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-tri-chloro-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°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.

Key words: cholinesterase; carboxylesterase; chlorpyrifos; fetal brain; dose response; butyrylcholinesterase; acetylcholinesterase.

The classical role of acetylcholinesterase (EC 3.1.1.7) is to cleave acetylcholine, thereby clearing the synaptic cleft of the ligand responsible for conducting cholinergic neurotransmission (Brown et al., 1936; reviewed by Silver, 1974). In contrast, butyrylcholinesterase (EC 3.1.1.8) is a cholinesterase without an identified physiologic function (Kutty, 1980; reviewed by Silver, 1974; Williams, 1985). Beyond the classical clearance function of acetylcholinesterase and the enigmatic existence of butyrylcholinesterase, these cholinesterases are thought to have distinct developmental roles in many phases of neurogenesis such as mitosis, axonal guidance, neurite outgrowth, synaptogenesis, and cell adhesion (Holmes et al., 1997; Lassiter et al., 1998a; Robitzki et al., 1997; Robitzki et al., 1998; Sternfeld et al., 1998) (reviews: Drews, 1975; Karczmar et al., 1973; Layer and Willbold, 1995; Small et al., 1996). Considering the accumulating evidence that the cholinesterases may be involved in brain development, there is concern that inhibition of these enzymes during the prenatal and neonatal periods may cause developmental neurotoxicity. This study focuses on total cholinesterase activity, the combined activities of butyrylcholinesterase and acetylcholinesterase, in maternal and fetal tissues following gestational exposure to an organophosphorus pesticide. Carboxylesterase (EC 3.1.1.1) activity does not represent the activity of a single enzyme, but rather the activities of several related isofoms (for a recent review, see Satoh and Hosokawa, 1998). The actual physiologic role of these carboxylesterases is unclear, but may involve lipid metabolism and steroidogenesis (Barr et al., 1998). The primary interest, however, in carboxylesterases for these present investigations is the capacity of these enzymes to detoxify organophosphorus compounds (e.g., Casida et al., 1963; Clement, 1984).

Chlorpyrifos [(O,O’-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothionate] is the most commonly used organophosphorus pesticide (Aspelin, 1997). Chlorpyrifos-oxon is a metabolite of chlorpyrifos that potently and irreversibly inhibits serine esterases (for general reviews see Ecobichon, 1996;
Fukuto, 1990). Although the neonatal rat is particularly sensitive to the acute toxicity of chlorpyrifos (Atterberry et al., 1997; Moser and Padilla, 1998; Pope et al., 1991; Whitney et al., 1995), in utero the fetal rat brain cholinesterases may appear to be protected from repeated gestational exposure to chlorpyrifos (Chanda and Pope, 1996; Lassiter et al., 1998b). The purpose of this dose response study was to determine which tissue and enzyme (cholinesterase or carboxylesterase) provides a sensitive biomarker of gestational chlorpyrifos exposure. The present chlorpyrifos study has in vivo and in vitro components. In the in vivo component, cholinesterase and carboxylesterase activity in multiple fetal and maternal tissues was measured following repeated gestational exposure to chlorpyrifos. It was possible to determine in vivo which enzyme and tissue were the most sensitive to inhibition following gestational chlorpyrifos exposure. In the in vitro component of this study, the sensitivity of the carboxylesterase activities to inhibition by a particular concentration of chlorpyrifos-oxon was measured. Carboxylesterases were of critical interest because of their capacity to stoichiometrically detoxify chlorpyrifos-oxon (Chambers et al., 1994; Chanda et al., 1997; reviewed in Maxwell, 1992). This component will determine in vitro which tissue had the most sensitive carboxylesterase activity. This sensitivity information could be important in interpreting or verifying the in vivo dose response profiles. Ultimately, these in vivo and in vitro profiles will be essential for achieving the level of understanding that is necessary to develop a physiologically-based model of gestational chlorpyrifos action and distribution.

