Bibliography of Selected Peer-Reviewed Journal Articles on Chlorpyrifos Insecticide Human and Environmental Exposure and Toxicity:

Aldridge, J. E., et al. (2003). "Serotonergic Systems Targeted by Developmental Exposure to Chlorpyrifos: Effects during Different Critical Periods." Environmental Health Perspectives **111**(14): 1736-1743.

> During brain development, serotonin (5HT) provides essential neurotrophic signals. In the present study, we evaluated whether the developmental neurotoxicity of chlorpyrifos (CPF) involves effects on 5HT signaling, as a potential mechanism underlying noncholinergic neuroteratogenic events. We evaluated four different treatment windows ranging from the neural tube stage [gestational days (GD) 9-12] and the late gestational period (GD17-20) through postnatal phases of terminal neuronal differentiation and synaptogenesis [postnatal days (PN) 1-4, PN11-14]. Exposure to CPF on GD9-12 elicited initial suppression, immediately followed by rebound elevation, of 5HT1A and 5HT2 receptors as well as the 5HT transporter, all at doses below the threshold for cholinergic hyperstimulation and the resultant systemic toxicity. In contrast, with GD17-20 exposure, the initial effect was augmentation of all three components by low doses of CPF. Sensitivity of these effects declined substantially when exposure was shifted to the postnatal period. We also identified major alterations in 5HT-mediated responses, assessed for the adenylyl cyclase signaling cascade. Although GD9-12 exposure had only minor effects, treatment on GD17-20 elicited supersensitivity to both stimulatory and inhibitory responses mediated by 5HT. Our results indicate that CPF affects 5HT receptors, the presynaptic 5HT transporter, and 5HT-mediated signal transduction during a discrete critical gestational window. These effects are likely to contribute to the noncholinergic component of CPF's developmental neurotoxicity.

Alleva, R., et al. (2016). "Organic honey supplementation reverses pesticide-induced genotoxicity by modulating DNA damage response." Molecular Nutrition and Food Research **60**(10): 2243-2255.

> SCOPE: Glyphosate (GLY) and organophosphorus insecticides such as chlorpyrifos (CPF) may cause DNA damage and cancer in exposed individuals through mitochondrial dysfunction. Polyphenols ubiquitously present in fruits and vegetables, have been viewed as antioxidant molecules, but also influence mitochondrial homeostasis. Here, honey containing polyphenol compounds was evaluated for its potential protective effect on pesticide-induced genotoxicity. METHODS AND RESULTS: Honey extracts from four floral organic sources were evaluated for their polyphenol content, antioxidant activity, and potential protective effects on pesticide-related mitochondrial destabilization, reactive oxygen and nitrogen species formation, and DNA damage response in human bronchial epithelial and neuronal cells. The protective effect of honey was, then evaluated in a residential population chronically exposed to pesticides. The four honey types showed a different polyphenol profile associated with a different antioxidant power. The pesticide-induced mitochondrial dysfunction parallels ROS formation from mitochondria (mtROS) and consequent DNA damage. Honey extracts efficiently inhibited pesticide-induced mtROS formation, and reduced DNA damage by upregulation of DNA repair through NFR2. Honey supplementation enhanced DNA repair activity in a residential population chronically exposed to pesticides, which resulted in a marked reduction of pesticide-induced

DNA lesions. CONCLUSION: These results provide new insight regarding the effect of honey containing polyphenols on pesticide-induced DNA damage response.

Alvi, A. H. K., et al. (2012). "Field Evolved Resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* Toxin Cry1Ac in Pakistan." Plos One **7**: 1-9.

Helicoverpa armigera (Hübner) is one of the most destructive pests of several field and vegetable crops, with indiscriminate use of insecticides contributing to multiple instances of resistance. In the present study we assessed whether *H. armigera* had developed resistance to Bt cotton and compared the results with several conventional insecticides. Furthermore, the genetics of resistance was also investigated to determine the inheritance to Cry1Ac resistance. To investigate the development of resistance to Bt cotton, and selected foliar insecticides, *H. armigera* populations were sampled in 2010 and 2011 in several cotton production regions in Pakistan. The resistance ratios (RR) for Cry1Ac, chlorpyrifos, profenofos, cypermethrin, spinosad, indoxacarb, abamectin and deltamethrin were 580-fold, 320-, 1110-, 1950-, 200-, 380, 690, and 40-fold, respectively, compared with the laboratory susceptible (Lab-PK) population. Selection of the field collected population with Cry1Ac in 2010 for five generations increased RR to 5440-fold. The selection also increased RR for deltamethrin, chlorpyrifos, profenofos, cypermethrin, spinosad, indoxacarb, abamectin to 125-folds, 650-, 2840-, 9830-, 370-, 3090-, 1330-fold. The estimated LC(50s) for reciprocal crosses were 105 µg/ml (Cry1Ac-SEL female × Lab-PK male) and 81 g µg/ml (Lab-PK female × Cry1Ac-SEL male) suggesting that the resistance to Cry1Ac was autosomal; the degree of dominance (D(LC)) was 0.60 and 0.57 respectively. Mixing of enzyme inhibitors significantly decreased resistance to Cry1Ac suggesting that the resistance to Cry1Ac and other insecticides tested in the present study was primarily metabolic. Resistance to Cry1Ac was probably due to a single but unstable factor suggesting that crop rotation with non-Bt cotton or other crops could reduce the selection pressure for *H. armigera* and improve the sustainability of Bt cotton.

Arcury, T. a., et al. (2007). Pesticide urinary metabolite levels of children in eastern North Carolina farmworker households. Environmental Health Perspectives. **115:** 1254-1260.

