The Effect of Route, Vehicle, and Divided Doses on the Pharmacokinetics of Chlorpyrifos and Its Metabolite Trichloropyridinol in Neonatal Sprague-Dawley Rats

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The objective of regulatory toxicity testing of pesticides is to identify the hazards posed by a pesticide, to characterize its dose-response relationship, and to use these data in a risk assessment paradigm to determine permissible exposure levels. One of the difficulties of risk assessment is the need to extrapolate adverse effects seen in experimental animals at high dose levels to potential effects seen in humans following low-level exposures to environmental chemicals. As pointed out in Conolly et al. (1999), “the onus is on the toxicologist to consider relevant dose levels during characterization of the dose-response curve.” Aside from dose selection, it also is the responsibility of toxicologists to use relevant dosing scenarios. Toxicity is driven by the internal dose of a chemical at the target site; therefore, exposure conditions that produce higher internal doses yield greater potential to detect adverse effects. However, the utility of toxicology data in risk assessment will be compromised if higher internal doses are achieved in experimental animals by doses or routes of exposure that depart appreciably from those expected in humans. To improve the utility of toxicological data for risk assessment, experiments using relevant exposure scenarios that include realistic dose levels should be employed.

As toxicological research evolves, dose and route of exposure are considered more carefully; however, in newer areas, such as neonatal toxicity studies, adherence to these principles is less stringent. Experiments using neonatal rats pose unique challenges with respect to dosing, such as the technical difficulties involved in repeatedly dosing young animals. Direct oral bolus administration of the test material to neonatal rats has been proposed as a means of supplemental dosing when materials are poorly transported through maternal milk and when investigators wish to precisely control exposure conditions or as a matter of experimental convenience. However, little information is available regarding the relationship of this direct bolus dosing and resulting systemic levels of a chemical. Understanding the relationship between dosing regimes and body burden in neonatal rats is critical in order to understand internal dosimetry and develop more relevant dosing paradigms for subsequent risk assessment.

One example of a material wherein multiple dosing scenarios have been used for neonatal toxicity studies is the organophosphate insecticide, chlorpyrifos (CPF). CPF has been extensively studied in humans and animals; its metabolism and
kinetics are well understood. A physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model in rats and humans has been developed for CPF and its major metabolites (Timchalk et al., 2002b) and has recently been extended to include preweanling rats (Timchalk et al., 2007). CPF has been administered to neonates via nursing of milk from treated dams (Mattsson et al., 2000) and by direct gavage dosing (Betancourt et al., 2006; Kacham et al., 2006; Zheng et al., 2000). Furthermore, several studies have investigated toxicological end points (e.g., neurotoxicity) after exposing neonatal rats to CPF in dimethyl sulfoxide (DMSO) by the sc route (Aldridge et al., 2005; Auman et al., 2000; Crumpton et al., 2000; Dam et al., 1999, 2000). The impact of these different exposure scenarios on neonatal body burden and toxicological outcome is unknown.

The primary objective of this study was to evaluate direct neonatal dosing methods to determine their impact on CPF pharmacokinetics (PK) relative to the most realistic neonatal exposure scenario for CPF, lactational exposure. Thus, neonatal body burdens following oral bolus gavage dosing (1 mg/kg, one time per day) or divided oral dosing (0.33 mg/kg, three times per day) on postnatal day (PND) 5 were compared with exposure via nursing of milk from dams exposed to test material (5 mg/kg/day) in the diet. The impact of different oral gavage vehicles (e.g., rat milk and corn oil) on PK parameters was investigated. These vehicles were selected as they have been used in previous neonatal dosing studies with CPF (e.g., Betancourt et al., 2006; Dam et al., 1999, 2000). Dose frequency and vehicle are important as changes in frequency and/or vehicle may lead to significant differences in the disposition of a xenobiotic to target organs and a corresponding change in toxicological outcome. Plasma levels of CPF and trichloropyridinol (TCP) following oral exposures were compared with levels achieved after sc administration of 1 mg/kg CPF in 1 ml/kg DMSO. Data from the current study will be used to further refine the model of CPF PK in neonatal rats. In addition, these data will be useful to evaluate different methodologies for supplemental dosing, which are needed to meet test guideline requirements (e.g., the Developmental Neurotoxicity Test Guideline [OPPTS 870.6300] as refined in the Federal Register 40 CFR Parts 152 and 158, wherein the U.S. Environmental Protection Agency [U.S. EPA] may propose PK in fetuses and/or young animals and direct gavage administration to pups during lactation).

**MATERIALS AND METHODS**

**Test materials and standards.** CPF (O,O-diethyl, O-(3,5,6-trichloro-2-pyridyl)phosphorothioate) was obtained from Dow AgroSciences, LLC (Indianapolis, IN). The test material was 97.6% pure as confirmed by liquid chromatography with ultra violet-visible spectroscopy (UV-VIS) detection and internal standards (unpublished data; Ghaoui, Kinnunen, Nelson, Babbit, Balcer, 1996; results confirmed in 2001). The identity of the major component was also confirmed with mass spectral analysis with electron impact ionization. Dosing solutions were prepared on the day of administration, and the concentration of CPF in the dosing solutions was confirmed using high-performance liquid chromatography with a UV detector. Stripped corn oil from Acros Organics (Morris Plains, NJ) was used as a vehicle in gavage experiments. An authentic standard of TCP was obtained from Dow AgroSciences, LLC. The chemical structures were confirmed as CPF and TCP by infrared spectroscopy and gas chromatograph-mass spectrometry (GC-MS), and purity was verified by differential scanning calorimetry and fast liquid chromatography. Isotopically labeled internal standards of CPF and TCP (13C2, 15N) were obtained from Dow AgroSciences, LLC. [Ring–4–2H4] CPF, with a specific activity of 5.0 Ci/mmol and radiochemical purity of 99.7%, was obtained from Amersham Biosciences (Piscataway, NJ).

**Animals.** For the direct neonatal dosing experiments, time-mated female rats (Crl:CD(SD)IGS BR rats; Charles River Laboratories, Portage, MI) were received on gestation days 3–5 (spornum position = gestation day 0). Animals were housed one per cage in rooms designed to maintain adequate conditions (temperature, light, and photoperiod). Dams were allowed to naturally deliver their litters (day of birth was designated as PND 0). On PND 4, litters were culled to eight pups per litter with a preference to save male offspring. On PND 5, select male pups were dosed by gavage with CPF. For the dietary study, adult male and female (nulliparous) rats were obtained at 10 and 8 weeks of age, respectively. These animals were singly housed and acclimated to the laboratory for approximately 7 days prior to the start of dietary dosing with CPF. Animals were provided LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO) in pelleted form (direct neonatal dosing) and meal form (dietary-exposed animals). Feed and municipal water were provided ad libitum. The animal activities required for the conduct of this study were approved by the Institutional Animal Care and Use Committee.

