APPENDIX 5.

MECHANISTIC STUDIES OF
CHLORPYRIFOS RELATED NEURODEVELOPMENTAL EFFECTS

INTRODUCTION

Identification of a rigorous neurodevelopmental point of departure for chlorpyrifos (CPF) would be strengthened by elucidation of the possible mechanistic underpinnings for its effects. While the studies reviewed in the preceding sections shed some light on the question of mechanism, the following paragraphs summarize studies that were designed to approach it directly. Investigations into CPF-induced neuroinflammation, as well as into its effects on neurotransmission in the endocannabinoid, dopaminergic, serotonergic and glutamatergic systems have been carried out by several laboratories recently and are given special attention in this section.

Mechanisms Associated with CPF-Related Disruption of Serine Hydrolases that Degrade Endocannabinoids after Perinatal Treatment

Recent research has shown that organophosphate (OP) pesticides, including CPF, block 30–50 % of all serine hydrolase activities in vivo in the brain beyond acetylcholinesterase (AChE) (Medina-Cleghorn et al. 2014). These included the serine hydrolases monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) that are responsible for the breakdown of endogenous cannabinoid signaling lipids 2-arachidonylglycerol and anandamide (2-AG and AEA). Blockade of MAGL and FAAH and disruption of signaling in the brain during the development can lead to cannabinoid receptor (CB1)-mediated behaviors result in long term behavioral deficits. CPF has been shown to inhibit MAGL and FAAH in to rat pups treated by gavage from postnatal day (PND) 10 to PND 16. Importantly, MAGL and FAHH inhibitions occur at doses lower than those inhibiting brain AChE (R.L. Carr et al. 2011; R. L. Carr et al. 2013).

CPF and its major metabolite, CPF-oxon, have both been shown to inhibit the CB1 receptor of male Swiss-Webster mouse whole brain membranes in vitro (Quistad et al. 2002). CPF -oxon inhibition was 2500 times more potent than CPF ethyl based on the concentration of inhibitor displacing 50% specific binding (IC_{50}) (IC_{50} mean $\pm$ S.E: 14$\pm$4 versus 35000 $\pm$ 6000 nM, respectively). In vivo, these mice showed CPF -oxon (3 mg/kg) and CPF (30 mg/kg) inhibited CB1 receptor at 24 ($\pm$7) and 35 ($\pm$6) percent, respectively. Since CPF is highly lipophilic it is also possible that it could diffuse into cells, circumventing the CB1 receptor (Smith et al. 2011; Smith et al. 2014). Calcium influx and K^+ efflux are necessary for neurotransmitter release (Elphick and Egertova 2001; Guo and Ikeda 2004; Twitchell et al. 1997); however, pre-synaptic agonist activation of CB1 leads to inhibition of adenylyl cyclase (AC) and inhibition of the conversion of ATP to cyclic AMP, resulting in direct stimulation of K+ channel opening (efflux) and inhibition of Ca^{+2} influx (Di Marzo 2008; Elphick and Egertova 2001; Howlett et al. 2002; Pertwee 2008).

CPF also inhibits the normal reabsorption and pre-synaptic breakdown of 2-AG by MAGL and FAAH degradation of AEA post-synaptically (Di Marzo 2011; Ohno-Shosaku and Kano 2014). When MAGL and FAAH are inhibited, the normal metabolic breakdown of 2-AG and anandamide is disrupted and endocannabinoids accumulate (R.L. Carr et al. 2011; R. L. Carr et al. 2013; R. L. Carr et al. 2014; R.L. Carr et al. 2015) resulting in inhibition of neurotransmitter release (i.e., GABA, glutamate, dopamine, norepinephrine, and acetylcholine). Depending on

dose, treatment regimen, tests performed, etc., both excitatory and inhibitory effects on behavior (anxiety and motor activity) may be detected after CPF treatment (R.L. Carr et al. 2017a; Lee et al. 2015; Silva et al. 2017). Continuous stimulation of the CB1 receptor and/or inhibition of FAAH and MAGL have been shown to have long term developmental effects in animals (Buntyn et al. 2017; R.L. Carr et al. 2015; Russell L Carr et al. 2017b; Mohammed et al. 2015).

