CHLORPYRIFOS AFFECTS IMMORTALIZED HYPOTHALAMIC MURINE GNRH NEURONS INTEGRITY AND EXPRESSION AT HUMAN RELEVANT EXPOSURE LEVELS

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Chlorpyrifos (CPF) is a widely used pesticide exerting several neurodevelopmental effects with severe consequences on children cognitive, behavioral and motor development. Human exposure occurs mainly through diet; pregnant women and children are subpopulations at higher risk. In brain, hypothalamus is one of the main target organ affected by pre- and postnatal CPF exposure. We previously demonstrated that developmental exposure to CPF alters oxytocin, vasopressin and Estrogen Receptor (ER) beta expression in mice. To better elucidate the mode of action of CPF in hypothalamus, at molecular level, we used the fully differentiated GT1-7 mouse hypothalamic cell line, analyzing a battery of endpoints at human relevant concentrations. GT1-7 cells were treated for 72h with CPF in a range of six 10-fold diluted concentrations (1 nM-100 μM) assessing cell proliferation, metabolic activity, apoptosis and necrosis. Cells treated at the three lowest CPF doses (1-10-100 nM) were used to assess Gonadotropin Releasing Hormone (GnRH) secretion by ELISA assay, gene expression of GnRH, ERα, ERβ, Androgen Receptor (AR), aromatase, oxytocin and oxytocin receptor by qPCR; in addition, we assessed protein expression profiles by Mass Spectrometry of all samples. Electronic Microscopy (TEM) was performed on GT1-7 treated with 100 nM CPF. CPF dose-dependently reduced metabolic activity and decreased cell proliferation only at the highest dose. At same dose, apoptosis was observed after 48h and 72h treatment. Both GnRH secretion and gene expression were repressed by CPF at 100 nM concentration. The selected genes were all up-regulated by CPF, with different patterns according to the concentration. TEM analysis evidenced severe mitochondrial damage, mitophagy, increased mielino-like figures and reduced cell-cell contact. By proteomic analysis we observed a higher number of differentially expressed proteins at the intermediate concentration; most significantly affected KEGG pathways were related to autophagy, immune-mediated diseases and dopamine signaling. Exposure of hypothalamic GT1-7 cells to CPF, at human relevant concentrations, demonstrated to impair mitochondria integrity also inducing neuroendocrine markers' expression and affecting relevant pathways possibly involved in neurological disorders. Such evidence further supports the concern for neurodevelopmental effects exerted by this pesticide.