BACKGROUND: In this investigation we documented the pesticide urinary metabolite levels of farmworker children in North Carolina, determined the number of different metabolites detected for each child, and delineated risk factors associated with the number of metabolites. METHODS: Urine samples were collected from 60 Latino farmworker children 1-6 years of age (34 female, 26 male). Interviews were completed by their mothers in Spanish. We analyzed urine samples for 14 pesticide metabolites, including the organophosphate pesticides chlorpyrifos, coumaphos, diazinon, isazaphos, malathion, pirimiphos, and parathion and its methyl counterpart; a common metabolite of at least 18 pyrethroid insecticides; the repellent DEET; and the herbicides 2,4,5-trichlorphenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, acetochlor, atrazine, and metolachlor. Predictors included measures of paraoccupational, residential, and environmental exposure, child characteristics, and mother characteristics. RESULTS: Thirteen metabolites were present in the urine samples. Organophosphate pesticide metabolites were detected in a substantial proportion of children, particularly metabolites of parathion/methyl parathion (90.0%; geometric mean 1.00 microg/L), chlorpyrifos/chlorpyrifos

methyl (83.3%; geometric mean 1.92 microg/L), and diazinon (55.0%; geometric mean 10.56 microg/L). The number of metabolites detected ranged from 0 to 7, with a mode of 4 detected (28.3%). Boys, children living in rented housing, and children with mothers working part-time had more metabolites detected. CONCLUSIONS: Children in farmworker homes experience multiple sources of pesticide exposure. Pesticides may remain in their environments for long periods. Environmental and occupational health changes are needed to address these exposures. Research is needed with more precise measures of exposure and on the health effects of concurrent exposure to multiple pesticides.

Bonifacio, A. F., et al. (2016). "Alterations in the general condition, biochemical parameters and locomotor activity in *Cnesterodon decemmaculatus* exposed to commercial formulations of chlorpyrifos, glyphosate and their mixtures." Ecological Indicators **67**: 88-97.

The Pampean region, an extensive area of South America is continuously impacted by agricultural activities and the pesticides related to them like chlorpyrifos and glyphosate. Both pesticides have been registered in freshwater bodies of the region. One of the most abundant and widely distributed fish species in Pampean streams is *Cnesterodon decemmaculatus*, which have to cope with this altered scenario. In the present study the toxicity of Clorfox® and Roundup Max®, the commercial formulations of chlorpyrifos and glyphosate, respectively, and their mixture where evaluated using a set of biomarkers at different biological organization levels in fish exposed to relevant environmentally pesticides concentrations. Somatic indexes such as the condition factor (K), and the hepato-somatic index (HSI), the locomotor activity through the distance traveled and the average speed, the enzymatic activities of acetylcholinesterase (AChE) in brain and muscle, catalase (CAT) in muscle and liver, glutathione-S-transferase (GST) in brain, liver, muscle and gills, aspartate amino-transferase (AST), alanine amino-transferase (ALT), AST/ALT ratio and alkaline phosphatase (ALP) in liver were measured on *C. decemmaculatus*. Adult females were exposed during 6 weeks to the following concentrations: 0.0084 µl/l and 0.00084 µl/l of Clorfox (CF), 0.2 and 2 mg/l of Roundup Max (RM) and all the combinations of these concentrations. The CF exposure caused a decrease in the condition factor and in the locomotor activity parameters and induced an increase brain AChE, liver CAT activity and AST/ALT ratio. On the other hand, the exposure to RM produced a decrease in liver GST, AST/ALT ratio and ALP activity. Finally, some pesticide combinations decrease general condition and liver GST activities, and increase brain GST and liver ALP activities. Different responses in biomarkers were observed in mixtures treatments, reflecting the complex interactions between these toxics and suggesting a suppressive action of RM on CF effects. Since the concentrations we tested are environmentally relevant and the overall fish health condition was affected, the presence of these pesticides in freshwater systems could impose a risk for populations by causing deleterious effects on *C. decemmaculatus* in Pampean region.

Casabe, N., et al. (2007). "Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field." Journal of Soils and Sediments **7**: 232-239.

Background, Aim and Scope. Continuous application of pesticides may pollute soils and affect non-target organisms. Soil is a complex ecosystem; its components can modulate the effects of

pesticides. Therefore, it is recommended to evaluate the potential environmental risk of these compounds in local conditions. We performed an integrated field-laboratory study on an Argentine soya field sprayed with glyphosate and chlorpyrifos under controlled conditions. Our aim was to compare the sensitivity of a series of endpoints for the assessment of adverse effects of the extensive use of these agrochemicals. Materials and Methods. A RR soya field in a traditional farming area of Argentina was sprayed with glyphosate (GLY) or chlorpyrifos (CPF) formulations at the commercially recommended rates, according to a randomized complete block design with 3 replicates. In laboratory assays, Eisenia fetida andrei were exposed to soil samples (0-10 cm depth) collected between the rows of soya. Endpoints linked to behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time NRRT; DNA damage - Comet assay) were tested. Field assays included litterbag and bait-lamina tests. Physico/chemical analyses were performed on soil samples. Results. GLY reduced cocoon viability, decreasing the number of juveniles. Moreover, earthworms avoided soils treated with GLY. No effects on either reproduction or on avoidance were observed at the very low CPF concentration measured in the soils sampled 10 days after treatment. Both pesticides caused a reduction in the feeding activity under laboratory and field conditions. NRRT was responsive to formulations of CPF and GLY. Comet assay showed significantly increased DNA damage in earthworms exposed to CPF treated soils. No significant differences in DNA migration were observed with GLY treated soils. Litterbag field assay showed no differences between treated and control plots. Discussion. The ecotoxicological effects of pesticides can be assessed by monitoring the status of communities in real ecosystems or through the use of laboratory toxicity tests. Litterbag field test showed no influence of the treatments on the organic matter breakdown, suggesting a scarce contribution of soil macrofauna. The bait-lamina test, however, seemed to be useful for detecting the effects of GLY and CPF treatments on the activity of the soil fauna. CPF failed to give significant differences with the controls in the reproduction test and the results were not conclusive in the avoidance test. Although the field population density of earthworms could be affected by multiple factors, the effects observed on the reproduction and avoidance tests caused by GLY could contribute to its decrease, with the subsequent loss of their beneficial functions. Biomarkers measuring effects on sub-organism level could be useful to predict adverse effects on soil organisms and populations. Among them, NRRT, a lysosomal destabilization biomarker, resulted in demonstrating more sensitivity than the reproduction and avoidance tests. The Comet assay was responsive only to CPE Since DNA damage can have severe consequences on populations, it could be regarded as an important indicator to be used in the assessment of soil health. Conclusions. Reproduction and avoidance tests were sensitive indicators of GLY exposure, with the former being more labor intensive. Bait-lamina test was sensitive to both CPF and GLY. NRRT and Comet assays revealed alterations at a subcellular level, and could be considered complementary to the biological activity tests. Because of their simplicity, some of these bioassays seemed to be appropriate pre-screening tests, prior to more extensive and invasive testing. Recommendations and Perspectives. This study showed deleterious effects of GLY and CPF formulations when applied at the nominal concentrations recommended for soya crops. Further validation is needed before these endpoints could be used as field monitoring tools in Argentine soya soils (ecotoxicological risk assessment - ERA tools).

