Physiologically Based Pharmacokinetic Model for the Inhibition of Acetylcholinesterase by Organophosphate Esters

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Organophosphate (OP) exposure can be lethal at high doses while lower doses may impair performance of critical tasks. The ability to predict such effects for realistic exposure scenarios would greatly improve OP risk assessment. To this end, a physiologically based model for diisopropylfluorophosphate (DFP) pharmacokinetics and acetylcholinesterase (AChE) inhibition was developed. DFP tissue/blood partition coefficients, rates of DFP hydrolysis by esterases, and DFP-esterase bimolecular inhibition rate constants were determined in rat tissue homogenates. Other model parameters were scaled for rats and mice using standard allometric relationships. These DFP-specific parameter values were used with the model to simulate pharmacokinetic data from mice and rats. Literature data were used for model validation. DFP concentrations in mouse plasma and brain, as well as AChE inhibition and AChE resynthesis data, were successfully simulated for a single iv injection. Effects of repeated, subcutaneous DFP dosing on AChE activity in rat plasma and brain were also well simulated except for an apparent decrease in basal AChE activity in the brain which persisted 35 days after the last dose. The physiologically based pharmacokinetic (PBPK) model parameter values specific for DFP in humans, for example, tissue/blood partition coefficients, enzymatic and nonenzymatic DFP hydrolysis rates, and bimolecular inhibition rate constants for target enzymes were scaled from rodent data or obtained from the literature. Good agreement was obtained between model predictions and human exposure data on the inhibition of red blood cell AChE and plasma butyrylcholinesterase after an intramuscular injection of 33 μg/kg DFP and at 24 hr after acute doses of DFP (10–54 μg/kg), as well as for repeated DFP exposures. The PBPK model for DFP was also adapted for the purpose of modeling parathion, including its metabolism to the toxic daughter product paraoxon. The development and validation of this PBPK model for two OPs provides a basis for studying the kinetics and in vivo metabolism of other bioactivated organophosphate pesticides and their pharmacodynamic effect in humans. — Environ Health Perspect 102(Suppl 11):51–60 (1994)

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Introduction

A variety of organophosphate (OP) esters inhibit acetylcholinesterase (AChE) in both central and peripheral nervous tissues resulting in excessive buildup of acetylcholine at neural receptors (1). AChE accumulation causes a spectrum of acute toxic effects mediated by the nicotinic, muscarinic, and central nervous system interactions of acetylcholine. These range, depending on dose, from subclinical performance decrements to convulsions, asphyxia, and death (2). A model capable of predicting the relationship between OP exposure and resultant toxic effects would be useful in risk assessment and the design of measures protective against exposure to cholinesterase inhibitors. Ideally, the model should be amenable to cross-species scaling and accurately predictive outside the dose range over which its primary validation studies were conducted. Physiologically based pharmacokinetic (PBPK) models meet these criteria (3–7). They consist of descriptions of the physiology of the exposed organism and the biochemical processes, which are quantitatively important determinants of toxicant disposition. This includes parameters describing tissue solubilities, metabolism, and binding of the toxicant. A number of PBPK models have been developed for solvents and relatively stable halogenated compounds (5,8,9). Additionally, an in vitro kinetic model for diisopropylfluorophosphate (DFP) in rat brain has been developed (10), and a PBPK model for single dose pharmacodynamics of soman in rats was recently published (11).

The goal of this study was to develop a quantitative, physiologically based model of OP pharmacokinetics and AChE inhibition. While AChE inhibition is not a toxic effect per se, it is an index of the probability that acute OP toxicity will occur (12). DFP is not itself commercially important, but was chosen for this study as its mechanism of action is thought to be representative of other highly toxic OPs (12). Parathion (PA) is an organophosphate insecticide that owes its toxicity to the oxidized product of parathion, paraoxon (PO). The metabolism of PA takes place predominantly in the liver,
but it is also thought that some metabolism of parathion occurs in extrahepatic tissues. The basic model structure described herein for DFP and parathion should be applicable to other OPs whose acute toxicity is mediated by inhibition of AChE.

**Development of the Physiologically Based Pharmacokinetic Model**

**Elements of Model Structure (Overview)**

The major determinants of DFP disposition *in vivo* are its hydrolysis by esterases, binding to esterases, tissue solubility, and volatility (leading to exhalation). DFP is metabolized (hydrolyzed) by A esterases (AEST) to a non-inhibitory product, diisopropylphosphoric acid (DIP). This organophosphate ester also binds to B esterases (BEST) and inhibits their enzymatic activity. The hydrolysis reaction is governed by Michaelis-Menten kinetics whose $V_{max}$ and $K_m$ were determined in *in vitro* assays (Appendix 1). Those compartments described in the model as having A esterase activity (DFPase) are shown in Figure 1 as shaded areas. The binding to and inhibition of B esterases were modeled as bimolecular reactions and were also determined from *in vitro* studies (Tables 1 and 2). All tissue compartments in the model except fat were described as having B esterase activity (13). The major determinants of parathion and paraxxon disposition in the model are metabolism of parathion to paraxxon, hydrolysis of paraxxon by esterases, binding of paraxxon to esterases, and the tissue solubility of the parent and daughter compound.