MATERIALS AND METHODS

Animals, Treatment, and Sample Collection

Experimental design. Time-pregnant, primiparous, Long-Evans rats determined to be sperm positive on gestational day 0 (GD0) were obtained from Charles River Laboratories CrI: (LE) BR (Portage, ME). Dams arrived on GD10 and were housed individually on a 12:12 h light:dark cycle, with food (Rodent Diet 5001; Lab Diet, Indianapolis, IN) and water provided ad libitum. Maternal body weight was assessed daily GD10–21. Dams were distributed among dosage groups on GD13 according to a ranking of body weight gain to achieve balanced body weights among time points and dosage groups. Dams were dosed on GD14–18 by gavage with 0, 3, 5, 7, or 10 mg/kg chlorpyrifos (n/dosage = 15) in corn oil (rate: 1 ml/kg). This subset of dams (n/dosage = 3) was sacrificed on GD18 for biochemical assessments, but a second subset of dams (n/dosage = 12) was allowed to deliver their pups. Indicators of maternal health status were assessed in this second subset: maternal weight GD14–21, postdosing cage-side observations, and neurobehavioral evaluation. The neurobehavioral testing consisted of an abbreviated functional observational battery (Moser et al., 1988; Moser, 1989) (protocol described in McDaniel and Moser, 1993). The dams were tested twice: 5 h after the last dose of chlorpyrifos and 1 day after parturition. The 3-mg/kg animals were not tested neurobehaviorally because the sample size for this group was not large enough for valid statistical analysis of neurobehavioral data.

Sample collection. On GD18, 5 h after the last dose (previously determined as the time of peak inhibition; Lassiter et al., 1998b) dams were anesthetized with CO₂ and sacrificed by decapitation for collection of tissue samples. Following collection of the trunk blood, each dam was perfused transectorially with saline. The perfusion continued until the normally dark liver was a very pale brown. This cleared the hepatic blood that could otherwise confound quantitation of liver enzyme activities. Maternal blood, liver, and brain were collected and frozen on dry ice. The uterus was removed, placed on ice, and the position of any resorptions recorded. Fetuses were removed from four locations: upper right horn, lower right horn, upper left horn, and lower left horn. The fetal livers and brains were collected individually and frozen on dry ice. The placenta corresponding to each fetus sampled was also collected and frozen on dry ice. Terminal body and brain weights were recorded for dams and fetuses. All tissues were stored at –80°C until biochemical analysis.

Biochemical Determinations

Sample homogenization. Total cholinesterase (acetylcholinesterase and butyrylcholinesterase) and carboxylesterase activities were measured on all the tissues collected. Samples were homogenized (larger samples, > 50 mg wet weight: 20 sec, setting 6, Polytron, Brinkman Industries, Westbury, NY) or sonicated on ice (smaller samples, < 50 mg wet weight: 10 sec, setting 3, Branson Sonifier 250, Branson Ultrasonics, Danbury, CT) in 0.1 M Na phosphate buffer (pH 8) containing 1% Triton X-100. Maternal liver was homogenized for 45 sec due to the high collagen content. Control activity was expressed as micromoles of the appropriate substrate hydrolyzed per minute per gram wet weight or per milliliter. Activity for the chlorpyrifos-treated subjects was expressed as a percent of the control activity.

Cholinesterase activity. Total cholinesterase activity was determined for maternal blood, maternal brain, placenta, and fetal brain using an Hitachi 911 Automatic Analyzer (Boehringer Mannheim Corp., Indianapolis, IN) according to the method outlined in Hunter et al. (1997). The Hitachi 911 is a spectrophotometer-equipped robot that appropriately dispenses sample, buffer, chromogen, and substrate to assay cholinesterase activity according to a variation of the Ellman et al. (1961) method. Considering the high sulfhydryl background present in the liver and resultant nonspecific reaction with the chromogen used in the Ellman assay, it was inappropriate to use this colorimetric technique on liver tissue. Therefore, fetal and maternal liver cholinesterase activity was assayed using the Johnson and Russell (1975) radiometric assay with a final substrate concentration of 1.2 mM. The [3H]acetate produced in the hydrolysis of [3H]acetylcholine was quantified using a Wallac 1410 liquid scintillation counter. All cholinesterase activities assayed were previously proven to be fully sensitive to inhibition both by eserine or a mixture of BW284c51 and iso-OMPA.