Colborn, T. (2006). "A case for revisiting the safety of pesticides: A closer look at neurodevelopment." Environmental Health Perspectives **114**: 10-17.

> The quality and quantity of the data about the risk posed to humans by individual pesticides vary considerably. Unlike obvious birth defects, most developmental effects cannot be seen at birth or even later in life. Instead, brain and nervous system disturbances are expressed in terms of how an individual behaves and functions, which can vary considerably from birth through adulthood. In this article I challenge the protective value of current pesticide risk assessment strategies in light of the vast numbers of pesticides on the market and the vast number of possible target tissues and end points that often differ depending upon timing of exposure. Using the insecticide chlorpyrifos as a model, I reinforce the need for a new approach to determine the safety of all pesticide classes. Because of the uncertainty that will continue to exist about the safety of pesticides, it is apparent that a new regulatory approach to protect human health is needed.

Colt, J. S., et al. (2004). "Comparison of pesticide levels in carpet dust and self-reported pest treatment practices in four US sites." Journal of Exposure Analysis and Environmental Epidemiology **14**(1): 74-83.

> Epidemiologic studies have used both questionnaires and carpet dust sampling to assess residential exposure to pesticides. The consistency of the information provided by these two approaches has not been explored. In a population-based case-control study of non-Hodgkin's lymphoma, carpet dust samples were collected from the homes of 513 control subjects in Detroit, Iowa, Los Angeles, and Seattle. The samples were taken from used vacuum cleaner bags and analyzed for 30 pesticides. Interviewers queried subjects about the types of pests treated in their home using a detailed questionnaire accompanied by visual aids. Geographic variations in pesticide levels were generally consistent with geographic differences in pest treatment practices. Los Angeles residents reported the most treatment for crawling insects, fleas/ticks, and termites, and Los Angeles dust samples had the highest levels of propoxur, chlorpyrifos, diazinon, permethrin, and chlordane. Iowa had the most treatment for lawn/garden weeds, and also the highest levels of 2,4-dichlorophenoxyacetic acid and dicamba. Although Seattle had the highest proportion of subjects treating for lawn/garden insects, the lawn/garden insecticides were higher in other sites. Multivariate linear regression revealed several significant associations between the type of pest treated and dust levels of specific pesticides. The strongest associations were between termite treatment and chlordane, and flea/tick treatment and permethrin. Most of the significant associations were consistent with known uses of the pesticides; few expected associations were absent. The consistency between the questionnaire data and pesticide residues measured in dust lends credibility to both methods for assessing residential exposure to pesticides. The combined techniques appear promising for epidemiologic studies. Interviewing is the only way to assess pesticide exposures before current carpets were in place. Dust sampling provides an objective measure of specific compounds to which a person may have been exposed through personal use of a pesticide or by drift-in or track-in from outside, and avoids recall bias.

Crane, a. L., et al. (2013). "Longitudinal assessment of chlorpyrifos exposure and effect biomarkers in adolescent Egyptian agricultural workers." Journal of Exposure Science and Environmental Epidemiology **8**: 23-24.

> Chlorpyrifos (CPF) is applied seasonally in Egypt by adolescent agricultural workers and the extent of occupational exposure and the potential for environmental CPF exposure in this population is poorly understood. Adolescent pesticide applicators (n=57; 12-21 years of age) and age-matched non-applicators (n=38) from the same villages were followed for 10 months in 2010, spanning pre-application through post-application. Eight urine and five blood samples were collected from participants within this time period. Blood acetylcholinesterase and butyrylcholinesterase (BChE; exposure/effect biomarker) and urine 3,5,6-trichloro-2-pyridinol (TCPy; exposure biomarker) were used to assess occupational CPF exposures in pesticide applicators and environmental exposures in non-applicators. Applicators demonstrated significantly higher TCPy concentration and BChE depression than non-applicators throughout CPF application. This difference persisted for 4-7 weeks after the cessation of agricultural spraying. However, both groups exhibited significantly elevated TCPy and depressed BChE, compared with their respective baseline. The peak TCPy levels during the spray season (95% confidence interval (CI)) for non-applicators and applicators reached 16.8 (9.87-28.5) and 137 (57.4-329) ug/g creatinine, respectively. BChE levels (95% CIs) during the spray were as follows: 1.47 (1.28-1.68) for non-applicators and 0.47 (0.24-0.94) U/ml for applicators. The longitudinal assessment of CPF biomarkers provided robust measures of exposure and effect throughout CPF application in adolescents and revealed significant exposures in both applicators and non-applicators. Biomarker data in the non-applicators, which mirrored that of the applicators, indicated that non-applicators received environmental CPF exposures. This suggests that similar exposures may occur in other residents of this region during periods of pesticide application.Journal of Exposure Science and Environmental Epidemiology advance online publication, 16 January 2013; doi:10.1038/jes.2012.113.