**Hydrolysis of Diisopropylfluorophosphate**

DFP is rapidly hydrolyzed by A esterases (14). These enzymes are present in virtually all tissues but often have high activity in the blood. As a result, blood AEST strongly affects the amount of DFP reaching AChE in target tissues. The activity of AEST was obtained from laboratory studies in the rat as described below. The Michaelis–Menten constants $K_m$ and $V_{max}$ were determined for AEST activity in rat brain, blood, liver, and kidney. The $K_m$ for AEST in each of these tissues was assumed to be the same for each of the corresponding mouse tissues. $V_{max}$ values for each of the corresponding mouse tissues were scaled to the 0.7 power of body weight (8). DFP also undergoes spontaneous hydrolysis in aqueous solutions. A pseudo-first-order rate of spontaneous hydrolysis was estimated in heat treated (30 min at 60°C) homogenates of each tissue (10) and was found to be insignificant (0.0046 min⁻¹ relative to the enzymatic rates of DFP hydrolysis (15).

**Hydrolysis of Paraxxon**

The hydrolysis of paraxxon by A esterases was described with Michaelis–Menten kinetics, using data from Wallace and Dargan (16), Chenmitius et al. (17), and Pla and Johnson (18).

**Diisopropylfluorophosphate Binding to Esterases**

The schema for model simulations of free B esterase, in this case AChE, is shown in Figure 2. The amount of free AChE is determined by the concentration of DFP and 4 different rate constants. The basal level of AChE results from a balance between basal degradation of AChE and synthesis of new enzyme. After exposure to DFP, the amount of free AChE is governed by the balance between the bimolecular rate of inhibition and the rate at which inhibited AChE is regenerated. Once

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**Table 1. Metabolic constants for pharmacokinetic model of diisopropylfluorophosphate.**

| Organ             | Mouse $V_{max}$ mg/hr | $K_m$ mg/l | Rat $V_{max}$ mg/hr | $K_m$ mg/l | Human $V_{max}$ mg/hr | $K_m$ mg/l |
|-------------------|------------------------|------------|---------------------|------------|------------------------|------------|
| Brain             | 2.28                   | 439.80     | 9.18                | 439.80     | 668                    | 440        |
| Liver             | 256.59                 | 237.36     | 1300.00             | 237.36     | 52474                  | 237        |
| Kidney            | 25.01                  | 134.3      | 103.32              | 134.3      | 5042                   | 134        |
| Richly perfused   | 2.72                   | 50.89      | 11.04               | 50.89      | 560                    | 51         |
| Venous blood      | 79.38                  | 198.70     | 320.18              | 198.70     | 616                    | 199        |
| Arterial blood    | 26.46                  | 198.70     | 106.73              | 198.70     | 216                    | 199        |

Bimolecular rate constants, $\mu$M⁻¹ hr⁻¹

- Acetylcholinesterase 14.16
- Butyrylcholinesterase
  - Fast 354.00
  - Slow 30.00
- Carboxylesterase
  - Fast 1.10
  - Slow 0.52

**Table 2. Metabolic constants for a pharmacokinetic model of parathion.**

| Organ            | Parathion desulfuration $V_{max}$ mg/hr | $K_m$ mg/l | Parathion dearylation $V_{max}$ mg/hr | $K_m$ mg/l | Paraxon hydrolysis $V_{max}$ mg/hr | $K_m$ mg/l |
|------------------|----------------------------------------|------------|--------------------------------------|------------|----------------------------------|------------|
| Brain            | ---                                    | ---        | ---                                  | ---        | 9.2                              | 440        |
| Liver            | 16.6                                   | 3.0        | 28.4                                 | 81.8       | 11                               | 50         |
| Kidney           | ---                                    | ---        | ---                                  | ---        | 103                              | 134        |
| Richly perfused  | ---                                    | ---        | ---                                  | ---        | 11                               | 50         |
| Venous blood     | ---                                    | ---        | ---                                  | ---        | 150                              | 170        |
| Arterial blood   | ---                                    | ---        | ---                                  | ---        | 100                              | 170        |
AChE is inhibited, it will either return to free AChE, or age and form a permanently inhibited enzyme.

B esterases (19) have a serine residue at the catalytic site to which OPs bind, inactivating the enzyme. BESTs include AChE, butyrylcholinesterase (BChE), and carboxylesterase (CaE). While AChE is the target in acute OP intoxication, DFP binding to BChE and CaE is without adverse physiological effect (20). Phosphorylated BESTs undergo spontaneous hydrolysis, leading to regeneration of the active enzyme (Figure 2). For DFP, however, this process is relatively slow (27) (Table 3). Phosphorylated BEST may also undergo aging (22), resulting in a permanent loss of activity (Figure 2). BEST activities were obtained from Maxwell et al. (13).

Basal activities of AChE, BChE, and CaE were defined by zero-order synthesis rates (moles binding sites/hour) and first-order loss rates (hr⁻¹; Table 3). First-order loss of rat brain AChE was obtained from Wenthold (23), who used radiolabeled precursors of AChE to determine enzyme degradation. Synthesis of rat plasma and brain AChE was determined from model optimization of the data of Michalek (24), Traina and Serpietri (25). The zero-order synthesis rates and first-order loss rates for BESTs in rat plasma and in mice were estimated by fitting simulations to data describing the rate of return of enzyme activity to normal levels over time after DFP dosing (see "Results"). The synthesis and loss rates used for AChE were also used for BChE and CaE since the rate of increase of these activities after OP inhibition is close to that of AChE (25,26).

