CHLORPYRIFOS

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of chlorpyrifos. Chlorpyrifos (CAS# 2921-88-2) is a broad-spectrum organophosphate insecticide effective in controlling a variety of insects. Residential use of chlorpyrifos has been banned in the U.S. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, as well as on lawns and ornamental plants.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. This information was used in a screen to select appropriate chemicals for presentation to the Developmental and Reproductive Toxicant Identification Committee as possible candidates for Committee consideration. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to environmental exposures to chlorpyrifos. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Nine epidemiologic studies of chlorpyrifos reporting increased risk of adverse developmental or reproductive outcomes were identified, six of which were analytical studies of adequate quality. Four meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes were also identified. One epidemiologic study reporting no increased risk of adverse developmental or reproductive outcomes was identified.

- Twenty-one animal studies of chlorpyrifos and one meeting abstract reporting reproductive or developmental toxicity were identified, as well as three animal studies that did not report reproductive or developmental toxicity. Forty-three related articles were also identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

***Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children.**
Rauh, V. A.; Garfinkel, R.; Perera, F. P.; Andrews, H. F.; Hoepner, L.; Barr, D. B.; Whitehead, R.; Tang, D.; Whyatt, R. W.
Pediatrics.2006; 118(6):e1845-59

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.

[*]Biomarkers in assessing residential insecticide exposures during pregnancy and effects on fetal growth.
Whyatt, R. M.; Camann, D.; Perera, F. P.; Rauh, V. A.; Tang, D.; Kinney, P. L.; Garfinkel, R.; Andrews, H.; Hoepner, L.; Barr, D. B.
Toxicol Appl Pharmacol. 2005; 206(2):246-54.

The Columbia Center for Children's Environmental Health is using a combination of environmental and biologic measures to evaluate the effects of prenatal insecticide exposures among urban minorities in New York City. Of the 571 women enrolled, 85% report using some form of pest control during pregnancy and 46% report using exterminators, can sprays, and/or pest bombs. Chlorpyrifos, diazinon, and propoxur were detected in 99.7-100% of 48-h personal air samples collected from the mothers during pregnancy (n = 394) and in 39-70% of blood samples collected from the mothers (n = 326) and/or newborns (n = 341) at delivery. Maternal and newborn blood levels are similar and highly correlated (r = 0.4-08, P < 0.001). Levels of insecticides in blood samples and/or personal air samples decreased significantly following the 2000-2001 U.S. Environmental Protection Agency's regulatory actions to phase out residential use of chlorpyrifos and diazinon. Among infants born prior to 1/1/01, birth weight decreased by 67.3 g (95% confidence interval (CI) -116.6 to -17.8, P = 0.008) and birth length decreased by 0.43 centimeters (95% CI, -0.73 to -0.14, P = 0.004) for each unit increase in log-transformed cord plasma chlorpyrifos levels. Combined measures of (ln)cord plasma chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length (P < /= 0.007). Birth weight averaged 215.1 g less (95% CI -384.7 to -45.5) among those with the highest exposures compared to those without detectable levels. No association was seen between birth weight and length and cord plasma chlorpyrifos or diazinon among newborns born after 1/1/01 (P > 0.8). Results support recent regulatory action to phase out residential uses of these insecticides.

***Biomarkers in assessing residential insecticide exposures during pregnancy and effects on fetal growth.**
Whyatt RM, Camann D, Perera FP, Rauh VA, Tang D, Kinney PL, Garfinkel R, Andrews H, Hoepner L, Barr DB.
Toxicol Appl Pharmacol. 2005 Aug 7;206(2):246-54.

The Columbia Center for Children's Environmental Health is using a combination of environmental and biologic measures to evaluate the effects of prenatal insecticide exposures among urban minorities in New York City. Of the 571 women enrolled, 85% report using some form of pest control during pregnancy and 46% report using exterminators, can sprays, and/or pest bombs. Chlorpyrifos, diazinon, and propoxur were detected in 99.7-100% of 48-h personal

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

air samples collected from the mothers during pregnancy (n = 394) and in 39-70% of blood samples collected from the mothers (n = 326) and/or newborns (n = 341) at delivery. Maternal and newborn blood levels are similar and highly correlated (r = 0.4-08, P < 0.001). Levels of insecticides in blood samples and/or personal air samples decreased significantly following the 2000-2001 U.S. Environmental Protection Agency's regulatory actions to phase out residential use of chlorpyrifos and diazinon. Among infants born prior to 1/1/01, birth weight decreased by 67.3 g (95% confidence interval (CI) -116.6 to -17.8, P = 0.008) and birth length decreased by 0.43 centimeters (95% CI, -0.73 to -0.14, P = 0.004) for each unit increase in log-transformed cord plasma chlorpyrifos levels. Combined measures of (ln)cord plasma chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length (P </= 0.007). Birth weight averaged 215.1 g less (95% CI -384.7 to -45.5) among those with the highest exposures compared to those without detectable levels. No association was seen between birth weight and length and cord plasma chlorpyrifos or diazinon among newborns born after 1/1/01 (P > 0.8). Results support recent regulatory action to phase out residential uses of these insecticides.

The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality.
Meeker JD, Ryan L, Barr DB, Herrick RF, Bennett DH, Bravo R, Hauser R.
Environ Health Perspect. 2004 Dec;112(17):1665-70.

Most of the general population is exposed to carbaryl and other contemporary-use insecticides at low levels. Studies of laboratory animals, in addition to limited human data, show an association between carbaryl exposure and decreased semen quality. In the present study we explored whether environmental exposures to 1-naphthol (1N), a metabolite of carbaryl and naphthalene, and 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos-methyl, are associated with decreased semen quality in humans. Subjects (n=272) were recruited through a Massachusetts infertility clinic. Individual exposures were measured as spot urinary concentrations of 1N and TCPY adjusted using specific gravity. Semen quality was assessed as sperm concentration, percent motile sperm, and percent sperm with normal morphology, along with sperm motion parameters (straight-line velocity, curvilinear velocity, and linearity). Median TCPY and 1N concentrations were 3.22 and 3.19microg/L, respectively. For increasing 1N tertiles, adjusted odds ratios (ORs) were significantly elevated for below-reference sperm concentration (OR for low, medium, and high tertiles = 1.0, 4.2, 4.2, respectively; p-value for trend =0.01) and percent motile sperm (1.0, 2.5, 2.4; p-value for trend = 0.01). The sperm motion parameter most strongly associated with 1N was straight-line velocity. There were suggestive, borderline-significant associations for TCPY with sperm concentration and motility, whereas sperm morphology was weakly and nonsignificantly associated with both TCPY and 1N. The observed associations between altered semen quality and 1N are consistent with previous studies of carbaryl exposure, although suggestive associations with TCPY are difficult to interpret because human and animal data are currently limited.

Urinary levels of insecticide metabolites and DNA damage in human sperm.
Meeker JD, Singh NP, Ryan L, Duty SM, Barr DB, Herrick RF, Bennett DH, Hauser R.
Hum Reprod. 2004 Nov;19(11):2573-80. Epub 2004 Aug 27.

BACKGROUND: Members of the general population are exposed to non-persistent insecticides at low levels. The present study explored whether environmental exposures to carbaryl and chlorpyrifos are associated with DNA damage in human sperm. METHODS: Subjects (n=260) were recruited through a Massachusetts infertility clinic. Individual exposures were measured as spot urinary metabolite concentrations of chlorpyrifos [3,5,6-trichloro-2-pyridinol (TCPY)] and carbaryl [1-naphthol (1N)], adjusted using specific gravity. Sperm DNA integrity was assessed by neutral comet assay and reported as comet extent, percentage DNA in comet tail (Tail%) and tail distributed moment (TDM). RESULTS: A statistically significant increase in Tail% was found for an interquartile range (IQR) increase in both 1N [coefficient=4.1; 95% confidence interval (CI) 1.9-6.3] and TCPY (2.8; 0.9-4.6), while a decrease in TDM was associated with IQR changes in 1N (-2.2; -4.9 to 0.5) and TCPY (-2.5; -4.7 to -0.2). A negative correlation between Tail% and TDM was present only when stratified by comet extent, suggesting that Tail% and TDM may measure different types of DNA damage within comet extent strata. CONCLUSIONS: Environmental exposure to carbaryl and chlorpyrifos may be associated with increased DNA damage in human sperm, as indicated by a change in comet assay parameters.

A related article with similar findings (Whyatt et al., 2005), is presented above.

***Prenatal insecticide exposures and birth weight and length among an urban minority cohort.**
Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP.
Environ Health Perspect. 2004 Jul;112(10):1125-32.

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

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

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.

*** In utero pesticide exposure, maternal paraoxonase activity, and head circumference**.
Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, Holzman IR, Wolff MS.
Environ Health Perspect. 2004 Mar;112(3):388-91.

Although the use of pesticides in inner-city homes of the United States is of considerable magnitude, little is known about the potentially adverse health effects of such exposure. Recent animal data suggest that exposure to pesticides during pregnancy and early life may impair growth and neurodevelopment in the offspring. To investigate the relationship among prenatal pesticide exposure, paraoxonase (PON1) polymorphisms and enzyme activity, and infant growth and neurodevelopment, we are conducting a prospective, multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City. In this report we evaluate the effects of pesticide exposure on birth weight, length, head circumference, and gestational age among 404 births between May 1998 and May 2002. Pesticide exposure was assessed by a prenatal questionnaire administered to the mothers during the early third trimester as well as by analysis of maternal urinary pentachlorophenol levels and maternal metabolites of chlorpyrifos and pyrethroids. Neither the questionnaire data nor the pesticide metabolite levels were associated with any of the fetal growth indices or gestational age. However, when the level of maternal PON1 activity was taken into account, maternal levels of chlorpyrifos above the limit of detection coupled with low maternal PON1 activity were associated with a significant but small reduction in head circumference. In addition, maternal PON1 levels alone, but not PON1 genetic polymorphisms, were associated with reduced head size. Because small head size has been found to be predictive of subsequent cognitive ability, these data suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity.

---

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

*** Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population.**
Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu YH, Diaz D, Dietrich J, Whyatt RM.
Environ Health Perspect. 2003 Feb;111(2):201-5.

Inner-city, minority populations are high-risk groups for adverse birth outcomes and also are more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), polycyclic aromatic hydrocarbons (PAHs), and pesticides. In a sample of 263 nonsmoking African-American and Dominican women, we evaluated the effects on birth outcomes of prenatal exposure to airborne PAHs monitored during pregnancy by personal air sampling, along with ETS estimated by plasma cotinine, and an organophosphate pesticide (OP) estimated by plasma chlorpyrifos (CPF). Plasma CPF was used as a covariate because it was the most often detected in plasma and was highly correlated with other pesticides frequently detected in plasma. Among African Americans, high prenatal exposure to PAHs was associated with lower birth weight ($p = 0.003$) and smaller head circumference ($p = 0.01$) after adjusting for potential confounders. CPF was associated with decreased birth weight and birth length overall ($p = 0.01$ and $p = 0.003$, respectively) and with lower birth weight among African Americans ($p = 0.04$) and reduced birth length in Dominicans ($p < 0.001$), and was therefore included as a covariate in the model with PAH. After controlling for CPF, relationships between PAHs and birth outcomes were essentially unchanged. In this analysis, PAHs and CPF appear to be significant independent determinants of birth outcomes. Further analyses of pesticides will be carried out. Possible explanations of the failure to find a significant effect of PAHs in the Hispanic subsample are discussed. This study provides evidence that environmental pollutants at levels currently encountered in New York City adversely affect fetal development.

Chlorpyrifos (Dursban)-associated birth defects: a proposed syndrome, report of four cases, and discussion of the toxicology.
Sherman JD
International Journal of Occupational Medicine and Toxicology 1995 Oct-Dec; 4(4):417-31

This report discusses four children with an unusual pattern of birth defects, affecting the eye, ear, palate, teeth, heart, feet, nipples, genitalia, and brain. The latter abnormalities include defects of the ventricles, corpus callosum, choroid plexus, and septum pellucidum. All four children have growth retardation and three have hypotonia profound mental retardation. Genital defects include undescended testes, microphallus, and fused labia. Exposures occurred in utero to Dursban, a product containing the organophosphate pesticide chlorpyrifos, and associated additives and components. A review of published literature and unpublished EPA documents shows similar defects in test animals and other children exposed to organophosphates. The pattern of defects in

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

these four children may represent a heretofore unrecognized syndrome, and may be the basis for diagnosis of other children similarly affected.

B. Meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes

Prenatal Chlorpyrifos Exposure and Neurodevelopment
Rauh, V. A.; Perera, F.; Whyatt, R.
Pediatr Res 2006;(2006 PAS Annual Meeting):4550.14

BACKGROUND: Over 900 million pounds of pesticides are used annually in the US, including organophosphates. It is well-known that pesticide poisonings in children result in neurological impairment; however, few studies have assessed the neurobehavioral functioning of children who have been exposed to low-levels of insecticides. Concern about the potentially harmful neurodevelopmental effects of prenatal exposure to organophosphate pesticides, in particular chlorpyrifos (CPF), is growing. CPF is a known neurodevelopmental toxicant in animal studies, and its adverse effects on human development may have gone largely undetected because of the absence of overt signs of toxicity. OBJECTIVE: This report examines cognitive and motor development through age 3 on the Bayley Scales of Infant Development and behavior problems as a function of CPF levels in umbilical cord plasma collected from newborns at delivery. DESIGN/METHODS: As part of a prospective cohort study in a non-smoking inner-city minority population, neurotoxicant effects of prenatal exposure to CPF were evaluated in 254 children through age 3. A previous report showed widespread prenatal CPF exposure and significant adverse impacts on birth weight and birth length (Whyatt et al., 2004, 2005). Models were adjusted for race/ethnicity, sex, length of gestation, maternal education, maternal IQ, prenatal secondhand smoke exposure, and quality of the home environment. RESULTS: Using clinically defined cut-points for developmental delay, we found that children prenatally exposed to high CPF levels (greater than 6.17 picograms/gram [pg/g] plasma) were significantly more likely than children exposed to low levels to experience delay in psychomotor (p=0.002) and cognitive (p=0.024) development at 36 months of age. Highly exposed children were significantly more likely than less exposed children to manifest symptoms of attentional disorders (p=0.009), ADHD (p=0.046), and pervasive personality disorder (p=0.025) at age 3, based on parental report using the Child Behavior Checklist. CONCLUSIONS: Prenatal exposure to chlorpyrifos is associated with developmental delay and behavior problems at age 3 in a sample of inner-city minority children.

Organophosphate Poisoning Associated With Fetal Death.
Sebe, A.; Satar, S.; Alpay, R.; Kozaci, N.; Hilal, A.; Avci, A.
Toxicol Lett 2005; 158 Suppl 1:S120

The increased use of organophosphorus insecticides in agriculture, inside homes and schools, and widespread existence in the environment poses a potential health hazard. As the use of these

agents expands, acute and chronic exposure has become more common. Like other organophosphates, chlorpyrifos kills insects and other animals, including human being itself because of its toxicity to the nervous system. Exposure of organophosphates in pregnant women is an important clinical entity because of its effects on two organisms, both mother and baby. There are limited reports about fetal toxicity of organophosphates in the literature because of relatively less reported cases. In this paper we reported a case with chlorpyrifos intoxication, an organophosphorus compound, during pregnancy, causing fetal death.

Neural Tube Defects And Maternal Residential Proximity To Agricultural Pesticide Applications.
Rull RP, Ritz B, Shaw GM
Epidemiology 2004 Jul;15(4):S188

We evaluated the effects of maternal environmental exposure to 59 agricultural pesticides on neural-tube defects (NTDs) ascertained in two birth cohorts born in California between 1987 and 1991. Maternal residential proximity within 1,000 meters of pesticide applications occurring around the month of conception was assessed using a geographic model based on linking California Pesticide Use Reports (PUR) and land-use survey maps to increase the spatial resolution of the PUR. To adjust for multiple comparisons and correlated pesticide exposures, we employed hierarchical logistic regression, assessing agents in subgroups with similar physicochemical properties and comparing the estimates to those obtained from conventional logistic models. In addition, we estimated separate exposure effects for anencephaly and spina bifida, the two main NTD subtypes, and employed a polytomous hierarchical model for these outcomes. The precision of effect estimates based on the hierarchical models substantially improved compared to conventional logistic models, as effect estimates were shrunk towards the means of subgroups. Exposure prevalence to individual pesticides was low (e.g., methomyl had the highest prevalence of exposure, with 8.6% of 731 cases and 5.6% of 940 controls), thus limiting our ability to detect effects for specific pesticides. For NTDs, we observed a moderate effect for benomyl, a fungicide identified as a developmental toxin and endocrine disruptor (hierarchical OR: 2.05; 95% CI: 0.92, 4.55). Examining NTD subtypes in a polytomous hierarchical model, we also observed associations for the following organophosphosus compounds: chlorpyrifos (OR: 1.54; 95% CI: 0.87, 2.73) with spina bifida, and glyphosate (OR: 1.55; 95% CI: 0.85, 2.85) and naled (OR: 1.95; 95% CI: 0.89, 4.28) with anencephaly. These are the first results from a human study to suggest an increased risk for NTDs from residential proximity to specific pesticides, including agents (e.g., benomyl) classified by the US and California Environmental Protection Agencies as developmental toxins. In toxicological studies of mammals and amphibians, benomyl and its metabolites have been demonstrated to inhibit microtubule formation, which is critical to the differentiation of neural tissue. Chlorpyrifos in rat embryos has been shown to induce apoptosis and inhibit mitosis of neuroepithelial cells during neurulation. Further study is needed to improve our understanding of the mechanisms for teratogenesis resulting from exposure to these agents at low doses in the environment.