Curwin, B. D., et al. (2007). "Pesticide dose estimates for children of Iowa farmers and non-farmers." Environmental Research **105**: 307-315.

> Farm children have the potential to be exposed to pesticides. Biological monitoring is often employed to assess this exposure; however, the significance of the exposure is uncertain unless doses are estimated. In the spring and summer of 2001, 118 children (66 farm, 52 non-farm) of Iowa farm and non-farm households were recruited to participate in a study investigating potential take-home pesticide exposure. Each child provided an evening and morning urine sample at two visits spaced approximately 1 month apart, with the first sample collection taken within a few days after pesticide application. Estimated doses were calculated for atrazine, metolachlor, chlorpyrifos, and glyphosate from urinary metabolite concentrations derived from the spot urine samples and compared to EPA reference doses. For all pesticides except glyphosate, the doses from farm children were higher than doses from the non-farm children. The difference was statistically significant for atrazine ($p<0.0001$) but only marginally significant for chlorpyrifos and metolachlor ($p=0.07$ and 0.1, respectively). Among farm children, geometric mean doses were higher for children on farms where a particular pesticide was applied compared to farms where that pesticide was not applied for all pesticides except glyphosate; results were significant for atrazine ($p=0.030$) and metolachlor ($p=0.042$), and marginally

significant for chlorpyrifos (p=0.057). The highest estimated doses for atrazine, chlorpyrifos, metolachlor, and glyphosate were 0.085, 1.96, 3.16, and 0.34 µg/kg/day, respectively. None of the doses exceeded any of the EPA reference values for atrazine, metolachlor, and glyphosate; however, all of the doses for chlorpyrifos exceeded the EPA chronic population adjusted reference value. Doses were similar for male and female children. A trend of decreasing dose with increasing age was observed for chlorpyrifos.

Engel, S. M., et al. (2011). "Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood." Environmental Health Perspectives **119**: 1182-1188.

BACKGROUND: Prenatal exposure to organophosphate pesticides has been shown to negatively affect child neurobehavioral development. Paraoxonase 1 (PON1) is a key enzyme in the metabolism of organophosphates. OBJECTIVE: We examined the relationship between biomarkers of organophosphate exposure, PON1, and cognitive development at ages 12 and 24 months and 6-9 years. METHODS: The Mount Sinai Children's Environmental Health Study enrolled a multiethnic prenatal population in New York City between 1998 and 2002 (n = 404). Third-trimester maternal urine samples were collected and analyzed for organophosphate metabolites (n = 360). Prenatal maternal blood was analyzed for PON1 activity and genotype. Children returned for neurodevelopment assessments ages 12 months (n = 200), 24 months (n = 276), and 6-9 (n = 169) years of age. RESULTS: Prenatal total dialkylphosphate metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanics. These associations appeared to be enhanced among children of mothers who carried the PON1 Q192R QR/RR genotype. In later childhood, increasing prenatal total dialkyl- and dimethylphosphate metabolites were associated with decrements in perceptual reasoning in the maternal PON1 Q192R QQ genotype, which imparts slow catalytic activity for chlorpyrifos oxon, with a monotonic trend consistent with greater decrements with increasing prenatal exposure. CONCLUSION: Our findings suggest that prenatal exposure to organophosphates is negatively associated with cognitive development, particularly perceptual reasoning, with evidence of effects beginning at 12 months and continuing through early childhood. PON1 may be an important susceptibility factor for these deleterious effects.

Ferre, D. M., et al. (2018). "Potential risks of dietary exposure to chlorpyrifos and cypermethrin from their use in fruit/vegetable crops and beef cattle productions." Environmental Monitoring and Assessment **190**(5): 292.

The active ingredients (a.i.) used as pesticides vary across regions. Diet represents the main source of chronic exposure to these chemicals. The aim of this study was to look at the pesticides applied in fruit, vegetable, and beef cattle productions in Mendoza (Argentina), to identify those that were simultaneously used by the three production systems. Local individuals (n = 160), involved in these productions, were interviewed. Glyphosate was the a.i. most often used by fruit-vegetable producers, and ivermectin by beef cattle producers. Chlorpyrifos (CPF) and cypermethrin (CYP) were the only a.i. used by the three production systems. The survey revealed that CPF, CYP, alpha CYP, and CPF+CYP were used by 22, 16, 4, and 20% of the fruit and vegetable producers, respectively. Regarding beef cattle, CYP was used by 90% of producers, CYP + CPF formulation by 8%, and alpha CYP by 2%. The second approach of this study was to

search the occurrence of CYP and CPF residues in food commodities analyzed under the National Plan for Residue Control (2012-2015). CYP residues found above the LOD were reported in 4.0% and CPF in 13.4% of the vegetable samples tested, as well as in 1.2 and 28.8%, respectively, of the fruit samples tested. Regarding beef cattle, CYP residues were reported in 2.3% and organophosphates (as a general pesticide class) in 13.5% of samples tested. In conclusion, consumers may be exposed simultaneously to CPF and CYP, from fruits, vegetables, and beef intake. Accordingly, the policy for pesticide residues in food and human risk assessment should account for the combined exposure to CPF and CYP. Moreover, appropriate toxicological studies of this mixture (including genotoxicity) are warranted.

Furlong, C. E., et al. (2006). "PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity." Pharmacogenetics and genomics **16**: 183-190.