Paraoxon Binding to Esterases
The activity of esterases which are inhibited by paraoxon were obtained from Maxwell (13).

Metabolism of Parathion to Paraoxon
The metabolism of parathion to its toxic daughter product paraoxon was described in the model to occur in the liver and the kidney by Michaelis–Menten kinetics. The initial $K_m$ and $V_{max}$ for this metabolism were obtained from the work of Wallace and Dargan (16). These rates of metabolism were optimized to provide better predictions of parathion and paraoxon pharmacokinetics.

Specification of Compartments
The PBPK model for DFP consisted of both organ-specific and lumped compartments (Figure 1; Appendix 2; Table 4).

Organ-specific compartments were used to describe those tissues directly involved in acute DFP toxicity (e.g., brain, lung, diaphragm) or those expected to significantly influence DFP pharmacokinetics (e.g., blood, fat). Blood, though not a target organ, is an important site of DFP metabolism, and blood AChE is a useful reflection of AChE activity in less accessible organs. Separate venous and arterial compartments were used because it has been shown experimentally that there can be significant arterial–venous differences in DFP concentrations (27). The kidney and liver are also sites of enzymatic hydrolysis of DFP for which tissue-specific metabolic parameters were readily obtainable. Lumped compartments (fat, rapidly perfused, etc.) were used to describe remaining tissues. The rapidly perfused lumped compartment represented viscera not explicitly described elsewhere. The slowly perfused compartment denoted mainly muscle tissue distributed throughout the organism. Use of lumped compartments helped to preserve a balance between parsimony of model structure, to maintain chemical mass balance, and to describe explicitly mammalian physiology and biochemistry that determine the pharmacokinetic behavior of OPs.

The PBPK model consisting of mass-balance differential equations and associated computer programs that describe the time-dependent pharmacokinetics of DFP and inhibition of AChE were written in Advanced Continuous Simulation Language (ACSL) (Mitchell and Gauthier Associates, Inc., Concord, MA). Simulations were run on a VAX 8530 (Digital Equipment Co., Maynard, MA).

**Table 3. Model parameters for AChE inhibition.**

| Parameter                        | Mouse | Rat | Human |
|----------------------------------|-------|-----|-------|
| Enzyme inhibition, μM⁻¹·hr⁻¹     | 14.16 | 14.16 | 14.16 |
| Enzyme regeneration, hr⁻¹        | 0.016 | 0.016 | 0.016 |
| Enzyme aging, hr⁻¹               | 0.297 | 0.263 | 0.150 |
| Enzyme synthesis, nmole/hr       | 0.0030d | 0.0019p | 0.053 |
| Brain                            | 0.0025d | 0.0014f | 0.0014 |
| Enzyme degradation, hr⁻¹         | 0.080d | 0.100d | 0.200 |
| Brain                            | 0.070d | 0.010d | 0.010 |

*Data from Jepson (10). b Data from Vandeker and Heath (21). c Data from Andersen et al. (40). d Data from Martin (34). e Data from Traina and Serpietri (25). f Data from Michalek et al. (24). g Data from Wenthold et al. (23). The model tracked the inhibition of B esterases in brain, plasma, lung, diaphragm, kidney, liver, and slow and fast compartments. Data and model simulations are shown only for brain and plasma.

**Table 4. Physiological parameters for a pharmacodynamic model of organophosphates.**

| Parameter                        | Mouse | Rat | Human |
|----------------------------------|-------|-----|-------|
| Body weight, kg                  | 0.03  | 0.22 | 80.6  |
| Alveolar ventilation, 1/hr       | 1.04  | 4.57 | 354   |
| Cardiac output, 1/hr             | 1.04  | 4.57 | 302   |
| Organ volumes, percentage of body weight |        |      |       |
| Brain                            | 1.47  | 1.16 | 2.14  |
| Liver                            | 6.17  | 4.00 | 4.00  |
| Kidney                           | 1.73  | 0.73 | 0.43  |
| Richly perfused                  | 2.00  | 2.00 | 3.43  |
| Fat                              | 8.00  | 7.00 | 17.00 |
| Slowly perfused                  | 65.74 | 68.66| 55.14 |
| Diaphragm                        | 0.30  | 0.030| 0.3   |
| Lung                             | 0.36  | 1.15 | 0.86  |
| Venous blood                     | 5.00  | 4.00 | 5.70  |
| Arterial blood                   | 2.50  | 2.00 | 2.00  |
| Organ blood flows, percentage of cardiac output |        |      |       |
| Brain                            | 3.00  | 3.00 | 13.40 |
| Liver                            | 25.00 | 25.00| 27.00 |
| Kidney                           | 20.00 | 20.00| 22.30 |
| Richly perfused                  | 28.00 | 27.96| 20.00 |
| Fat                              | 9.00  | 9.00 | 5.60  |
| Slowly perfused                  | 14.40 | 14.40| 13.10 |
| Diaphragm                        | 0.60  | 0.60 | 0.60  |

They were housed in plastic shoe box cages with hardwood chip bedding, two per cage, on a 12-hr light and dark cycle. Food (Purina Formula #5008) and water were available ad libitum. Animals were quarantined for 2 weeks to allow for acclimatization and for quality assurance procedures designed to ascertain animal health.