**Study overview.** The goal of this study was to compare the CPF body burden of neonatal rats following dosing by different routes (e.g., single bolus oral gavage dosing, split-dose oral gavage dosing, and sc injection) and in different vehicles (e.g., oral rat milk, oral corn oil, and sc DMSO). These dosing paradigms were compared with lactational exposure of pups nursing milk from dams exposed to CPF in the diet. CPF is known to be transferred through rat milk (Mattsson et al., 2000).

**Milk collection.** In order to directly expose the pups to CPF in milk by oral bolus gavage, milk was collected from untreated lactating dams. Time-mated dams were ordered on gestation day 13 and allowed to deliver (day of delivery is lactation day 0). Whenever possible, litters were culled to 12 pups on lactation day 4. Litters with less than 12 pups were not culled. On lactation days 9–10, pups were removed from the dams early in the morning and humanely euthanized. In the afternoon, dams were anesthetized and milk was collected (Mattsson et al., 2000). A terminal blood sample was collected from the dams and served as a negative control in subsequent analyses. Dams were humanely euthanized prior to recovery from anesthesia. Milk was stored at –80°C until used to prepare dosing solutions. A small aliquot of milk was stored at –80°C until analyzed as a CPF-negative control sample.

**Experimental design: direct neonatal dosing by gavage.** The experimental design used in this study is illustrated in Figure 1. The dose groups and routes of exposure were as follows: group 1, bolus gavage dose (1 mg CPF/kg) in corn oil (5 ml/kg); group 2, divided gavage doses in corn oil (0.33 mg CPF/kg in corn oil × 5 ml/kg, given three times in 24 h, once every 8 h); group 3, bolus gavage dose (1 mg CPF/kg) in milk (5 ml/kg); group 4, divided gavage doses in milk (0.33 mg CPF/kg/milk × 5 ml/kg, given three times in 24 h, once every 8 h); and group 5, bolus sc injection (1 mg CPF/kg) in DMSO (1 ml/kg). The rationale for selecting these vehicles and routes is that gavage dosing with corn oil and sc injections with DMSO have been used in previous neonatal dosing studies with CPF (e.g., Betancourt et al., 2006; Dam et al., 1999, 2000). Milk was included for comparison as it is normally consumed by PND 5 rat pups. Divided doses were designed to model episodic CPF exposures. Basically, PND 5 male pups in groups 1, 3, and 5 were treated with a single bolus dose of CPF; then blood levels of CPF and TCP were determined at multiple time points postexposure (0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h).
Other PND 5 pups (groups 2 and 4) received CPF (1 mg/kg total dose) administered as three divided doses over a 24-h period with blood levels of CPF and TCP determined at the same time points (i.e., 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h) after the last dose. In the lactational experiments (C), PND 5 pups were exposed to CPF via lactating dams receiving 5 mg/kg/day CPF in the diet. Dams were at steady state, and dam blood and milk samples were collected at 6:00 A.M., 11:00 A.M., and 5:00 P.M. Pup blood was collected at 12:00 A.M., 7:00 A.M., and 1:00 P.M. n = 3-4 pups per time point per dosing scenario.

**Experimental design: direct neonatal dosing of \(^3\)H-CPF in DMSO by sc injection.** Initial data from the sc DMSO dosing experiment indicated low recovery, either as CPF or as TCP, of the total administered dose of CPF. Because of rapid absorption from DMSO, we hypothesized that CPF levels may have increased in the blood prior to our first collection time at 30 min. Thus, an additional experiment was undertaken to better characterize the kinetics of sc exposure at early time points. For this experiment, \(^3\)H-CPF was used. Basically, a separate group of PND 5 pups were weighed, then a circular injection site was outlined on the back of each pup in the area between the scapulas. Each pup was given a single bolus sc injection of 1 mg \(^3\)H-CPF/kg in DMSO into the center of the designated area between 6:00 and 8:00 A.M. The DMSO dose volume was the same as that used in the previous sc experiment, 1 ml/kg dose volume. Pups (three male pups per group per time point) were euthanized at the designated times (5, 10, 15, and 30 min, 1 and 2 h postexposure), blood was collected into heparinized tubes, and blood levels of the radiolabel were determined. In addition, the designated injection site (dermal layers and a thin layer of underlying tissue), the carcass, and other tissues (liver, brain, and heart) were collected and analyzed for the presence of \(^3\)H radioactivity. The injection site was analyzed to determine the qualitative rate at which the administered dose penetrated beyond the injection site.
Experimental design: indirect neonatal dosing via milk from dams given dietary CPF. Aside from the groups outlined above, samples were collected from PND 5 pups exposed to CPF through maternal milk. Dams were maintained on a CPF-containing diet for greater than 4 weeks to ensure that steady-state levels were achieved at the time of lactation (Saghiri et al., 2006). Thus, males and females were exposed to CPF at 5 mg/kg/day in the diet for 1 week, then housed (one male:one female) for either 2 weeks or until mating had occurred. Positive signs of mating include a vaginal plug or a vaginal smear positive for sperm. On the day that the females were considered sperm positive, they were removed from the males’ cages. Dosing of the males was discontinued. Females continued to receive CPF in the diet throughout gestation, delivery, and lactation. Dietary CPF did not appear to cause systemic toxicity in either dams or pups as dams gained weight during gestation and lactation and there were no clinical signs of toxicity in either dams or pups. Pup body weights were not recorded as pups were not dosed directly. At three time points on PND 5 (12:00 A.M., 7:00 A.M., and 1:00 P.M.), male pups were randomly selected and euthanized (three to four pups per time point), and blood was collected as described above. Once pups had been removed for sample collection, all remaining pups in the dietary CPF litters were humanely euthanized. Approximately 4–6 h after removing pups, dams were anesthetized for terminal milk and blood collection. Data from these animals were used to establish actual exposure levels for pups ingesting milk from CPF-exposed dams. These data were compared to the various direct dosing paradigms and also compared to previous data showing levels of CPF in milk after gavage exposure to the dams (Mattsson et al., 2000).

Analytical procedures. Samples of whole blood were collected into glass containers and transferred into tared vials containing extraction solvent (ethyl acetate, methanol, and acetonitrile containing 62 ng CPF and 65 ng TCP internal standards). The blood samples were weighed, vortexed, and separated into layers by centrifugation (20 min at 570 × g). An aliquot of the organic phase was removed, dried under nitrogen, reconstituted in toluene, and derivatized with N-methyl-N-[tert-butyldimethyl-silyl]trifluoroacetimide. The derivatized sample was mixed and heated 1 h at 60°C and then analyzed by GC-MS, as per Brzak et al. (1998).