Carboxylesterase activity. Carboxylesterase activity was determined using the Chanda et al. (1997) microassay, which is a variation of the Clement and Erhardt (1990) method. Briefly, the carboxylesterase activity measures the para-nitrophenyl acetate (pNPA) hydrolyzing capacity of the sample in the presence of 1 mM EGTA in 50 mM Tris buffer. The EGTA was necessary to prevent A-esterase from hydrolyzing pNPA (Aldridge and Reiner, 1975). Carboxylesterases cleave pNPA, producing para-nitrophenol. Production of para-nitrophenol increased the spectrophotometric absorbance of the reaction mixture at 405 nm (ThermoMax Microtiter Plate Reader, Molecular Devices, Menlo Park, CA).

Chlorpyrifos-oxon IC₅₀ s for carboxylesterase. Control fetal and maternal tissue homogenates were preincubated for 30 min with an appropriate concentration of chlorpyrifos-oxon and EGTA (to inhibit A-esterase by chelating Ca²⁺) at 26°C. Following this preincubation, the remaining carboxylesterase activity was assayed as described previously. For each tissue, several concentrations of chlorpyrifos-oxon were tested to delineate a full profile of sensitivity. An IC₅₀ was determined for the carboxylesterase activity in each tissue using the Pharmacologic Calculation Program (Tallarida and Murray, 1981).

Statistics

Litter was considered the smallest unit of analysis, meaning that end points recorded for individual fetuses from a single litter were averaged as replicates.
(reviewed in Holson and Pearce, 1992). All data analyses were performed on untransformed data. To analyze maternal body weight, a repeated measures, two-way ANOVA was performed on absolute maternal weight from GD10–21. Following a significant treatment by gestational day interaction, step-down one-way ANOVAs were performed for each gestational day, followed by a Tukey-Kramer Honestly Significant Difference (Kramer, 1956; Tukey, 1953) post hoc analysis ($\alpha \leq 0.05$). To analyze the functional observational battery data, an ANOVA was also used to test for treatment-related differences. The neurobehavioral end points were either rank (scored using defined criteria) or incidence data. These data were analyzed using a categorical modeling procedure (CATMOD, SAS, 1990). This procedure fits linear models to functions of response frequencies, which can then be analyzed by weighted regression (Creason, 1989). When a significant overall treatment effect was obtained ($\alpha \leq 0.05$), comparisons of each treatment group to control were conducted using $t$-test contrasts. A two-way ANOVA was performed for cholinesterase or carboxylesterase activity (not percent of control). Following a significant interaction in the two-way ANOVA, a step-down one-way ANOVA was performed on the cholinesterase or carboxylesterase activity for each tissue followed by a Tukey-Kramer Honestly Significant Difference test as a post hoc analysis ($\alpha \leq 0.05$).

RESULTS

Enzyme Activity in the Control Animals

Cholinesterase activity. The cholinesterase activity values for control fetuses and dams are presented in the legend of Figure 1. Some tissue-related and age-related differences in the control cholinesterase activities were evident. Maternal brain had the most cholinesterase activity (11.0 $\mu$moles of acetylthiocholine hydrolyzed/min/g wet weight), whereas fetal brain cholinesterase activity was only 10% of the maternal brain activity. Maternal liver (2.9 moles of $[^3]$H]acetylcholine hydrolyzed/min/g wet weight) had 3-fold more cholinesterase activity than fetal liver. Maternal blood cholinesterase activity was 9- and 2-fold lower than maternal brain and liver activity respectively, but comparable to the lower level of cholinesterase activity in the placenta.

Carboxylesterase activity. The carboxylesterase activity levels for the fetal and maternal control tissues are presented in the legend of Figure 2. Activity was 20 to 60-fold higher in the maternal liver than in any other tissue assayed. Specifically, maternal liver carboxylesterase activity was 23-fold higher than fetal liver activity and 22-fold higher than maternal brain activity. This maternal brain carboxylesterase activity was still double the activity assayed in fetal brain. Fetal brain, placenta, and maternal blood carboxylesterase activities were comparable and lower than all other tissues assayed.