Oxidative-reduction (redox) potential alterations occur during neurogenesis and mitochondrial respiration in differentiated neurons. Redox signaling regulates hippocampal neuroprogenitor cell proliferation, differentiation and function (Hebert-Chatelain et al. 2016; Le Belle et al. 2011). Neural stem cells have a higher oxidative state with reactive oxygen species (ROS: e.g., hydrogen peroxide) than adult cells because high ROS levels are necessary for self-renewal and neurogenesis. Low doses of CPF can result in oxidative stress in rodent models (Kopjar et al. 2018). Post-weaning male Wistar rats treated with CPF at 0 (ethanol), 0.01, 0.015 and 0.16 mg/kg/d for 28 days showed no effects on plasma, RBC or brain ChE however there was an increase in superoxide dismutase in the brain at 0.16 mg/kg/d, indicating that CPF was inducing oxidative stress in developing animals at very low doses.

Control of many neuronal processes is initiated by CB1-receptor agonist activation of mitochondrial CB1 receptors (mtCB1) on the mitochondrial membranes. Mitochondria regulate normal cell function through ATP production, generation of reactive oxygen species (ROS), calcium buffering and metabolism of neurotransmitters in the CNS (Djeungoue-Petga and Hebert-Chatelain 2017). When mtCB1 are activated, cAMP is decreased and adenylyl cyclase and protein kinase A are inhibited which results in decreased complex I phosphorylation (NADH dehydrogenase (Hebert-Chatelain et al. 2016). Complex I is the first enzyme of the mitochondrial electron transport chain for the production of ATP and when it is decreased, the result is decreased energy production and disruption of mitochondrial Ca^{2+} inner membrane potential (Bénard et al. 2012; Djeungoue-Petga and Hebert-Chatelain 2017). MtCB1 directly increases the closure of N- and P/Q-type voltage activated Ca^{2+} channels in neurons, preventing Ca^{2+} release, preventing release of neurotransmitters at GABAergic synapses in the hippocampus and glutamatergic synapses in the dorsal striatum (Pankratov et al. 2002). Exposure to the active metabolite of CPF-oxon, results in over-expression of gene sets involved in mitochondrial dysfunction and oxidative stress in the rat cerebellum (Cole et al. 2011); and the antioxidant vitamin E has been shown to mediate the anti-proliferative effect of CPF in PC12 cells (Slotkin et al. 2007).

CPF effects on neuronal pathway development and differentiation, as well as synaptogenesis and dendritogenesis that are stimulated by various growth factors (neurotropins). CPF inhibits neurite outgrowth in vitro by affecting the cAMP pathway and nerve growth factor (NGF) (Eaton et al. 2008). NGF binds to and activates tropomyosin receptor kinase A (TrkA) and the PI3 Kinase Sl to stimulate neurogenesis, plasticity, and axonal growth (Dalton and Howlett 2012; Keimpema et al. 2013). TrkA can also increase expression of diacyglycerol lipases (DAGL), MAGL, and the CB1 receptor (Berghuis 2007; Keimpema et al. 2013).

Fibroblast growth factor (FGF) in the CNS functions as a regulator of neural stem cell proliferation, in addition to neurogenesis, axon growth, and differentiation (Rash et al. 2011; Rash et al. 2013). Postnatal exposure of Sprague-Dawley rats to 1 mg/kg/d CPF on post-natal

days 1-4 altered expression of the neurotropin fibroblast growth factor (FGF) (Slotkin et al. 2007; Slotkin et al. 2008). The FGF receptor signal activates phospholipase Cγ pathway to produce diacylglycerol (DAG) post-synaptically (Williams et al. 2003). In early development, diacylglycerol lipase-β (DAGL) catalyzes DAG to produce 2-AG (Figure 3) (Ahn et al. 2008; Jung et al. 2011). Depending on the cell-state-specific developmental stage, 2-AG (DAGL-dependent) synthesis and subsequent interaction with CB1 receptor signal transduction has been shown to be regulated by FGF signaling cascades (Maison et al. 2009). Disruption of FGF by CPF can adversely affect 2-AG synthesis as well as cellular differentiation into neural pathways (Keimpema et al. 2010).

