CHAPTER

46

Organophosphate Pharmacokinetics

Charles Timchalk
Pacific Northwest National Laboratory

46.1 BACKGROUND

In this chapter, an overview will be presented of the pharmacokinetic principles that are of major importance in understanding the toxicology of organophosphate (OP) insecticides in animals and humans. The approach will not entail a comprehensive review of the extensive literature, but rather a focused presentation highlighting important principles by utilizing specific examples for this class of insecticide.

Organophosphates constitute a large family of insecticides that are structurally related, pentavalent phosphorus acid esters. Their insecticidal as well as toxicological mode of action is primarily associated with their ability to target and inhibit the enzyme acetylcholinesterase (AChE) (Sultatos, 1994). In this regard, the acute toxic effects of OP insecticides are associated with the capacity of the parent chemical or an active metabolite to inhibit AChE enzyme activity within nerve tissue (Murphy, 1986; Sultatos, 1994). The three major classes of OP insecticides are the phosphorothionates, the phosphorodithioates, and the phosphoroamidothiolates (Chambers, 1992; Mileson *et al.*, 1988). As an example, phosphorothionate insecticides such as chlorpyrifos, parathion, and diazinon are weak inhibitors of AChE, but once they undergo metabolic activation (desulfuration) to their corresponding oxygen analogs (oxon), they become extremely potent inhibitors. This enhanced toxicity is due to the oxon having a high affinity and potency for phosphorylating the serine hydroxyl group within the active site of AChE (Mileson *et al.*, 1988; Sultatos, 1994). The toxic potency is dependent on the balance between a delivered dose to the target site and the rates of bioactivation and/or detoxification as illustrated in Fig. 46.1 (Calabrese, 1991). The pharmacokinetics and biochemical interactions between OPs and AChE and the toxicological implications of AChE inhibition are well understood. To further illustrate this point, a diagram relating OP toxicity with pharmacokinetic disposition and the formation of key OP metabolites is presented in Figs. 46.2 and 46.3. The thionophosphate pesticide diazinon [*O,O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] is being utilized for illustration purposes; however, based on a common mode of action, this scheme is readily extended to other OPs.

Organophosphate insecticides, like all chemical contaminants, can gain entry into the body and, based on the detection of low levels of OP metabolites in urine within human populations, there is good evidence for widespread although low level, exposures (Aprea *et al.*, 1999; Hill *et al.*, 1995). These exposures can come from numerous sources. For example, ingestion of pesticide residues on foods may account for some of the low-level body burdens detected, whereas accidental or intentional ingestion of OP insecticides is associated with acute poisoning, resulting in significantly higher blood, tissue, and urine concentrations of relevant OP metabolites (Drevenkar *et al.*, 1993). Dermal exposure represents a potential exposure route during the mixing, loading, and application of OP insecticides or from skin contact with contaminated surfaces (Knaak *et al.*, 1993). Likewise, inhalation of airborne insecticide is feasible either during an application or as the result of exposure associated with chemical drift (Vale and Scott, 1974). Once the OP arrives at a portal of entry, it is available for absorption and, based on the bioavailability of a given OP and the exposure route, a systemic dose of the parent compound (Fig. 46.3, #1) will enter the systemic circulation. Although localized portal of entry metabolism (i.e., lung, intestines, skin) is feasible, the bulk of the metabolic activation as well as detoxification reactions occur within the liver (Sultatos, 1988; Sultatos *et al.*, 1984). As previously mentioned, phosphorothionates like diazinon do not directly inhibit AChE, but must first be metabolized to the corresponding oxygen analog (oxon; Fig. 46.2, #2) (Iverson *et al.*, 1975; Mücke *et al.*, 1970; Murphy, 1986; Sultatos, 1994). Activation to the oxon metabolite (#2) is mediated by cytochrome P450 mixed-function oxidases (CYP450) primarily within the liver, although extrahepatic metabolism has been reported in other tissues, including the brain (Chambers and Chambers, 1989; Guengerich, 1977). In addition, oxidative dearylation of the parent compound, forming both 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP, #3) and diethylthiophosphate (DETP, #4), represents a competing detoxification pathway that is likewise mediated by hepatic CYP450 (Ma and Chambers, 1994). These initial activation/detoxification reactions are believed to share a common phosphooxythiran intermediate and represent the critical biotransformation steps required for toxicity (Neal, 1980). Differences in the ratio