Milk samples were collected into glass tubes and transferred into tared vials containing acidified (2.5 N acetic acid) saturated NaCl solution (Brzak et al., 1998) and mixed. Samples were spiked with 10 µl CPF internal standard (332 µg/ml). Hexane was added, and the milk samples were mixed and centrifuged (~20 min at ~1500 × g). The hexane layer was transferred to a clean vial and concentrated to dryness using nitrogen. The residue was reconstituted in toluene and analyzed by GC-MS as above.

Solvent standards were prepared in solution and analyzed for quantitation of the analytes. Appropriate fortified samples were prepared in blood or milk from control animals to determine recoveries of the analytes. Recoveries for milk spikes were 99.8–100%, whereas recoveries for whole blood spikes were 96.7 ± 2.41% (n = 4). The limits of quantification (LOQ) for CPF ranged from 3.24 to 3.70 ng/g blood for neonatal samples and 3.56–4.43 ng/g blood for adult samples. Milk was not analyzed for TCP; however, previous data (McKellar et al., 1976 and unpublished data; Kuper) indicate that TCP is transferred through milk in amounts similar to CPF in other species.

Data analysis. Means and SDs were calculated with Microsoft Excel using full precision mode accuracy. Area under the curve (AUC) and absorption and elimination half-life (t1/2) values for plasma CPF and TCP concentration-time curves were estimated from 0 to 24 h for the direct bolus dose groups using PK Solutions (Summit Research Services, Montrose, CO). Only average AUC and t1/2 values were calculated, as all blood samples were collected via serial sacrifice of study animals. The relative SD for the TCP and CPF concentrations at all time points averaged 44 and 28%, respectively, which is consistent with TCP and CPF blood determinations from other serial sacrifice studies (28 and 61%, respectively; Mattsson et al. 2000). Resulting data for oral gavage exposure of pups to CPF in corn oil or milk also were simulated utilizing a CPF PBPK/PD model, which was recently refined to include young animals (Timchalk et al., 2006, 2007). This model incorporates age-dependent changes in metabolism to simulate dosimetry and cholinesterase inhibition dynamics in the preweanling rat.

RESULTS

Pups were exposed to CPF through multiple routes: via milk from dams exposed to 5 mg/kg/day CPF in the diet, direct oral dosing with 1 mg/kg CPF either as a single bolus (one time per day) or as divided (three times per day) doses using either rat milk or corn oil as vehicles, or 1 mg/kg CPF administered as a single bolus dose sc to pups in DMSO. Each of these factors (route, dose rate, and vehicle) likely contributed to differences in neonatal internal dosimetry.

CPF and TCP Levels Following Lactational Exposure from Diet-Exposed Dams

CPF and TCP in the blood and milk of dams exposed to dietary CPF (5 mg/kg/day for 6–7 weeks) and pup blood levels for lactationally exposed offspring are summarized in Table 1. Dams were exposed to dietary CPF during the prebreeding, breeding, gestation, and lactation periods and, therefore, were considered to be at steady-state levels for CPF. Three dams were sacrificed at different time points on lactation day 5 to examine diurnal variations in CPF levels. Maternal blood levels of CPF were nonquantifiable at all time points with dietary exposure (LOQ = 3.56–4.43 ng CPF/g blood). Milk CPF was fairly consistent at the three time points during 24 h, at about 118 ng/g milk, indicating that dietary exposure provided reasonably stable blood levels of CPF (Table 1). Lactating dam’s TCP levels from dietary CPF at 5 mg/kg/day varied fourfold over the course of the day, ranging from 281 ng/g blood at 6:00 A.M. to 1263 ng/g blood at 11:00 A.M. Intermediate maternal blood levels were seen at 5:00 P.M.

| TABLE 1 |

| CPF and TCP Concentrations in the Blood and Milk of Dams Exposed to 5 mg/kg/day CPF in the Diet and Pup Blood Levels Following Lactational Exposure (PND 5) |
|---|---|---|---|---|
| Sample time | Animal | CPF (ng/g blood) | TCP (ng/g blood) | Estimated CPF (ng/g blood)* |
| 12:00 A.M. | Neonates | NQ | 48.63 ± 3.09 | — |
| 6:00 A.M. | Dam | NQ | 280.86 | 111.42 | 1.1 |
| 7:00 A.M. | Neonates | NQ | 54.93 ± 2.10 | — |
| 11:00 A.M. | Dam | NQ | 1262.83 | 117.08 | 1.1 |
| 1:00 P.M. | Neonates | NQ | 71.11 ± 8.00 | — |
| 5:00 P.M. | Dam | NQ | 566.40 | 127.28 | 1.2 |

*Estimated to be 1.1 ng CPF/g blood (see “Results” section). Assumes milk:blood partitioning of 104 from Mattsson et al. (2000).

**n = 3–4 neonates per group; mean ± SD for TCP blood concentrations.

**Nonquantifiable; LOQ in neonatal blood ranged from 3.24 to 3.70 ng CPF/g blood.

**n = 1 dam/group.

**Nonquantifiable; LOQ in dam blood ranged from 3.56 to 4.43 ng CPF/g blood.
CPF was not detected in the blood of PND 5 pups exposed via nursing of dams given 5 mg/kg/day CPF by diet (LOQ = 4 ng/g, this study). Neonatal blood levels of TCP were more consistent across time points than diet-exposed dam TCP levels, varying only by a factor of 1.5. Consistent with the dams, neonatal TCP levels were highest midday, with values of 71 ng/g blood at 1:00 A.M. The lowest level in pups, 49 ng/g blood, was detected at 12:00 A.M. At all time points, pup blood levels of TCP were considerably lower than dam blood levels. On average, the PND 5 blood TCP levels in pups were 12 times lower than their dam’s. TCP concentrations in the milk of dams exposed to CPF in the diet at 5 mg/kg were not measured, but it was assumed that TCP would partition into milk and that a substantial amount of the pup TCP was from milk. This assumption is based on previous data (McKellar and unpublished data; Kuper) showing the transfer of TCP through milk in other species.

**Neonatal CPF and TCP Levels Following Oral Gavage Exposures**

PK parameters for pup blood levels of CPF and TCP following direct exposure to 1 mg/kg CPF via oral gavage are shown in Table 2. AUC values for bolus doses were calculated from time 0 (first dose) to 24 h after the dose was administered. In each of the direct oral dosing scenarios, the time to maximum blood concentrations (Tmax) was the same, at 2 h postdosing for CPF and 4 h postdosing for TCP. The maximum CPF and TCP concentrations (Cmax) were highest in pups given the single bolus dose of CPF compared with pups given split doses. Compared with the bolus milk group, bolus dosing in corn oil yielded the highest total exposure based on both Cmax and AUC values. Absorption t1/2 values for the two single oral dose groups were calculated from the TCP blood data and were found to be comparable at 1.0 h for both the corn oil and the milk vehicle groups (data not shown).

