addition, these studies were done with n=2-3 according to the Methods, so a value of 60% (at 120 minutes at the injection site) cannot be distinguished from a value of 20%. In fact, the 95% confidence limit actually goes above 100%, which is biologically impossible.

The variability in these results may be explained by the authors' report of animals "writhing" when injected in DMSO, a finding which they discuss as a confound of the vehicle. To my knowledge, none of the hundreds of papers in the literature where DMSO was used as an injection vehicle for water-insoluble agents has ever reported the "writhing" seen by Marty et al., (2007). In 38 years of experience, we have never seen this effect. In my estimation, this effect may have been caused by the pain of injection if an inappropriate, large gauge

needle were used; the needle size appropriate for an adult rat (200 g) is decidedly too large for a neonatal rat (6 g). When the needle is too large, it produces a large hole, with resultant pain, as well as leakage of the injection out through the hole, thus producing what Dow staff has reported, namely poor absorption with material appearing to remain at the site. Further, this type of technical inexperience in injecting newborn rats could readily produce injection into the underlying tissue instead of the subcutaneous site, again resulting in pain and poor absorption. Either of these events would certainly the produce enormous variability for absorption evident in the data of Figure 2 in Marty et al. (2007). The authors themselves provide some evidence of technical incompetence, as they report that they punctured one of the pup's esophagi while performing oral gavage, a problem that should not occur with such a routine procedure unless the technical staff is incompetent.

In summary, the actual data presented by Marty et al., (2007) illustrate that the subcutaneous DMSO injection is a near-perfect pharmacokinetic mimic of oral gavage in milk, despite the opposite conclusions stated in their abstract and discussion. A previous paper funded by Dow Chemical also hypothesized that administration in DMSO would produce very high peak levels, much higher than by gavage. Despite their initial criticisms about subcutaneous absorption being too fast and producing peak levels (Zhao et al., 2006), followed by criticisms that the absorption would be slow and incomplete (Marty et al., 2007), their own experimental data confirm the validity of the subcutaneous route of administration.

4. Summary

In summary, I urge the Panel to review recent epidemiological studies which provide ample scientific evidence that non-symptomatic exposures of humans to chlorpyrifos lead to adverse neurological and development endpoints in humans that are predicted by animal studies. Second, given the abundant data illustrating non-cholinergic mechanisms of chlorpyrifos that result in developmental neurotoxicity outcomes, the Agency would be remiss if such mechanisms, and resulting outcomes, were not included in an aggregate assessment of risk associated with chlorpyrifos exposure. Finally, despite industry claims to the contrary, numerous studies using different routes of administration have resulted in virtually identical outcomes, illustrating that different routes of administration are equally valid. The Panel should include studies using different routes of administration in their assessment of the safety of chlorpyrifos, rather than focusing on only a single route.

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