The objective was to determine PON1 status as a predictor for organophosphorus insecticide sensitivity in a cohort of Latina mothers and newborns from the Salinas Valley, California, an area with high levels of organophosphorus insecticide use. PON1 status was established for 130 pregnant Latina women and their newborns using a high-throughput two substrate activity/analysis method which plots rates of diazoxon (DZO) hydrolysis against rates of paraoxon (PO) hydrolysis. Arylesterase activity (AREase) was determined using phenylacetate as a substrate, allowing comparison of PON1 levels across PON1192 genotypes in mothers and children. Phenylacetate hydrolysis is not affected by the Q192R polymorphism. Among newborns, levels of PON1 (AREase) varied by 26-fold (4.3-110.7 U/ml) and among mothers by 14-fold (19.8-281.4 U/ml). On average, children's PON1 levels were four-fold lower than the mothers' PON1 levels ($P<0.001$). Average PON1 levels in newborns were comparable with reported hPON1 levels in transgenic mice expressing human PON1Q192 or PON1R192, allowing for prediction of relative sensitivity to chlorpyrifos oxon (CPO) and DZO. The predicted range of variability in sensitivity of mothers and children in the same Latino cohort was 65-fold for DZO and 131 to 164-fold for CPO. Overall, these findings indicate that many of the newborns and some of the mothers in this cohort would be more susceptible to the adverse effects of specific organophosphorus pesticide exposure due to their PON1 status. Of particular concern are exposures of pregnant mothers and newborns with low PON1 status.

Grandjean, P. and P. J. Landrigan (2014). "Neurobehavioural effects of developmental toxicity." The Lancet Neurology **13**: 330-338.

Neurodevelopmental disabilities, including autism, attention-deficit hyperactivity disorder, dyslexia, and other cognitive impairments, affect millions of children worldwide, and some diagnoses seem to be increasing in frequency. Industrial chemicals that injure the developing brain are among the known causes for this rise in prevalence. In 2006, we did a systematic review and identified five industrial chemicals as developmental neurotoxicants: lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene. Since 2006, epidemiological studies have documented six additional developmental neurotoxicants-manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and the polybrominated diphenyl ethers. We postulate that even more neurotoxicants remain undiscovered. To control the pandemic of developmental neurotoxicity, we propose a global prevention strategy.

Untested chemicals should not be presumed to be safe to brain development, and chemicals in existing use and all new chemicals must therefore be tested for developmental neurotoxicity. To coordinate these efforts and to accelerate translation of science into prevention, we propose the urgent formation of a new international clearinghouse. © 2014 Elsevier Ltd.

Greenlee, A. R., et al. (2004). "Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos." Environ Health Perspect **112**(6): 703-709.

Occupational exposures to pesticides may increase parental risk of infertility and adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, and congenital anomalies. Less is known about residential use of pesticides and the risks they pose to reproduction and development. In the present study we evaluate environmentally relevant, low-dose exposures to agrochemicals and lawn-care pesticides for their direct effects on mouse preimplantation embryo development, a period corresponding to the first 5-7 days after human conception. Agents tested were those commonly used in the upper midwestern United States, including six herbicides [atrazine, dicamba, metolachlor, 2,4-dichlorophenoxyacetic acid (2,4-D)], pendimethalin, and mecoprop), three insecticides (chlorpyrifos, terbufos, and permethrin), two fungicides (chlorothalonil and mancozeb), a desiccant (diquat), and a fertilizer (ammonium nitrate). Groups of 20-25 embryos were incubated 96 hr in vitro with either individual chemicals or mixtures of chemicals simulating exposures encountered by handling pesticides, inhaling drift, or ingesting contaminated groundwater. Incubating embryos with individual pesticides increased the percentage of apoptosis (cell death) for 11 of 13 chemicals (p <or= 0.05) and reduced development to blastocyst and mean cell number per embryo for 3 of 13 agents (p <or= 0.05). Mixtures simulating preemergent herbicides, postemergent herbicides, and fungicides increased the percentage of apoptosis in exposed embryos (p <or= 0.05). Mixtures simulating groundwater contaminants, insecticide formulation, and lawn-care herbicides reduced development to blastocyst and mean cell number per embryo (p <or= 0.05). Our data demonstrate that pesticide-induced injury can occur very early in development, with a variety of agents, and at concentrations assumed to be without adverse health consequences for humans.

Horton, M. K., et al. (2012). "Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory?" Neurotoxicology and Teratology **34**: 534-541.

Prenatal exposure to chlorpyrifos (CPF), an organophosphorus insecticide, has long been associated with delayed neurocognitive development and most recently with decrements in working memory at age 7. In the current paper, we expanded the previous work on CPF to investigate how additional biological and social environmental factors might create or explain differential neurodevelopmental susceptibility, focusing on main and moderating effects of the quality of the home environment (HOME) and child sex. We evaluate how the quality of the home environment (specifically, parental nurturance and environmental stimulation) and child sex interact with the adverse effects of prenatal CPF exposure on working memory at child age 7. years. We did not observe a remediating effect of a high quality home environment (either parental nurturance or environmental stimulation) on the adverse effects of prenatal CPF exposure on working memory. However, we detected a borderline significant interaction

between prenatal exposure to CPF and child sex (B (95% CI) for interaction term = -1.714 (-3.753 to 0.326)) suggesting males experience a greater decrement in working memory than females following prenatal CPF exposure. In addition, we detected a borderline interaction between parental nurturance and child sex (B (95% CI) for interaction term =1.490 (-0.518 to 3.499)) suggesting that, in terms of working memory, males benefit more from a nurturing environment than females. To our knowledge, this is the first investigation into factors that may inform an intervention strategy to reduce or reverse the cognitive deficits resulting from prenatal CPF exposure. ?? 2012 Elsevier Inc.

Lu, C., et al. (2008). "Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children." Environmental Health Perspectives **116**: 537-542.