Comparing all groups, the highest concentration of CPF detected in neonatal blood was 49 ± 31 ng/g blood (or 0.14 ± 0.09 μmol/l) (mean ± SD) at 2 h following single oral bolus gavage administration of 1 mg CPF/kg bw in 5 ml/kg corn oil. This spike in CPF concentration from “corn oil bolus” dosing also occurred in blood CPF profiles of adult rats (Fig. 3, Timchalk et al., 2002b). In contrast, concentrations of CPF were less than 9 ng/g blood (0.025 μmol/l or less) following single bolus administration in 5 ml/kg milk and split dose in corn oil and split dose in milk administrations. This approximately sixfold increase in plasma Cmax via bolus dosing in corn oil versus milk was substantially higher than the 2.3-fold increase in plasma AUC for CPF from these dose groups. It was also substantially higher than the 1.65-fold and 1.33-fold increases in TCP Cmax and AUC values seen for the corn oil versus milk vehicle groups. This specific increase in the Cmax parameter for the corn oil group suggests a decrease in the high rate of metabolism when CPF is administered in this dose vehicle.

The ability of corn oil vehicle to markedly increase Cmax and AUC values appeared to be dose dependent because both CPF and TCP Cmax values were similar for pups receiving CPF divided doses of 0.33 mg/kg each in either vehicle (corn oil or milk). Thus, neonatal doses in excess of 0.33 mg/kg of CPF in corn oil vehicle appear to give a disproportionately higher Cmax when compared to a milk vehicle.

The PND 5 CPF plasma elimination half-life (t1/2) was calculated to be 3.0 h following administration of a single 1 mg CPF/kg bw dose in corn oil. Longer half-lives were found for the single dose in rat milk (8.3 h). No half-life was determined for the divided dose in corn oil or rat milk as an insufficient number of data points were available. The reason for the longer half-life from milk vehicle compared with corn oil dose is unknown but may be related to different distribution profiles for the parent compound administered in this vehicle.

**TABLE 2**

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<th>Vehicle</th>
<th>Dose route</th>
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<th>AUCb</th>
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*a* A single dose of 1 mg CPF/kg or three doses of 0.33 mg CPF/kg administered 8 h apart.

*b* Mean concentration ± SD.

* Calculated from 0 to 24 h after last administered dose.

*d* Not determined.

*e* n = 2.
These experiments were conducted with three pups per time point per dose group. At some time points, large variability in the blood concentrations of CPF and TCP was observed between animals. This variability appeared unrelated to technical errors during dose administration. The condition of the esophagus was examined after blood collection by exposing the esophagus and injecting dye into it to evaluate its integrity. In this study, there were only two esophageal observations of concern noted at necropsy. One pup (18-h divided corn oil group) had a perforated esophagus at sacrifice; this animal’s data were excluded from analysis. One pup (8-h divided milk group) had a thin esophageal wall; however, this animal’s data were included in the analysis.

**CPF and TCP Levels Following sc Exposure**

PK parameters describing the time course of CPF and TCP following sc administration of CPF as a single dose in DMSO are also presented in Table 2. The time to maximum concentration (Cmax) of CPF in the blood was 2 h, the same time at which Cmax occurred with direct oral exposures. The calculated AUC for parent CPF was 82 ng h/ml following sc administration, and the half-life for parent CPF was 8.3 h. Thus, the CPF Cmax, AUC, and ½ values were similar to those seen with bolus oral exposure in a milk vehicle. However, the time to TCP Cmax was 6 h rather than 4 h as was seen with the other exposure routes. Furthermore, the fate and disposition of CPF are not fully understood with sc administration. The AUC for TCP following the sc exposure was less than the TCP AUCs via the oral route. This suggests that less test material was absorbed via this exposure route. This is consistent with the results of a follow-up study, wherein radiolabeled CPF was administered sc in DMSO. Blood samples were taken soon after dosing until 2 h postdosing to characterize the rapid absorption of CPF that was expected because of the use of DMSO vehicle. However, the majority of radiolabel remained at the injection site up to 2 h, coincident with peak blood CPF levels for this group (Fig. 3). The ultimate fate of this CPF “depot” is not known as the radiolabel experiments were terminated at 2 h. The blood kinetic data for CPF administered sc fit poorly into the existing PBPK model for juvenile animals (Timchalk et al., 2006). Together, the longer TCP Tmax, the lower absorbed dose, the CPF depot at the injection site, and the poor PBPK model fit suggest that the PK via this route differ substantially from CPF administered orally. Whether these PK differences are due to the route of exposure (sc), vehicle (DMSO), or both cannot be determined with the data presently available.

**PBPK Simulations**

The adult CPF PBPK model (Timchalk et al., 2002a,b) was modified to simulate age-dependent changes in metabolism to enable simulation of dosimetry in preweanling rats (Timchalk et al., 2007). The model then was used to generate simulations of CPF and TCP blood levels following direct dosing with CPF using either a corn oil or milk matrix (Fig. 2). Overall, the PBPK model reasonably described rat pup dosimetry under a broad range of exposure scenarios. The model was reasonably consistent with the experimental data in its prediction of CPF and TCP blood kinetics following direct oral exposure for either a single bolus or a split-dose design. However, it is interesting to note that for the single dose simulation, the model was not able to accurately simulate the high peak CPF concentration obtained following the corn oil administration, yet accurately simulated the CPF time course using the milk matrix. Attempts were also made to simulate the CPF and TCP kinetics following the sc dosing study; however, the model was not able to accurately simulate these data without substantial optimization of model parameters (data not shown).

**DISCUSSION**

In order to accurately assess the potential risks to children from low-level environmental CPF exposures, study designs should model relevant childhood exposure scenarios. Parameters selected in each study design (dose, route of exposure, vehicle, dose rate, etc.) affect the internal dose received by the test species and, hence, the toxicological outcome. Thus, it is important to understand both the PK and the PD of toxicants under the experimental conditions used. This study was designed to examine how different dosing scenarios can affect systemic CPF dose in neonatal rat pups. The CPF doses selected were higher than environmentally relevant exposures but were typical for doses commonly used in contemporary rodent studies. The impact of these experimental parameters on risk assessment is addressed briefly.

A More Relevant Exposure Paradigm: Neonatal Exposure via Lactation

Conolly et al. (1999) stated, “The relevance of experiments using doses that are many multitudes of conceivable human exposures and unrealistic routes of exposure is, at most, quite dubious.” Thus, exposure scenarios used in animal studies should be relevant to realistic human exposures so that the resulting data are useful in risk assessment. Based on the age of the rat pups, it was concluded that lactational exposure of rat pups represents a “more realistic” exposure scenario than bolus dosing by gavage or bolus sc administration.