Quantitation of Diisopropylfluorophosphate Metabolism

**Enzymatic Hydrolysis.** $V_{max}$ and $K_m$ estimates were obtained by measuring fluoride ion appearance in rat tissue–saline homogenates (1:9 w/v) stirred constantly at 37°C (Table 3). Standards were prepared in distilled water. Fluoride was measured with an Orion 701 digital ionicalyzer equipped with an Orion 96-09 combination fluoride...
 electrode (10). Heat-treated (30 min at 60°C) homogenates of each tissue were used to measure nonenzymatic DFP hydrolysis. \( V_{\text{max}} \) and \( K_m \) were estimated from Lineweaver-Burk plots of the data (28). \( V_{\text{max}} \) values for DF metabolism determined in rat tissues were scaled for mice and humans by 0.7 bodily weight (8,29). The rat \( K_m \) was used without adjustment for mouse and human simulations. 

**Bimolecular Inhibition Rate Constants.** The rate of reaction of DFP with AChE, BChE, and CaE, corresponding to the rate of esterase inhibition, was obtained by measuring decreases in tissue homogenate esterase activity after addition of DFP (Table 1). AChE activity was assayed with a modification of the method of Ellman (30). Assays were initiated by adding 20 \( \mu \)l of tissue-saline homogenate (1:3 w/v) to 2.0 ml of substrate solution (0.3 mM acetylthiocholine [Calbiochem, San Diego, CA], 0.3 mM di-thionitrobenzoic acid [Calbiochem, San Diego, CA] in 0.05 M potassium phosphate buffer [Pflatz and Bauer, Stanford, CT]) that had been previously warmed in a water bath at 37°C. After rapid mixing, 0.3 ml of the homogenate/substrate mixture was immediately added to a temperature-controlled cuvette (37) in a Gilford 2600 spectrophotometer (Oberlin, OH). The cuvette was held for 1 min and then absorbance was read at 0.1-min intervals for 1 min. DFP (Sigma Chemical Co., St. Louis, MO) was then added and the absorbance read at 412 nm at 0.1-min intervals for 5 min. The bimolecular inhibition rate constant was evaluated as

\[
-k_2 = \ln(V/V_0)/t(K_m + [S])/K_m \times [DFP]
\]  

where \( k_2 \) is the bimolecular rate constant (M\(^{-1}\) min\(^{-1}\)); \([S]\) is the substrate concentration (M); \( V \) is the reaction velocity (mole/min/g tissue), \( V_0 \) is the AChE activity at time zero (mole/min/g tissue); \( t \) is the duration (min) of incubation; [DFP] is the concentration of DFP (M), \( K_m \) is the Michaelis-Menten constant (M). The appropriate volume of DFP was added and there was a plot of absorbance at 412 nm versus time obtained. Slopes of tangents to the curve provided the velocity of the substrate hydrolysis reaction. The bimolecular rate constants for BChE and CaE were determined as for AChE, except that the substrates were butyrylthiocholine and \( p \)-nitrophenylacetate, respectively. The concentration of tissues used in tissue homogenates was not a factor in these calculations as inhibition reaction rates were determined per gram of tissue. 

**Partition Coefficients**

Partition coefficients for DFP (Table 5) were determined by the dial equilibration technique (31,32). DFP was injected into a closed vial containing heat-treated (60°C, 1 hr) rat blood or tissue homogenate at 37°C. After equilibration (2 hr), head space DFP space was quantitated by gas chromatography. Reference vials containing saline were treated identically. Tissue-blood partition coefficients were calculated by dividing the tissue–air value by that for blood–air. Partition coefficients determined for rat tissues were also determined for simulations of mouse and human data. The muscle–blood partition was used for the lumped, slowly perfused compartment and the liver–blood partition for the lumped richly perfused compartment. The partitioning of parathion in the brain compartment was estimated from the data of Eigenberg et al. Parathion and paraxoxon partitions in other tissues were determined in vitro by equilibrium dialysis (33).

### Table 5. Partition coefficients.

| Tissue          | DFP   | Parathion | Paraxoxon |
|-----------------|-------|-----------|-----------|
| Blood-air       | 12.57 | NA        | NA        |
| Brain-blood     | 0.67  | 4.56\(^a\) | 2.31\(^a\) |
| Liver-blood     | 1.53  | 5.21      | 6.62      |
| Kidney-blood    | 1.63  | 5.21      | 6.62      |
| Richly perfused |       |           |           |
| blood           | 0.67  | 5.21      | 6.62      |
| Fat-blood       | 17.6  | 101.2     | 10.22     |
| Slowly perfused |       |           |           |
| blood           | 0.77  | 2.55      | 3.62      |
| Diaphragm-blood | 0.77  | 2.55      | 3.62      |

Results

**Diospropylfluorophosphate Pharmacokinetics and Acetylcholinesterase Inhibition in Mice**

Several published studies of DFP pharmacokinetics and AChE inhibition in mice and rats were analyzed with the simulation model. This included both single and multi...
multiple dosing regimens. Martin (34) injected Dublin ICR male mice (Dominion Laboratories, Dublin, VA) via the tail vein with 1 mg tritium [3H]DFP/kg. To measure free, bound, and metabolized [3H]DFP, tissues from treated animals were homogenized, centrifuged, and extracted with ethyl acetate. The ethyl acetate extracts were then either counted with liquid scintillation for total radioactivity, concentrated under nitrogen (N2), and subjected to thin-layer chromatography, or the aqueous portion that remained after extraction was solubilized and counted for radioactivity.