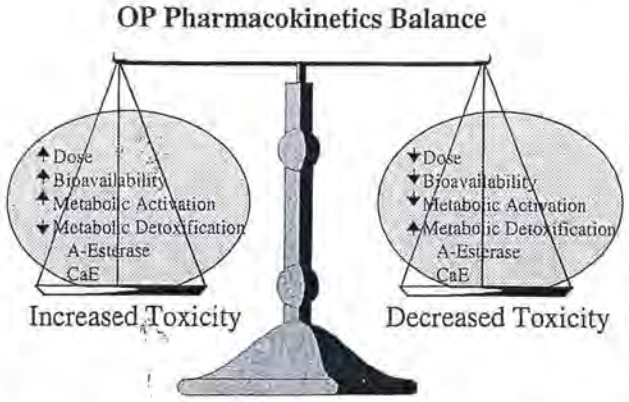


Figure 46.1 Parameters impacting the organophosphate (OP) insecticide toxicity balance.

of activation to detoxification are associated with chemical-, species-, gender-, and age-dependent sensitivity to OPs (see Fig. 46.1) (Ma and Chambers, 1994). Hepatic and extrahepatic (i.e., blood and tissue) A-esterase can effectively metabolize the oxon metabolite (#2), forming IMHP (#3) and diethylphosphate (DEP, #4) metabolites. Likewise, B-esterases such as carboxylesterase (CaE) and butyrylcholinesterase (BChE) that are also well distributed across tissues can metabolize the oxon; however, these B-esterases become irreversibly bound (1:1 ratio) to the oxon and thereby become inactivated (Chanda *et al.*, 1997; Clement, 1984). It is likewise clear from both tissue distribution and partitioning studies that phosphothionate OPs are generally well distributed in tissue throughout the body (Tomokuni *et al.*, 1985; Wu *et al.*, 1996). Finally, due to the extensive metabolism, little, if any, parent phosphothionate or oxon is available for excretion; however, more stable metabolites such as DEP, DETP, and IMHP are readily excreted in the urine (Iverson *et al.*, 1975; Mücke *et al.*, 1970).

Numerous pharmacokinetic approaches have been applied to OP insecticides, including:

1. Application of pharmacokinetics to understand the overall disposition and clearance of OPs
2. Development and application of pharmacokinetic models for quantitative biological monitoring to assess OP insecticide exposure in humans
3. Studies that facilitate extrapolation of dosimetry and biological response from animals to humans and the assessment of human health risk

To illustrate the utility of pharmacokinetics in addressing the health concerns associated with OP insecticides, several examples of these types of pharmacokinetic studies with OP insecticides will be used to illustrate both their utility and their limitations.

46.2 PHARMACOKINETIC PRINCIPLES OF IMPORTANCE TO ORGANOPHOSPHATE INSECTICIDES

Pharmacokinetics are concerned with the quantitative integration of those processes associated with the absorption, distribution, metabolism, and excretion (ADME) of drugs and xenobiotics within the body (Renwick, 1994). Studies on the pharmacokinetics of a xenobiotic provide critically useful insights into the toxicological response associated with a given agent. In this regard, pharmacokinetics provides quantitative data on the amount of toxicant delivered to a target site as well as species-, age-, and gender-specific and dose-dependent differences in biological response. An important application of pharmacokinetics within toxicology has been to provide a realistic estimate of risk by providing a means to quantitatively estimate the absorbed dose of a chemical under realistic exposure conditions (Clewell, 1995).