Relationship Between Prenatal Environ-Mental Exposures, Birth Outcomes And Cognitive Development In An Urban Minority Cohort.
Rauh VA, Tang D, Barr DB, Camann D, Kinney PL, Andrews H, Whyatt RM
Neurotoxicology 2004 Jun;25(4):667-8

The Columbia Center for Children's Environmental Health (CCCEH) is evaluating effects of environmental exposures during pregnancy on fetal growth and infant neurocognitive development in a cohort of African American and Dominican mothers and infants in New York City. Exposures include polycyclic aromatic hydrocarbons (PAH), environmental tobacco smoke (ETS), and pesticides. A battery of data collection strategies includes personal air monitoring of the mother during pregnancy, detailed questionnaires and laboratory analyses of b iomarkers in blood samples collected from the mothers and newborns at delivery. To date, over 550 pregnant nonsmoking women have been enrolled and their infants are being followed prospectively through school age. Key results include the findings that high prenatal exposure to PAH as estimated by personal air monitoring of the mother was associated with lower birth weight ($p = 0.003$) and smaller head circumference ($p=0.01$) among African American newborns, after adjusting for potential confounders. Among both African Americans and Dominicans ETS was associated with decreased head circumference at birth ($p=0.04$); and there was a significant interaction between prenatal exposure to ETS and PAH-DNA adducts in cord blood such that the combined exposure to high ETS and high adducts had a significant multiplicative effect on birth weight ($p=0.04$) and head circumference ($p=0.01$) after adjusting for potential confounders. In addition, ETS exposure and material hardship during pregnancy were significantly inversely associated with infant cognitive development measured at two years ($p < 0.05$), controlling for gestational age and other confounders. Compared to non-exposed children, those with prenatal ETS exposure were twice as likely to be classified as significantly delayed; and those with both ETS and material hardship were four times as likely to be delayed. Finally, levels of the insecticide chlorpyrifos in umbilical cord blood samples were inversely associated with birth weight and length ($p < 0.05$), controlling for potential confounders. Combined measures of the insecticides chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length (p 0.03). The associations between birth weight/length and cord plasma chlorpyrifos and diazinon were highly significant (p 0.007) among newborns born Prior to the 2000-01 U.S. Environmental Protection Agency's regulatory actions to phase out residential use of these insecticides, but not among newborns born after 1/1/01 ($p > 0.8$). These results indicate that interactions between toxicants as well as between toxicants and socioeconomic stressors during pregnancy impair fetal growth and/or child cognitive development in this minority cohort. They support recent regulatory action to phase out residential uses of chlorpyrifos and diazinon and indicate the need for further preventive measures to reduce ETS and PAH exposures.

C. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population.
Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT.
Environ Health Perspect. 2004 Jul;112(10):1116-24.

Although pesticide use is widespread, little is known about potential adverse health effects of in utero exposure. We investigated the effects of organophosphate pesticide exposure during pregnancy on fetal growth and gestational duration in a cohort of low-income, Latina women living in an agricultural community in the Salinas Valley, California. We measured nonspecific metabolites of organophosphate pesticides (dimethyl and diethyl phosphates) and metabolites specific to malathion (malathion dicarboxylic acid), chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphoro-thioate], and parathion (4-nitrophenol) in maternal urine collected twice during pregnancy. We also measured levels of cholinesterase in whole blood and butyryl cholinesterase in plasma in maternal and umbilical cord blood. We failed to demonstrate an adverse relationship between fetal growth and any measure of in utero organophosphate pesticide exposure. In fact, we found increases in body length and head circumference associated with some exposure measures. However, we did find decreases in gestational duration associated with two measures of in utero pesticide exposure: urinary dimethyl phosphate metabolites [beta(adjusted) = -0.41 weeks per log10 unit increase; 95% confidence interval (CI), -0.75 -- -0.02; p = 0.02], which reflect exposure to dimethyl organophosphate compounds such as malathion, and umbilical cord cholinesterase (beta(adjusted) = 0.34 weeks per unit increase; 95% CI, 0.13-0.55; p = 0.001). Shortened gestational duration was most clearly related to increasing exposure levels in the latter part of pregnancy. These associations with gestational age may be biologically plausible given that organophosphate pesticides depress cholinesterase and acetylcholine stimulates contraction of the uterus. However, despite these observed associations, the rate of preterm delivery in this population (6.4%) was lower than in a U.S. reference population.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Transplacental disposition and teratogenic effects of chlorpyrifos in rats.
Akhtar N, Srivastava MK, Raizada RB. J Toxicol Sci. 2006 Dec;31(5):521-7

Rats were orally fed with different doses viz. 9.6, 12 and 15 mg/kg/d from GD 0-20 and examined for evidence of fetotoxicity and teratogenicity to evaluate the potential effects of technical chlorpyrifos (97%). Fetotoxic effects were not observed at tested dose levels as evidenced by number of implantations, number of corpora lutea and live fetuses/dam, but significant alterations were noted in percent delta resorption and fetal weight. There were no major malformations, but some minor anomalies such as reduced parietal ossification and absence of phalanges found significant in high dose were not considered as compound-related effects. On the other hand the accumulation of chlorpyrifos residue in dams was more in brain (0.0328 micro g/g) than in liver (0.0071 micro g/g).The level of residue in fetuses was in the following order : liver (0.0531 micro g/g) > brain (0.0364 micro g/g) >placenta (0.040 micro g/g) > amniotic fluid (0.0010 micro g/g). Although, the total residue was higher in fetuses (0.0447 micro g/g) than in dams (0.0120 micro g/g), the absence of abnormalities in fetal gross morphology, visceral and skeleton suggest that technical chlorpyrifos at tested dose levels is non-teratogenic in rats.

A social recognition test for female mice reveals behavioral effects of developmental chlorpyrifos exposure.
Venerosi A, Calamandrei G, Ricceri L. Neurotoxicol Teratol. 2006 Jul-Aug;28(4):466-71.

CD-1 mice were exposed to the organophosphate pesticide chlorpyrifos (CPF) both prenatally (gestational days 15-18; doses 0, 3 or 6 mg/kg) and postnatally (postnatal days 11-14, doses 0, 1 or 3 mg/kg). When four-month-olds, females underwent a social recognition test in which ultrasound vocalizations (USVs) and social investigation behavior emitted by a resident female in the presence of a female partner were measured during two subsequent 3 min sessions (interval between the two sessions 45 min). Throughout the social recognition test a marked increase in USVs was found in females prenatally treated with the highest CPF dose; USV increase was also paralleled by a selective increase in frequency and not in duration of social investigation. These results confirm that developmental exposure to CPF induces long-lasting alterations in the social behavior repertoire of the mouse, thus extending our previous observations on the effects of postnatal CPF on male agonistic behavior to the female sex. They also suggest that social recognition can be easily and rapidly assessed in the female mouse making it possible to evaluate, primarily by means of USV emission, even subtle alteration of social behavioral patterns dissociated from cognitive components of individual recognition.

Developmental neurotoxicity of organophosphorous pesticides: fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini P, Fortuna S, Calamandrei G. Toxicol Sci. 2006 Sep;93(1):105-13.

Developmental exposure to the organophosphorous insecticide chlorpyrifos (CPF) induces long-term effects on brain and behavior in laboratory rodents. We evaluated in adult mice the behavioral effects of either fetal and/or neonatal CPF exposure at doses not inhibiting fetal and neonatal brain cholinesterase. CPF (3 or 6 mg/kg) was given by oral treatment to pregnant females on gestational days 15-18 and offspring were treated sc (1 or 3 mg/kg) on postnatal days (PNDs) 11-14. Serum and brain acetylcholinesterase (AChE) activity was evaluated at birth and 24 h from termination of postnatal treatments. On PND 70, male mice were assessed for spontaneous motor activity in an open-field test and in a socioagonistic encounter with an unfamiliar conspecific. Virgin females underwent a maternal induction test following presentation of foster pups. Both sexes were subjected to a plus-maze test to evaluate exploration and anxiety levels. Gestational and postnatal CPF exposure (higher doses) affected motor activity in the open field and enhanced synergically agonistic behavior. Postnatal CPF exposure increased maternal responsiveness toward pups in females. Mice of both sexes exposed to postnatal CPF showed reduced anxiety response in the plus-maze, an effect greater in females. Altogether, developmental exposure to CPF at doses that do not cause brain AChE inhibition induces long-term alterations in sex-specific behavior patterns of the mouse species. Late neonatal exposure on PNDs 11-14 was the most effective in causing behavioral changes. These findings support the hypothesis that developmental CPF may represent a risk factor for increased vulnerability to neurodevelopmental disorders in humans.

Effect of chlorpyrifos-methyl on steroid and thyroid hormones in rat F0- and F1-generations.
Jeong SH, Kim BY, Kang HG, Ku HO, Cho JH. Toxicology. 2006 Mar 15;220(2-3):189-202.

Chlorpyrifos-methyl (CPM) suppressed androgenic activity in Hershberger assay using castrated rats. Acute oral lowest-observed-adverse-effect-level (LOAEL) and no-observed-adverse-effect-level (NOAEL) was evaluated as 12 and 0.1 mg/kg bw, respectively, based on its major effect of cholinesterase inhibition. Also, repeated oral NOAEL was 0.1 mg/kg bw/day based on adrenal damage in rats. We investigated one-generation reproductive toxicity of CPM focusing on endocrine-disrupting effects by the administration of 1, 10 and 100 mg/kg bw/day CPM to mature SD rats (F0) through pre-mating, mating, gestation and lactation period and to their offspring (F1) until 13 weeks age via gavage. A group treated with corn oil served as vehicle control. In F0 rats, the most affected organs were adrenal glands as increased in weight at all doses of CPM in males and at 10 and 100 mg/kg CPM in females and adrenal vacuolation at CPM 10 and 100 mg/kg. The relative and absolute ovaries and the absolute seminal vesicle weights were decreased but the weights of liver, spleen or kidneys were increased at 100 mg/kg

CPM. Parameters representing reproductive performances as mating ratio, gestation length and delivery index were not affected, except for decreased fertility index and numbers of implantation and born pups and a higher male sex ratio of pups at CPM 100 mg/kg. F1 pups exposed to CPM 100 mg/kg in utero and via maternal milk showed lower body weight with changes of absolute or relative weights of brain, ovary, liver, spleen and epididymis and decreased absolute not relative anogenital distance at weanling time. The time of vaginal patency and preputial separation and estrous cycling pattern of F1 rats were not impacted by CPM. After further 10 weeks oral administration until 13 weeks old, adrenal glands, brain, liver, spleen or kidneys tended to be increased, while thyroid gland, testes and ventral prostate of F1 male rats were decreased at CPM 10 or 100 mg/kg. Histopathologically, necrosis or vacuolation of thyroid follicular epithelial cells and adrenal cortical cells were observed at all doses of CPM. Serum levels of estradiol, testosterone, T4 and T3 were significantly lower while TSH and cholesterol were higher in both F1 female and male rats treated with CPM though dose-responsiveness was not clear in F1 females. Decreased sperm were counted in F1 rats at CPM 100 mg/kg. As a whole, LOAEL and NOAEL was evaluated as 10 and 1 mg/kg bw, respectively, based on decreased estradiol and T4 and increased TSH in serum of F1 male rats, and when considering histopathological alteration of adrenal and thyroid glands, LOAEL assumed to be lower than 1 mg/kg bw. This study elucidates that CPM exhibit weak reproductive toxicity in F0 rats exposed at adulthood and negligible effects in F1 offspring exposed in utero and via lactation at weanling, but induce anti-androgenic effect and hypothyroidism after long term exposure from in utero through sexual maturation of F1 rats.

Alterations in central nervous system serotonergic and dopaminergic synaptic activity in adulthood after prenatal or neonatal chlorpyrifos exposure.

Aldridge JE, Meyer A, Seidler FJ, Slotkin TA. Environ Health Perspect. 2005 Aug;113(8):1027-31.

Exposure to chlorpyrifos (CPF) alters neuronal development of serotonin (5HT) and dopamine systems, and we recently found long-term alterations in behaviors related to 5HT function. To characterize the synaptic mechanisms underlying these effects, we exposed developing rats to CPF regimens below the threshold for systemic toxicity, in three treatment windows: gestational days (GD) 17-20, postnatal days (PN) 1-4, or PN11-14. In early adulthood (PN60), we assessed basal neurotransmitter content and synaptic activity (turnover) in brain regions containing the major 5HT and dopamine projections. CPF exposure on GD17-20 or PN1-4 evoked long-term increases in 5HT turnover across multiple regions; the effects were not secondary to changes in neurotransmitter content, which was unaffected or even decreased. When the treatment window was shifted to PN11-14, there were no long-term effects. Dopamine turnover also showed significant increases after CPF exposure on GD17-20, but only when the dose was raised above the threshold for overt toxicity; however, hippocampal dopamine content was profoundly subnormal after exposures below or above the acute, toxic threshold, suggesting outright neurotoxicity. These results indicate that, in a critical developmental period, apparently nontoxic

exposures to CPF produce lasting activation of 5HT systems in association with 5HT-associated behavioral anomalies.

Teratogenicity and developmental toxicity of chlorpyrifos. Maternal exposure during organogenesis in mice.
Tian Y, Ishikawa H, Yamaguchi T, Yamauchi T, Yokoyama K. Reprod Toxicol. 2005 Jul-Aug;20(2):267-70.

Chlorpyrifos, an organophosphate pesticide, was evaluated for potential teratogenicity and developmental toxicity in mice. Pregnant females were given a single intraperitoneal injection (40 or 80 mg/kg) on day 10 of gestation and fetuses were evaluated on gestation day 17. At 80 mg/kg, chlorpyrifos treatment resulted in a significant reduction in numbers of live fetuses, and increase in resorptions, versus control litters. There was no indication of maternal toxicity. External and skeletal malformations were observed at 80 mg/kg, but not 40 mg/kg. Rates of fetuses with cleft palate increased significantly ($p<0.05$) following 80 mg/kg chlorpyrifos (5.97%) versus control litters (0.97%). Similarly, the absence of thoracic vertebrae was increased and the number of caudal vertebrae was significantly decreased. It is suggested that chlorpyrifos is teratogenic and embryotoxic in mice at doses below those that cause significant maternal toxicity.

Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats.
Roy TS, Sharma V, Seidler FJ, Slotkin TA. Brain Res Dev Brain Res. 2005 Mar 22;155(1):71-80.

Neurochemical and behavioral studies indicate that the widely used organophosphorus insecticide, chlorpyrifos (CPF), evokes neurobehavioral teratogenicity with a wide window of vulnerability, ranging from embryonic life through postnatal development. Few studies have detailed morphological damage that corresponds to the operational deficits. We administered 5 mg/kg of CPF sc daily on postnatal days (PN) 11-14, a regimen that is devoid of systemic toxicity, but that elicits long-term cognitive impairment and disruption of cholinergic, catecholaminergic, and serotonergic synaptic function. On PN15 and 20, we conducted quantitative morphologic examinations of neurons and glia in CA1, CA3, and dentate gyrus regions of the hippocampus. Although hippocampal morphology after CPF exposure was normal on gross observation, morphometric analysis revealed a significant overall reduction in the total number of neurons and glia. Superimposed on this basic effect, CPF elicited a delayed-onset increase in the neuron/glia ratio that emerged by PN20, connoting selective gliotoxicity. The alterations in cell numbers were accompanied by significant perikaryal swelling and by enhanced development of astrocytic processes. Layer thickness also showed delayed-onset effects of CPF, with thinning of the CA1 and CA3 layers and enlargement of the dentate gyrus. Our results indicate that there are subtle morphological changes in the juvenile rat brain after neonatal CPF

exposure that are detectable only with quantitative analysis and that correlate with regional and cell-specific targets identified earlier in neurochemical studies. The simultaneous targeting of neurons and glia by CPF is likely to play an important role in its developmental neurotoxicant effects.

Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation
Icenogle, L. M., Christopher, N. C., Blackwelder, W. P., Caldwell, D. P., Qiao, D., Seidler, F. J., Slotkin, T. A., and Levin, E. D.
Neurotoxicol Teratol 2004;26(1):95-101

The widely used organophosphate insecticide, chlorpyrifos (CPF), elicits neurobehavioral abnormalities after apparently subtoxic neonatal exposures. In the current study, we administered 1 or 5 mg/kg/day of CPF to pregnant rats on gestational days 9-12, the embryonic phase spanning formation and closure of the neural tube. Although there were no effects on growth or viability, offspring showed behavioral abnormalities when tested in adolescence and adulthood. In the CPF-exposed groups, locomotor hyperactivity was noted in early T-maze trials, and in the elevated plus-maze; alterations in the rate of habituation were also identified. Learning and memory were adversely affected, as assessed using the 16-arm radial maze. Although all CPF-exposed animals eventually learned the task, reference and working memory were impaired in the early training sessions. After training, rats in the CPF group did not show the characteristic amnestic effect of scopolamine, a muscarinic acetylcholine antagonist, suggesting that, unlike the situation in the control group, muscarinic pathways were not used to solve the maze. These results indicate that apparently subtoxic CPF exposure during neurulation adversely affects brain development, leading to behavioral anomalies that selectively include impairment of cholinergic circuits used in learning and memory. The resemblance of these findings to those of late gestational or neonatal CPF exposure indicates a prolonged window of vulnerability of brain development to CPF.

Chlorpyrifos exposure during neurulation: cholinergic synaptic dysfunction and cellular alterations in brain regions at adolescence and adulthood
Qiao, D., Seidler, F. J., Abreu-Villaca, Y., Tate, C. A., Cousins, M. M., and Slotkin, T. A.
Brain Res Dev Brain Res 2004;148(1):43-52

The developmental neurotoxicity of chlorpyrifos (CPF) involves multiple mechanisms, thus rendering the immature brain susceptible to adverse effects over a wide window of vulnerability. Earlier work indicated that CPF exposure at the neural tube stage elicits apoptosis and disrupts mitotic patterns in the brain primordium but that rapid recovery ensues before birth. In the current study, we assessed whether defects in cholinergic synaptic activity emerge later in development. CPF was given to pregnant rats on gestational days 9-12, using regimens devoid of overt maternal or fetal toxicity. We then examined subsequent development of acetylcholine

systems and compared the effects to those on general biomarkers of cell development. Choline acetyltransferase (ChAT), a constitutive marker for cholinergic nerve terminals, was increased in the hippocampus and striatum in adolescence and adulthood. In contrast, hemicholinium-3 (HC-3) binding to the presynaptic choline transporter, an index of nerve impulse activity, was markedly subnormal. Furthermore, m2-muscarinic cholinergic receptor binding was significantly reduced, instead of showing the expected compensatory upregulation for reduced neural input. CPF also elicited delayed-onset alterations in biomarkers of cell packing density, cell number, cell size and neuritic projections, involving brain regions both with and without reductions in indices of cholinergic activity. In combination with earlier results, the current findings indicate that the developing brain, and especially the hippocampus, is adversely affected by CPF regardless of whether exposure occurs early or late in brain development, and that defects emerge in adolescence or adulthood even in situations where normative values are initially restored in the immediate post-exposure period.

Developmental toxicity study of chlorpyrifos in rats
Farag, A. T., El Okazy, A. M., and El-Aswed, A. F.
Reprod Toxicol 2003;17(2):203-8

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate) was evaluated for potential developmental toxicity. Groups of 30 bred female Fischer 344 rats were given 0, 5, 15, and 25mg/kg per day by gavage on gestation days 6-15; the fetuses were evaluated on gestation day 21. Clinical signs of toxicity attributed to chlorpyrifos were noted in dams receiving 15 and 25mg/kg per day. Maternal effects in these groups also included depressed body weight and acetylcholinesterase activity. Fetal weight and viability were decreased, and fetal death and early resorption were increased at the 25mg/kg per day maternal dose. Visceral, skeletal, and external variations were also increased in this group. Chlorpyrifos showed fetotoxic and teratogenic effects at a maternal dose of 25mg/kg per day, a dose that also produced maternal toxicity.

Micronucleus formation in 3-day mouse embryos associated with maternal exposure to chlorpyrifos during the early preimplantation period
Tian, Y. and Yamauchi, T.
Reprod Toxicol 2003;17(4):401-5

Chlorpyrifos, an organophosphate pesticide, was evaluated for its ability to induce cytogenetic damage in preimplantation embryos after maternal exposure. Pregnant female mice were intraperitoneally (i.p.) administered a single dose of chlorpyrifos (40 or 80 mg/kg) at 10:00 h on Day 0 of pregnancy. On Day 3 of gestation, blastocysts were collected and evaluated for gross morphology, micronucleus (MN) frequency, and cell number. A significant increase in MN frequency indicating cytogenetic damage was observed in the treatment groups in comparison to control. The MN frequency revealed a clear dose-dependent increase. There was also a significant decrease in the embryo cell number in the 80 mg/kg treated group. A simultaneous

decrease in the cell number and increase in MN frequency may reflect an embryonic developmental disadvantage resulting from maternal treatment with chlorpyrifos.

Prenatal chlorpyrifos exposure in rats causes persistent behavioral alterations
Levin, E. D., Addy, N., Baruah, A., Elias, A., Christopher, N. C., Seidler, F. J., and Slotkin, T. A.
Neurotoxicol Teratol 2002;24(6):733-41

Use of chlorpyrifos (CPF) has been curtailed due to its developmental neurotoxicity. In rats, postnatal CPF administration produces lasting changes in cognitive performance, but less information is available about the effects of prenatal exposure. We administered CPF to pregnant rats on gestational days (GD) 17-20, a peak period of neurogenesis, using doses (1 or 5 mg/kg/day) below the threshold for fetal growth impairment. We then evaluated performance in the T-maze, Figure-8 apparatus and 16-arm radial maze, beginning in adolescence and continuing into adulthood. CPF elicited initial locomotor hyperactivity in the T-maze. Females showed slower habituation in the Fig. 8 maze; no effects were seen in males. In the radial-arm maze, females showed impaired choice accuracy for both working and reference memory and again, males were unaffected. Despite the deficits, all animals eventually learned the maze with continued training. At that point, we challenged them with the muscarinic antagonist, scopolamine, to determine the dependence of behavioral performance on cholinergic function. Whereas control females showed impairment with scopolamine, CPF-exposed females did not, implying that the delayed acquisition of the task had been accomplished through alternative mechanisms. The differences were specific to muscarinic circuits, as control and CPF groups responded similarly to the nicotinic antagonist, mecamylamine. Surprisingly, adverse effects of CPF were greater in the group receiving 1 mg/kg as compared to 5 mg/kg. Promotional effects of acetylcholine (ACh) on cell differentiation may thus help to offset CPF-induced developmental damage that occurs through other noncholinergic mechanisms. Our results indicate that late prenatal exposure to CPF induces long-term changes in cognitive performance that are distinctly gender-selective. Additional defects may be revealed by similar strategies that subject the animals to acute challenges, thus, uncovering the adaptive mechanisms that maintain basal performance.

Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat
Breslin, W. J., Liberacki, A. B., Dittenber, D. A., and Quast, J. F.
Fundam Appl Toxicol 1996;29(1):119-30

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate), an organophosphate insecticide, was evaluated for its potential to produce developmental and reproductive toxicity in rats following oral exposure. Pregnant Fischer 344 rats were given doses of 0 (corn oil vehicle), 0.1, 3.0, or 15 mg chlorpyrifos/kg/day, by gavage, on Gestation Days 6 through 15. Maternal effects noted at the two higher dose levels included decreased cholinesterase levels at 3.0 mg/kg/day and cholinergic signs (excessive salivation and tremors), decreased cholinesterase

levels, and decreased body weight gain at 15 mg/kg/day. No maternal effects were apparent at 0.1 mg/kg/day. Although maternal toxicity was observed at these two higher exposure levels, no developmental effects were noted at any dose. In a two-generation reproduction study, Sprague-Dawley rats were maintained on diets supplying 0, 0.1, 1.0, or 5.0 mg chlorpyrifos/kg/day. Parental effects included decreased plasma and erythrocyte cholinesterase at 1.0 mg/kg/day, and decreased plasma, erythrocyte, and brain cholinesterase and histopathologic alterations of the adrenal zona fasciculata at 5.0 mg/kg/day. The histopathologic alterations of the adrenal were characterized as very slight to slight vacuolation (consistent with fatty change) in males, and very slight vacuolation and/or altered tinctorial properties in females. No effects on the reproductive or fertility indices or on the histopathology of reproductive tissues were observed at any dose level, and no neonatal effects were observed at 0.1 or 1.0 mg/kg/day in the F1 or F2 litters. Parental toxicity at the high dose was accompanied by decreased pup body weight and increased pup mortality in the F1 litters only. These data show that oral administration of chlorpyrifos to rats at parentally toxic dose levels was not embryolethal, embryo/fetotoxic, or teratogenic and did not adversely affect fertility or the function or structure of the reproductive organs. Although effects on neonatal growth and survival were observed at a maternally toxic dose level in one generation, this effect was not observed in the subsequent generation and, therefore, may not have been related to treatment.

Comparative developmental and maternal neurotoxicity following acute gestational exposure to chlorpyrifos in rats
Chanda, S. M., Harp, P., Liu, J., and Pope, C. N.
J Toxicol Environ Health 1995;44(2):189-202

Chlorpyrifos (CPF), an organophosphorus (OP) insecticide, exerts toxicity through inhibition of acetylcholinesterase (AChE). In the present study, pregnant Sprague-Dawley rats were given CPF (200 mg/kg, sc) as a single dose on gestation d 12 (GD12) and then sacrificed on either GD16, GD20, or postnatal d 3 (PND3) for measurement of maternal and developmental indicators of toxicity. While most CPF-treated rats exhibited no overt signs, a subset (4/28) showed moderate to severe signs of "cholinergic" toxicity at 2-3 d after treatment, and these rats were omitted from further studies. Extensive AChE inhibition (82-88%) was noted in maternal brain at all three time points following acute exposures. At GD16 and GD20, fetal brain AChE activity was inhibited 42-44%. While some degree of recovery in AChE activity was noted in pup brain by PND3, AChE activity was still inhibited (30%) in treated pups cross-fostered to control dams. In vitro inhibition of maternal and fetal (GD20) brain AChE activity by the active metabolite, chlorpyrifos oxon, suggested that the prenatal brain AChE activity was somewhat more sensitive (IC50 at 37.0 degrees C, 20 min: dam, 26.6 +/- 1.8 x 10(-9) M; fetus, 6.7 +/- 0.4 x 10(-9) M). Maternal brain muscarinic receptor binding was more extensively reduced (30-32%) at GD20 and PND3 as compared to the developing brain at GD20 (16%) and PND3 (11%). A simple postnatal reflex test (righting reflex) was transiently altered by CPF. The results suggest that CPF exposure to dams during gestation produces more extensive neurotoxicological effects in the dam relative to the developing fetus.

Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to DURSBAN
Muto, M. A., Lobelle, F. Jr, Bidanset, J. H., and Wurpel, J. N.
Vet Hum Toxicol 1992;34(6):498-501

DURSBAN (DB; active ingredient chlorpyrifos) is a widely-used organophosphate insecticide. The teratogenic and neurotoxic potential of DB was evaluated in rats in utero by exposing embryos on days 0-7 or days 7-21 of development. These prenatal exposures to DB (0.03, 0.1 or 0.3 mg chlorpyrifos/kg, ip) induced physical abnormalities and embryotoxicity. Rat pups which had been exposed to 0.3 mg chlorpyrifos/kg prenatally demonstrated significant behavioral neurotoxicity on postnatal day 16 in the rotorod test compared to time-matched saline-infused litters. Exposure to DB on postnatal day 3, 10 or 12 also caused neurotoxicity as evaluated by the rotorod test. Our studies suggest prenatal exposure to relatively low concentrations of DB may be associated with embryotoxicity, fetal lethality and behavioral neurotoxicity.

Effect of Dursban 44 on semen output of Holstein bulls
Everett, R. W.
J Dairy Sci 1982;65(9):1781-94

Dursban 44, an insecticide for lice control, was applied to 185 Holstein bulls 9 to 52 mo of age. These sires were in various stages of progeny testing at an artificial insemination center. Application of this product killed 7 bulls, and the remaining bulls exhibited varying severity of illness with 6 classified as very sick. This study evaluated the effect of this illness on semen production. Semen output on 40,950 ejaculates from 583 Holstein bulls collected from July 1, 1975, through March 31, 1981, was analyzed to establish normal semen production and to estimate the effect of illness caused by Dursban 44 treatment. Ejaculate number, days between collections by previous number of ejaculates, calendar months, years, and ages of bulls affected the semen output characteristics, original volume, sperm concentration, percent motile sperm, total sperm per ejaculate, percent prefreeze discards, percent postthaw sperm motility, and percent postthaw discards. Ejaculate volume, motility, total percent prefreeze discards, and percent postthaw discards were influenced negatively on the 6 very sick bulls. Percent postthaw discards were higher on all bulls treated with Dursban 44 for up to 6 mo post-treatment.

Three Generation Reproduction and Teratology Study in the Rat Following Prolonged Dietary Exposure to Dursban, O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate
Dow Chemical 1971; Thompson, D.J., Gerbig, C.G., and Warner, S.D.
Summary from California Department of Pesticide Regulation. Chlorpyrifos, purity and grade not specified. Doses for the main portion of the reproduction study were 0, 0.1, 0.3, and 1.0 mg/kg/day in diet. ChE inhibition NOEL = 0.3 mg/kg/day. General adult toxicity NOEL = 1.0 mg/kg/day (HDT). Reproductive NOEL = 0.3 mg/kg/day (slightly increased pup mortality in first 5 days post-partum).

Developmental neurotoxicity of chlorpyrifos: what is the vulnerable period?
Qiao, D., Seidler, F. J., Padilla, S., and Slotkin, T. A.
Environ Health Perspect 2002;110(11):1097-103

Previously, we found that exposure of neonatal rats to chlorpyrifos (CPF) produced brain cell damage and loss, with resultant abnormalities of synaptic development. We used the same biomarkers to examine prenatal CPF treatment so as to define the critical period of vulnerability. One group of pregnant rats received CPF (subcutaneous injections in dimethyl sulfoxide vehicle) on gestational days (GD) 17-20, a peak period of neurogenesis; a second group was treated on GD9-12, the period of neural tube formation. In the GD17-20 group, the threshold for a reduction in maternal weight gain was 5 mg/kg/day; at or below that dose, there was no evidence (GD21) of general fetotoxicity as assessed by the number of fetuses or fetal body and tissue weights. Above the threshold, there was brain sparing (reduced body weight with an increase in brain/body weight ratio) and a targeting of the liver (reduced liver/body weight). Indices of cell packing density (DNA per gram of tissue) and cell number (DNA content) similarly showed effects only on the liver; however, there were significant changes in the protein/DNA ratio, an index of cell size, in fetal brain regions at doses as low as 1 mg/kg, below the threshold for inhibition of fetal brain cholinesterase (2 mg/kg). Indices of cholinergic synaptic development showed significant CPF-induced defects but only at doses above the threshold for cholinesterase inhibition. With earlier CPF treatment (GD9-12), there was no evidence of general fetotoxicity or alterations of brain cell development at doses up to the threshold for maternal toxicity (5 mg/kg), assessed on GD17 and GD21; however, augmentation of cholinergic synaptic markers was detected at doses as low as 1 mg/kg. Compared with previous work on postnatal CPF exposure, the effects seen here required doses closer to the threshold for fetal weight loss; this implies a lower vulnerability in the fetal compared with the neonatal brain. Although delayed neurotoxic effects of prenatal CPF may emerge subsequently in development, our results are consistent with the preferential targeting of late developmental events such as gliogenesis, axonogenesis, and synaptogenesis.

Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers emerge postnatally and continue into adolescence and adulthood
Qiao, D., Seidler, F. J., Tate, C. A., Cousins, M. M., and Slotkin, T. A.
Environ Health Perspect 2003;111(4):536-44

Fetal and childhood exposures to widely used organophosphate pesticides, especially chlorpyrifos (CPF), have raised concerns about developmental neurotoxicity. Previously, biomarkers for brain cell number, cell packing density, and cell size indicated that neonatal rats were more sensitive to CPF than were fetal rats, yet animals exposed prenatally still developed behavioral deficits in adolescence and adulthood. In the present study, we administered CPF to pregnant rats on gestational days 17-20, using regimens devoid of overt fetal toxicity. We then examined subsequent development of acetylcholine systems in forebrain regions involved in

cognitive function and compared the effects with those on general biomarkers of cell development. Choline acetyltransferase, a constitutive marker for cholinergic nerve terminals, showed only minor CPF-induced changes during the period of rapid synaptogenesis. In contrast, hemicholinium-3 binding to the presynaptic choline transporter, which is responsive to nerve impulse activity, displayed marked suppression in the animals exposed to CPF; despite a return to nearly normal values by weaning, deficits were again apparent in adolescence and adulthood. There was no compensatory up-regulation of cholinergic receptors, as m2-muscarinic cholinergic receptor binding was unchanged. CPF also elicited delayed-onset alterations in biomarkers for general aspects of cell integrity, with reductions in cell packing density, increases in relative cell size, and contraction of neuritic extensions; however, neither the magnitude nor timing of these changes was predictive of the cholinergic defects. The present findings indicate a wide window of vulnerability of cholinergic systems to CPF, extending from prenatal through postnatal periods, occurring independently of adverse effects on general cellular neurotoxicity.