A dominant characteristic of DFP pharmacokinetics well illustrated by this study is rapid clearance of free DFP from blood and brain. Free DFP in plasma had largely disappeared within 1 min of iv injection (Figure 3a). In brain, free DFP at approximately 1 min post injection had fallen to a small fraction of its peak concentration (Figure 3b). The PBPK model accurately simulated these data (Figure 3a,b). At time points greater than 5 min though, a less rapid disappearance of DFP from blood and brain than was predicted by the model was measured experimentally (Figure 3c,d). However, the area under the plasma and brain concentration–time curves at these later time points represented less than 3% of the total free DFP dose to these tissues. It is possible that the apparent failure of the model to accurately track the free DFP concentration at later time points may be due to the measurement of [3H] activity as a surrogate for the actual concentration of free DFP. Some [3H] activity inferred to be free DFP may, in fact, have been a DFP metabolite or products of covalent binding.

Martin (34) also measured AChE inhibition in mice after iv injection of DFP. The nadir of AChE activity occurred within 1 min in plasma and at about 5 min in brain (Figures 4a,5a). After optimization of AChE resynthesis, PBPK model simulations of these data were reasonably accurate from the time of injection through 24 hr (Figures 4b,5b). The PBPK model was initially structured so that plasma and brain tissue AChE activities would return to 100% of control levels in about 3.75 days. Martin (34) found, however, that 7 days after a single iv injection of 1 mg DFP/kg, brain AChE activity in the mouse was only about 80% of the preexposure level (Figure 5c).

Acetylcholinesterase Inhibition in Rat Plasma and Brain after Repeated Dosing with Diisopropylfluorophosphate

Michalek (24) injected male Wistar rats sc with DFP every second day for 22 days. The first dose was 1.1 mg DFP/kg, and subsequent doses were 0.7 mg/kg. Five rats (four DFP and one vehicle exposed) were sacrificed for measurement of brain AChE activity at 1.5 and 24 hr after the 1st, 2nd, 4th, 6th, 9th, and 12th DFP doses and at various intervals (48 and 72 hr; days 7, 14, 28, and 35) after the last dose. Traina and Serpieri (25) measured the effect of DFP on plasma AChE activity using the same rat strain and dosing regimen as Michalek (24).

Traina and Serpieri (25) found that plasma AChE activity in the rat fell rapidly after sc injection of DFP (Figure 6a). AChE activity was about 15% of control 1.5 hr after the initial dosing and about 70% of control at 24 hr. Simulations of these data (Figure 6a) were obtained after optimization of the rate of absorption of DFP into blood following a single sc injection. Simulation of the initial fall in plasma AChE activity was particularly sensitive to this parameter. The rate of AChE synthesis and first-order loss were also visually optimized against these data. This was critical to successful simulation of the return of AChE activity towards control levels obtained after a single sc injection. Once obtained in this manner the model was used for simulation of the multiple dosing scenario (Figure 6b).

Plasma AChE activities measured (25) after the last subcutaneous dose of DFP (day 22) are suggestive of an overshoot of the control level. Plasma AChE activity...
had returned to a stable 100% of the control level by 5 to 6 days after the last DFP dose (Figure 6b). There was no provision in the PBPK model for simulation of such an overshoot, though this phenomenon has been modeled for other systems, as in the case of glutathione replacement after depletion by toxicant exposure (35,36).

The pattern of brain AChE activity after subcutaneous DFP dosing (24) was similar to that seen in plasma (25), though the extent of recovery between doses was smaller (Figure 7a,b). The recovery of brain AChE activity after termination of DFP dosing was prolonged. For example, activity was only about 65% of control 28 days after the last dose of DFP. When the basal rates of AChE degradation and synthesis were optimized for acceptable simulation of brain AChE activity during dosing (Figure 7a), simulation of AChE activity after termination of dosing predicted a more rapid return than was observed (Figure 7b). Model structure dictated a relatively rapid and monophasic return of brain AChE activity to the original control level. The data suggest, however, that a full return of brain AChE activity to the control level is a multiphasic process.

**Acetylcholinesterase Inhibition in Humans**

Simulations of both the acute and repeated DFP exposures provide predictions of AChE and BChE inhibition that are very near the experimentally determined human values. The time-course of AChE and BChE inhibition after an acute dose of 33 µg DFP/kg bw resulted in a maximum AChE inhibition at 24 hr and maximum BChE inhibition at 4 hr (Figure 8a). This difference in the degree and time to maximum enzyme inhibition is a direct reflection of the 20-fold difference in the bimolecular inhibition rate constants for the two enzymes. The simulation provided a good prediction of AChE inhibition for all the acute time-course inhibition data. Simulations of red blood cell (RBC)–AChE and plasma BChE inhibition in four people injected at four different doses of DFP covering 10 to 54 µg DFP/kg bw compared favorably to the actual enzyme activity measured in these individuals at 24 hr (Figure 8b). The greatest discrepancy between the simulations and data occurred for the RBC–AChE at 54 µg/kg dose and the 10 µg/kg plasma BChE values (Figure 8b).