The expression of CB1, MAGL, FAAH, and DAGL has been reported in neuroprogenitor cells (Berghuis 2007). CB1 activation promotes progenitor cell proliferation, while genetic deletion of CB1 decreases cortical progenitor proliferation in the embryonic brain. Deletion of FAAH increases neural progenitor proliferation. A DAGL antagonist inhibits the *in vitro* proliferation of neural stem cells, and the proliferation of neuroprogenitor cells is impaired in DAGL knockout mice (Gao et al. 2010).

Several lines of evidence suggest a potential CPF effect on proliferation, differentiation, and migration in neuroprogenitor cells. CPF was found to alter the proliferation, differentiation, and histone modifications of human neuroprogenitor cells (Kim et al. 2016). In the hippocampus, most of the CB1-expressing neurons are cholecystokinin-expressing interneurons (CCK- INTs) (Antypa et al. 2011; Morozov et al. 2009). Exposure to CPF evoked a robust upregulation of cholecystokinin in PC12 cells (Slotkin and Seidler 2010). CPF and CPF oxon can directly bind to muscarinic cholinergic receptors (mAChR) M2 at concentrations below those that result in AChE inhibition (Huff et al. 1994; Ward et al. 1993). This supports a potential developmental neurotoxicity mechanism associated with the morphogenetic roles of acetylcholine (Borodinsky and Belgacem 2016; Lauder and Schambra 1999)
The endocannabinoid system controls the guidance of axonal growth in connecting the thalamus and cerebral cortex (Keimpema et al. 2010). Corticofugal axons are CB1 positive, whereas thalamocortical axons are CB1 negative but MAGL positive. The autocrine 2-AG signaling in corticofugal axons promotes their elongation, while MAGL guides the axonal growth by limiting the spatial spread of 2-AG. After synapses are formed, MAGL is overexpressed to provide a 'stop' signal at the pre-synapses. CPF and CPF-oxon were shown to alter cell and axonal growth in a mouse neuroblastoma × rat glioma hybrid cell line and zebrafish, respectively (Campanha et al. 2014; Yang et al. 2011).

Perinatal disruption of synaptogenesis by CPF can result in detrimental consequences in later life. Several published reviews report the association of various adverse developmental health outcomes and potential, estimated, or quantified exposure to CPF during pregnancy (see Epidemiology Epidemiological Studies Related to Neurodevelopmental Effects for an in depth presentation of effects on humans).

Other CPF Mechanisms for Developmental Neurotoxicity Related to Disruption of the Adenylyl Cyclase, Serotonergic Pathways

CPF has been shown to disrupt the serotonergic and dopaminergic systems; however, the low doses used in the above studies were at the threshold of RBC AChE inhibition. 5HT is critical to the control of neural differentiation and organization of the developing brain (Dreyfus 1998; Lauder 1985; Levitt et al. 1997; Turlejski 1996; Weiss and Wagner 1998; Whitaker-Azmitia 1991, 2001). A possible mechanism for developmental neurotoxicity may be via disruption of cell signaling through the serotoninergic system. One of the most potent effects noted for CPF is the ability to control cAMP-dependent cell differentiation through inhibition of adenylyl cyclase (AC) (Crumpton et al. 2000; Garcia et al. 2001; Schuh et al. 2002).

1. AC was inhibited in during gestational neurulation (GD9-12 and GD 17-21) which may lead to later effects on 5HT receptor signaling.
2. CPF treatment in the immediate perinatal period (PND1-4: neuronal differentiation and synaptogenesis) is the most sensitive for detecting decreases in 5HT receptors and 5HTT that persist into adulthood (PND60).
3. Degree of effects on 5HT receptors and 5HTT is dependent on period of treatment and brain region.
4. The 5HT and 5HTT decrements from CPF treatment in the immediate post-natal period were associated with deficits in learning, memory and signs of depression, based on anhedonia, in adulthood (Aldridge et al. 2005b).