Pyrinex teratogenicity study in the rat
Rubin, Y., Gal, N., Waner, T., and Nyska, A.
1987

Summary from California Department of Pesticide Regulation. Three groups of 32 mated CD rats were dosed with Pyrinex in maize oil by oral (intragastric) intubation from Day 6 to Day 15 post coitum, inclusive, at dosages of 0.5, 2.5, and 15 mg/kg/day, respectively. A fourth group served as a vehicle control.
Ten rats from each group were bled at the end of the dosing period for plasma cholinesterase determination and discarded. The remaining rats were sacrificed on Day 20 post coitum for examination of the uterine contents. Ten of these rats per group were bled prior to sacrifice for terminal plasma cholinesterase determination.
Maternal toxic response to Pyrinex administration consisted of reduced body weight gain and a transient decrease in food consumption among animals dosed at 15 mg/kg/day and dosage related depression in plasma cholinesterase activity in all treated groups. Terminal (Day 20 p.c.) cholinesterase activity returned to normal levels among low and intermediate dosage groups. Post-implantation fetal loss was more frequent among high dosage animals than among controls. This difference was statistically significant when arcsine transformed data were tested under parametric assumptions, but not when data were tested non-parametrically, by rank order methods.
Fetuses in the high dosage group were significantly larger than controls, and showed enhanced skeletal development. The toxicological significance of these findings is not apparent.
Pyrinex is concluded not to have shown a potential for teratogenicity and not to have caused fetal toxicity in the absence of maternal toxicity in the current test system.

The effects of chlorpyrifos and xylene on embryonal and fetal development in the rat
Nimphius, M. J.
1995;

Chlorpyrifos (0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl)-phosphorothioate) is the active ingredient in DURSBAN and LORSBAN insecticides. The most common carrier solvent of these pesticides is xylene. The purpose of this study was to assess the potential of chlorpyrifos and xylene to adversely affect embryonal and fetal development. This study also determined the distribution of the chlorpyrifos and xylene through out the dam and fetus. Cholinesterase levels in the maternal blood, brain, and fetus were also determined.
Pregnant Sprague Dawley rats were given wither chlorpyrifos, xylene, or chlorpyrifos with xylene. Doses were 0, 0.3, 3.0, and 10 mg/kg by subcutaneous injection on days 0 through 7 of gestation. No evidence of maternal toxicity was noted. There were no deaths among the dams and no apparent aborted litters in any of the treatment groups. The dams were sacrificed on days 19 and 20 of gestation. The maternal blood and brain was collected for analysis. The fetuses were removed, weighed, and measured (crown to rump) and examined for external malformations. No obvious malformations were observed. A decrease in fetal body weights were noted in 95% of the chloropyrifos treated groups. A decrease in fetal body weights was seen in the higher xylene concentration dosage groups as well. This decrease in fetal weights indicate that chlorpyrifos and xylene are potential developmental toxins.
Maternal plasma cholinesterase inhibition was observed at the 10 mg/kg dose of chlorpyrifos. Maternal erythrocyte cholinesterase inhibition was observed at the 3.0 and 10 mg/kg chlorpyrifos dose. The maternal brain tissue cholinesterase was inhibited at the 10 mg/kg chlorpyrifos dose. The fetal tissue demonstrated inhibition at the 3.0 and 10 mg/kg chlorpyrifos dose. None of the 0.3 mg/kg chlorpyrifos or chlorpyrifos/xylene treated groups demonstrated cholinesterase inhibition in any of the tissues.
Chlorpyrifos was detected in all of the tissues that exhibited cholinesterase inhibition except it was not detected in the 3.0 mg/kg chlorpyrifos/xylene even though cholinesterase inhibition was observed. Xylene did not attenuate the concentration of chlorpyrifos or the enzyme inhibition.

B. Meeting abstracts reporting developmental or reproductive toxicity

Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers
Qiao, D., Seidler, F. J., and Slotkin, T. A.
Toxicologist 2003;72(S-1):124

Fetal and childhood exposures to the widely-used organophosphate pesticide, chlorpyrifos (CPF), have raised concerns about developmental neurotoxicity. Previously, biomarkers for brain cell number, cell packing density and cell size indicated that neonatal rats were more sensitive to CPF than were fetal rats, yet animals exposed prenatally still developed behavioral deficits in adolescence and adulthood. We administered CPF to pregnant rats on gestational days 17-20, using regimens devoid of overt maternal or fetal toxicity. We then examined subsequent

development of acetylcholine systems in forebrain regions, and compared the effects to those on general biomarkers of cell development. Choline acetyltransferase, a marker for cholinergic nerve terminals, showed only minor CPF-induced changes. In contrast, hemicholinium-3 binding to the presynaptic choline transporter, which is responsive to nerve impulse activity, displayed marked suppression that persisted into adulthood. There was no compensatory upregulation of cholinergic receptors, as m2-muscarinic cholinergic receptor binding was unchanged. CPF also elicited alterations in biomarkers of cell development, with reductions in cell packing density, increases in relative cell size and contraction of neuritic extensions; however, neither the magnitude nor timing of these changes were predictive of the cholinergic defects. The current findings indicate a wide window of vulnerability of cholinergic systems to CPF, extending from prenatal through postnatal periods, occurring independently of adverse effects on general cellular neurotoxicity.

C. Studies reporting no developmental or reproductive toxicity

Dursban Insecticide: Assessment of Neonatal Survival In A Two-Generation Reproduction Study In Rats
Dietz, F. K., Mensik, C. A., Hinze, B. L., Rachunek, and Taylor, H. W.
1983;

The purpose of this study was to evaluate the potential effects of DURSBAN (a trademark of The Dow Chemical Company) insecticide on the reproductive capability, and neonatal growth and survival of rats fed the test material over two generations. Particular emphasis was placed upon evaluating effects of treatment on neonatal survival since equivocal results from a previous reproduction study (Thompson et al., 1971) have been interpreted as demonstrating a treatment-related increase in neonatal mortality. Male and female Sprague-Dawley rats, approximately 6 weeks of age, were fed diets providing 0, 0.5, 0.8, or 1.2 mg DURSBAN insecticide/kg/day for 135 days and then bred to produce f1 litters. On day 21 of lactation, pups for the following generation (f1) were randomly selected and the remaining pups were sacrificed. The f1 weanlings were dosed in a similar fashion for 120 days prior to their breeding to produce the f2 litters. These pups were also weaned on day 21 of lactation. All adult animals were sacrificed at the termination of each generation.
No overt signs of toxicity were observed in any animals throughout the study. Treatment-related effects noted in the adult generations were limited to a decrease in body weights during the post-mating period for f1 males. These effects were generally limited to the 1.2 mg/kg/day f1 group, with slight effects observed at 0.8 mg/kg/day and no significant effects noted at 0.5 mg/kg/day. Adult food consumption was unaffected by the administration of DURSBAN insecticide at all three dose levels. No adverse effects on fertility, reproduction, or neonatal growth were observed among animals exposed to the test material for two generations. In addition, the dietary administration of up to 1.2 mg DURSBAN insecticide/kg/day did not adversely affect the survival of pups from f1 or f2 litters during the lactation period.
In conclusion, these data demonstrate that the dietary ingestion of 0.5, 0.8, or 1.2 mg DURSBAN

insecticide/kg/day by male and female rats for two successive generations did not result in any adverse effects on the ability of rats to mate, reproduce, or nurse their pups. Neonatal growth and survival were also unaffected by administration of the test material. Based on these results, 1.2 mg DURSBAN insecticide/kg/day is considered to be a no-observable-effect level for reproductive parameters in the Sprague-Dawley rat.

Chlorpyrifos: Oral Teratology Study in Fisher 344 Rats
Ouellette, J. H., Dittenber, D. A., Kloes, P. M. , and John, J. A.
1983;

Summary from California Department of Pesticide Regulation. Chlorpyrifos, 96.6%. 0, 0.1, 3.0, and 15 mg/kg/day (gavage). Maternal NOEL (excluding cholinesterase (ChE) inhibition_ = 3.0 mg/kg/day (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma and RBC ChE). Developmental toxicity NOEL = 15 mg/kg/day (HDT).

The effect of Pyrinex (chlorpyrifos) on reproductive function of two generations in the rat
James, P., Stubbs, A., Parker, C. A., Offer, J. M., and Anderson, A.
1988;

Summary provided by California Department of Pesticide Regulation. Crl:CD**7**(SD)BR rats received diets containing 0, 2, 10, or 50 ppm chlorpyrifos (95% purity) in diets over 2 generations (1 litter per generation). Parental rats numbered 28/sex/group in the F0 generation, and 24/sex/group in the F1 generation. Protocol was that of a standard reproduction study, with a few pre-weaning developmental evaluations added (surface righting, air righting, and startle responces; and pupil reflex). There were **no definitive treatment-related effects** (report attributes 3 high dose deaths to treatment, however there were deaths in other groups and no evident unique symptoms in high dose decedents).

D. Related articles

Paradoxical effects of prenatal acetylcholinesterase blockade on neuro-behavioral development and drug-induced stereotypies in reeler mutant mice.
Laviola G, Adriani W, Gaudino C, Marino R, Keller F.Psychopharmacology (Berl). 2006 Aug;187(3):331-44.
INTRODUCTION: Epidemiological and experimental studies support a link between genetic and epigenetic factors in vulnerability to develop enduring neurobehavioral alterations. We studied the interplay between genetic vulnerability and the prenatal exposure to a neurotoxic compound. Chlorpyrifos, a potent and reversible acetylcholinesterase blocker used as a pesticide, and the "reeler" mouse, lacking the extracellular-matrix protein Reelin, were used. MATERIALS AND METHODS: Homozygous reeler (RL), heterozygous (HZ), and wild-type (WT) mice were prenatally exposed to chlorpyrifos-oxon (CPF-O), the active metabolite of chlorpyrifos, or to

vehicle (prenatal controls) on gestation days 14-16, that is, during a peak period of neurogenesis in the cerebral cortex. The offspring was reared by the natural dam and tested during infancy and at adulthood for global consequences of the prenatal exposure. CONCLUSION: The results are consistent with complex interactions between genetic (reeler genotype) and epigenetic (prenatal exposure to CPF-O) factors. In the case of some "genetically modulated" parameters (ultrasound vocalization, amphetamine-induced locomotion, and stereotypy), exposure to CPF-O paradoxically reverted the effects produced by progressive reelin absence. Conversely, for an "epigenetically modulated" parameter (grasping reflex maturation), the effects of CPF-O exposure were counteracted by progressive reelin absence. Finally, for parameters apparently untouched by either factor alone (righting reflex latency, scopolamine-induced locomotor activity), prenatal CPF-O exposure unmasked an otherwise latent genotype dependency. This complex picture also points to reciprocal adaptations within cholinergic and dopaminergic systems during development. Data are interesting in view of recently discovered cholinergic abnormalities in autism and schizophrenia, and may suggest new avenues for early intervention.

Interaction between organophosphate compounds and cholinergic functions during development.

Aluigi MG, Angelini C, Falugi C, Fossa R, Genever P, Gallus L, Layer PG, Prestipino G, Rakonczay Z, Sgro M, Thielecke H, Trombino S. Chem Biol Interact. 2005 Dec 15;157-158:305-16.

Organophosphate (OP) compounds exert inhibition on cholinesterase (ChE) activity by irreversibly binding to the catalytic site of the enzymes. For this reason, they are employed as insecticides for agricultural, gardening and indoor pest control. The biological function of the ChE enzymes is well known and has been studied since the beginning of the XXth century; in particular, acetylcholinesterase (AChE, E.C. 3.1.1.7) is an enzyme playing a key role in the modulation of neuromuscular impulse transmission. However, in the past decades, there has been increasing interest concerning its role in regulating non-neuromuscular cell-to-cell interactions mediated by electrical events, such as intracellular ion concentration changes, as the ones occurring during gamete interaction and embryonic development. An understanding of the mechanisms of the cholinergic regulation of these events can help us foresee the possible impact on environmental and human health, including gamete efficiency and possible teratogenic effects on different models, and help elucidate the extent to which OP exposure may affect human health. The chosen organophosphates were the ones mainly used in Europe: diazinon, chlorpyriphos, malathion, and phentoate, all of them belonging to the thionophosphate chemical class. This research has focused on the comparison between the effects of exposure on the developing embryos at different stages, identifying biomarkers and determining potential risk factors for sensitive subpopulations. The effects of OP oxonisation were not taken into account at this level, because embryonic responses were directly correlated to the changes of AChE activity, as determined by histochemical localisation and biochemical measurements. The identified biomarkers of effect for in vitro experiments were: cell proliferation/apoptosis as well

as cell differentiation. For in vivo experiments, the endpoints were: developmental speed, size and shape of pre-gastrula embryos; developmental anomalies on neural tube, head, eye, heart. In all these events, we had evidence that the effects are mediated by ion channel activation, through the activation/inactivation of acetylcholine receptors (AChRs).

Alpha7 nicotinic acetylcholine receptors targeted by cholinergic developmental neurotoxicants: nicotine and chlorpyrifos
Slotkin, T. A., Southard, M. C., Adam, S. J., Cousins, M. M., and Seidler, F. J.
Brain Res Bull 2004;64(3):227-35

Alpha7 nicotinic acetylcholine receptors (nAChRs) play a role in axonogenesis, synaptogenesis and synaptic plasticity, and are therefore potential targets for developmental neurotoxicants. We administered nicotine to neonatal rats during discrete periods spanning the onset and peak of axonogenesis/synaptogenesis, focusing on three brain regions with disparate distributions of cell bodies and neural projections: brainstem, forebrain and cerebellum. Nicotine treatment on postnatal days (PN) 1-4 had little or no effect on alpha7 nAChRs but treatment during the second (PN11-14) or third (PN21-24) weeks elicited significant decrements in receptor expression in brainstem and cerebellum, regions containing cell bodies that project to the forebrain. Exposure to chlorpyrifos, a neurotoxicant pesticide that acts partially through cholinergic mechanisms, also elicited deficits in alpha7 nAChRs during the second postnatal week but not the first week. For both nicotine and chlorpyrifos, the effects on alpha7 nAChRs were distinct from those on the alpha4beta2 subtype. Continuous prenatal nicotine exposure, which elicits subsequent, postnatal deficits in axonogenesis and synaptogenesis, also produced delayed-onset changes in alpha7 nAChRs, characterized by reductions in the forebrain and upregulation in the brainstem and cerebellum, a pattern consistent with impaired axonogenesis/synaptogenesis and reactive sprouting. Males were more sensitive to the persistent effects of prenatal nicotine exposure on alpha7 nAChRs, a pattern that mimics neurobehavioral deficits resulting from this treatment. The present findings reinforce the mechanistic involvement of alpha7 nAChRs in the actions of developmental neurotoxicants, and its biomarker potential for neuroteratogens that target neuritic outgrowth.

Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation
Icenogle, L. M., Christopher, N. C., Blackwelder, W. P., Caldwell, D. P., Qiao, D., Seidler, F. J., Slotkin, T. A., and Levin, E. D.
Neurotoxicol Teratol 2004;26(1):95-101

The widely used organophosphate insecticide, chlorpyrifos (CPF), elicits neurobehavioral abnormalities after apparently subtoxic neonatal exposures. In the current study, we administered 1 or 5 mg/kg/day of CPF to pregnant rats on gestational days 9-12, the embryonic phase spanning formation and closure of the neural tube. Although there were no effects on growth or

viability, offspring showed behavioral abnormalities when tested in adolescence and adulthood. In the CPF-exposed groups, locomotor hyperactivity was noted in early T-maze trials, and in the elevated plus-maze; alterations in the rate of habituation were also identified. Learning and memory were adversely affected, as assessed using the 16-arm radial maze. Although all CPF-exposed animals eventually learned the task, reference and working memory were impaired in the early training sessions. After training, rats in the CPF group did not show the characteristic amnestic effect of scopolamine, a muscarinic acetylcholine antagonist, suggesting that, unlike the situation in the control group, muscarinic pathways were not used to solve the maze. These results indicate that apparently subtoxic CPF exposure during neurulation adversely affects brain development, leading to behavioral anomalies that selectively include impairment of cholinergic circuits used in learning and memory. The resemblance of these findings to those of late gestational or neonatal CPF exposure indicates a prolonged window of vulnerability of brain development to CPF.