Toxicology studies are designed to provide a quantitative assessment of toxicity based on what the chemical agent does to the test animals. In contrast, pharmacokinetics focuses on what the animal does to the chemical. Clearly, toxicity and pharmacokinetics are integrally related because the extent of absorption, retention, metabolic activation, or detoxification is ultimately responsible for delivering a dose to a target tissue, resulting in observed effects. Pharmacokinetics represent a critically important tool that, if used correctly, can quantitatively establish a unifying model that describes both dosimetry and biological response across exposure routes, species, and chemical agents. This approach is particularly useful for OP insecticides because they share a common mode of action through their capability to inhibit AChE activity (Mileson *et al.*, 1988). Pharmacokinetic strategies for quantitating dosimetry can be developed to measure the parent compound and its active (i.e., oxon) or inactive metabolites. It is also feasible to link dosimetry with biologically based pharmacodynamic (PD) response models based on a common mode of action (i.e., AChE inhibition). In general, pharmacokinetic modeling approaches can be characterized as empirical or physiologically based and both types of models have been applied to understand the toxicological response to OP chemicals in multiple species (Brimer *et al.*, 1994; Gearhart *et al.*, 1990; Pena-Egido *et al.*, 1988; Sultatos, 1990; Tomokuni *et al.*, 1985; Wu *et al.*, 1996).

46.2.1 COMPARTMENTAL PHARMACOKINETIC MODELS

Compartmental models have formed the cornerstone of pharmacokinetic analysis and as such have been extensively utilized to assess bioavailability, tissue burden, and elimination kinetics in various species, including humans. All pharmacokinetics are concerned with the time course by which a chemical is absorbed into the systemic circulation, distributed throughout the body, altered through metabolic transformation, and eliminated. Compartmental models are empirical and as such

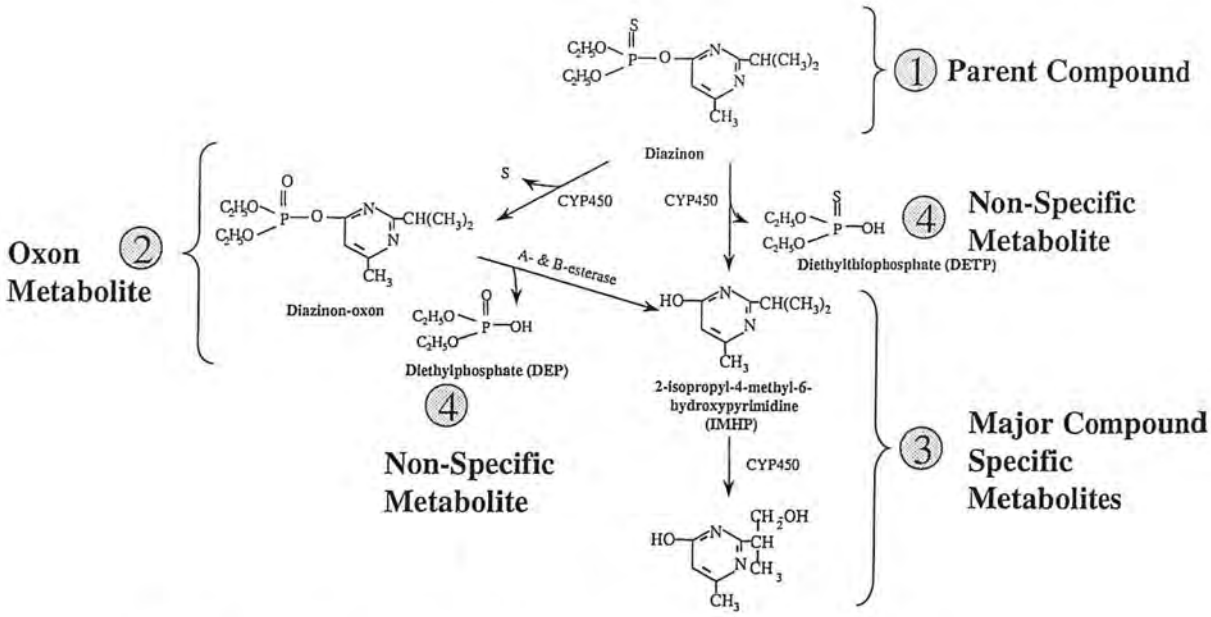


Figure 46.2 Metabolic scheme for the metabolism of the organophosphate (OP) insecticide diazinon.