This study was conducted using rats less than 10 days of age, similar to many other CPF studies (e.g., Betancourt et al., 2006; Dam et al., 1999, 2000). When using neonatal rats, investigators should consider the extrapolation of these data to humans; thus, it is incumbent upon researchers to consider relevant routes of exposure and how these dosing scenarios impact internal dosimetry. Relative to humans, rat pups are altricial at the time of birth. Conservative estimates place the nervous system in a newborn human as equivalent to a rat pup on PND 10 or later (Bayer et al., 1993; Vidair, 2004; Watson et al., 1993; Vidair, 2004; Watson et al., 2000).
et al., 2006; West, 1987). Thus, given the relatively altricial state at which rat pups are born, the most realistic exposure scenario during PND 1–10 in the rat pup would be an exposure equivalent to a continuous in utero exposure in humans. Due to technical difficulties, this approach is seldom implemented in practice. The second most relevant exposure scenario would be lactational exposure following dietary exposure to the dams as this scenario provides an opportunity for continuous exposure as would occur in utero. Lactational exposure also may be relevant for premature infants.

Exposure to food residues of CPF would occur at older ages (>10 days of age for rat pups) and at later stages of development; thus, the kinetics of dietary administration may differ from the current study. When these food residue exposures occur, infant exposures would generally be disbursed making split doses more relevant than a bolus exposure. Thus, the relevance of the dosing scenarios for rat pups on PND 5 would be in utero equivalent dose > lactational dose > direct oral exposure via divided doses > direct oral exposure to bolus doses > direct exposure to bolus doses by sc injection. While these and other dosing scenarios may be used in neonatal studies, the PK of the dosing regimen in rat pups of the appropriate age should be considered in order to establish its relevance to realistic human exposures.

However, even with lactational exposures, selection of study parameters will impact neonatal dosimetry. Maternal PK factors can influence neonatal exposures. Based on the time course for milk CPF concentrations the degree of partitioning of CPF into milk and estimated milk consumption by PND 5 pups, it was estimated that pups were exposed to 0.1 mg/kg/day CPF when lactating dams were given 5 mg/kg/day by gavage in corn oil (Mattsson et al., 2000). This is approximately fourfold higher than the estimated exposure to pups through milk on PND 5 from dams exposed to 5 mg/kg/day CPF in the diet (0.024 mg/kg/day). This value is based on estimated pup intake of 2.35 ml/day (Sampson and Jansen, 1984) and the mean body weight of PND 5 pups used in this study (mean body weight = 11.50 g). These data support dietary exposure to dams and subsequent lactational exposure of pups as the most relevant exposure scenario for risk assessment.

With maternal dietary exposure at 5 mg/kg/day, milk contained about 118 ng/g of CPF (Table 1), while milk from dams given CPF by gavage in corn oil had about 1534 ng/g milk (Mattsson et al., 2000), a ratio of 1 to 13. Maternal blood levels of CPF were nonquantifiable at all time points (LOQ = 3.6–4.4 ng CPF/g blood) in this dietary study and also nonquantifiable in male F344 rats administered CPF at the same dose level in diet (LOQ = 0.3 ng/ml; Saghir et al., 2006). However, blood levels from dietary exposure were estimated at 1.1 ng/g based on examination of the chromatograms and the estimated milk:blood partitioning ratio of 104:1 reported in Mattsson et al. (2000). In contrast, CPF blood levels were 14.8 ng/g in PND 5 lactating dams 4 h after CPF by gavage in corn oil at 5 mg/kg/day, and the 24-h average concentration of CPF was estimated at 3.8 ng/g blood (derived from Mattsson et al., 2000). Thus, the estimated CPF blood levels from dietary exposure at 5 mg/kg/day were about 13 times lower than peak levels in lactating dams tested 4 h after CPF gavage in corn oil at 5 mg/kg/day (i.e., 1.1 vs. 14.8 ng/ml blood). Thus, milk levels correlate with maternal blood levels with both exposure paradigms.

Similar results were seen with maternal blood TCP levels. Lactating dams given dietary CPF at 5 mg/kg/day had similar
plasma TCP levels to male Fischer 344 rats exposed to CPF in the diet at 5 mg/kg/day (daily range 400–1060 ng/ml plasma; Saghir et al., 2006). These dietary blood TCP values were lower than peak blood TCP levels of approximately 2000 ng/g in PND 5 lactating dams 4 h after administration of 5 mg/kg CPF by gavage in corn oil (Mattsson et al., 2000). Interestingly, the data indicate that exposure to CPF at 5 mg/kg/day in the diet versus gavage (in corn oil) has an appreciably greater impact on blood levels of CPF than on blood levels of TCP. Consistent with maternal blood levels, gavage of CPF in corn oil resulted in a 13-fold higher milk CPF level (at 4 h) than the steady-state levels from diet. Gavage of CPF in corn oil also resulted in approximately two times higher levels of blood TCP in pups than from diet, but this difference may have been influenced by a transiently elevated TCP level from the bolus effect of gavage (Table 2).

Whether dams were dosed by diet or gavage, CPF was not detected in the blood of PND 5 pups exposed via nursing from dams given 5 mg/kg/day CPF (LOQ = 4 and 1 ng/g, respectively, Mattsson et al., 2000). PND 5 pup blood levels of TCP were approximately 12 times lower on average than dam blood levels at all time points in the current study. Similar results were seen by Mattsson et al. (2000), where TCP levels in PND 5 dam blood were 2048 ng/ml versus 49 ng/ml in pup blood.

Thus, the current data illustrate that the exposure paradigm (diet vs. gavage) affects internal dose in the dam and thereby alters lactational exposure of the offspring. The impact of gavage versus dietary exposure on internal dose has been reported previously. One example is the study by Yuan et al. (1995), who reported higher benzoic acid concentrations in plasma in rats and mice gavaged with benzyl acetate in corn oil compared with those given benzyl acetate in the diet. In this study, daily doses of benzyl acetate were similar, but exposure via gavage resulted in saturation of clearance pathways, resulting in a peak in plasma levels of the major metabolite, benzoic acid. In chronic studies, gavage administration of benzyl acetate has been associated with tumors of the clitoral gland in rats and liver and forestomach in mice, whereas there was no increase in neoplasms in chronic dietary studies with benzyl acetate.

Based on these points and the developmental stage of the animals, exposure of nursing rat pups via lactation is the most relevant exposure scenario for human infants. In laboratory studies, rat pups could be exposed to CPF via their dam’s milk at doses that exceed expected human exposures and thereby allow a conservative estimate of hazard. Furthermore, the dam’s dietary doses could be readily adjusted to provide nursing exposure levels that overlap expected human exposures. The advantages of this approach are clear: dose levels to pups would be adequate for risk assessment, and the route and rate of exposure would be far more realistic than gavage or sc injection exposure of pups. In addition, the pups would not be stressed by the gavage or injection procedures.