Comparative teratogenicity of chlorpyrifos and malathion on Xenopus laevis development
Bonfanti, P., Colombo, A., Orsi, F., Nizzetto, I., Andrioletti, M., Bacchetta, R., Mantecca, P., Fascio, U., Vailati, G., and Vismara, C.
Aquat Toxicol 2004;70(3):189-200

The embryotoxic potential of chlorpyrifos (CPF) and malathion (MTN), two organophosphorus insecticides (OPs), was evaluated by modified Frog Embryo Teratogenesis Assay-Xenopus (FETAX). CPF and MTN were not embryolethal even at the highest concentration tested (6000 microg/l), but both exhibited a powerful teratogenicity. The probit analysis of malformed larva percentages showed a TC(50) of 161.54mug/l for CPF, and a TC(50) of 2394.01 microg/l for MTN. Therefore, CPF teratogenicity was about 15 times higher than MTN. Larvae of both exposed groups were mainly affected by ventral and/or lateral tail flexure coupled with abnormal gut coiling. Histopathological diagnosis displayed abnormal myotomes and myocytes with marked hypertrophies localized at the cell extremity, probably due to a break away of myofibril extremities at the intersomitic junction level. We speculate that this muscular damage was related to inhibition of acetylcholinesterase that showed a clear concentration-response in CPF and MTN exposed larvae. The teratogenic effects of these anti-cholinesterase compounds on Xenopus laevis myogenesis suggest a possible role played by OPs on induction of congenital muscular dystrophy.

Cholinergic synaptic signaling mechanisms underlying behavioral teratogenicity: effects of nicotine, chlorpyrifos, and heroin converge on protein kinase C translocation in the intermedial part of the hyperstriatum ventrale and on imprinting behavior in an avian model
Izrael, M., Van der Zee, E. A., Slotkin, T. A., and Yanai, J.
J Neurosci Res 2004;78(4):499-507

A wide variety of otherwise unrelated neuroteratogens elicit a common set of behavioral defects centering around cholinergic contributions to cognitive function. We utilized the developing chick to overcome confounds related to maternal effects and compared the actions of nicotine, chlorpyrifos, and heroin on cholinergic signaling in the intermedial part of the hyperstriatum ventrale (IMHV), which controls imprinting behavior. Chicken eggs were injected with nicotine (10 mg/kg of egg), chlorpyrifos (10 mg/kg of egg), or heroin (20 mg/kg of egg; all doses below the threshold for dysmorphology) on incubation days (ID) 0 and 5, and then tests were conducted posthatching. All three compounds elicited significant deficits in imprinting behavior. We also found defects in cholinergic synaptic signaling specifically involving the muscarinic receptor-mediated membrane translocation of protein kinase C (PKC)-gamma and in the basal levels of both PKCgamma and PKCbetaII, the two isoforms known to be relevant to behavioral performance. In contrast, there were no alterations in the response of PKCalpha, an isoform that does not contribute to the behavior, nor were cytosolic levels of any of the isoforms affected. Taken together with similar results obtained in rodents, our findings suggest that disparate neuroteratogens all involve signaling defects centering on the ability of cholinergic receptors to elicit PKCgamma translocation/activation and that this effect is direct, i.e., not mediated by maternal confounds. The chick thus provides a suitable model for the rapid screening of neuroteratogens and elucidation of the mechanisms underlying behavioral anomalies.

Critical periods for chlorpyrifos-induced developmental neurotoxicity: alterations in adenylyl cyclase signaling in adult rat brain regions after gestational or neonatal exposure
Meyer, A., Seidler, F. J., Aldridge, J. E., Tate, C. A., Cousins, M. M., and Slotkin, T. A.
Environ Health Perspect 2004;112(3):295-301

Developmental exposure to chlorpyrifos (CPF) alters the function of a wide variety of neural systems. In the present study we evaluated the effects in adulthood of CPF exposure of rats during different developmental windows, using the adenylyl cyclase (AC) signaling cascade, which mediates the cellular responses to numerous neurotransmitters. Animals were exposed on gestational days (GD) 9-12 or 17-20 or on postnatal days (PN) 1-4 or 11-14 and assessed at PN60. In addition to basal AC activity, we evaluated the responses to direct AC stimulants (forskolin, Mn2+) and to isoproterenol, which activates signaling through ss-adrenoceptors coupled to stimulatory G-proteins. CPF exposure in any of the four periods elicited significant changes in AC signaling in a wide variety of brain regions in adulthood. In general, GD9-12 was the least sensitive stage, requiring doses above the threshold for impaired maternal weight gain, whereas effects were obtained at subtoxic doses for all other regimens. Most of the effects were

heterologous, involving signaling elements downstream from the receptors, and thus shared by multiple stimulants; superimposed on this basic pattern, there were also selective alterations in receptor-mediated responses, in G-protein function, and in AC expression and subtypes. Exposures conducted at GD17-20 and later all produced sex-selective alterations. These results suggest that developmental exposure to CPF elicits long-lasting alterations in cell-signaling cascades that are shared by multiple neurotransmitter and hormonal inputs; the resultant abnormalities of synaptic communication are thus likely to occur in widespread neural circuits and their corresponding behaviors.

Developmental effects of chlorpyrifos extend beyond neurotoxicity: critical periods for immediate and delayed-onset effects on cardiac and hepatic cell signaling
Meyer, A., Seidler, F. J., and Slotkin, T. A.
Environ Health Perspect 2004;112(2):170-8

The fetal and neonatal neurotoxicity of chlorpyrifos (CPF) and related insecticides is a major concern. Developmental effects of CPF involve mechanisms over and above cholinesterase inhibition, notably events in cell signaling that are shared by nonneural targets. In the present study, we evaluated the immediate and long-term effects of CPF exposure of rats during different developmental windows [gestational days (GD) 9-12 or 17-20, postnatal days (PN) 1-4 or 11-14] on the adenylyl cyclase (AC) signaling cascade in the heart and liver. In addition to basal AC activity, we assessed the responses to direct AC stimulants (forskolin, Mn2+); to isoproterenol and glucagon, which activate signaling through specific membrane receptors; and to sodium fluoride, which activates the G-proteins that couple the receptors to AC. Few immediate effects on AC were apparent when CPF doses remained below the threshold for systemic toxicity. Nevertheless, CPF exposures on GD9-12, GD17-20, or PN1-4 elicited sex-selective effects that emerged by adulthood (PN60), whereas later exposure (PN11-14) elicited smaller, nonsignificant effects, indicative of closure of the window of vulnerability. Most of the effects were heterologous, involving signaling elements downstream from the receptors, and thus were shared by multiple inputs; superimposed on this basic pattern, there were also selective alterations in receptor-mediated responses. These results suggest that the developmental toxicity of CPF extends beyond the nervous system, to include cell signaling cascades that are vital to cardiac and hepatic homeostasis. Future work needs to address the potential implications of these effects for cardiovascular and metabolic disorders that may emerge long after the end of CPF exposure.

Developmental chlorpyrifos effects on hatchling zebrafish swimming behavior
Levin, E. D., Swain, H. A., Donerly, S., and Linney, E.
Neurotoxicol Teratol 2004;26(6):719-23

Chlorpyrifos (CPF), a widely used organophosphate insecticide and potent acetylcholinesterase inhibitor, interferes with neurobehavioral development. Rat models have been key in demonstrating that developmental CPF exposure causes learning deficits and locomotor activity

alterations, which persist into adulthood. Complementary nonmammalian models can be useful in determining the neurodevelopmental mechanisms underlying these persisting behavioral effects. Zebrafish (Danio rerio) with their clear chorion and extensive developmental information base provide an excellent model for assessment of molecular processes of toxicant-impacted neurodevelopment. We have developed methods for assessing spatial discrimination learning in adult zebrafish and have documented persisting effects of developmental CPF exposure on swimming activity and learning after low and high doses of CPF (10 and 100 ng/ml) administered to zebrafish embryos on Days 1-5 postfertilization (pf). In the current study, we developed methods for behavioral assessment of CPF exposure on swimming activity in newly hatched zebrafish. An equal area segmented annular grid (concentric circles divided into quadrants through the diameter) was made in a 16-mm diameter cylinder. The test area was placed on a heating device secured to an Olympus SZH10 dissecting scope stage. Zebrafish embryos were exposed to 10 ng/ml CPF, 100 ng/ml CPF, or vehicle control (25 microl/ml DMSO) (n=8-10/treatment group). Each treatment group was kept in a total volume of 25 ml of egg water (60 mg/ml Instant Ocean) including DMSO with or without CPF mixed to above dilutions in an incubator set at 28.5 degrees C. CPF dilutions or vehicle were changed daily with exposure ending on Day 5 pf. Testing of larval zebrafish was performed on Days 6 and 9 pf. The fish were placed in the test cylinder with 1.5 ml of egg H(2)O (28.5 degrees C). After a 2-min acclimation period, the swimming activity of the fish was measured for a 3-min testing session. The 100 ng/ml CPF dose caused significant slowing of swimming activity on Days 6 and 9 pf and had persisting effects of impairing spatial discrimination and decreasing response latency in adulthood. Developmental exposure to 10 ng/ml of CPF did not cause a significant change in locomotor activity during the period soon after hatching. CPF exposure during early development caused clear behavioral impairments detectable during the posthatching period. In a previous study, we found that early developmental CPF exposure caused behavioral alterations in zebrafish, which lasted throughout adulthood. The molecular mechanisms by which early developmental CPF exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebrafish model.

Developmental exposure to chlorpyrifos elicits sex-selective alterations of serotonergic synaptic function in adulthood: critical periods and regional selectivity for effects on the serotonin transporter, receptor subtypes, and cell signaling
Aldridge, J. E., Seidler, F. J., and Slotkin, T. A.
Environ Health Perspect 2004;112(2):148-55

During brain development, serotonin (5HT) provides essential neurotrophic signals, and in earlier work, we found that developmental exposure to chlorpyrifos (CPF) elicits short-term changes in 5HT systems. In the present study, we evaluated the effects in adulthood after CPF exposures from the neural tube stage [gestational days (GD) 9-12] and the late gestational period (GD17-20) through postnatal neuronal differentiation and synaptogenesis [postnatal days (PN) 1-4 and 11-14], using treatments below the threshold for systemic toxicity. With exposure on GD9-12, CPF elicited global elevations in 5HT1A and 5HT2 receptors and in the 5HT presynaptic

transporter. The GD17-20 treatment elicited larger effects that displayed selectivity for regions with 5HT nerve terminals and that were preferential for males. Although similar receptor up-regulation was seen after PN1-4 exposure, the effects were larger in regions with 5HT cell bodies; in addition, the presynaptic transporter was down-regulated in the nerve terminal zones of females. The PN11-14 exposure had much smaller effects on receptors but still elicited transporter suppression with the same regional and sex selectivity. Although CPF exposure on GD17-20, PN1-4, or PN11-14 altered the ability of 5HT to modulate adenylyl cyclase, this change did not correspond with the effects on 5HT receptors, suggesting an additional set of effects on proteins that transduce the 5HT signal. Our results indicate that CPF elicits long-lasting changes in 5HT receptors, the presynaptic 5HT transporter, and 5HT-mediated signal transduction after exposure in discrete developmental windows that range from the neural tube stage through synaptogenesis. These effects are likely to contribute to neurobehavioral teratology of CPF.

Maternal exposure to nicotine and chlorpyrifos, alone and in combination, leads to persistently elevated expression of glial fibrillary acidic protein in the cerebellum of the offspring in late puberty

Abdel-Rahman, A., Dechkovskaia, A. M., Mehta-Simmons, H., Sutton, J. M., Guan, X., Khan, W. A., and Abou-Donia, M. B.

Arch Toxicol 2004;78(8):467-76

We previously showed that maternal exposure to nicotine, alone or in combination with chlorpyrifos, caused an increase in glial fibrillary acidic protein (GFAP) immunostaining in the CA1 subfield of hippocampus and cerebellum in postnatal day (PND) 30 offspring. In the present study, PND 60 offspring were evaluated for histopathological and cholinergic effects following maternal exposure to nicotine and chlorpyrifos, alone and in combination. Timed-pregnant Sprague-Dawley rats (300-350 g) were treated daily with nicotine (1 mg/kg, s.c., in normal saline) or chlorpyrifos (0.1 mg/kg, dermal, in ethanol) or a combination of nicotine and chlorpyrifos from gestational days (GD) 4 to 20. Control animals were treated with saline and ethanol. On PND 60, the offspring were evaluated for cholinergic changes and pathological effects. Plasma butyrylcholinesterase (BChE) activity in the female offspring from chlorpyrifos treated mothers showed a significant increase (approximately 183% of control). Male offspring from mothers treated with either chlorpyrifos or nicotine alone showed a significant increase in the acetylcholinesterase (AChE) activity in the brainstem while female offspring from mothers treated with either nicotine or a combination of nicotine and chlorpyrifos showed a significant increase (approximately 134 and 126% of control, respectively) in AChE activity in the brainstem. No significant changes were observed in the ligand binding densities for alpha4beta2 and alpha7 nicotinic acetylcholine receptors in the cortex. Histopathological evaluation using cresyl violet staining showed a significant decrease in surviving Purkinje neurons in the cerebellum of the offspring from nicotine treated mothers. An increase in GFAP immunostaining in cerebellar white matter was observed in the offspring from the mothers treated with nicotine. These results suggest that maternal exposure to real-life levels of nicotine and/or chlorpyrifos

causes differential regulation of brainstem AChE activity. Also, nicotine caused a decrease in the surviving neurons and an increased expression of GFAP in cerebellar white matter of the offspring on PND 60. These changes can lead to long-term neurological adverse health effects later in life.

Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos
Greenlee, A. R., Ellis, T. M., and Berg, R. L.
Environ Health Perspect 2004;112(6):703-9

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 ($p \leq 0.05$) and reduced development to blastocyst and mean cell number per embryo for 3 of 13 agents ($p \leq 0.05$). Mixtures simulating preemergent herbicides, postemergent herbicides, and fungicides increased the percentage of apoptosis in exposed embryos ($p \leq 0.05$). Mixtures simulating groundwater contaminants, insecticide formulation, and lawn-care herbicides reduced development to blastocyst and mean cell number per embryo ($p \leq 0.05$). 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.

Morphologic effects of subtoxic neonatal chlorpyrifos exposure in developing rat brain: regionally selective alterations in neurons and glia
Roy, T. S., Seidler, F. J., and Slotkin, T. A.
Brain Res Dev Brain Res 2004;148(2):197-206

The widely used organophosphate insecticide, chlorpyrifos (CPF), elicits neurobehavioral teratogenesis with exposure windows ranging from the embryonic neural tube stage through postnatal development. To explore the morphologic changes occurring in late-stage exposure, newborn rats were given 5 mg/kg of CPF s.c. daily on postnatal days (PN) 11-14, a regimen that

is devoid of systemic toxicity, but that elicits long-term cognitive impairment. On PN15 and 20, we examined the septal nucleus, striatum and somatosensory cortex. Across all three regions, CPF elicited a significant decrease in the number of glial cells. Superimposed on this basic pattern, there were region-specific alterations in the number and type of neurons, and neuronal perikaryal dimensions. In the septal nucleus, the CPF group exhibited an increase in the number of neurons on PN20, representing a delay in the normal maturational decline; there was a parallel decrease in the glial/neuronal ratio. In the striatum, the number of neurons per unit area was reduced in the CPF group, accompanied by perikaryal hypertrophy, as evidenced by an increase in the average neuronal cell diameter. In the somatosensory cortex, the distribution of cell sizes indicated a decrease in the proportion of small, nonpyramidal cells. Thus, there are subtle morphological changes in the juvenile rat brain after neonatal CPF exposure that are detectable with quantitative analysis and that correlate with later emergence of behavioral alterations. Furthermore, the current findings support the hypothesis that CPF interferes with gliogenesis, a relatively late event in brain development; accordingly, the vulnerable period for adverse effects of CPF is likely to extend into childhood or adolescence.

Neurochemical effects of repeated gestational exposure to chlorpyrifos in developing rats
Richardson, J. R. and Chambers, J. E.
Toxicol Sci 2004;77(1):83-90

The neurochemical effects in developing rats exposed during gestation to the anticholinesterase organophosphorus insecticide chlorpyrifos (CPS) were determined. Pregnant rats were dosed daily with CPS (0, 3, or 7 mg/kg) in corn oil from gestation days (GD) 6-20. Pups were euthanized on postnatal days (PND) 1, 3, 6, 9, 12, and 30 for the determination of brain cholinesterase (ChE) and choline acetyltransferase (ChAT) activities, along with muscarinic receptor (mAChR) densities, the levels of the high-affinity choline uptake (HACU) system, and the vesicular acetylcholine transporter (VAChT). ChE activities were inhibited about 15 and 30% on PND 1, in the low- and high-dosage groups, respectively, and were not different from control values by PND 6. mAChR densities on PND 1 were reduced in the high-dosage group by about 18, 21, and 17%, using 3H-N-methylscopolamine, 3H-quinuclidinyl benzilate, and 3H-4-DAMP, respectively, as ligands, and were not different from control levels by PND 6. ChAT activity was decreased by approximately 12% in the high-dosage group on PND 9, 12, and 30. HACU levels, using 3H-hemicholinium-3 as the ligand, were reduced by approximately 25% on PND 6 in the low- and high-dosage groups, and by approximately 14 and 21% on PND 12 and 30, only in the high-dosage group. Levels of the VAChT were reduced by a range of 13-31% on PND 3 through 30 in the high-dosage group, using 3H-AH5183 (vesamicol) as the ligand. These data suggest that gestational exposure to 7 mg/kg/day CPS results in long-term alterations of presynaptic cholinergic neurochemistry.

Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination
Levin, E. D., Chrysanthis, E., Yacisin, K., and Linney, E.
Neurotoxicol Teratol 2003;25(1):51-7

Chlorpyrifos (CPF) is a widely used insecticide, which has been shown to interfere with neurobehavioral development. Rat models have been key in demonstrating that prenatal CPF exposure causes choice accuracy deficits and motor alterations, which persist into adulthood. Complementary nonmammalian models can be useful in determining the molecular mechanisms underlying the persisting behavioral effects of developmental CPF exposure. Zebrafish with their clear chorion and extensive developmental information base provide an excellent model for assessment of molecular processes of toxicant impacted neurodevelopment. To facilitate the use of the zebrafish model and to compare it to the more typical rodent models, the behavioral phenotype of CPF toxicity in zebrafish must be well characterized. Our laboratory has developed methods for assessing spatial discrimination learning in zebrafish, which can differentiate response latency from choice accuracy in a three chambered fish tank. Low and high doses of CPF (10 and 100 ng/ml on days 1-5 postfertilization) both had significant persisting effects on both spatial discrimination and response latency over 18 weeks of testing. The high, but not the low dose, significantly accelerated mortality rates of the fish during the study from 20-38 weeks of age. Developmental exposure to either 10 or 100 ng/ml of CPF caused significant spatial discrimination impairments in zebrafish when they were adults.

Developmental neurotoxicity elicited by prenatal or postnatal chlorpyrifos exposure: effects on neurospecific proteins indicate changing vulnerabilities
Garcia, S. J., Seidler, F. J., and Slotkin, T. A.
Environ Health Perspect 2003;111(3):297-303

The developmental neurotoxicity of the organophosphate pesticide chlorpyrifos (CPF) is thought to involve both neurons and glia, thus producing a prolonged window of vulnerability. To characterize the cell types and brain regions involved in these effects, we administered CPF to developing rats and examined neuroprotein markers for oligodendrocytes (myelin basic protein, MBP), for neuronal cell bodies (neurofilament 68 kDa, NF68), and for developing axons (neurofilament 200 kDa, NF200). Prenatal CPF administration on gestational days (GDs) 17-20 elicited an immediate (GD21) enhancement of MBP and NF68; by postnatal day (PN) 30, however, there were deficits in all three biomarkers, with the effect restricted to females. Exposure in the early postnatal period, PN1-4, did not evoke significant short-term or long-term changes in the neuroproteins. However, with treatment on PN11-14, we found reductions in MBP in the immediate posttreatment period (PN15, PN20) throughout the brain, and deficiencies across all three proteins emerged by PN30. With this regimen, males were targeted preferentially. The sex-selective effects seen here for the GD17-20 and PN11-14 regimens match those reported earlier for subsequent behavioral performance. These results indicate a shift in the populations of neural cells targeted by CPF, dependent upon the period of exposure. Similarly,

developmental differences in the sex selectivity of the biochemical mechanisms underlying neurotoxicant actions are likely to contribute to discrete behavioral outcomes.

Developmental toxicity study of chlorpyrifos in rats
Farag, A. T., El Okazy, A. M., and El-Aswed, A. F.
Reprod Toxicol 2003;17(2):203-8

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate) was evaluated for potential developmental toxicity. Groups of 30 bred female Fischer 344 rats were given 0, 5, 15, and 25mg/kg per day by gavage on gestation days 6-15; the fetuses were evaluated on gestation day 21. Clinical signs of toxicity attributed to chlorpyrifos were noted in dams receiving 15 and 25mg/kg per day. Maternal effects in these groups also included depressed body weight and acetylcholinesterase activity. Fetal weight and viability were decreased, and fetal death and early resorption were increased at the 25mg/kg per day maternal dose. Visceral, skeletal, and external variations were also increased in this group. Chlorpyrifos showed fetotoxic and teratogenic effects at a maternal dose of 25mg/kg per day, a dose that also produced maternal toxicity.

Effects of gestational exposure to chlorpyrifos on postnatal central and peripheral cholinergic neurochemistry
Richardson, J. and Chambers, J.
J Toxicol Environ Health A 2003;66(3):275-89

The effects of gestational exposure to the commonly used organophosphorus insecticide chlorpyrifos (O,O-diethyl O-[3,5,6-trichloro-2-pyridinyl]phosphorothioate) on postnatal central and peripheral cholinergic neurochemistry were investigated. Pregnant rats were orally dosed daily with chlorpyrifos (0, 3, 5, or 7 mg/kg) in corn oil from gestation day 6 to 20. Pups were sacrificed on postnatal days 1, 3, 6, 9, and 12 for the determination of brain, heart, lung, and serum cholinesterase, and brain choline acetyltransferase activities, along with liver carboxylesterase activity. Exposure to chlorpyrifos did not produce signs of overt toxicity to the dams or developing offspring. Cholinesterase activities were inhibited in a dose-related manner, with brain cholin-esterase inhibition of about 26%, 32%, and 45% on postnatal day 1. Inhibition of brain cholineste-rase persisted in all treatment groups until postnatal day 6 and in the medium and high-dosage groups through postnatal day 9. Liver carboxylesterase activity was also inhibited in a dose-related manner, with a recovery profile parallel to that of brain cholinesterase. Choline acetyltransferase activity was decreased by about 13% in the high-dosage group on postnatal days 9 and 12. These results indicate that gestational exposure to chlorpyrifos results in relatively persistent inhibition of brain cholinesterase and a delayed depression of choline acetyltransferase at a time when brain cholinesterase activity had returned to control levels in the high-dosage group.
The impairment caused by 10 ng/ml was seen during early but not later testing, while the impairment caused by 100 ng/ml became more pronounced with continued testing. The higher

dose caused a more pervasive impairment. The 10 and 100 ng/ml doses had opposite effects on response latency. The low 10 ng/ml dose significantly slowed response latency, while the high 100 ng/ml dose significant increased response latency. Both of these effects diminished with continued testing. CPF exposure during early development caused clear behavioral impairments, which lasted throughout adulthood in zebrafish. The molecular mechanisms by which early developmental CPF exposure produces these behavioral impairments expressed in adulthood can now be studied in the zebrafish model.

Increased expression of glial fibrillary acidic protein in cerebellum and hippocampus: differential effects on neonatal brain regional acetylcholinesterase following maternal exposure to combined chlorpyrifos and nicotine

Abdel-Rahman, A., Dechkovskaia, A., Mehta-Simmons, H., Guan, X., Khan, W., and Abou-Donia, M.

J Toxicol Environ Health A 2003;66(21):2047-66

Cigarette smoking and environmental exposure to chlorpyrifos during pregnancy could lead to developmental toxicity in the offspring. In the present study, pregnant female Sprague-Dawley rats (300-350 g) were treated daily with nicotine (1 mg/kg, sc) or chlorpyrifos (0.1 mg/kg, dermal) or a combination of nicotine and chlorpyrifos from gestational days (GD) 4-20. Control animals were treated with saline and ethanol. Male offspring from the mothers treated with nicotine alone gained significantly less weight on postnatal day (PND) 30 as compared to control. On PND 7, there was a significant increase in brain acetylcholinesterase (AChE) activity in pups from nicotine- and chlorpyrifos-treated dams, whereas plasma butyrylcholinesterase (BChE) activity was significantly elevated in pups of mothers treated with either chlorpyrifos alone or pesticide combined with nicotine. On PND 30 there was a significant increase in AChE activity in brainstem and cerebellum in all treated male pups. In female pups on PND 30 there was a significant rise in AChE activity in brainstem of chlorpyrifos alone and in cerebellum of the combination nicotine and chlorpyrifos group. Histopathological evaluation demonstrated an increased neuronal cell death in the cerebellum granular cell layer of female offspring from nicotine or combined nicotine with chlorpyrifos group. A rise in glial fibrillary acidic protein (GFAP) immunostaining was observed in the CA1 subfield of hippocampus and cerebellum on PND 30 in female and male offspring of mothers treated with either nicotine or nicotine in combination with chlorpyrifos, but to a lesser extent in males. Data suggest that maternal exposure to nicotine and chlorpyrifos, alone or in combination, produces differential alterations in brain regional AChE activity and expression of GFAP in cerebellum and hippocampus in offspring on PND 30.

Biochemical effects of chlorpyrifos on two developmental stages of Xenopus laevis
Richards, S. M. and Kendall, R. J.
Environ Toxicol Chem 2002;21(9):1826-35

Abstract-The effects of a 96-h static exposure to chlorpyrifos were examined in two developmental stages of larval Xenopus laevis (premetamorph and metamorph). Measures of effect included mortality, deformity, cholinesterase (ChE) activity, and DNA and protein concentration. All parameters indicated that metamorphs were more sensitive than were premetamorphs. For larvae exposed as premetamorphs, the median lethal concentration and median effective concentration were 14.6 mg/L and 1.71 mg/L; for those exposed as metamorphs, values were 0.56 mg/L and 0.24 mg/L, respectively. Cholinesterase activity was the most sensitive biochemical parameter. Exposure to chlorpyrifos at 0.01 mg/L caused significant decreases in the ChE activity of metamorphs; 0.1 mg/L significantly decreased premetamorph ChE activity. Metamorph DNA was significantly decreased at 0.1 mg/L; premetamorph DNA was not reduced until exposure to 1.0 mg/L. Whole-body protein was the least sensitive biochemical measure of effect. Premetamorphs did not experience a reduction in protein concentrations. Metamorph protein concentration was significantly decreased at 1.0 mg/L. Based on current surface water data, the most sensitive effect would not have a high probability (< or = 4.2%) of occurring in the environment.

Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein
Garcia, S. J., Seidler, F. J., Qiao, D., and Slotkin, T. A.
Brain Res Dev Brain Res 2002;133(2):151-61

The organophosphate pesticide, chlorpyrifos (CPF), is a developmental neurotoxicant. In cell cultures, CPF affects gliotypic cells to a greater extent than neuronotypic cells, suggesting that glial development is a specific target. We administered CPF to developing rats and examined the levels of glial fibrillary acidic protein (GFAP), an astrocytic marker. Prenatal CPF exposure (gestational days 17-20) elicited an increase in GFAP levels in fetal brain, but the effect was seen only at high doses that elicited maternal and fetal systemic toxicity. Early postnatal (PN) CPF treatment (PN1-4) elicited effects only in the cerebellum of male rats; GFAP was suppressed initially (PN5) and showed a rebound elevation (PN10) before returning to normal values by PN30. In contrast, when we administered CPF during the peak of gliogenesis and glial cell differentiation (PN11-14), GFAP was initially decreased across all brain regions and in both sexes; in males, subsequent elevations were seen on PN30, with the largest effect in the striatum; females also showed an increase in striatal GFAP. Our results indicate that CPF disrupts the pattern of glial development in vivo, with the maximum effect corresponding to the peak period of gliogenesis and glial cell differentiation. As glia are responsible for axonal guidance, synaptogenesis and neuronal nutrition, glial targeting suggests that these late-occurring developmental processes are vulnerable to CPF, extending the critical period for susceptibility into stages of synaptic plasticity, myelination, and architectural modeling of the developing brain.

Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro
Andersen, H. R., Vinggaard, A. M., Rasmussen, T. H., Gjermandsen, I. M., and Bonefeld-Jorgensen, E. C.
Toxicol Appl Pharmacol 2002;179(1):1-12

Twenty-four pesticides were tested for interactions with the estrogen receptor (ER) and the androgen receptor (AR) in transactivation assays. Estrogen-like effects on MCF-7 cell proliferation and effects on CYP19 aromatase activity in human placental microsomes were also investigated. Pesticides (endosulfan, methiocarb, methomyl, pirimicarb, propamocarb, deltamethrin, fenpropathrin, dimethoate, chlorpyriphos, dichlorvos, tolchlofos-methyl, vinclozolin, iprodion, fenarimol, prochloraz, fosetyl-aluminum, chlorothalonil, daminozid, paclobutrazol, chlormequat chlorid, and ethephon) were selected according to their frequent use in Danish greenhouses. In addition, the metabolite mercaptodimethur sulfoxide, the herbicide tribenuron-methyl, and the organochlorine dieldrin, were included. Several of the pesticides, dieldrin, endosulfan, methiocarb, and fenarimol, acted both as estrogen agonists and androgen antagonists. Prochloraz reacted as both an estrogen and an androgen antagonist. Furthermore, fenarimol and prochloraz were potent aromatase inhibitors while endosulfan was a weak inhibitor. Hence, these three pesticides possess at least three different ways to potentially disturb sex hormone actions. In addition, chlorpyrifos, deltamethrin, tolclofos-methyl, and tribenuron-methyl induced weak responses in one or both estrogenicity assays. Upon cotreatment with 17beta-estradiol, the response was potentiated by endosulfan in the proliferation assay and by pirimicarb, propamocarb, and daminozid in the ER transactivation assay. Vinclozolin reacted as a potent AR antagonist and dichlorvos as a very weak one. Methomyl, pirimicarb, propamocarb, and iprodion weakly stimulated aromatase activity. Although the potencies of the pesticides to react as hormone agonists or antagonists are low compared to the natural ligands, the integrated response in the organism might be amplified by the ability of the pesticides to act via several mechanism and the frequent simultaneous exposure to several pesticides.

Functional alterations in CNS catecholamine systems in adolescence and adulthood after neonatal chlorpyrifos exposure
Slotkin, T. A., Tate, C. A., Cousins, M. M., and Seidler, F. J.
Brain Res Dev Brain Res 2002;133(2):163-73

Chlorpyrifos (CPF), one of the most widely used pesticides, is a neurobehavioral teratogen in animals. We administered CPF to neonatal rats on postnatal days (PN) 1-4 (1 mg/kg) or PN11-14 (5 mg/kg), regimens devoid of overt systemic toxicity. We then examined the impact on catecholaminergic systems in adolescence (PN30) and adulthood (PN60), assessing basal neurotransmitter content and transmitter utilization rates (turnover) in brain regions comprising the major noradrenergic and dopaminergic projections. Although CPF had only sporadic effects on basal norepinephrine and dopamine content, it profoundly suppressed norepinephrine turnover across multiple regions, indicative of net reductions in presynaptic activity. Dopamine

turnover showed less consistent effects, with subnormal turnover in some regions and activation in others. We also evaluated whether CPF exposure altered the ability of catecholamine systems to respond to acute cholinergic stimulation, elicited by administration of a single challenge dose of nicotine. In the normal brain, nicotine increases the utilization of norepinephrine and dopamine. With only a few exceptions, animals receiving neonatal CPF exposure showed lasting desensitization of the nicotine response; not only was the activation by nicotine blunted in the CPF group, but in some regions the nicotine response was reversed, eliciting a reduction in transmitter turnover. These results indicate that neonatal CPF exposure produces widespread deficiencies in catecholaminergic synaptic function that persist into adulthood, and that are best revealed by dynamic measures of synaptic activity and responsiveness, as opposed to static markers like basal transmitter levels. The effects seen here are likely to contribute to alterations in behavioral performance that persist or emerge long after the termination of CPF exposure.

Inhibition and recovery of maternal and fetal cholinesterase enzymes following a single oral dose of chlorpyrifos in rats

Ashry, K. M., Abu-Qare, A. W., Saleem, F. R., Hussein, Y. A., Hamza, S. M., Kishk, A. M., and Abou-Donia, M. B.