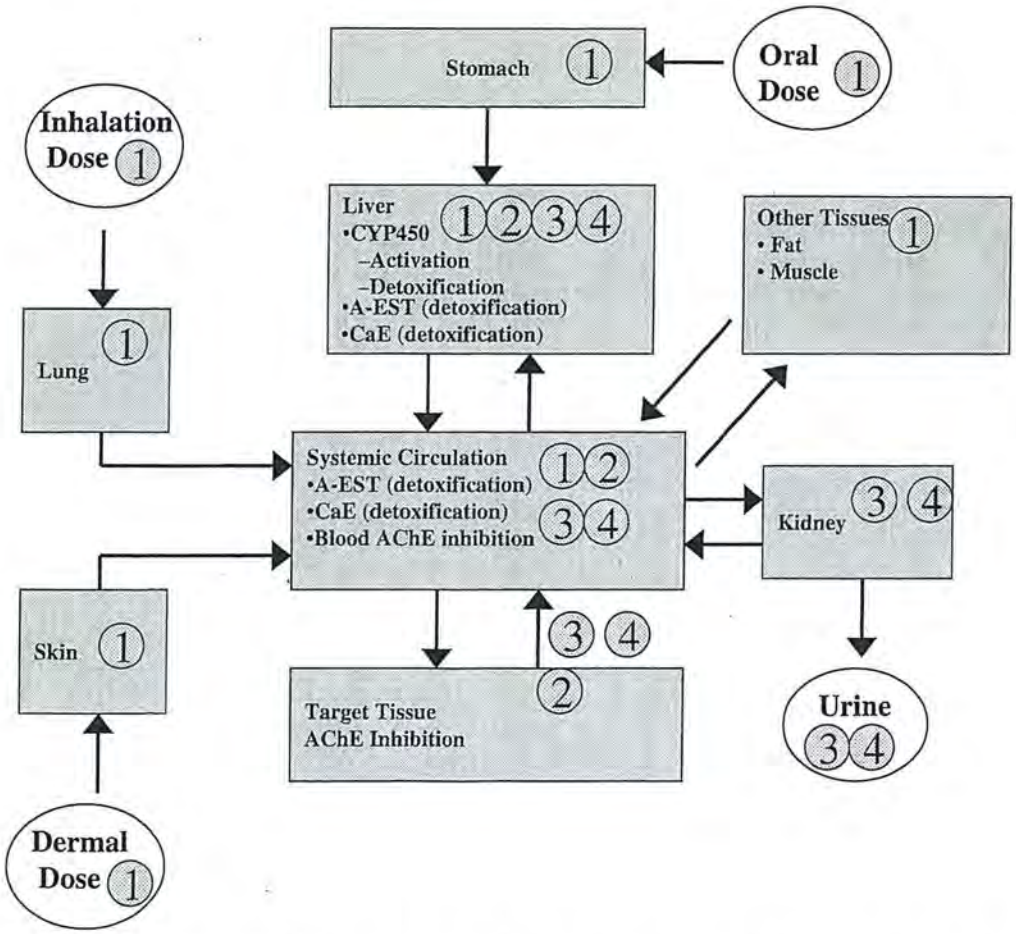
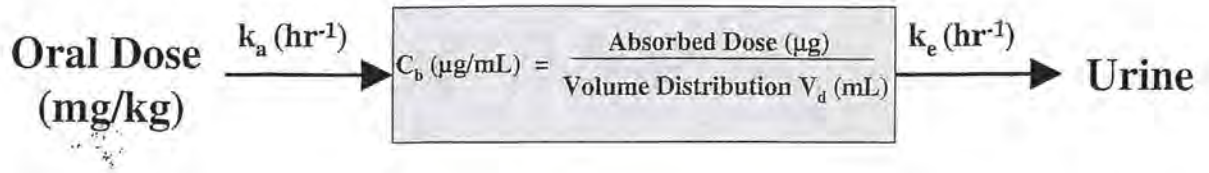


Figure 46.3 Compartmental flow diagram illustrating the critical tissue compartments associated with absorption, distribution, metabolism and excretion of organophosphate (OP) insecticides. The circled numbers (1–4) correspond to the parent compound and major metabolic products associated with metabolism of diazinon (see Fig. 46.2) that are most likely found within each compartment.