The critical effects occurring from CPF exposure were altered neuronal development of 5HT receptor subtypes, 5HTT as well as AC at 1.0 mg/kg/d (lowest dose tested). Severity of effects differed by brain region. However, 1.0 mg/kg/day is also the threshold for AChE inhibition, so it is difficult to separate non-cholinergic from cholinergic effects. The Aldridge et al. studies did not co-examine AChE for comparison and they used the subcutaneous (s.c.) route of exposure which is not a representative route in humans. The results suggest that gestational and neonatal CPF exposures can cause persistent changes in brain synaptic activity based on observed changes to 5-HT levels and turnover. The greatest sensitivity to the above affects occurs prior to the second postnatal week. These effects had gender selectivity and were observed below the threshold for cholinergic symptoms.

The dopaminergic system was disrupted in pups exposed perinatally to CPF as shown by Mohammed et al. (2015) and Aldridge et al. (2005a). At 0.5 mg/kg/d CPF administered by gavage in corn oil, male and female Sprague-Dawley rats showed increased DA metabolism in the amygdala that was associated with decreased anxiety (Mohammed et al., 2015). Aldridge et al. (2005a) showed DA levels and turnover at PND60 were either increased or decreased depending on brain region when pups were treated by s.c. injection PND1-4 at 1.0 mg/kg/d CPF. DA levels and turnover in the cerebral cortex but were increased in the striatum and only turnover was increased in the midbrain. Effects in Mohammed et al. (2015) occurred below the general threshold for RBC AChE inhibition (1 mg/kg/day) when CPF was administered by gavage, although it should be noted that the former study used an atypical route of administration.

Included in Appendix 5, Table 1, below, is an evaluation of studies reporting age-dependent, serotonergic effects of CPF based on published evidence for this MOA. All of the reported serotonergic effects occurred at the lowest dose levels of their corresponding studies and at dose-levels where cholinergic effects were either seen or expected.

Appendix 5. Table 1. Individual End-point Data from Published Studies Reviewed to Evaluate Potential Age Susceptibility to Serotonergic Effects Related to Exposures to CPF

Reference	Test System	Route/Dose Levels (mg/kg/day)	Treatment Period	Endpoint Type	Endpoint	Endpoint Timing	Effects of CPF Treatment	Conclusion(s)
(Mohammed et al. 2015)	Rat Pups	Oral Gavage; 0, 0.5, 0.75, 1; m/f: 17-18/12-16	PND10-16 (pre-adolescence)	Changes to emergence behavior as emotional reactivity (ER) or anxiety	Time-to-emergence from cup	PND16	M and F: ↓ER	The results suggest that CPF targets the endocannabinoid system of the developing brain by disrupting endocannabinoid-mediated dopaminergic signaling.
(Mohammed et al. 2015)	Rat Pups	Oral Gavage; 0, 0.5, 0.75, 1; m/f: 17-18/12-16	PND10-16 (pre-adolescence)	Changes to brain monoamine neurotransmitter (MNT) signaling related to emotional behavior	MNT levels in the hippocampus and amygdala. MNTs included dopamine, serotonin, and metabolites	PND16	Hippocampus: ↑ NE, 5-HT, and 5-HIAA levels Amygdala: ↑DOPAC and HVA	Effects were observed at doses ≥ 0.5 mg/kg/day.
(Aldridge et al. 2003)	Rats: pregnant dams and pups	Subcutaneous injection; 0, 1, 2, and 5	GD9-12 (neurulation)	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of pups.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	GD17 and 21	Whole Brain: ↓5-HTR and 5-HTT binding (GD17). Brainstem: ↑5-HTR and 5-HTT binding (GD21).	The results suggest that CPF targets the 5-HT system of the developing brain at the level of the cell. CPF likely targets the development and function of signaling molecules (5-HTRs and