Arch Toxicol 2002;76(1):30-9

Pregnant Sprague-Dawley rats (14-18 days of gestation) were treated with a single dose of 50 mg/kg (61% of oral LD50 in female rats) of chlorpyrifos (0,0-diethyl- 0-3,5,6-trichloro-2-pyridyl phosphorothioate) by oral gavage. Animals treated on day 18 of gestation were sacrificed at 1, 2, 4, 12 h after dosing. Animals treated on days 17, 16, 15, and 14 of gestation were sacrificed at 24, 48, 72, and 96 h after dosing, respectively. Maternal and fetal brain acetylcholinesterase (AchE) and plasma butyrylcholinesterase (BuChE) activities were significantly inhibited 1 h after treatment. Activity of fetal brain AChE and plasma BuChE recovered faster than that of the maternal enzymes. Peak inhibition of maternal spinal cord AChE and BuChE activities occurred 2 h and 1 h after dosing, respectively. Maternal spinal cord BuChE activity was totally recovered by 96 h compared to the partial recovery of spinal cord AChE activity. Maternal liver BuChE activity was significantly decreased within 1 h of dosing. The individual molecular forms (10S and 4S) of maternal and fetal brain AChE and BuChE activities were significantly decreased 1 h after treatment. Recovery of both forms of fetal brain AChE activity was much faster than the maternal forms. Activity of the 10S form of maternal control brain AChE was significantly higher than in the fetus control. The rapid recovery of cholinesterase enzymes in the fetus is attributed to the de novo synthesis of AChE enzymes in the fetus compared to the mother.

Pharmacokinetic profile and placental transfer of a single intravenous injection of
Abdel-Rahman, A. A., Blumenthal, G. M., Abou-Donia, S. A., Ali, F. A., Abdel-Monem, A. E., and Abou-Donia, M. B.
Arch Toxicol 2002;76(8):452-9

The pharmacokinetics and placental transfer of a single intravenous dose of 5.0 mg/kg (10 micro Ci/kg) ring-labeled [(14)C]chlorpyrifos were investigated in pregnant Sprague-Dawley rats at 11-13 days of gestation. Three rats were killed at 5, 15 or 30 min, or 1, 2, 4, 8, 12, 18, 24, 36, 48, 72 or 96 h after dosing. Radioactivity and 3,5,6-trichloropyridinol (TCP) were detected in all tissues 5 min after dosing. Chlorpyrifos was only found in maternal plasma and liver. Peak maternal plasma concentration of radioactivity (micro g chlorpyrifos equivalents/ml) was 157 at 5 min, compared with 1.9 for fetal plasma at 15 min. The maximum concentrations of radioactivity (micro g chlorpyrifos equivalents/g), detected in most tissues within 12 h of dosing, were, in descending order: liver (30), brain (29), placenta (21), and fetus (2). All peaks occurred at 5 min except for fetus and fetal plasma, which were at 15 min. TCP was detected by HPLC as the major compound identified in plasma and tissues. The maximum concentration detected was in plasma, at 12.4 micro g/ml, and for the following tissues was: liver 4.3 ng/g fresh tissue, fetus 4 ng/g, placenta 2.97 ng/g, brain 1.68 ng/g, and fetal plasma 0.52 ng/g. All TCP peaks occurred at 5 min except for fetus at 30 min and fetal plasma at 15 min. Parent chlorpyrifos was detected in maternal plasma and liver at maximum concentrations of 5.1 micro g/ml and 0.40 micro g/g, respectively, at 5 min. Chlorpyrifos was detectable in maternal plasma up to 36 h after dosing, and in liver up to 24 h after dosing. Pharmacokinetic analysis best described radioactivity, chlorpyrifos, and TCP as disappearing biexponentially from plasma and tissues. The terminal elimination half-lives of radioactivity, chlorpyrifos and TCP from maternal plasma were 16, 18, and 16 h, respectively. The results indicate that (1). chlorpyrifos undergoes a rapid metabolism to its major metabolites (TCP); (2). chlorpyrifos and its metabolites are distributed to all maternal and fetal tissues and plasma; and (3). the elimination of chlorpyrifos and TCP is slow, with redistribution from lipid stores a likely determinant of elimination rates.

Does the developmental neurotoxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells
Garcia, S. J., Seidler, F. J., Crumpton, T. L., and Slotkin, T. A.
Brain Res 2001;891(1-2):54-68

The widespread use of chlorpyrifos (CPF) has raised major concerns about its potential to cause fetal or neonatal neurobehavioral damage, even at doses that do not evoke acute toxicity. CPF has been shown to inhibit replication of brain cells, to elicit alterations in neurotrophic signaling governing cell differentiation and apoptosis, and to evoke oxidative stress. However, the specific cell types targeted by CPF have not been clarified, an issue of vital importance in establishing the boundaries of the critical period in which the developing brain is vulnerable. In the current study, we evaluated the effects of CPF on C6 glioma cells, a well-established glial model. In

undifferentiated C6 cells, CPF inhibited DNA synthesis in a concentration-dependent manner, with greater potency than had been seen previously with neuronal cell lines. Just as found after in vivo CPF treatment or with neuronal cell lines, the effects on cell replication were independent of cholinergic stimulation, as cholinergic antagonists did not block CPF-induced inhibition. CPF interfered with cell signaling mediated through adenylyl cyclase at the level of G-protein function; the effects again were greater in undifferentiated C6 cells but were still detectable in differentiating cells. In contrast, differentiation enhanced the ability of CPF to elicit the formation of reactive oxygen species and to evoke deficits in Sp1, a nuclear transcription factor essential for differentiation. These results indicate that glial-type cells are targeted by CPF through the same multiple mechanisms that have been demonstrated for the effects of CPF on brain development in vivo. Because glial development continues long after the conclusion of neurogenesis, and given that CPF targets events in both glial cell replication and the later stages of differentiation, the vulnerable period for developmental neurotoxicity of CPF is likely to extend well into childhood.

Inhibition of cholinesterase enzymes following a single dermal dose of chlorpyrifos and methyl parathion, alone and in combination, in pregnant rats
Abu-Qare, A. W., Abdel-Rahman, A., Brownie, C., Kishk, A. M., and Abou-Donia, M. B.
J Toxicol Environ Health A 2001;63(3):173-89

Pregnant Sprague-Dawley rats (14-18 d of gestation) were treated with either a single dermal subclinical dose of 30 mg/kg (15% of dermal LD50) chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridinyl] phosphorothioate) or a single dermal subclinical dose of 10 mg/kg (15% of dermal LD50) methyl parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate) or the two in combination. Chlorpyrifos inhibited maternal and fetal brain acetylcholinesterase (AChE) activity within 24 h of dosing, (48% and 67% of control activity, respectively). Following application of methyl parathion, peak inhibition of maternal and fetal brain AChE activity occurred at 48 h and 24 h after dosing (17% and 48% of control activity, respectively). A combination of chlorpyrifos and methyl parathion produced peak inhibition of maternal and fetal brain AChE activity at 24 h postdosing (35% and 73% of control activity, respectively). Maternal and fetal brain AChE activity recovered to various degrees of percentage of control 96 h after dosing. Application of methyl parathion or chlorpyrifos alone or in combination significantly inhibited maternal plasma butyrylcholinesterase (BuChE) activity. No significant inhibition of fetal plasma BuChE activity was detected. Peak inhibition of maternal liver BuChE occurred 24 h after application of methyl parathion or chlorpyrifos alone or in combination (64%, 80%, and 61% of control activity, respectively). Significant inhibition of placental AChE occurred within 24 h after application of methyl parathion or chlorpyrifos alone or in combination. The results suggest that methyl parathion and chlorpyrifos, alone or in combination, were rapidly distributed in maternal and fetal tissues, resulting in rapid inhibition of cholinesterase enzyme activities. The lower inhibitory effect of the combination could be due to competition between chlorpyrifos and methyl parathion for cytochrome P-450 enzymes, resulting in inhibition of the formation of the potent cholinesterase inhibitor oxon forms. The faster recovery of fetal plasma BuChE is

attributed to the de novo synthesis of cholinesterase by fetal tissues compared to maternal tissues.

Comparison of oxime-initiated reactivation of organophosphorous-inhibited acetylcholinesterase in brains of avian embryos
Lesser, J., Blodgett, D., and Ehrich, M.
J Toxicol Environ Health A 2000;59(1):57-66

Organophosphorous (OP) insecticide-induced inhibition and oxime reactivation of acetylcholinesterase (AChE) was determined in whole-brain homogenates prepared from 15-d-old chick embryos. Doses of chlorpyrifos, parathion, acephate, and trichlorfon that inhibited AChE >70% were administered to the embryos. Following insecticide exposure, an in vitro system compared the capability of the oximes pralidoxime (2-PAM), obidoxime, TMB-4, and HI-6 to reactivate the OP-inhibited AChE. Concentration-related increases in AChE activities were noted in embryo brains reactivated with 2-PAM, TMB, and HI-6. 2-PAM was the most effective reactivator of trichlorfon-inhibited AChE; 2-PAM and obidoxime were relatively similar in effectiveness for reactivation of AChE inhibited with the other OP insecticides used as test agents. All oximes were similarly effective against acephate, but HI-6 was the least effective reactivator of AChE in chick embryo brain homogenates inhibited by the other OP insecticides. These results suggest that both the OP insecticide inhibiting AChE and the oxime reactivating this enzyme can contribute to the effectiveness of the avian brain AChE reactivation.

Developmental toxicity studies in rats and rabbits with 3,5,6-trichloro-2-pyridinol, the major metabolite of chlorpyrifos
Hanley, T. R. Jr, Carney, E. W., and Johnson, E. M.
Toxicol Sci 2000;53(1):100-8

3,5,6-Trichloro-2-pyridinol (TCP), the primary metabolite of chlorpyrifos and chlorpyrifos-methyl, was evaluated for potential developmental toxicity. Groups of 32-34 bred female Fischer 344 rats were given 0, 50, 100, or 150 mg TCP/kg/day by gavage on gestation days 6-15; the fetuses were evaluated on gestation day 21. Similarly, groups of 16 inseminated female New Zealand White rabbits were given 0, 25, 100, or 250 mg TCP/kg/day by gavage on gestation days 7-19, and fetuses were evaluated on gestation day 28. No clinical signs of toxicity attributed to TCP were noted in either species. In rats, at 150 mg/kg/day, maternal effects included slight decreases in feed consumption, significantly depressed body weight gain (25% relative to controls) resulting in significantly lower maternal terminal body weights, and increased relative liver weight. At 100 mg/kg/day, maternal body weight gain in rats was depressed approximately 22%. Among rabbits, maternal effects were limited to the group given 250 mg/kg/day, which lost an average of approximately 70 g during the treatment period (vs. 140 g in the controls). There were no effects on fetal weight or viability, nor were there significant increases in any fetal alteration in either species. A slightly higher (not statistically significant) than usual incidence of

central nervous system anomalies occurred in rabbits, but these anomalies were found in both treated and control groups in this study as well as contemporaneous studies of unrelated compounds. This, and the fact that these anomalies were not seen with the parent compound, chlorpyrifos, suggest that their origin was spontaneous. Thus, TCP was not considered fetotoxic or teratogenic in either rats or rabbits, even at dose levels that produced maternal toxicity.

Lack of selective developmental neurotoxicity in rat pups from dams treated by gavage with chlorpyrifos
Maurissen, J. P., Hoberman, A. M., Garman, R. H., and Hanley, T. R. Jr
Toxicol Sci 2000;57(2):250-63

Pregnant Sprague-Dawley rats were given chlorpyrifos (O:, O-diethyl-O:-[3,5,6-trichloro-2-pyridinyl] phosphorothioate; CPF) in corn oil by gavage from gestation day 6 (GD 6) through lactation day 10 (LD 10) at dosages of 0, 0.3, 1, or 5 mg/kg/day in a developmental neurotoxicity study that conformed to U.S. Environmental Protection Agency 1991 guidelines. GD 0 was the day when evidence of mating was observed and postnatal day 0 (PND 0) was the day of birth. Toxicity was limited to the highest dosage level (5 mg/kg/day) and, in the dams, consisted of muscle fasciculation, hyperpnea, and hyperreactivity. A nonsignificant overall trend toward weight gain and feed consumption was also observed in the high-dosage dams, with a statistically significant Group x Time interaction for reduced weight gain in the 5-mg/kg/day group near the end of gestation. Although many developmental indices were normal, pups from high-dosage dams had increased mortality soon after birth, gained weight more slowly than controls, and had several indications of slightly delayed maturation. The early deaths and delayed maturation were attributed to maternal toxicity, though a possible contributing role of direct pup toxicity in delayed development cannot be eliminated. In spite of the apparent delay in physical development, high-dosage pups tested just after weaning had normal learning and memory as tested on a T-maze spatial delayed-alternation task. Habituation, a primitive form of learning, was tested in 2 tasks (motor activity and auditory startle) and was not affected. No overt effects were noted in either dams or pups at 1 or 0.3 mg/kg/day. Based on these data, chlorpyrifos produced maternal and developmental toxicity in the 5-mg/kg/day-dosage group. There was no evidence of selective developmental neurotoxicity following exposure to chlorpyrifos.

Lack of differential sensitivity to cholinesterase inhibition in fetuses and neonates compared to dams treated perinatally with chlorpyrifos
Mattsson, J. L., Maurissen, J. P., Nolan, R. J., and Brzak, K. A.
Toxicol Sci 2000;53(2):438-46

Pregnant Sprague-Dawley rats were exposed to chlorpyrifos (CPF; O,O-diethyl-O-[3,5,6-trichloro-2-pyridinyl] phosphorothioate) by gavage (in corn oil) from gestation day (GD) 6 to postnatal day (PND) 10. Dosages to the dams were 0 (control), 0.3 (low), 1.0 (middle) or 5.0

mg/kg/day (high). On GD 20 (4 h post gavage), the blood CPF concentration in fetuses was about one half the level found in their dams (high-dose fetuses 46 ng/g; high-dose dams 109 ng/g). CPF-oxon was detected only once; high-dose fetuses had a blood level of about 1 ng/g. Although no blood CPF could be detected (limit of quantitation 0.7 ng/g) in dams given 0.3 mg/kg/ day, these dams had significant inhibition of plasma and red blood cell (RBC) ChE. In contrast, fetuses of dams given 1 mg/kg/day had a blood CPF level of about 1.1 ng/g, but had no inhibition of ChE of any tissue. Thus, based on blood CPF levels, fetuses had less cholinesterase (ChE) inhibition than dams. Inhibition of ChE occurred at all dosage levels in dams, but only at the high-dose level in pups. At the high dosage, ChE inhibition was greater in dams than in pups, and the relative degree of inhibition was RBC approximately plasma > or = heart > brain (least inhibited). Milk CPF concentrations were up to 200 times those in blood, and pup exposure via milk from dams given 5 mg/kg/day was estimated to be 0.12 mg/kg/day. Therefore, the dosage to nursing pups was much reduced compared to the dams exposure. In spite of exposure via milk, the ChE levels of all tissues of high-dosage pups rapidly returned to near control levels by PND 5.

Gestational exposure to chlorpyrifos: dose response profiles for cholinesterase and carboxylesterase activity
Lassiter, T. L., Barone, S. Jr, Moser, V. C., and Padilla, S.
Toxicol Sci 1999;52(1):92-100

This study investigates the in vivo dose response profiles of the target enzyme cholinesterase (ChE) and the detoxifying enzymes carboxylesterase (CaE) in the fetal and maternal compartments of pregnant rats dosed with chlorpyrifos [(O,O'-diethyl O-3,5,6-trichloro-2-pyridyl) phosphorothionate], a commonly used organophosphorus insecticide. Pregnant rats were dosed daily (po) with chlorpyrifos in corn oil (0, 3, 5, 7, or 10 mg/kg) on gestational days (GD) 14-18. Animals were sacrificed 5 h after the last chlorpyrifos dose (time of maximum brain cholinesterase inhibition) for analysis of ChE and CaE activity in maternal blood, liver, brain, placenta, and fetal liver and brain. The in vitro sensitivity (i.e., IC50, 30 min, 26 degrees C) of CaE also was determined by assaying the activity remaining after incubation with a range of chlorpyrifos-oxon concentrations. In vivo exposure to 10 mg/kg chlorpyrifos from GD14-18 caused overt maternal toxicity, with dose-related decreases in ChE activity more notable in maternal brain than fetal brain. Dose-related effects were also seen with chlorpyrifos-induced inhibition of fetal liver ChE and maternal brain CaE activities. Gestational exposure caused no inhibition of placental ChE or CaE, fetal brain CaE, or maternal blood CaE. ChE activities in the maternal blood and liver, as well as fetal and maternal liver CaE, however, were maximally inhibited by even the lowest dosage of chlorpyrifos. The in vitro sensitivity profiles of CaE to chlorpyrifos-oxon inhibition were valuable in predicting and verifying the in vivo CaE response profiles. Both the in vivo and in vitro findings indicated that fetal liver CaE inhibition was an extremely sensitive indicator of fetal chlorpyrifos exposure.