$$C_b\ (\mu g/mL) = \frac{K_a \times dose \times F}{V_d \times (k_a - k_e)} \times \exp(k_e \times time - k_a \times time) \quad (1)$$

$$\text{Urinary Excretion Rate } (\mu g/hr) = C_b \times k_e \times V_d \times \text{Body wt.} \quad (2)$$

Figure 46.4 Single compartment model used to describe the blood and urine time-course of 3,5,6-trichloropyridinol (TCP) a major metabolite of the organophosphate (OP) insecticide chlorpyrifos (CPF). Equations adapted from Nolan *et al.* (1984).

consider the organism as a single- or multicompartment homogeneous system. The number and behavior of the compartments are primarily determined by the equations chosen to describe the time-course data and not the physiological characteristics of the organism (Krishnan and Andersen, 1994). In these models, the net transfer between compartments is directly proportional to the difference in chemical concentration between compartments. However, the rate constants associated with the transfer between compartments cannot be experimentally determined (Srinivasan *et al.*, 1994).

Compartmental models range from simple well-mixed single-compartment models to more complicated multicompartment models that are used to describe the blood and/or plasma time course of a chemical or drug. These simple compartmental approaches have been broadly utilized to model the pharmacokinetics of OP insecticides and their major metabolites (Braeckman *et al.*, 1983; Drevenkar *et al.*, 1993; Nolan *et al.*, 1984; Wu *et al.*, 1996). For example, Nolan *et al.* (1984) developed a one-compartment pharmacokinetic model that accurately describes the blood and urine time course of 3,5,6-trichloropyridinol (TCP), a major metabolite of the OP insecticide chlorpyrifos, in human volunteers. A diagram of this single-compartment model is illustrated in Fig. 46.4. In this model, the blood TCP concentration and urinary excretion data were simultaneously fit to a single-compartment model using the equations shown in the figure. Absorption (k_a) and elimination (k_e) are handled as first-order processes, the blood TCP concentration is represented by C_b, and F and V_d represent the fractional absorption and the volume of distribution, respectively. To develop this model, male volunteers were orally administered a dose of 0.5 mg chlorpyrifos/kg of body weight. Then blood and urine specimens were collected at specified intervals and analyzed for TCP. The model parameters used to describe the time course of TCP and the model fit of the experimental data are presented in Table 46.1 and Fig. 46.5. The model provides an excellent fit of the experimental data and, based on the model parameters, it was determined that approximately 72% of the ingested dose was absorbed and eliminated in the urine with a half-life of 27 h. Based on this model, Nolan *et al.* (1984) suggested that the blood TCP concentration and/or urinary excretion rate could be utilized to quantify the amount of chlorpyrifos absorbed under actual use conditions.

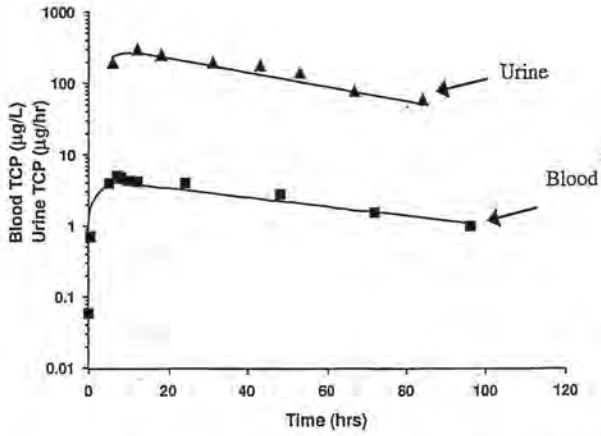


Figure 46.5 Time-course of 3,5,6-trichloropyridinol (TCP) in the blood and urine of male volunteers orally administered a 0.5 mg chlorpyrifos (CPF)/kg of body weight dose. Figure adapted from Nolan *et al.* (1984).