The model predictions of AChE and BChE inhibition resulting from repeated im injections of 20 µg DFP/kg bw were representative of the overall trend of enzyme inhibition (Figure 8c). During the first 5 days of repeated dosing, the actual AChE activity dropped rapidly from 100% of baseline to approximately 50% activity and then at a much slower rate to a final enzyme activity of 30%. The simulation of these data predicted an inhibition of approximately 40% during the initial 5 days of dosing that was sustained throughout the rest of the exposure period. BChE activity dropped immediately during the first 5 days to 15% of the basal enzyme activity and remained at this level during the exposure period (Figure 8c). The simulation predicted a 10 to 20% greater initial drop in enzyme activity than was actually measured.

**Parathion and Paraaxon Kinetics in the Rat**

Eigenberg (37) measured concentrations of PA and PO in brain, liver, and blood, as well as PA in fat, following an iv injection of PA at a dose of 3 mg/kg. Simulation of PA and PO kinetics in brain, liver, and blood after iv injection of 3.0 mg PA/kg were in general agreement with the published data (Figures 9,10). The simulation of PA kinetics in fat tissue (Figure 11) required the addition of diffusion limitation to this compartment to achieve agreement with the experimental data.

**Discussion**

The Michaelis–Menten parameters used to describe DFP hydrolysis and the bimolecular inhibition rate constants describing BEST inhibition were obtained in vitro. The success of simulations of in vivo DFP pharmacokinetics data (Figure 3a,b) suggests that the parameter values measured in vitro are reasonable estimates of the corresponding in vivo values. In vitro estimation of in vivo parameter values was also used by Dedrick (3). They developed a PBPK model for arabinofuranosylycysteine (ARA-C) and
its metabolite, arabinofuranosyluracil in four species and used rates of ARA-C metabolism measured in vitro. However, Reitz (38) found significant in vitro and in vivo differences in the rates of methylene chloride metabolism. Clearly, this problem must be considered on a case-by-case basis. Good agreement between in vitro and in vivo metabolic rates might be expected when the enzyme is soluble and its activity is not directly dependent on cofactor concentration (e.g., phosphorylphosphatas) (17). In such cases it should be relatively easy to create an in vitro milieu functionally similar to the in vivo milieu. On the other hand, some enzymes are embedded in cellular membranes, have specific orientations with respect to other enzymes that pre-process substrates, and have activities highly dependent on cofactor concentrations (e.g., cytochrome P450) (39). Activities of the latter type may be greatly affected by tissue homogenization and dilution in buffer. The results obtained in this study and in the studies of Dedrick (3) and Reitz (38) are consistent with this expectation.

![Graph 1](https://via.placeholder.com/150)

**Figure 8.** (a) Time-course of red blood cell acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activities in a human after an im injection with 33 μg diisopropylfluorophosphate (DFP)/kg. (b) Twenty-four hour values for red blood cell AChE and plasma BChE activities in four humans after an im injection with from 10 to 54 μg DFP/kg. (c) Average values for the time-course of red blood cell AChE and plasma BChE activities in 35 human subjects after daily im injections with an average of 20 μg DFP/kg/day. (d) Predicted inhibition of human brain AChE activity after an acute im dose of 20 μg DFP/kg/day. Data for a, b, and c are expressed as a fraction of control activity. Solid lines depict computer simulation. Data from Grob et al. (47).

The rate of AChE inhibition is a function of the bimolecular rate constant, $k_i$. The $k_i$ for AChE was determined in rat brain homogenate and this value was then used in all model compartments having this enzyme activity. The rat $k_i$ for AChE was also used for mice. This approach was also used for the DFP-esterase inhibition rate constants for CaE and BChE (see section on model development). The ability of the PBPK model to simulate AChE inhibition in mice and rats (Figures 6-7) indicates that the variance in these rate constants among tissues and species is not large. Andersen (40) found the rates of inhibition for solubilized AChE from the cerebral cortex of mice was within 20% of the rate for rats.

One goal of this research effort was to predict the kinetics and AChE inhibition not only after an acute exposure to DFP, but also after repeated doses. A good simulation of the experimental data for plasma and brain AChE inhibition in rats during repeated subcutaneous administration of DFP was achieved (Figures 6, 7). The experimentally measured return of brain AChE activity was much slower than the rate of enzyme resynthesis that was used in the model. If the brain AChE resynthesis rate was decreased in the model to agree with the rate of experimentally determined postexposure synthesis, then predictions of brain AChE activity during DFP dosing would be underestimated.

The experimentally determined activity of AChE in brain after each DFP dose was reproducible, yet the levels of AChE at the 48- and 72-hr time points at the end of all repeated dosing indicated there was an alteration in the mechanisms controlling enzyme synthesis. For the model to predict the return of brain AChE to normal levels of activity after repeated dosing, it would be necessary to address the effects of down regulation of AChE receptors on AChE enzyme synthesis rates. The simulation of AChE resynthesis used the preexposed level of AChE activity as a reference in setting the rate of resynthesis. This assumed that the

![Graph 2](https://via.placeholder.com/150)

**Figure 9.** Simulated concentration of diisopropylfluorophosphate in human brain (mg/l) after a 5-min inhalation at 50 ppm. The level of plasma A for esterates for curve A was 1.5 times the scaled activity; for curve B it was the scaled level of activity; and for C, it was 50% of the scaled activity.