BACKGROUND: The widespread use of organophosphorus (OP) pesticides has led to frequent exposure in adults and children. Because such exposure may cause adverse health effects, particularly in children, the sources and patterns of exposure need to be studied further. OBJECTIVES: We assessed young urban/suburban children's longitudinal exposure to OP pesticides in the Children's Pesticide Exposure Study (CPES) conducted in the greater Seattle, Washington, area, and used a novel study design that allowed us to determine the contribution of dietary intake to the overall OP pesticide exposure. METHODS: Twenty-three children 3-11 years of age who consumed only conventional diets were recruited for this 1-year study conducted in 2003-2004. Children switched to organic diets for 5 consecutive days in the summer and fall sampling seasons. We measured specific urinary metabolites for malathion, chlorpyrifos, and other OP pesticides in urine samples collected twice daily for a period of 7, 12, or 15 consecutive days during each of the four seasons. RESULTS: By substituting organic fresh fruits and vegetables for corresponding conventional food items, the median urinary metabolite concentrations were reduced to nondetected or close to non-detected levels for malathion and chlorpyrifos at the end of the 5-day organic diet intervention period in both summer and fall seasons. We also observed a seasonal effect on the OP urinary metabolite concentrations, and this seasonality corresponds to the consumption of fresh produce throughout the year. CONCLUSIONS: The findings from this study demonstrate that dietary intake of OP pesticides represents the major source of exposure in young children.

Lu, C., et al. (2006). "Organic diets significantly lower children's dietary exposure to organophosphorus pesticides." Environmental Health Perspectives **114**: 260-263.

We used a novel study design to measure dietary organophosphorus pesticide exposure in a group of 23 elementary school-age children through urinary biomonitoring. We substituted most of children's conventional diets with organic food items for 5 consecutive days and collected two spot daily urine samples, first-morning and before-bedtime voids, throughout the 15-day study period. We found that the median urinary concentrations of the specific metabolites for malathion and chlorpyrifos decreased to the nondetect levels immediately after the introduction of organic diets and remained nondetectable until the conventional diets were reintroduced. The median concentrations for other organophosphorus pesticide metabolites were also lower in the organic diet consumption days; however, the detection of those metabolites was not frequent enough to show any statistical significance. In conclusion, we

were able to demonstrate that an organic diet provides a dramatic and immediate protective effect against exposures to organophosphorus pesticides that are commonly used in agricultural production. We also concluded that these children were most likely exposed to these organophosphorus pesticides exclusively through their diet. To our knowledge, this is the first study to employ a longitudinal design with a dietary intervention to assess children's exposure to pesticides. It provides new and persuasive evidence of the effectiveness of this intervention.

Perera, F. P., et al. (2005). "A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures." NeuroToxicology **26**: 573-587.

Inner-city minority populations are high-risk groups for adverse birth outcomes and also more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), benzo[a]pyrene B[a]P, other ambient polycyclic aromatic hydrocarbons (global PAHs), and residential pesticides. The Columbia Center for Children's Environmental Health (CCCEH) is conducting a prospective cohort study of 700 northern Manhattan pregnant women and newborns to examine the effects of prenatal exposure to these common toxicants on fetal growth, early neurodevelopment, and respiratory health. This paper summarizes results of three published studies demonstrating the effects of prenatal ETS, PAH, and pesticides on birth outcomes and/or neurocognitive development [Perera FP, Rauh V, Whyatt RM, Tsai WY, Bernert JT, Tu YH, et al. Molecular evidence of an interaction between prenatal environment exposures on birth outcomes in a multiethnic population. Environ Health Perspect 2004;12:630-62; Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicol Teratol 2004;26:373-85; Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, et al. Prenatal insecticide exposures, birth weight and length among an urban minority cohort. Environ Health Perspect, in press]. To evaluate the effects of prenatal exposure to ETS, PAHs, and pesticides, researchers analyzed questionnaire data, cord blood plasma (including biomarkers of ETS and pesticide exposure), and B[a]P-DNA adducts (a molecular dosimeter of PAHs). Self-reported ETS was associated with decreased head circumference (P = 0.04), and there was a significant interaction between ETS and adducts such that combined exposure had a significant multiplicative effect on birth weight (P = 0.04) and head circumference (P = 0.01) after adjusting for confounders. A second analysis examined the neurotoxic effects of prenatal ETS exposure and postpartum material hardship (unmet basic needs in the areas of food, housing, and clothing) on 2-year cognitive development. Both exposures depressed cognitive development (P < 0.05), and there was a significant interaction such that children with exposure to both ETS and material hardship exhibited the greatest cognitive deficit (7.1 points). A third analysis found that 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). These results underscore the importance of policies that reduce exposure to ETS, air pollution, and pesticides with potentially adverse effects on fetal growth and child neurodevelopment. ?? 2004 Elsevier Inc. All rights reserved.

Quirós-Alcalá, L., et al. (2012). "Organophosphorous pesticide breakdown products in house dust and children's urine." Journal of Exposure Science and Environmental Epidemiology **22**: 559-568.

Human exposure to preformed dialkylphosphates (DAPs) in food or the environment may affect the reliability of DAP urinary metabolites as biomarkers of organophosphate (OP) pesticide exposure. We conducted a study to investigate the presence of DAPs in indoor residential environments and their association with children's urinary DAP levels. We collected dust samples from homes in farmworker and urban communities (40 homes total, n=79 samples) and up to two urine samples from resident children ages 3-6 years. We measured six DAPs in all samples and eight DAP-devolving OP pesticides in a subset of dust samples (n=54). DAPs were detected in dust with diethylphosphate (DEP) being the most frequently detected (≥60%); detection frequencies for other DAPs were ≤50%. DEP dust concentrations did not significantly differ between communities, nor were concentrations significantly correlated with concentrations of chlorpyrifos and diazinon, the most frequently detected diethyl-OP pesticides (Spearman ρ=-0.41 to 0.38, P>0.05). Detection of DEP, chlorpyrifos, or diazinon, was not associated with DEP and/or DEP+diethylthiophosphate detection in urine (Kappa coefficients=-0.33 to 0.16). Finally, estimated non-dietary ingestion intake from DEP in dust was found to be ≤5% of the dose calculated from DEP levels in urine, suggesting that ingestion of dust is not a significant source of DAPs in urine if they are excreted unchanged.

Rauh, V., et al. (2011). "Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide." Environmental Health Perspectives **119**: 1196-1201.