Although compartment modeling is extremely useful for interpolation within the confines of the test species and experimental conditions (i.e., exposure routes and dose levels), these

Table 46.1
Selected Model Parameters Describing Blood Concentrations and Urinary Excretion of 3,5,6-Trichloropyridinol by Individual Volunteers Following Oral Administration of the Organophosphate Insecticide Chlorpyrifos

Parameter	Body weight (kg)	Absorption lag time (h)	Absorption rate constant k_a (h^{-1})	Absorption half-life (h)	Volume distribution V_d (ml/kg)	Elimination rate constant (h^{-1})	Elimination half-life k_e (h)	Model predicted percentage dose absorbed	Percentage dose recovered in urine
Range	72–102	0.9–1.9	0.1–2.7	0.4–6.9	160–204	0.02–0.03	21–32	52–84	49–81
Mean ± SD	83.3 ± 10.3	1.3 ± 0.4	1.5 ± 1.2	0.5	181 ± 18	0.026 ± 0.005	26.9	72 ± 11	70 ± 11

Data obtained from six male volunteers.
Data adapted from Nolan *et al.* (1984).

models are limited in their capability to extrapolate across dose levels, species, and exposure routes (Krishnan and Andersen, 1994). To enable extrapolation, physiologically based pharmacokinetic (PBPK) models have emerged as an important tool that has seen broad applications in toxicology and more specifically in human health risk assessment (Andersen, 1995; Clewell and Andersen, 1996; Krishnan and Andersen, 1994; Leung and Paustenbach, 1995; Mason and Wilson, 1999).

46.2.2 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Unlike compartment modeling approaches, PBPK models utilize biologically meaningful compartments that represent individual organs such as liver and kidney or groups of organ systems (i.e., well perfused/poorly perfused) (Mason and Wilson, 1999). The general model structure is based on an understanding of comparative physiology and xenobiotic metabolism, a chemical's physical properties that define tissue partitioning, the rates of biochemical reactions determined from both *in vivo* and *in vitro* experimentation, and the physiological characteristics of the species of interest (Krishnan and Andersen, 1994). PBPK models have been developed to describe target tissue dosimetry for a broad range of environmental contaminants such as solvents, heavy metals, and pesticides, including OP insecticides (Andersen *et al.*, 1987a; Corley *et al.*, 1990; Gearhart *et al.*, 1990; O'Flaherty, 1995; Sultatos, 1990). A number of reviews have been published on the development, validation, application, and limitations of PBPK models in human health risk assessment (Andersen, 1995; Clewell, 1995; Clewell and Andersen, 1996; Frederick, 1995; Krishnan and Andersen, 1994; Leung and Paustenbach, 1995; Mason and Wilson, 1999; Slob *et al.*, 1997).

To illustrate the application of this modeling approach to OP insecticides, a PBPK model that also incorporates a pharmacodynamic (PD) component to describe AChE inhibition following diisopropylfluorophosphate exposure in rodents will be described (Gearhart *et al.*, 1990). Gearhart *et al.* (1990) developed a basic PBPK/PD model structure that described target tissue dosimetry and AChE inhibition following an acute exposure to diisopropylfluorophosphate in mice and rats. In developing this model, the authors were primarily interested in building a structure that could readily be extended to describe the acute effects for a broad range of commercially important OP insecticides. A diagram of the PBPK/PD model for diisopropylfluorophosphate in rats is illustrated in Figs. 46.6 and 46.7. The conceptual representation of the PBPK model for diisopropylfluorophosphate is based on the anatomical and physiological characteristics of the rat and the major determinants of diisopropylfluorophosphate disposition, which include esterase binding and hydrolysis, tissue partitioning, and diisopropylfluorophosphate volatility (Gearhart *et al.*, 1990; Krishnan and Andersen, 1994). Because this OP ester does not require metabolic activation, like thionophosphate OPs, the hydrolysis of diisopropylfluorophosphate by blood and tissue A-esterase is a major factor in determining the protection against AChE inhibition.