Overt Toxicity of Gestational Chlorpyrifos Treatment

Maternal weight is represented graphically as the daily percentage weight gain over the initial weight on GD10 (Fig. 3). On GD19, one day after the last dose of chlorpyrifos, the dams in the 10 mg/kg exposure group weighed less than any other group. With the exception of exophthalmus in less than 25% of the dams in the 10 mg/kg dosage group, dams did not display any other signs of an overt cholinergic crisis (i.e., smacking, lacrimation, diarrhea, or tremors) in cage-side observations conducted 2–5 h after any dose of chlorpyrifos. Additionally, 5 h after the last dose of chlorpyrifos or corn oil vehicle, a

![FIG. 1. Maternal and fetal cholinesterase activity profiles following chlorpyrifos exposure. Panels A, B, and C each present the cholinesterase activity remaining in a single maternal tissue and a single fetal tissue following repeated, oral exposure to chlorpyrifos on GD14-18. The cholinesterase control activities for those tissues measured spectrophotometrically in $\mu$moles of acetylthiocholine hydrolyzed/min/g wet weight were: maternal brain, 11.00 ± 0.38; fetal brain, 1.11 ± 0.05; maternal blood, 1.24 ± 0.10; placenta, 1.92 ± 0.18. Liver cholinesterase was measured radiometrically as $\mu$moles of $[^3]$Hacetylcholine hydrolyzed/min/g wet weight. The control activities were: maternal liver, 2.87 ± 0.56; and fetal liver, 0.87 ± 0.08.](image)
functional observational battery indicated that gestational chlorpyrifos exposure caused a treatment-related decline in maternal health status primarily in those dams receiving the 10 mg/kg dosage (Table 1). Exposure to 10 mg/kg chlorpyrifos on GD14–18 caused significant alterations in activity level, gait characteristics, increased incidence of tremors, failed pupillary response, and miosis. Although the 7 mg/kg dosage level decreased open field activity and increased the incidence of miosis, no other component of this functional observational battery was affected (i.e., lacrimation, salivation, arousal, reactivity to handling, ease of removal from the cage, or smacking). All functional effects observed on GD18 were reversible; when the dams were tested one day after parturition they were not different from control dams. In addition, resorptions, fetal body weights, fetal brain weights, and litter size also were unaffected by any dosage of chlorpyrifos following this exposure schedule.

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Enzyme Activity in Chlorpyrifos-Treated Animals

Cholinesterase activity. Following gestational chlorpyrifos exposure, maternal brain cholinesterase was inhibited in a dose-dependent manner. Maternal brain cholinesterase activity was 67, 30, 14, and 11% of control levels at 3, 5, 7, and 10 mg/kg chlorpyrifos, respectively (Fig. 1A). As expected from previous work (Lassiter et al., 1998b), maternal brain cholinesterase activity was more inhibited (2.4- to 4.8-fold more) than fetal brain cholinesterase activity following repeated exposure to 5, 7, or 10 mg/kg chlorpyrifos. The fetal brain cholinesterase activity also decreased as the gestational dosage of chlorpyrifos increased. Fetal brain cholinesterase activity decreased from 86% to 53% of control activity as the dosage increased from 3 to 10 mg/kg of chlorpyrifos (Fig. 1A). In both the fetal and maternal brain, the severity of cholinesterase inhibition did not change as the dosage increased from 7 to 10 mg/kg. Cholinesterase activity in the blood of chlorpyrifos-exposed dams was markedly inhibited as compared to the placental activity (Fig. 1B). Maternal whole blood cholinesterase activity was inhibited completely (4–12% of control activity) at every dosage level of chlorpyrifos. In stark contrast, placental cholinesterase activity was not inhibited at any dosage level. Note that this marked difference in blood and placental cholinesterase inhibition is convincing evidence that the placental tissue was not contaminated with maternal blood. Maternal liver cholinesterase activity was 11–22% of control activity at all chlorpyrifos

![Graph showing sensitivity of carboxylesterase activity in maternal and fetal tissues to inhibition by chlorpyrifos-oxon. These in vitro sensitivity curves were generated by assaying the residual activity of tissue homogenates following a 30 min incubation with increasing concentrations of chlorpyrifos-oxon at 26°C (n = 3). Fetal (GD18) and maternal liver contained the most sensitive carboxylesterase contingent. The maternal brain and placental carboxylesterase isozymes had similar, intermediate chlorpyrifos-oxon sensitivity, but fetal brain and maternal blood were relatively insensitive to inhibition. Based on these sensitivity curves, an IC₅₀ was calculated for each tissue. In addition, the absolute activity and percentage of inhibition resistant carboxylesterase are also important considerations and are summarized in Table 2.](image)