**Direct Dosing of Neonates: The Impact of Dose Rate, Vehicle, and Route**

Due to the complexity of quantifying lactational exposures in neonatal rats, some researchers prefer direct neonatal dosing. Gavage of pups is convenient, provides a known time of dose administration, and provides a known quantity of dose. However, gavage studies pose unique and challenging interpretive issues that must be addressed. In addition to the stress of gavage, a particular concern in gavage studies is an unrealistic Cmax that may impact toxicity outcome or transiently alter maternal or pup behavior. To address this, direct dosing studies should include measurements of internal concentrations so the data can be more readily compared to other dose routes and extrapolated to more realistic human exposures during the risk assessment process.

In this study, direct bolus gavage dosing produced higher CPF Cmax values than estimated pup exposures via lactation or direct administration of divided doses to pups. This result is not surprising as numerous examples exist which have shown that dose rate can affect toxicological outcome (e.g., Corley et al., 2005). Bolus administration can affect the concentration-time pattern of ingested chemicals and therefore affect potential toxic responses. Toxic responses may occur due to high peak concentrations, and toxic responses may affect maternal or pup behavior, which has its own panoply of consequences. Thus, toxicity may be accentuated by bolus gavage dosing. In the case of food residue exposures with CPF, a direct dosing exposure scenario using divided doses throughout the day is more realistic than a single bolus dose by oral gavage but still suffers from the bolus effect and even more stress of gavage (Branchi et al., 2005).

Both Cmax and AUC values are sensitive to dose rate (Corley et al., 1994). The effect of dose rate on CPF internal dosimetry and plasma cholinesterase inhibition was examined using computer simulations (Timchalk et al., 2002b), wherein adult rats were administered 10 mg/kg CPF by bolus gavage or 10 mg/kg CPF given by gavage in three doses over a 24-h period (3 × 3.3 mg/kg at 8-h intervals). In adults, dividing the dose decreased Cmax by 60% and blunted the plasma cholinesterase inhibition from 17% remaining activity for a single dose to 27% of control activity for divided doses. The simulations indicated that administration of a large bolus dose of CPF resulted in greater cholinesterase inhibition than the same dose administered over a longer period, suggesting that dose rate could impact CPF dosimetry, as well as CPF dynamic response. The current results in rat pups suggest that a similar response would be expected with bolus and divided doses in young animals. Understanding the impact of bolus versus divided doses on internal dosimetry becomes more important when one considers that younger animals exhibit greater sensitivity to cholinesterase inhibition (Timchalk et al., 2006).

Timchalk et al. (2006) examined the PK and cholinesterase inhibition with CPF administered by bolus dose gavage (1 or 10 mg/kg/day in corn oil) to pups at 5, 12, and 17 days of age.
Pups were euthanized at 0, 3, 6, and 24 h after dosing. In the Timchalk study, the CPF Cmax in 5-day-old pups given 1 mg/kg CPF was 3 h, which differs slightly from the 2 h reported in the current study. This may be related to the time at sacrifice (3 h was the earliest time point in the Timchalk study) and/or state of the pups at the time of CPF dosing (i.e., when pups last nursed relative to CPF dosing). A similar difference was noted in peak TCP levels in these pups, which occurred at 6 h in Timchalk et al. (2006) compared with 4 h in this study. The Cmax value for CPF (0.07 µmol/l) was slightly lower than the Cmax in the current study (0.135 µmol/l), whereas the TCP Cmax (3.84 µmol/l at 6 h) was higher than the TCP Cmax in this study (1.53 µmol/l at 4 h). Timchalk et al. (2006) reported decreased cholinesterase activity in 5-day-old pups given 1 mg/kg CPF with a maximum inhibition to 78% of control brain cholinesterase values at 3–6 h after dosing (i.e., tissue sensitivity was plasma > RBC > brain).

The vehicle used in toxicity studies also impacts the absorption characteristics of toxicants. In this study, the Cmax and AUC of plasma CPF and TCP from a bolus dose of CPF in a corn oil vehicle were greater than for the pups given CPF in rat milk. One must consider the relevance of the vehicle used during direct neonatal dosing experiments; a bolus of corn oil is clearly not a normal dietary substrate for neonatal rats. Intuitively, any vehicle other than maternal milk should raise some concerns because milk is a pup’s sole source of nutrition during much of lactation (Hanley and Watanabe, 1985). To support these concerns, data from this study show that PK are different in pups gavaged with CPF in corn oil compared with pups gavaged with CPF in dam’s milk. Our conclusion is that a dam’s milk vehicle is preferable over a corn oil vehicle when administering a gavage dose to neonates.

Although gavage administration of CPF with a milk vehicle is more realistic than other vehicles, this scenario is more difficult to implement. Collection of rat milk is labor intensive, and the lactational time points at which to collect milk must be carefully considered due to changes in milk composition over the course of lactation (e.g., milk fat content declines over the course of lactation; Nicholas and Hartmann, 1991). In this study, milk from lactation day 9–10 dams was used in order to ensure sufficient milk yields to meet dosing requirements. This milk would be reasonably representative of milk consumed by pups on lactation day 5 (the day of dosing) because rat milk fat content remains relatively constant from lactation day 5 through the remainder of lactation (Nicholas and Hartmann, 1991).

Gavage in corn oil has been criticized as an unrealistic method of exposure because of the potential to deliver chemical to the target site at an excessive rate or in an excessive amount (Conolly et al., 1999). This may be due to the enhanced rate and extent of absorption from the gastrointestinal (GI) tract of both hydrophilic and lipophilic materials. Corn oil can enhance absorption across the GI mucosa, particularly if a chemical is sparingly soluble in water, like CPF. Flow of lymph can be increased with corn oil. This can lead to an increase in the quantity of the lipophilic material absorbed through the lymphatics and reduce the effect of hepatic portal circulation. This mechanism may account for the enhancement of the maximum plasma levels, bioavailability, and an increase in the duration and extent of absorption of the antibiotic, griseofulvin, when administered in corn oil as compared to administration as an aqueous suspension (Bates and Carrigan, 1975). Furthermore, the acute toxicity of dichloroethylene has also been shown to be greatly enhanced when the test material is administered in corn oil versus an aqueous vehicle (Chieco et al., 1981). The PK profiles of other halogenated hydrocarbons also are altered when administered in a corn oil versus aqueous vehicle (Staats et al., 1991; Withey et al., 1983). Thus, corn oil as a vehicle for oral gavage should be rigorously justified before use and the limitations relative to extrapolation for use in risk assessment clearly noted.