In a longitudinal birth cohort study of inner-city mothers and children (Columbia Center for Children's Environmental Health), we have previously reported that prenatal exposure to chlorpyrifos (CPF) was associated with neurodevelopmental problems at 3 years of age.

Rauh, V. a., et al. (2006). "Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children." Pediatrics **118**: e1845-e1859.

OBJECTIVE: The purpose of this study was to investigate the impact of prenatal exposure to chlorpyrifos on 3-year neurodevelopment and behavior in a sample of inner-city minority children. METHODS: As part of an ongoing prospective cohort study in an inner-city minority population, neurotoxicant effects of prenatal exposure to chlorpyrifos were evaluated in 254 children through the first 3 years of life. This report examined cognitive and motor development at 12, 24, and 36 months (measured with the Bayley Scales of Infant Development II) and child behavior at 36 months (measured with the Child Behavior Checklist) as a function of chlorpyrifos levels in umbilical cord plasma. RESULTS: Highly exposed children (chlorpyrifos levels of >6.17 pg/g plasma) scored, on average, 6.5 points lower on the Bayley Psychomotor Development Index and 3.3 points lower on the Bayley Mental Development Index at 3 years of age compared with those with lower levels of exposure. Children exposed to higher, compared with lower, chlorpyrifos levels were also significantly more likely to experience Psychomotor Development Index and Mental Development Index delays, attention problems, attention-deficit/hyperactivity disorder problems, and pervasive developmental disorder problems at 3 years of age. CONCLUSIONS: 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.

Shelton, J. F., et al. (2014). "Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study." Environ Health Perspect **122**(10): 1103-1109.

BACKGROUND: Gestational exposure to several common agricultural pesticides can induce developmental neurotoxicity in humans, and has been associated with developmental delay and autism. OBJECTIVES: We evaluated whether residential proximity to agricultural pesticides during pregnancy is associated with autism spectrum disorders (ASD) or developmental delay (DD) in the Childhood Autism Risks from Genetics and Environment (CHARGE) study. METHODS: The CHARGE study is a population-based case-control study of ASD, DD, and typical development. For 970 participants, commercial pesticide application data from the California Pesticide Use Report (1997-2008) were linked to the addresses during pregnancy. Pounds of active ingredient applied for organophophates, organochlorines, pyrethroids, and carbamates were aggregated within 1.25-km, 1.5-km, and 1.75-km buffer distances from the home. Multinomial logistic regression was used to estimate the odds ratio (OR) of exposure comparing confirmed cases of ASD (n = 486) or DD (n = 168) with typically developing referents (n = 316). RESULTS: Approximately one-third of CHARGE study mothers lived, during pregnancy, within 1.5 km (just under 1 mile) of an agricultural pesticide application. Proximity to organophosphates at some point during gestation was associated with a 60% increased risk for ASD, higher for third-trimester exposures (OR = 2.0; 95% CI: 1.1, 3.6), and second-trimester chlorpyrifos applications (OR = 3.3; 95% CI: 1.5, 7.4). Children of mothers residing near pyrethroid insecticide applications just before conception or during third trimester were at greater risk for both ASD and DD, with ORs ranging from 1.7 to 2.3. Risk for DD was increased in those near carbamate applications, but no specific vulnerable period was identified. CONCLUSIONS: This study of ASD strengthens the evidence linking neurodevelopmental disorders with gestational pesticide exposures, particularly organophosphates, and provides novel results of ASD and DD associations with, respectively, pyrethroids and carbamates.

Shelton, J. F., et al. (2014). "Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The CHARGE Study." Environmental Health Perspectives: 1-4.

BACKGROUND: Gestational exposure to several common agricultural pesticides can induce developmental neurotoxicity in humans, and has been associated with developmental delay and autism.OBJECTIVES: To evaluate whether residential proximity to agricultural pesticides during pregnancy is associated with autism spectrum disorders (ASD) or developmental delay (DD) in the Childhood Autism Risks from Genetics and Environment (CHARGE) Study.METHODS: The CHARGE study is a population-based case-control study of ASD, developmental delay (DD), and typical development. For 970 participants, commercial pesticide application data from the California Pesticide Use Report (1997-2008) were linked to the addresses during pregnancy. Pounds of active ingredient applied for organophophates, organochlorines, pyrethroids, and carbamates were aggregated within 1.25km, 1.5km, and 1.75km buffer distances from the home. Multinomial logistic regression was used to estimate the odds ratio (OR) of exposure comparing confirmed cases of ASD (n = 486) or DD (n = 168) with typically developing referents

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Vera-Candioti, J., et al. (2013). "Single-cell gel electrophoresis assay in the ten spotted live-bearer fish, *Cnesterodon decemmaculatus* (Jenyns, 1842), as bioassay for agrochemical-induced genotoxicity." Ecotoxicology and environmental safety **98**: 368-373.

The ability of two 48 percent chlorpyrifos-based insecticides (Lorsban* 48E(R) and CPF Zamba(R)), two 50 percent pirimicarb-based insecticides (Aficida(R) and Patton Flow(R)), and two 48 percent glyphosate-based herbicides (Panzer(R) and Credit(R)) to induce DNA single-strand breaks in peripheral blood erythrocytes of *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces, Poeciliidae) exposed under laboratory conditions was evaluated by the single-cell gel electrophoresis (SCGE) assay. In those fish exposed to Lorsban* 48E(R), CPF Zamba(R), Aficida(R), Patton Flow(R), Credit(R), and Panzer(R), a significant increase of the genetic damage was observed for all formulations regardless of the harvesting time. This genotoxic effect was achieved by an enhancement of Type II-IV comets and a concomitant decrease of Type 0-I comets over control values. A regression analysis revealed that the damage varied as a negative function of the exposure time in those Lorsban* 48E(R)- and Aficida(R)-treated fish. On the other hand, a positive correlation between damage increase and exposure time was achieved after Patton Flow(R) and Credit(R) treatment. Finally, no correlation was observed between increase in the genetic damage and exposure time after treatment with CPF Zamba(R) or Panzer(R). These results highlight that all agrochemicals inflict primary genotoxic damage at the DNA level at sublethal concentrations, regardless of the exposure time of the aquatic organisms under study, at least within a period of 96 h of treatment.