**TABLE 2**

Information Critical for Interpretation of the in Vitro Carboxylesterase Sensitivity Profiles

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control Activity</th>
<th>IC₅₀ ± S.E.</th>
<th>% IRE&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal blood</td>
<td>1.8 ± 0.1</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Maternal liver</td>
<td>102.6 ± 27.2</td>
<td>3.1nM ± 0.2</td>
<td>16%</td>
</tr>
<tr>
<td>Maternal brain</td>
<td>4.6 ± 0.3</td>
<td>26.5nM ± 1.1</td>
<td>49%</td>
</tr>
<tr>
<td>Placenta</td>
<td>2.4 ± 0.1</td>
<td>19.9nM ± 0.8</td>
<td>53%</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>4.4 ± 0.4</td>
<td>0.9nM ± 0.2</td>
<td>38%</td>
</tr>
<tr>
<td>Fetal brain</td>
<td>2.2 ± 0.1</td>
<td>19.1µM ± 2.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>µmoles of p-nitrophenol hydrolyzed/min of wet weight or ml.
<sup>b</sup>Inhibitor resistant esterase, i.e., percentage of carboxylesterase activity resistant to inhibition by chlorpyrifos-oxon, *in vitro*; see Discussion for details.
<sup>c</sup>Not determined.
gestational levels, whereas inhibition of fetal liver cholinesterase showed a dose response that declined to nearly the same level of cholinesterase inhibition as maternal liver as the dosage level approached 7–10 mg/kg chlorpyrifos (Fig. 1C).

Carboxylesterase activity. Following gestational chlorpyrifos exposure there was more carboxylesterase inhibition in the maternal liver than in the fetal liver. Maternal liver activity was less than 18% of control activity at 3–10 mg/kg chlorpyrifos (Fig. 2A). Fetal liver carboxylesterase activity also was inhibited maximally at all dosages, but with less severity than maternal liver: 45–50% of control activity (Fig. 2A). Maternal brain carboxylesterase activity was inhibited in a dose-dependent manner (100, 85, 71, and 65% of control carboxylesterase activity at 3, 5, 7, and 10 mg/kg of chlorpyrifos), whereas fetal brain carboxylesterase activity showed no inhibition at any dosage (Fig. 2B). Placental and maternal blood carboxylesterase activities were not inhibited by repeated, gestational doses of chlorpyrifos even at 10 mg/kg (Fig. 2C).