								5-HTTs).
(Aldridge et al. 2003)	Rats: pregnant dams and pups	Subcutaneous injection: 0, 1, 2, and 5	GD9-12 (neurulation)	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT and +/- forskolin	GD17 and 21	↓5-HT-mediated stimulation. ↑5-HT-mediated inhibition (+forskolin).	The critical window for CPF effects ranged from the neural tube stage to the stages of terminal differentiation and synaptogenesis.
(Aldridge et al. 2003)	Rats: pregnant dams and pups	Subcutaneous injection: 0, 1, 2, 5, 10, 20, 40	GD17-20 (late gestation)	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of pups.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	GD21	Brainstem: ↑5-HTR and 5-HTT binding. Forebrain: ↑5-HTR and ↑↓5-HTT binding.	
(Aldridge et al. 2003)	Rats: pregnant dams and pups	Subcutaneous injection: 0, 1, 2, 5, 10, 20, 40	GD17-20 (late gestation)	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT and +/- forskolin	GD21	↑5-HT-mediated stimulation. ↑5-HT-mediated inhibition (+forskolin).	These effects had gender specificity and were observed below the threshold for cholinergic symptoms.
(Aldridge et al. 2003)	Rat pups	Subcutaneous injection: 0 and 1	PND1-4	Changes to the levels of 5-HTRs (1A and 2) in the brains of pups.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2)	PND5 and 10	PND5 Brainstem (M/F): ↑5-HTR binding. Forebrain (M/F): ↑5-HTR binding. PND10 Brainstem (M/F): ↑/↓5-HTR binding Forebrain (M/F): ↑/↓ and ↑5-HTR binding.	Effects were observed at doses ≥ 1.0 mg/kg/day.

(Aldridge et al. 2003)	Rat pups	Subcutaneous injection: 0 and 5	PND11-14	Changes to the levels of 5-HTRs (1A and 2) in the brains of pups.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2)	PND15 and 20	PND15 Brainstem (M/F): ↓/↑ 5-HTR binding. Forebrain (M/F): ↑ and ↓/↑ 5-HTR binding. PND20 Brainstem (M/F): ↓5-HTR binding Forebrain (M/F): ↓5-HTR binding.	
(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection; 0, 1, and 5	GD9-12 (neurulation)	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of adult rats.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	PND60 (adulthood)	Cerebral Cortex, Midbrain, and Brainstem (M/F): ↑5-HTR and 5-HTT binding.	The results suggest that CPF acts to alter the development program for 5-HT innervation in specific synaptic populations. The period of greatest sensitivity was from late gestation to early post-natal corresponding the second trimester of

(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection; 0, 1, and 5	GD17-20 (late gestation)	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of adult rats.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	PND60 (adulthood)	Cerebral Cortex, Hippocampus, Striatum, Midbrain, and Brainstem (M/F): ↑↓5-HTR and 5-HTT binding. Effects were greater for males than females.	human fetal development. These effects had gender specificity and were observed below the threshold for cholinergic symptoms.
(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection: 0 and 1	PND1-4	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of adult rats.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	PND60 (adulthood)	Cerebral Cortex, Hippocampus, Striatum, Midbrain, and Brainstem (M/F): ↑↓5-HTR and 5-HTT binding. Effects were greater for males than females.	Effects were observed at doses ≥ 1.0 mg/kg/day.
(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection: 0 and 5	PND11-14	Changes to the levels of 5-HTRs (1A and 2) and 5-HTT in the brains of adult rats.	5-HT binding to *ex-vivo* 5-HTRs (1A and 2) and 5-HTT	PND60 (adulthood)	Cerebral Cortex, Hippocampus, Striatum, Midbrain, and Brainstem (M/F): ↑↓5-HTR and 5-HTT binding.	

(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection; 0, 1, and 5	GD9-12 (neurulation)	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT	PND60 (adulthood)	Gender-specific changes in basal AC activity.	
(Aldridge et al. 2004)	Rats: pregnant dams and adult progeny	Subcutaneous injection; 0, 1, and 5	GD17-20 (late gestation)	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT	PND60 (adulthood)	↓Forskolin-stimulated AC activity.	
(Aldridge et al. 2004)	Rat pups	Subcutaneous injection; 0, 1, and 5	PND1-4	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT (+/- forskolin)	PND60 (adulthood)	Gender-specific changes in basal AC activity.	
(Aldridge et al. 2004)	Rat pups	Subcutaneous injection; 0, 1, and 5	PND11-14	Changes to the Adenylyl cyclase (AC) response to 5HT in the brains of pups.	Ratio of AC activity +/- 5-HT (+/- forskolin)	PND60 (adulthood)	↓Basal AC activity. ↓Forskolin-stimulated AC activity.	