Vogt, R., et al. (2012). "Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: a risk assessment." Environmental health : a global access science source **11**: 83.

BACKGROUND: In the absence of current cumulative dietary exposure assessments, this analysis was conducted to estimate exposure to multiple dietary contaminants for children, who are more vulnerable to toxic exposure than adults. METHODS: We estimated exposure to multiple food contaminants based on dietary data from preschool-age children (2-4 years, n=207), school-age children (5-7 years, n=157), parents of young children (n=446), and older adults (n=149). We compared exposure estimates for eleven toxic compounds (acrylamide, arsenic,

lead, mercury, chlorpyrifos, permethrin, endosulfan, dieldrin, chlordane, DDE, and dioxin) based on self-reported food frequency data by age group. To determine if cancer and non-cancer benchmark levels were exceeded, chemical levels in food were derived from publicly available databases including the Total Diet Study. RESULTS: Cancer benchmark levels were exceeded by all children (100%) for arsenic, dieldrin, DDE, and dioxins. Non-cancer benchmarks were exceeded by >95% of preschool-age children for acrylamide and by 10% of preschool-age children for mercury. Preschool-age children had significantly higher estimated intakes of 6 of 11 compounds compared to school-age children ($p<0.0001$ to $p=0.02$). Based on self-reported dietary data, the greatest exposure to pesticides from foods included in this analysis were tomatoes, peaches, apples, peppers, grapes, lettuce, broccoli, strawberries, spinach, dairy, pears, green beans, and celery. CONCLUSIONS: Dietary strategies to reduce exposure to toxic compounds for which cancer and non-cancer benchmarks are exceeded by children vary by compound. These strategies include consuming organically produced dairy and selected fruits and vegetables to reduce pesticide intake, consuming less animal foods (meat, dairy, and fish) to reduce intake of persistent organic pollutants and metals, and consuming lower quantities of chips, cereal, crackers, and other processed carbohydrate foods to reduce acrylamide intake.

Whyatt, R. M., et al. (2004). "Prenatal insecticide exposures and birth weight and length among an urban minority cohort." Environmental Health Perspectives **112**: 1125-1132.

We reported previously that insecticide exposures were widespread among minority women in New York City during pregnancy and that levels of the organophosphate chlorpyrifos in umbilical cord plasma were inversely associated with birth weight and length. Here we expand analyses to include additional insecticides (the organophosphate diazinon and the carbamate propoxur), a larger sample size (n = 314 mother-newborn pairs), and insecticide measurements in maternal personal air during pregnancy as well as in umbilical cord plasma at delivery. Controlling for potential confounders, we found no association between maternal personal air insecticide levels and birth weight, length, or head circumference. For each log unit increase in cord plasma chlorpyrifos levels, birth weight decreased by 42.6 g [95% confidence interval (CI), -81.8 to -3.8, p = 0.03] and birth length decreased by 0.24 cm (95% CI, -0.47 to -0.01, p = 0.04). Combined measures of (ln)cord plasma chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length ($p < 0.05$). Birth weight averaged 186.3 g less (95% CI, -375.2 to -45.5) among newborns with the highest compared with lowest 26% of exposure levels (p = 0.01). Further, the associations between birth weight and length and cord plasma chlorpyrifos and diazinon were highly significant ($p <$ or = 0.007) among newborns born before the 2000-2001 U.S. Environmental Protection Agency's regulatory actions to phase out residential use of these insecticides. Among newborns born after January 2001, exposure levels were substantially lower, and no association with fetal growth was apparent ($p > 0.8$). The propoxur metabolite 2-isopropoxyphenol in cord plasma was inversely associated with birth length, a finding of borderline significance (p = 0.05) after controlling for chlorpyrifos and diazinon. Results indicate that prenatal chlorpyrifos exposures have impaired fetal growth among this minority cohort and that diazinon exposures may have contributed to the effects. Findings support recent regulatory action to phase out residential uses of the insecticides.

Yang, D., et al. (2008). "Chlorpyrifos and chlorpyrifos-oxon inhibit axonal growth by interfering with the morphogenic activity of acetylcholinesterase." Toxicology and Applied Pharmacology **228**: 32-41.

A primary role of acetylcholinesterase (AChE) is regulation of cholinergic neurotransmission by hydrolysis of synaptic acetylcholine. In the developing nervous system, however, AChE also functions as a morphogenic factor to promote axonal growth. This raises the question of whether organophosphorus pesticides (OPs) that are known to selectively bind to and inactivate the enzymatic function of AChE also interfere with its morphogenic function to perturb axonogenesis. To test this hypothesis, we exposed primary cultures of sensory neurons derived from embryonic rat dorsal root ganglia (DRG) to chlorpyrifos (CPF) or its oxon metabolite (CPFO). Both OPs significantly decreased axonal length at concentrations that had no effect on cell viability, protein synthesis or the enzymatic activity of AChE. Comparative analyses of the effects of CPF and CPFO on axonal growth in DRG neurons cultured from AChE nullizygous (AChE-/-) versus wild type (AChE+/+) mice indicated that while these OPs inhibited axonal growth in AChE+/+ DRG neurons, they had no effect on axonal growth in AChE-/- DRG neurons. However, transfection of AChE-/- DRG neurons with cDNA encoding full-length AChE restored the wild type response to the axon inhibitory effects of OPs. These data indicate that inhibition of axonal growth by OPs requires AChE, but the mechanism involves inhibition of the morphogenic rather than enzymatic activity of AChE. These findings suggest a novel mechanism for explaining not only the functional deficits observed in children and animals following developmental exposure to OPs, but also the increased vulnerability of the developing nervous system to OPs.