Gestational exposure to chlorpyrifos: comparative distribution of trichloropyridinol in the fetus and dam
Hunter, D. L., Lassiter, T. L., and Padilla, S.
Toxicol Appl Pharmacol 1999;158(1):16-23

Chlorpyrifos (O,O'-diethyl O-[3,5,6-trichloro-2-pyridyl] phosphorothionate) is a commonly used anticholinesterase insecticide, and therefore the potential for human exposure is high. The present time course and dose response studies were conducted to delineate the toxicokinetics of chlorpyrifos and its metabolites in the pregnant rat and fetus. Time-pregnant, Long-Evans rats were treated orally with chlorpyrifos during late gestation (Gestational Days 14-18). Following euthanasia the level of chlorpyrifos and its metabolites, chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP), were measured in both fetal and maternal brain and liver (limits of quantitation: 59.2, 28.8, and 14.0 ng/g tissue, respectively). In addition, cholinesterase inhibition was also measured in the same tissues for comparison. TCP was the only component detected. The highest level of TCP and the lowest level of cholinesterase activity showed the same time of peak effect: 5 h after the last dose. The concentration of TCP in the maternal liver was approximately fivefold higher than the TCP concentration in fetal liver, but, paradoxically, the concentration of TCP in the fetal brain was two- to fourfold higher than the TCP concentration in the maternal brain. The half-life of the TCP was identical in all tissues examined (12-15 h). These toxicokinetic results suggest that the fetal nervous system may be exposed to a higher concentration of chlorpyrifos than the maternal nervous system when the dam is orally exposed to chlorpyrifos during late gestation.

Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development
Bigbee, J. W., Sharma, K. V., Gupta, J. J., and Dupree, J. L.
Environ Health Perspect 1999;107 Suppl 1(81-7

Acetylcholinesterase (AChE) is the enzyme that hydrolyzes the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions. However, results from our laboratory and others indicate that AChE has an extrasynaptic, noncholinergic role during neural development. This article is a review of our findings demonstrating the morphogenic role of AChE, using a neuronal cell culture model. We also discuss how these data suggest that AChE has a cell adhesive function during neural development. These results could have additional significance as AChE is the target enzyme of agricultural organophosphate and carbamate pesticides as well as the commonly used household organophosphate chlorpyrifos (Dursban). Prenatal exposure to these agents could have adverse effects on neural development by interfering with the morphogenic function of AChE.

Chlorpyrifos elicits mitotic abnormalities and apoptosis in neuroepithelium of cultured rat embryos
Roy, T. S., Andrews, J. E., Seidler, F. J., and Slotkin, T. A.
Teratology 1998;58(2):62-8

Chlorpyrifos is used increasingly as a pesticide in place of more toxic alternatives such as parathion. Although chlorpyrifos is not a potent dysmorphogen, recent reports that fetal or infant exposures may exceed acceptable limits have raised concern about the potentially more subtle effects on brain development. In the current study, whole rat embryo culture was used to study the effects of chlorpyrifos at the neural tube stage of development. On embryonic day 9.5, embryos were exposed to 0.5, 5, or 50 micrograms/ml of chlorpyrifos. After 48 hr (embryonic day 11.5), embryos were examined for dysmorphogenesis and were then processed for light microscopic examination of the neuroepithelium. Examination of 1-micron-thick toluidine blue-stained sections of the forebrain and hindbrain region revealed reduced and altered mitotic figures, with dispersion and disorientation of the mitotic layer. In addition, cytotoxicity was evidenced by cytoplasmic vacuolation, enlargement of intercellular spaces, and the presence of a significant number of apoptotic cells. These alterations were evident even at the lowest concentration of chlorpyrifos, which produced no dysmorphogenesis. The effects were intensified at higher concentrations, which were just at the threshold for dysmorphogenesis; the neuroepithelial abnormalities, however, were still present in embryos that were not dysmorphogenic. Our results in rat embryo culture support the idea that chlorpyrifos specifically targets brain development at low concentrations, indicating the need to reevaluate the safety of this compound for exposure in vivo.

Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration
Dam, K., Seidler, F. J., and Slotkin, T. A.
Brain Res Dev Brain Res 1998;108(1-2):39-45

Despite the fact that they recover more rapidly from cholinesterase inhibition than do adults, developing animals are more sensitive to delayed neurotoxicity caused by chlorpyrifos exposure. Previous studies indicate that acute, high dose chlorpyrifos exposure of developing rats interferes with synthesis of brain macromolecules, dependent upon a critical maturational stage and upon regional disparities in cholinergic innervation. In order to determine whether chronic, lower level chlorpyrifos exposure targets similar developmental events, rats were treated daily on postnatal days 1-4, using a dose (1 mg/kg, s.c.) that caused no mortality or weight deficits and that produces minimal cholinesterase inhibition. At the end of the treatment period, we examined macromolecule synthesis in three brain regions possessing disparate maturational profiles and cholinergic innervation: the brainstem, which undergoes its primary phase of neurogenesis prenatally and develops prominent cholinergic innervation, the forebrain, which develops somewhat later but also becomes cholinergically enriched, and the cerebellum, which undergoes neurogenesis postnatally and remains poor in cholinergic innervation. Four h after the last

chlorpyrifos treatment, no effects were seen for DNA, RNA or protein synthesis. However, on postnatal day 5 (24 h after the last treatment), robust deficits in DNA synthesis were observed in brainstem and forebrain, with lesser effects on the cerebellum. Although the brain regional selectivity is compatible either with differences in critical maturational phases or with targeting of cholinergically-enriched brain regions, we found no significant effects in the heart, despite the fact that it is also receives cholinergic innervation. Effects on DNA synthesis were not evident 4 h after the last dose, but then appeared after 24 h. As the 4-h point is 28 h after the third dose, this suggests that a cumulative threshold needs to be exceeded in order for the delayed neurotoxicity to appear. At the point at which DNA synthesis was inhibited in brainstem and forebrain, no effects were seen for RNA or protein synthesis, indicating selectivity for macromolecule synthesis associated with cell replication. These data indicate that otherwise subtoxic, chronic exposures to chlorpyrifos nevertheless target DNA synthesis, and by inference, cell replication, in selective brain cell populations, early events that are likely contributors to the deficits in cell number that appear several days later.

Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes

Rawlings, N. C., Cook, S. J., and Waldbillig, D.
J Toxicol Environ Health A 1998;54(1):21-36

Many pesticides are used in the agricultural environment, and some may have the potential to disrupt reproductive or endocrine function. Ewes, in separate groups of 6, received orally into their rumen either empty gelatin capsules or capsules containing chlorpyrifos (12.5 mg/kg), trifluralin (17.5 mg/kg), lindane (2.5 mg/kg), or pentachlorophenol (2 mg/kg) 2 times per week for 43 d. Dimethoate (0.2 mg/kg), carbofuran (0.30 mg/kg), 2,4-dichlorophenoxyacetic acid (10 mg/kg), or triallate (5 mg/kg) was given 3 times per week. After 36 d of treatment, blood samples were taken every 12 min for 6 h for hormone analysis. Ewes were euthanized at the end of the study for necropsy and histopathology. No overt signs of toxicity were seen, and body weight was not affected by treatment. Carbofuran caused a significant increase in serum concentrations of thyroxine compared to control ewes, but all other pesticides, except trifluralin, resulted in a marked decrease in thyroxine concentrations. Serum concentrations of cortisol were significantly increased by trifluralin and chlorpyrifos. Concentrations of insulin in serum were markedly increased in ewes given dimethoate, lindane, trifluralin, triallate, and pentachlorophenol, and concentrations of estradiol were also significantly increased in ewes given lindane and trifluralin. Mean serum concentrations of LH were markedly decreased by trifluralin, and basal LH concentrations were significantly decreased by lindane, dimethoate, and trifluralin but increased by triallate. Both pentachlorophenol and triallate caused a significant increase in severity of oviductal intraepithelial cysts in ewes. Data suggest that several currently used pesticides could influence serum concentrations of reproductive and metabolic hormones, particularly thyroxine, the major secretory product of the thyroid and a principal regulator of metabolism.

Gestational exposure to chlorpyrifos: apparent protection of the fetus?
Lassiter, T. L., Padilla, S., Mortensen, S. R., Chanda, S. M., Moser, V. C., and Barone, S. Jr
Toxicol Appl Pharmacol 1998;152(1):56-65

Previous studies have shown that, in general, young, postnatal animals are more sensitive than adults to the toxic effects of anticholinesterase (antiChE) pesticides. Paradoxically, often fetal brain cholinesterase (ChE) is less inhibited than maternal brain after gestational exposure to an antiChE, presumably due to placental and fetal detoxification of the antiChE. The present investigation was designed to study selected toxicokinetic and toxicodynamic factors surrounding the toxicity of chlorpyrifos (CPF; [O,O'-diethyl O-3,5,6-trichloro-2-pyridyl] phosphorothionate) in pregnant rats dosed repeatedly or singly during late gestation. Dams were dosed daily (po) with CPF in corn oil (0 or 7 mg/kg) on gestational days (GD) 14 to 18. Animals were euthanized at 2 to 120 h after the last dose and tissues were collected for enzyme analysis. Using this dosing regimen, we found that (1) the time of maximal ChE inhibition was the same (i.e., 5-10 h after dosing) for both maternal and fetal brain, (2) the degree of fetal brain ChE inhibition was 4.7 times less than maternal brain inhibition, and (3) the detoxification potential (i.e., carboxylesterase and chlorpyrifos-oxonase) of the fetal tissues was very low compared to the maternal tissues. A separate group of experiments showed that if pregnant dams received only one oral dose of 7 or 10 mg/kg CPF on GD18, the degree of ChE inhibition in the fetal brain was comparable to the maternal brain ChE inhibition. Taking into consideration the net increase (more than fourfold) in fetal brain ChE activity from GD14 to 18 in control animals, and the fact that maternal brain ChE was inhibited more than fetal brain ChE only in a repeated-dosing regimen, we conclude that the fetus is not genuinely protected from the toxic effects of a given dose of CPF. We propose that fetal brain ChE is simply able to recover more fully between each dose as compared to maternal brain ChE, giving the illusion that the fetal compartment is less affected than the maternal compartment.

Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat
Breslin, W. J., Liberacki, A. B., Dittenber, D. A., and Quast, J. F.
Fundam Appl Toxicol 1996;29(1):119-30

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate), an organophosphate insecticide, was evaluated for its potential to produce developmental and reproductive toxicity in rats following oral exposure. Pregnant Fischer 344 rats were given doses of 0 (corn oil vehicle), 0.1, 3.0, or 15 mg chlorpyrifos/kg/day, by gavage, on Gestation Days 6 through 15. Maternal effects noted at the two higher dose levels included decreased cholinesterase levels at 3.0 mg/kg/day and cholinergic signs (excessive salivation and tremors), decreased cholinesterase levels, and decreased body weight gain at 15 mg/kg/day. No maternal effects were apparent at 0.1 mg/kg/day. Although maternal toxicity was observed at these two higher exposure levels, no developmental effects were noted at any dose. In a two-generation reproduction study, Sprague-Dawley rats were maintained on diets supplying 0, 0.1, 1.0, or 5.0 mg chlorpyrifos/kg/day. Parental effects included decreased plasma and erythrocyte cholinesterase at 1.0 mg/kg/day, and

decreased plasma, erythrocyte, and brain cholinesterase and histopathologic alterations of the adrenal zona fasciculata at 5.0 mg/kg/day. The histopathologic alterations of the adrenal were characterized as very slight to slight vacuolation (consistent with fatty change) in males, and very slight vacuolation and/or altered tinctorial properties in females. No effects on the reproductive or fertility indices or on the histopathology of reproductive tissues were observed at any dose level, and no neonatal effects were observed at 0.1 or 1.0 mg/kg/day in the F1 or F2 litters. Parental toxicity at the high dose was accompanied by decreased pup body weight and increased pup mortality in the F1 litters only. These data show that oral administration of chlorpyrifos to rats at parentally toxic dose levels was not embryolethal, embryo/fetotoxic, or teratogenic and did not adversely affect fertility or the function or structure of the reproductive organs. Although effects on neonatal growth and survival were observed at a maternally toxic dose level in one generation, this effect was not observed in the subsequent generation and, therefore, may not have been related to treatment.

Comparative developmental and maternal neurotoxicity following acute gestational exposure to chlorpyrifos in rats
Chanda, S. M., Harp, P., Liu, J., and Pope, C. N.
J Toxicol Environ Health 1995;44(2):189-202

Chlorpyrifos (CPF), an organophosphorus (OP) insecticide, exerts toxicity through inhibition of acetylcholinesterase (AChE). In the present study, pregnant Sprague-Dawley rats were given CPF (200 mg/kg, sc) as a single dose on gestation d 12 (GD12) and then sacrificed on either GD16, GD20, or postnatal d 3 (PND3) for measurement of maternal and developmental indicators of toxicity. While most CPF-treated rats exhibited no overt signs, a subset (4/28) showed moderate to severe signs of "cholinergic" toxicity at 2-3 d after treatment, and these rats were omitted from further studies. Extensive AChE inhibition (82-88%) was noted in maternal brain at all three time points following acute exposures. At GD16 and GD20, fetal brain AChE activity was inhibited 42-44%. While some degree of recovery in AChE activity was noted in pup brain by PND3, AChE activity was still inhibited (30%) in treated pups cross-fostered to control dams. In vitro inhibition of maternal and fetal (GD20) brain AChE activity by the active metabolite, chlorpyrifos oxon, suggested that the prenatal brain AChE activity was somewhat more sensitive (IC50 at 37.0 degrees C, 20 min: dam, 26.6 +/- 1.8 x 10(-9) M; fetus, 6.7 +/- 0.4 x 10(-9) M). Maternal brain muscarinic receptor binding was more extensively reduced (30-32%) at GD20 and PND3 as compared to the developing brain at GD20 (16%) and PND3 (11%). A simple postnatal reflex test (righting reflex) was transiently altered by CPF. The results suggest that CPF exposure to dams during gestation produces more extensive neurotoxicological effects in the dam relative to the developing fetus.

Effects of chlorpyrifos on neuronal development in rat embryo midbrain micromass cultures
Cosenza, M. E. and Bidanset, J.
Vet Hum Toxicol 1995;37(2):118-21

Chlorpyrifos (CPF) is a broad-spectrum organophosphate insecticide used to control mosquitos and household insects. In man it has toxic effects on the central nervous system, the cardiovascular system and the respiratory system. This study investigated the toxicity of CPF on nervous system development using the midbrain micromass culture system. Chlorpyrifos was tested as a marketed formulation and in 3 solvents. All demonstrated toxicity in midbrain micromass cells with IC50 values below 30 ug/mL, indicating a potent teratogen.

Evaluation of the genotoxic and embryotoxic potential of chlorpyrifos and its metabolites in vivo and in vitro
Muscarella, D. E., Keown, J. F., and Bloom, S. E.
Environ Mutagen 1984;6(1):13-23

The genotoxicity and embryotoxicity of chlorpyrifos (CPF) and two metabolites were evaluated using the chick embryo, Chinese hamster ovary cells, and by examining blastocysts from superovulated cows crossed to chlorpyrifos-treated bulls. Chlorpyrifos and metabolites were dissolved in acetone and administered to 3-day embryos by the air cell method. The LD50 was 1,500 micrograms/embryo when mortality was checked through and including 17 days of development. The metabolites were more embryotoxic than the parent compound, CPF. Chlorpyrifos and metabolites did not increase the sister chromatid exchange (SCE) frequency above background at any dosage in the 3-day chick embryo assay. Similarly, none of these compounds increased SCE frequencies in three-point dosage tests (1, 10, 100 micrograms/ml) using Chinese hamster ovary cells. Controls in these assays consisted of the solvent carrier acetone (7.0 +/- 2.5 SCE/cell) and 8.6 micrograms/ml methyl methane sulfonate (30.5 +/- 7.4 SCE/cell). Studies of bovine blastocysts obtained from superovulated cows crossed with Dursban 44 treated bulls did not reveal evidence of chromosome aberrations or developmental anomalies associated with pesticide application. However, reproductive performance of breeders may be subnormal as a result of severe poisoning. This underscores the limitations of short-term assays and emphasizes the need to perform thorough toxicological assays of a chemical according to actual usage patterns in the species of concern.

Effect of Dursban 44 on semen output of Holstein bulls
Everett, R. W.
J Dairy Sci 1982;65(9):1781-94

Dursban 44, an insecticide for lice control, was applied to 185 Holstein bulls 9 to 52 mo of age. These sires were in various stages of progeny testing at an artificial insemination center.

Application of this product killed 7 bulls, and the remaining bulls exhibited varying severity of illness with 6 classified as very sick. This study evaluated the effect of this illness on semen production. Semen output on 40,950 ejaculates from 583 Holstein bulls collected from July 1, 1975, through March 31, 1981, was analyzed to establish normal semen production and to estimate the effect of illness caused by Dursban 44 treatment. Ejaculate number, days between collections by previous number of ejaculates, calendar months, years, and ages of bulls affected the semen output characteristics, original volume, sperm concentration, percent motile sperm, total sperm per ejaculate, percent prefreeze discards, percent postthaw sperm motility, and percent postthaw discards. Ejaculate volume, motility, total percent prefreeze discards, and percent postthaw discards were influenced negatively on the 6 very sick bulls. Percent postthaw discards were higher on all bulls treated with Dursban 44 for up to 6 mo post-treatment.