An alternate route and vehicle used in this study involved the direct dosing of neonates to 1 mg CPF/kg bw by the sc route of administration using DMSO as a vehicle. Notably, the sc route is not the preferred method of dosing because it is not a realistic route of exposure; however, this dosing paradigm has been used in several studies examining the neonatal neurotoxicity of CPF (e.g., Campbell et al., 1997; Crompton et al., 2000; Whitney et al., 1995). Pups injected sc with CPF in 1 ml/kg bw DMSO showed signs of discomfort (“squirming”) for several seconds to a minute or two. Pain or discomfort, even transitory, raises concerns from two perspectives. First, there is an obligation to explore alternative routes of administration that would result in less pain or discomfort. This obligation is especially important for unusual routes of administration of test material in hazard evaluation studies, particularly when the utility of the resulting data for risk assessment of environmental exposures to chemicals is limited. Second, even transient pain can alter the chemistry of the developing brain, sometimes for long periods of time (Bhutta et al., 2001; Puchalski and Hummel, 2002; Ruda et al., 2000). CPF is known to be a mild irritant to the skin (WHO, 2007). Thus, in addition to the prompt signs of discomfort, the delay in absorption of sc CPF in DMSO raises questions of local irritation from this CPF depot. Swarm et al. (2001) state “Although the complexity of the CNS response to nociceptor input is only beginning to be understood, it is evident that there is marked CNS response plasticity; … not only does the CNS integrate and modify the transmitted nociceptive signals, but also the CNS itself is altered, sometimes fundamentally, by that signal processing.” In addition, irritation may trigger an inflammatory response with numerous secondary effects (Aggarwal et al., 2006; Chatterjee et al., 2006).

With sc administration of 1 mg CPF/kg in DMSO, the AUC for the parent material was similar to that observed with the oral route using a milk vehicle and less than the AUC when using bolus corn oil gavage (Table 2). The plasma half-life for CPF following sc injection in DMSO also was similar with the half-life seen after bolus exposure in milk. However, the Tmax
for TCP was 6 h compared with 4 h after exposure via the oral route. These data indicate that parental CPF may be available for a longer period than from gavage to interact with target sites when administered under these dosing conditions. Initial studies using radiolabeled CPF in DMSO showed that the majority of the radioactivity (56 ± 36%) remained at the site of injection 2 h after sc injection (Fig. 3). Thus, the transport properties of CPF in DMSO from the injection site are not understood, and the effect of repeated sc injections in DMSO on CPF PK is not known. Furthermore, the PBPK model for CPF, while showing good predictive values for parent CPF, under predicts the TCP concentration following sc dosing in DMSO. Thus, the kinetics of CPF following sc dosing in DMSO should not be assumed to mimic oral exposures.

With respect to the vehicle used, DMSO itself has been reported to have pharmacological and toxicological properties that may interfere with end points in toxicological studies (for review, see Brayton, 1986; Jacob and Herschler, 1986; Santos et al., 2003). DMSO has been shown to decrease neuronal conduction (Cavaletti et al., 2000; Evans et al., 1993) and inhibit enzymes (Prasanna et al., 1987; Waddell et al., 1989) including cholinesterase (Jagota, 1992; Watts and Hoogmoed, 1984). DMSO scavenges reactive oxygen species (Ogura et al., 1995), alters protein expression, cell cycle progression (Darling et al., 1989; Sawai et al., 1990), and apoptosis (Lin et al., 1995; Liu et al., 2001). It interferes with cationic and anionic cellular signaling (Santos et al., 2002) and some adenosine triphosphatases (Benaim and de Meis, 1989). Several reports state that DMSO alters permeability and/or transport at the blood-brain barrier (Broadwell et al., 1982; Scheld, 1989), although this observation is contentious (Ziylan et al., 1988). DMSO also facilitates the penetration of chemicals across biological membranes (Brayton, 1986). Given the goal of hazard assessment to be relevant for risk assessment (Conolly et al., 1999), it can be readily seen that neonatal sc administration of high doses of CPF (1 mg/kg/day) in biologically active doses of DMSO (1 ml/kg/day) must be accompanied by a very persuasive set of experiments to determine its relevance for risk assessment. The opportunities for confounding from sc injections and from large doses of DMSO are considerable.

Relevant Exposures in Children

In order to accurately assess the risks to children from low-level environmental CPF exposures, study designs should model and mimic, to the extent possible, relevant childhood exposure scenarios. Recently published studies by the U.S. EPA indicate that children’s aggregate exposure to CPF (food, air, indoor floor dust, outdoor soil, handwipes, and transferable residues) in the United States in 1997 and 2000/2001 was on the order of 0.03 μg/kg bw/day (Morgan et al., 2005; Wilson et al., 2003). The maximum estimate for any one child of CPF aggregate exposure was 0.329 μg/kg/day (Wilson et al., 2003). Although the rate of exposure was not evaluated in Wilson et al. (2003) or Morgan et al. (2005), the nature of aggregate exposure implies several different sources of exposure during a day, with Morgan et al. (2005) indicating that the majority of CPF exposure was from food.

In contrast to children’s CPF aggregate exposures that sum to less than 1 μg/kg/day, studies in rat pups to determine children’s risk to CPF are commonly conducted at doses that do not overlap (i.e., they exceed) environmental exposures. Exposures in rat pups are commonly 100 mg/kg or higher.

FIG. 3. PBPK model simulations showing the time course for CPF and TCP in pup blood following bolus exposure to 1 mg/kg in corn oil (A) or milk (B) or after split dosing (1 mg/kg administered as 0.333 mg/kg three times per day) in corn oil (C) or milk (D). The model could not adequately simulate the data from sc injections, which differed markedly from oral exposures. Experimental data are denoted with symbols, and simulations are marked with lines. The data represent the mean ± SD for three pups per time point per group. The original PBPK model was developed by Timchalk et al. (2002b) and recently has been extended to include preweanling rat pups (Timchalk et al., 2007).
### TABLE 3
PK Summaries from Various Studies Examining CPF and TCP in Neonatal Rat Pups on PND 5