Fetal and Maternal Carboxylesterase IC₅₀'s for Chlorpyrifos-Oxon

In vitro carboxylesterase IC₅₀ curves were generated for the fetal and maternal tissues characterized in the in vivo portion of this study. The sensitivity of the carboxylesterase activity to inhibition by chlorpyrifos-oxon depends on the complement of carboxylesterase isoforms contained in a particular tissue. The in vitro inhibition curves are presented in Figure 4 and summarized in Table 2. To characterize fully the in vitro sensitivity curves, three values are necessary: 1) the amount of carboxylesterase activity present in the tissue, 2) the IC₅₀ of the sensitive carboxylesterase(s), and 3) the proportion of carboxylesterase activity that is insensitive to inhibition. The amount of carboxylesterase activity present in the various control tissues was described at the beginning of the Results section. As for the calculated IC₅₀ values of the sensitive carboxylesterases, fetal liver had the lowest IC₅₀ (0.91 nM) of the tissues assayed. Maternal liver was the most sensitive tissue in the dam (IC₅₀ = 3.1 nM). The carboxylesterase isoforms in the placenta and maternal brain demonstrated comparable sensitivities (IC₅₀ = 19.9 versus 26.5 nM, respectively). Among the calculated IC₅₀ values, fetal brain carboxylesterases were the most insensitive to inhibition by chlorpyrifos-oxon (19.1 μM). The highest chlorpyrifos concentration used (0.5 mM) only marginally inhibited carboxylesterase activity in maternal blood; 63% of control activity; consequentially, no IC₅₀ value was calculated. The third value necessary to characterize fully the in vitro sensitivity curves was the percentage of the total carboxylesterase activity that is insensitive to inhibition by chlorpyrifos-oxon. Derived from the in vitro inhibition curves, inhibitor resistance was defined as carboxylesterase activity not inhibited an additional 6% (or more) when the chlorpyrifos-oxon concentration was increased 6- to 10-fold. Applying this definition, placenta and maternal brain had the largest percentage of inhibitor-resistant carboxylesterases: 53 and 49% of total activity, respectively. In the fetal liver, 38% of the carboxylesterase activity was resistant to inhibition by chlorpyrifos-oxon. The smallest percentage of resistant carboxylesterases was found in the maternal liver (16%). The percentage of inhibitor resistant carboxylesterases in the maternal blood and fetal brain was not determined because of the relative insensitivity of the carboxylesterase in these tissues. Overall, maternal blood carboxylesterases were only marginally sensitive to inhibition. The entire carboxylesterase complement in the fetal brain was essentially resistant to inhibition through 3 μM, but thereafter declined steadily from 3 μM to the highest concentration tested (0.5 mM); thus, the percentage of inhibitor resistant carboxylesterases could not be determined.

DISCUSSION

As expected, the control cholinesterase activities demonstrated age-related (Lassiter et al., 1998a; Mortensen et al., 1998; Silver, 1974) and tissue-related differences (Edwards and Brimijoin, 1982; Lassiter et al., 1998b). The control levels of carboxylesterase activity were also age and tissue dependent (Lassiter et al., 1998b; Morgan et al., 1994; Moser et al., 1998; Moser and Padilla, 1998; Pond et al., 1995; reviewed by Satoh and Hosokawa, 1998). In comparing in vitro fetal and maternal carboxylesterase profiles there were some interesting maturational profiles. As the liver matured from late gestation to adulthood, the amount of carboxylesterase activity increased 23-fold, the IC₅₀ tripled, and the percentage of inhibitor-resistant carboxylesterase activity decreased. In the brain, however, the amount of carboxylesterase activity only doubled, but the IC₅₀ decreased approximately 1000-fold.

Maternal health status is a matter of concern in gestational exposure studies where the fetus is the focal element. Maternal toxicity can be a confounding variable when attributing the adverse effects observed in the fetus to a gestationally administered agent. Several techniques are available to evaluate maternal health (as reviewed by Khera, 1987; Rogers, 1987; and Schardein, 1987). For this investigation maternal toxicity was defined as a statistically significant decrement in body weight. According to this definition, 10 mg/kg chlorpyrifos administered orally from GD14–18 was a maternally toxic dosage due to decreased body weight on GD19. Even though maternal toxicity can be a confounding factor in developmental neurotoxicity studies, the effects of such dosages on fetal and neonatal brain development should not be dismissed completely. The effects on the fetus resulting from non-maternally toxic dosages, however, are often more informative than the possibly nonspecific effects of compromised maternal health.

A single, gestational exposure to an anticholinesterase compound causes comparable degrees of cholinesterase inhibition in the fetal and maternal brain, whereas repeated gestational exposure to an anticholinesterase causes less cholinesterase inhibition in the fetal brain than in the maternal brain (Bisso et
1) no inhibition at any dosage tested, 2) sure to chlorpyrifos can be categorized for descriptive purposes chlorpyrifos. The response profiles following gestational expo-
positioning a toxicokinetic/dynamic model of gestational exposure to pyrifos exposure. This information will contribute to establish-
ment of these activities as indicators of gestational chlor-
ity in a number of fetal and maternal tissues, allowing
does fetal brain cholinesterase activity.