![Graph 3](https://via.placeholder.com/150)

**Figure 10.** Time-course of parahex in blood, liver, and brain in (μg/ml) for 8 hr after an iv injection of parahex at a dose of 3 mg/kg. Each data point represents the mean of four rats. Data from Eigenberg et al. (37).
receptor affinity and impetus to return AChE to control tissue levels was unaltered. If the receptor numbers and affinities are affected by repeated dosing with DFP, it would alter the rates of AChE synthesis experimentally, which would require a change in the model parameter controlling AChE synthesis. This effect is believed to be a response to excessive tissue concentrations of acetylcholine after AChE inhibition (41).

Clarification of the mechanism of this tolerance has involved the measurement of receptor binding and numbers. Binding of quinuclidinyl benzilate, a muscarinic affinity label, has been shown to decrease in affinity and density in the striatum of rats following chronic cholinesterase inhibition with DFP (42). Yamada (43) was able to demonstrate a correlation between and a dose-dependent decrease in muscarinic receptors, AChE activity, and choline uptake in regions of the brain and gastrointestinal tract of guinea pigs treated repeatedly with DFP. This effect was antagonized by phystostigmine and atropine.

Simulations of the 24-hr recovery of brain AChE after repeated DFP dosing were well within one standard deviation of the data during the series of DFP doses and only failed to predict brain AChE levels after dosing had ceased. This could be due to the experimental protocol chosen by Michalek (24), where 48 hr were allowed between each successive dose of DFP. Ehler (42) has shown the half-time for the loss of muscarinic binding sites to be approximately 1.6 days. The difference between the frequency of DFP dosing Michalek (24) used (i.e., which we have simulated) and the rate of change in receptor numbers and affinity may have been lessened by the repeated dosing until after the completion of dosing.

While there was a good prediction of DFP pharmacokinetics shortly after injection that accounted for greater than 95% of the material injected, the simulation and data became more divergent at longer times after dosing. After the initial injection of DFP, the major model parameter affecting the disappearance of compound from the animal is AEST hydrolysis activity. It would be expected that enzymatic hydrolysis of DFP would continue to decrease blood and tissue concentrations until there is total disappearance of the compound. The experimental data showed no difference in plasma DFP concentrations between 15 and 30 min after injection and only a decrease in DFP concentration of 32% in brain. Those BEST enzymes with the highest affinity for DFP already would have become inhibited at this time and their low molar concentration then would have very little effect on the overall kinetics of DFP. Because the model and data both predict the amount of DFP present in blood and brain to be in the picogram per milligram range within minutes of injection, it is possible that the difference between the data and simulations are due to inexact compound identification involved in using the combination of radiolabel and chemical extraction to quantitate very low concentrations of DFP.

The PBPK model initially developed to describe the kinetics of DFP and inhibition of AChE in the rodent was successfully scaled to simulate data from humans repeatedly treated therapeutically with DFP. It was possible to predict the time course of inhibition of RBC AChE and plasma BChE in a human male human injected intramuscularly with a 33 μg/kg dose of DFP and these enzyme activities at 24 hr in four different humans injected with DFP at doses from 10 to 54 μg/kg. By using the data from human studies to validate the model, it is possible to simulate the amount of AChE inhibition in other target organs for which there are no human data (Figure 12). This is significant, because it provides a means of predicting OP effects in the target organs of humans for which there will probably never be data. The development of this model to predict the human response to organophosphate exposure provides a method of modeling possible therapeutic or prophylactic approaches for organophosphate exposure in humans.

The model was exercised for a hypothetical inhalation exposure of humans to 50 ppm DFP for 5 min. Because CaE has been shown to be an important detoxification route for soman (20), a simulation of human exposure to DFP was conducted with the activity of CaE decreased by a factor of 10, which is the activity expected in humans (44). The effect of this alteration of CaE levels on the kinetics and inhibition of AChE was negligible due to the much lower affinity of CaE for DFP relative to the other enzymes present (Table 1). The parameter that did have an effect on DFP blood and brain kinetics was AEST activity in the blood. The levels of AEST in blood were decreased by one-half and increased by one-half over the amount that was scaled by (body weight)0.7 from rats to humans. A 50% decrease in AEST activity in blood caused a 16% increase in the peak concentration of DFP in brain. In contrast, increasing DFP AEST activity by 50% caused an 9.5% decrease of DFP in blood and a 13% decrease in brain.

The PBPK model for DFP was successfully modified to describe the time-course of parathion and paraaxon in blood, liver, and brain, and the time-course for parathion in fat. A preliminary validation of the model was conducted with data obtained from the literature and collected in laboratory studies. It was possible to provide a reasonably good simulation of in vivo data obtained from the literature, but this required adjustment of enzymatic values obtained in the literature. With further validation of the model, it will be possible to predict the inhibition of AChE in the different tissue compartments of the target organs and use this model to provide predictions of the degree of AChE inhibition in occupational situations where humans may be exposed. This will then provide a basis for using the model to perform risk assessments of different exposure scenarios.

The PBPK model for OPs is in effect a quantitative hypothesis specifying the factors controlling OP pharmacokinetics in
rats, mice, and humans. Once its parameters have been set to realistic values, the
ability of the model to simulate real data becomes a test of the hypothesis. Failure to
accurately simulate data suggests that model structure, the hypothesis, needs refinement.
Iterations of this process (i.e., model refinement, predictive simulation, laboratory
experimentation) can be exploited to advance the understanding of the biological
determinates of OP biodistribution. This general paradigm has recently been discussed
by Clewell and Andersen (45).