<table>
<thead>
<tr>
<th>End point</th>
<th>CPF in maternal blood (LD 5)</th>
<th>TCP in maternal blood (dams on LD 5)</th>
<th>ChE inhibition(^a) (dams on LD 5)</th>
<th>CPF in milk</th>
<th>Milk CPF ratio to 5 mg/kg gavage to dam</th>
<th>Estimated CPF exposures via milk</th>
<th>Pup exposure relative to dam</th>
<th>CPF in pup blood (Cmax)</th>
<th>TCP in pup blood (Cmax)</th>
<th>ChE inhibition(^a) (pups on LD 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Gavage to dam: 0.3 mg/kg in corn oil</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>123 ng/g</td>
<td>13.5 ng/g(^c)</td>
<td>0.009</td>
<td>0.003</td>
<td>mg/kg/day</td>
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<td>mg/kg/day</td>
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<tr>
<td><strong>B</strong> Gavage to dam: 1.0 mg/kg in corn oil</td>
<td>NQ at 4 h(^h) (estimated 0.8 ng/g from milk data)</td>
<td>418 ng/g</td>
<td>Yes</td>
<td>81.8 ng/g(^e)</td>
<td>0.05</td>
<td>0.017</td>
<td>0.017 mg/kg/day(^d)</td>
<td>0.17 NQ(^b) — 2 h(^e)</td>
<td>— NQ(^f) — 2 h(^e)</td>
<td>— No</td>
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<td>mg/kg/day</td>
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<tr>
<td><strong>C</strong> Diet given dam: 5.0 mg/kg/day</td>
<td>NQ (estimated 1.1 ng/g from milk data)</td>
<td>281–1263 ng/g</td>
<td>Yes(^e)</td>
<td>111–127 ng/g</td>
<td>0.08</td>
<td>0.024</td>
<td>0.005 mg/kg/day(^d)</td>
<td>49–21 ng/g(^g)</td>
<td>—</td>
<td>— Residual from in utero exposure</td>
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<td>mg/kg/day</td>
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<tr>
<td><strong>D</strong> Gavage to dam: 5 mg/kg/day in corn oil</td>
<td>14.8 ng/g at 4 h</td>
<td>2048 ng/g</td>
<td>Yes(^e)</td>
<td>1534 ng/g(^e)</td>
<td>1</td>
<td>0.1 mg/kg/day(^d)</td>
<td>0.02 NQ(^b) — 2 h(^e)</td>
<td>— 49</td>
<td>—</td>
<td>— Not evaluated</td>
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<td>mg/kg/day</td>
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<tr>
<td><strong>E</strong> Neonatal sc injection: 1 mg/kg in DMSO</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>9.5 mg/ml—2 h(^h)</td>
<td>171.3 ng/ml—6 h(^h)</td>
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<td>h/ml(^d)</td>
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<td><strong>F</strong> Neonatal gavage (bolus): 1 mg/kg in rat milk</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>8.4 mg/ml—2 h(^h)</td>
<td>90.1 ng/ml—4 h(^h)</td>
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<td>h/ml(^d)</td>
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<tr>
<td><strong>G</strong> Neonatal gavage (bolus): 1 mg/kg in corn oil</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>47.5 mg/ml—2 h(^h)</td>
<td>320 ng/ml—4 h(^h)</td>
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<td>h/ml(^d)</td>
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</table>

**Note:** LD, lactation day.

\(^a\)Plasma and/or red blood cell cholinesterase (ChE) inhibition at 4 h postgavage dosing of dams; Mattsson et al. (2000).

\(^b\)Nonquantifiable with LOQ = 0.7 ng/g; Mattsson et al. (2000).

\(^c\)Milk samples collected 4 h after dosing the dam via gavage, the time of peak blood concentrations in treated dams.

\(^d\)Calculated based on concentration of CPF in milk \(\times\) 2.35 ml milk consumed on PND 5 \(\times\) bw (~11.5 g).

\(^e\)Peak blood levels for CPF were estimated to be at 4 h postexposure in dams.

\(^f\)Nonquantifiable with LOQ = 10 ng/g; Mattsson et al. (2000).

\(^g\)Predicted response based on results from other studies cited in the table.

\(^h\)Nonquantifiable with LOQ = 3.2–3.7 ng/g (current study).

\(^i\)Peak time cannot be determined due to continuous exposure of pups via milk ingestion from CPF-exposed dams.

\(^j\)ChE inhibition is uncertain; pups from dams given 5 mg/kg/day by gavage had ChE inhibition with lower TCP values, but TCP values were likely underestimated (samples were collected at 2 h; peak at 4 h).

\(^k\)Mattsson et al. (2000).

\(^l\)Calculated from zero to infinity.

\(^m\)Song et al. (1997) with multiple doses.

\(^n\)Timchalk et al. (2006).
(Moser and Padilla, 1998; Zheng et al., 2000) with administration by gavage in corn oil or sc injection in DMSO (e.g., Aldridge et al., 2005; Auman et al., 2000; Crumpton et al., 2000; Dam et al., 1999, 2000). Because dose determines toxicity, the relevance of these dosing scenarios remains to be established. As stated by Conolly et al. (1999), “predicted risks based on studies conducted at excessive doses may have little or no relationship to real world risks.”

Dose, dose rate, and route of exposure affect internal dosimetry in neonatal rat studies. Table 3 illustrates some of the available PK data reported in the current study as well as previous studies. Rows A–D illustrate CPF and TCP levels measured in dams’ blood, milk, and pup blood after dosing dams with 0.3–5 mg/kg/day CPF by bolus gavage in corn oil or 5 mg/kg/day in the diet. Rows E–G illustrate that direct bolus dosing of neonatal rat pups with 1 mg/kg/day CPF in any vehicle exceeded the CPF and TCP blood levels achieved when pups were exposed lactationally following maternal exposures up to 5 mg/kg/day. Overall, these data illustrate the importance of kinetic data, including internal dosimetry, to put various dosing regimens and their toxicological outcome into perspective.

CONCLUSION

When conducting a neonatal/juvenile toxicity study, it is important to understand the PK of a test material and it is recommended that concentrations of the test material in dam milk and blood, as well as neonatal blood, be measured in order to understand internal dosimetry. For materials where supplemental dosing is needed, dose rate and vehicle must be considered carefully.

To make toxicological data useful for risk assessment and when establishing allowable daily intake limits, it is incumbent upon toxicologists to avoid irrelevant exposure routes and high dose levels that are often employed for the sake of experimental convenience (Conolly et al., 1999). Such data are likely to be misleading for predicting hazard at environmentally relevant levels of exposure.

FUNDING

The PBPK model that was used for dosimetry simulations was developed by Charles Timchalk under the partial support of (R01 OH003629-03 and R01 OH008173-01) Centers for Disease Control and Prevention and the U.S. Environmental Protection Agency’s STAR program through grant (R829608).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the following individuals for assistance during these studies: A. Liberacki, I. Gloden, J. Hammond, J. Passage, T. Card, J. Lacher, D. V. M., E. Mullen, M. Knoerr, A. Clark, A. Mendrala, J. Fairchild, J. Wachner, J. Beyer, J. Sushynski, K. Treadway, E. Harris, K. Brzak, S. Saghir, B. Marable, and J. Maurissen. The PBPK model contents are solely the responsibility of the authors and have not been subject to any review by Centers for Disease Control and Prevention (CDC) or EPA and therefore do not necessarily represent the official view of CDC or EPA, and no official endorsement should be inferred.

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following oral exposure to the organophosphorus insecticide chlorpyrifos. 