This dose response study characterizes the shape of the response profiles of cholinesterase and carboxylesterase activ-
ity in a number of fetal and maternal tissues, allowing in vivo assessment of these activities as indicators of gestational chlor-
pyrifos exposure. This information will contribute to establish-
ing a toxicokinetic/dynamic model of gestational exposure to chlorpyrifos. The response profiles following gestational ex-
posure to chlorpyrifos can be categorized for descriptive purposes as: 1) no inhibition at any dosage tested, 2) a classical dose response, or 3) maximal inhibition at all dosages tested. The term maximal refers to the most severe degree of inhibition achievable, but does not necessarily correspond to total or complete inhibition of enzyme activity. Among the tissue cholinesterases assayed, only the placental cholinesterase ac-

tivity fits into the first category; it was unaffected by gesta-
tional chlorpyrifos exposure at any dosage. The tissues that
demonstrated no carboxylesterase inhibition following in vivo exposure included fetal brain, placenta, and maternal blood. The tissues that demonstrated classic dose response profiles included fetal brain and liver cholinesterase, as well as mater-

nal brain cholinesterase and carboxylesterase. The tissues that
demonstrated maximal cholinesterase inhibition at all gesta-
tional dosages of chlorpyrifos tested included maternal blood and maternal liver cholinesterase activity. Fetal and maternal liver carboxylesterase activity was also maximally inhibited by the 3 mg/kg dosage level. Even though fetal and maternal carboxylesterase activities were both maximally inhibited at 3–10 mg/kg, the degree of inhibition in the maternal liver was twice as severe as the degree of inhibition in the fetal liver. There appeared to be an invisible floor that prevented further inhibition of the fetal or maternal liver carboxylesterase activ-
ity (discussed below). Both the in vivo and in vitro profiles suggested that liver carboxylesterase activity was the most
sensitive of the carboxylesterases assayed. The in vitro sensitivity profile indicated that fetal liver carboxylesterase activity was possibly more sensitive than maternal liver carboxylester-
ase (IC50 = 0.9 versus 3.1 nM). Both the in vivo and in vitro sensitivity profiles demonstrate that fetal liver carboxylesterase inhibition is an extremely sensitive indicator of fetal chlorpyr-
ifos exposure.

The in vitro IC50 values and the in vivo inhibition profiles demonstrated considerable continuity. As stated above, regard-
less of administered dosage, maternal liver carboxylesterase activity was maximally inhibited: ~15% of control activity. This suggested that 15% of the carboxylesterase isozymes in the maternal liver were insensitive to inhibition by chlorpyri-
fos-oxon. This pattern was supported by the observation that increasing the concentration of chlorpyrifos-oxon in vitro failed to decrease maternal liver carboxylesterase activity be-
low 16% of control activity. This provided additional in vitro evidence of this insensitive population of carboxylesterases. Similar parallels were evident for fetal liver with 48% (in vivo) and 38% (in vitro) of the tissue carboxylesterase complement being insensitive to inhibition by chlorpyrifos-oxon. The vir-
tual insensitivity of fetal brain carboxylesterase activity in vivo was also predicted by the extremely high IC50 for carboxyles-
terases in this tissue. The in vitro sensitivity profiles for car-

boxylesterase activity (Fig. 2) in every case, with possible exception of the placenta.

This study provides important information regarding the dose response profiles of cholinesterase and carboxylester-
ase activity following oral exposure to chlorpyrifos during late gestation. These esterase response profiles (both in vivo and in vitro) provide the framework critical for assessing the relative toxicologic responses of the fetus and the dam to gestational chlorpyrifos exposure. In the target tissue, brain cholinesterase activity fell in a dose-dependent manner in both the fetus and the dam. Moreover, the in vitro IC50 determinations for carboxylesterase, for the most part, pre-
dicted the inhibition pattern that unfolded after in vivo dosing. One of the most interesting and useful outcomes of this study is the realization that an extremely sensitive measure of chlorpyrifos exposure in the fetus appears to be inhibition of liver carboxylesterase activity. There are other pieces of information critical to this model that remain to be delineated, such as a detailed toxicokinetic profile for chlor-
pyrifos and its metabolites in the pregnant rat, and informa-
tion regarding the capacity of fetal versus maternal tissues to activate chlorpyrifos to chlorpyrifos-oxon. This dose re-
ponse information, coupled with time course information (Lassiter et al., 1998b), will be integral for establishing a physiologically-based dose response model in the pregnant rat that has potential for extrapolation to humans.
REFERENCES