The present model for OPs does not simulate toxicity per se. Instead, AChE
inhibition is simulated as an index of the likelihood of toxic effect (2). The acute
toxicities of some OP agents may not be due to acetycholine overload but rather to
actions at sites other than AChE (46). In these cases, inhibition of the concentration of
free OP in target tissues would be expected to provide an index correlated with
toxicity. For OPs whose primary mechanism of acute toxicity is not AChE
inhibition, it would be desirable to extend the current PBPK model to explicitly
describe the OP–tissue interaction most directly correlated with toxicity.

PBPK models are useful in risk assessment because their structure is amenable to
cross-species scaling or to simulate exposure scenarios that cannot be tested otherwise. A
PBPK model validated for experimental animals and appropriately scaled to humans is
teoretically capable of simulating pharmacokinetic behavior in humans. This
assumes that the model structure appropriate for the experimental species is also
appropriate for humans. Physiological parameters such as organ volumes, organ blood
flows, and pulmonary ventilation rates are well characterized for humans as well as for
common experimental animals. Scaling of toxicant-specific parameters can be more
problematical. Some success in scaling of PBPK models has been achieved by assuming
that metabolic rates (e.g., $V_{max}$, scale in proportion to body surface area (8)). With
respect to the DFP model, it is not clear a priori how the DFP–esterase bimolecular
inhibition rate constants should scale from rodents to humans. The ability demonstrated
in rats and mice to successfully use in vitro estimates of parameter values for
simulation of in vivo data suggested the same approach would work in the human
version of the model. Moreover, the similarity of inhibition rates for solubilized
AChE from the cerebral cortex of mice and rats (40) implies that some parameter values
may be relatively insensitive to large changes in body weight.

In summary, the model structure used in the present study is clearly relevant to humans for a variety of OPs (2) and the data required for scaling is explicitly specified by this structure. Application of the present model to other OPs of concern
for human exposure may, therefore, be a relatively straightforward task.

Appendix I: Conversion of In Vitro Measurements for In Vivo Modeling

The two calculations shown here provide an example of the dimensional analysis to
convert in vitro DFPase values of $V_{max}$ and $K_m$ for extrapolation to in vivo modeling.
The values in Table 3 for $V_{max}$ are reported in milligrams per hour. These values were
determined in vitro in grams per minute per gram of tissue. The in vitro value for $K_m$
was determined in nanomoles per liter. An example of converting in vitro values to in
vitro values for the liver is, for $V_{max}$, 9.02 mole DFP/min/g liver x 60 min/hr 40 g
liver/kg bw x 0.184 g/umole x mg/10 g = 3983 mg/hr x kg and, for $K_m$, 1.29
mmoles/l x 184 mg/mmol = 237.36 mg/l.

Appendix II: Description of Pharmacokinetic Model

The mass balance differential equation for DFP in brain is $V_{Br} \times dC_{Br}/dt = Q_{Br} \times$ 
(CABr – CVBr) – ($V_{max}^{Br} \times CVBr$) / ($K_{m}^{Br} + CVBr$) – KAEBr x CAEBR x CBr – 
CAE x CCEBr x CBr – KBChE x CBEBr x CBr, where $V_{Br}$ = volume of brain (liter);
$C_{Br}$ = concentration of DFP in the brain compartment (mg/l); $Q_{Br}$ = blood flow to
the brain (l/h); $CABr$ = DFP in arterial brain (mg/l); $CVBr$ = DFP in venous blood
leaving brain (mg/l); $V_{max}^{Br}$ = maximum rate of DFPase hydrolysis (mg/hr); $K_{m}^{Br}$ =
Michaelis-Menten constant for DFPase in brain (mg/l); KACE = bimolecular rate
constant for DFP reaction with AChE (M-hr)$^{-1}$; CAEBr = AChE concentration in
brain (M); KAEC = bimolecular rate constant for DFP reaction with CaE (M-hr)$^{-1}$;
CCEBr = CaE concentration in brain (M); KBChE = bimolecular rate constant for
DFP reaction with ChE (M-hr)$^{-1}$; and CBEBr = ChE concentration in brain (M).
This equation is also used to describe DFP in the liver, kidney, rapidly perfused, as well
as venous and arterial tissue compartments. In the remaining compartments (lungs, fat,
slowly perfused, and diaphragm), the differential equation for DFP is $VT \times dC_{T}/dt =$
$Q_{T} \times (CAB - CT_{PT})$, where $VT$ = volume of tissue (liter); $dC_{T}/dt$ = change in tissue
concentration with time (mg/hr); $Q_{T}$ = blood flow to tissue (l/h); $CAB$ = arterial
blood concentration (mg/l); $CT$ = tissue concentration (mg/l); and $PT$ = tissue-to-
blood partition coefficient.

The differential equation to calculate inhibited AChE activity in the different
tissue compartments is $V_{Br} \times dAEBR/\mathrm{dt}$ = 
(KACE x CAEBR x CBRM) – (KABR x AEBR) - (KAABR x AEBR), where $V_{Br}$ =
volume of brain (l); KACE = bimolecular inhibition rate constant of AChE (M-hr)$^{-1}$;
CAEBR = free AChE in brain (M); CBRM = DFP in brain (M); KABR = rate of
regeneration of inhibited AChE (hr$^{-1}$); AEBR = inhibited AChE (M); and KAABR
= rate of aging of inhibited AChE (hr$^{-1}$).

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