(Aldridge et al. 2005b)	Rat pups	Subcutaneous injection; CPF: 0 and 1	PND1-4	Changes to elevated plus maze navigation parameters to test for depression-like behaviors known to be mediated by 5-HT deficiencies.	Percentage of time spent in open arms and locomotive activity (center crossings).	PND52-53	M: ↑Time in open-arms. M: ↑Activity.	The results suggest that neonatal CPF exposures can cause persistent behavioral effects associated with rodent models of depression likely mediated by changes in 5-HT signaling.
(Aldridge et al. 2005b)	Rat pups	Subcutaneous injection; CPF: 0 and 1	PND1-4	Changes to chocolate milk consumption preference to test for anhedonia known to be mediated by 5-HT deficiencies.	Milk:Water preference ratio.	PND54	M and F: ↓Preference for chocolate milk.	These effects had gender selectivity but not specificity and were observed below the threshold for cholinergic symptoms.
(Aldridge et al. 2005b)	Rat pups	Subcutaneous injection; CPF: 0 and 1	PND1-4	Changes to radial-arm maze navigation parameters to test working and reference memory.	Working and reference memory error rates in locating food.	PND64	Working and Reference Memory: M: ↑ error rate. F: ↓ error rate. Effects eliminated characteristic sex differences observed in controls	Effects were observed at doses ≥ 1.0 mg/kg/day.

(Aldridge et al. 2005b)	Rat pups	Subcutaneous injection; CPF: 0 and 1 Ketanserin: 0, 0.5, 1 and 2	PND1-4	Changes to radial-arm maze navigation parameters to test working and reference memory and the role played by 5-HT.	Working and reference memory error rates in locating food.	PND64	M and F (combined): ↑Error rate. F: ↓Error rate. Effects in working memory > reference memory.	
(Aldridge et al. 2005a)	Rats: pregnant dams and adult progeny	Subcutaneous injection; CPF: 0, 1 and 5	GD17-20	Changes to brain 5-HT and DA signaling (synaptic activity) and its relationship to observed behaviors similar to those for rodent models of depression.	5-HT levels and turnover in the brain. Turnover is the ratio parent MNT to its metabolites. MNTs included dopamine, serotonin, and metabolites	PND60	M and F: Net ↓5-HT content Net ↑5-HT turnover Net - DA content Net ↑DA turnover	The results suggest that gestational and neonatal CPF exposures can cause persistent changes in brain synaptic activity based on observed changes to 5-HT levels and turnover. The greatest sensitivity to the above affects occurs prior to the second postnatal week.

(Aldridge et al. 2005a)	Rats: pregnant dams and adult progeny	Subcutaneous injection; CPF: 0 and 1	PND1-4	Changes to brain 5-HT signaling and its relationship to observed behaviors similar to those for rodent models of depression.	5-HT levels and turnover in the brain. Turnover was the ratio parent MNT to its metabolites. MNTs included dopamine, serotonin, and metabolites	PND60	M: Net -5-HT content Net ↑5-HT turnover F: Net ↓5-HT content Net ↑5-HT turnover	These effects had gender selectivity and were observed below the threshold for cholinergic symptoms. Effects were observed at doses ≥ 1.0 mg/kg/day.
(Aldridge et al. 2005a)	Rats: pregnant dams and adult progeny	Subcutaneous injection; CPF: 0 and 5	PND11-14	Changes to brain 5-HT signaling and its relationship to observed behaviors similar to those for rodent models of depression.	5-HT levels and turnover in the brain. Turnover was the ratio parent MNT to its metabolites. MNTs included dopamine, serotonin, and metabolites	PND60	M and F: Net -5-HT content Net -5-HT turnover	

APPENDIX 5. REFERENCES

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