Diisopropylfluorophosphate binds to and inhibits B-esterases, including AChE, BChE, and CaE. Although binding to AChE is associated with acute neurotoxicity, the binding to BChE and CaE is without adverse physiological effect and as such represents a detoxification pathway (Clement, 1984; Fonnum *et al.*, 1985; Pond *et al.*, 1995). The PBPK/PD model compartments included those tissues associated with toxicological response (i.e., brain, lung, and diaphragm), those containing high A-esterase activity (i.e., liver, kidney, and blood), a fat compartment having the highest tissue/blood partitioning, and the remaining tissues being collectively lumped (Gearhart *et al.*, 1990). To develop this model, tissue partitioning coefficients (PCs) were determined by the vial equilibration technique (Gargas *et al.*, 1989; Sato and Nakajima, 1979). In general, the tissue : blood PCs ranged from 0.77 to 1.63; however, the fat : blood partitioning was the highest with a coefficient of 17.6. The generalized mass balance differential equation for calculating diisopropylfluorophosphate tissue concentration and AChE tissue inhibition are also presented in Figs. 46.6 and 46.7. Within each tissue compartment, the net concentration of diisopropylfluorophosphate (mg/l) is a function of blood flow to the tissue, chemical partitioning from the blood into the tissue, and the loss of diisopropylfluorophosphate due to hydrolysis by A-esterase and inhibition of B-esterases (AChE, BChE, and CaE).

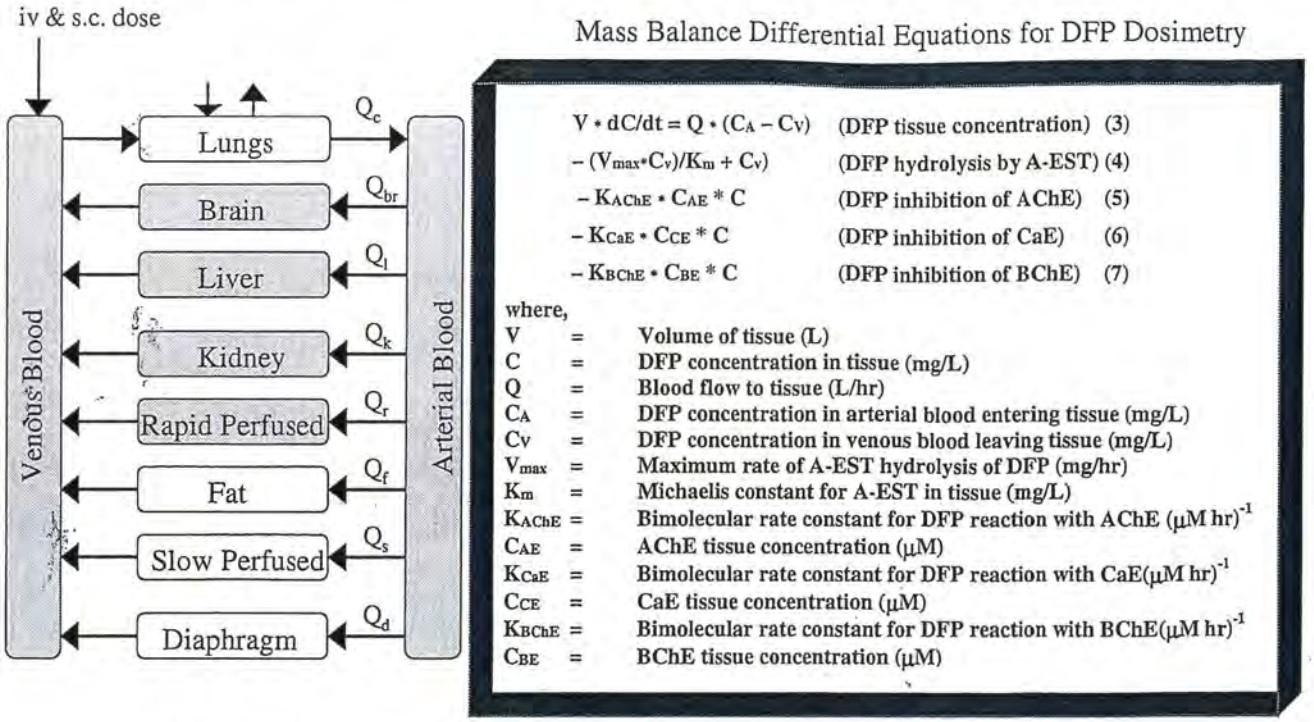


Figure 46.6 Physiologically based pharmacokinetic (PBPK) model structure and mass balance differential equations describing the distribution of diisopropylfluorophosphate (DFP) in the rat. Figure adapted from Gearhart *et al.* (1990).

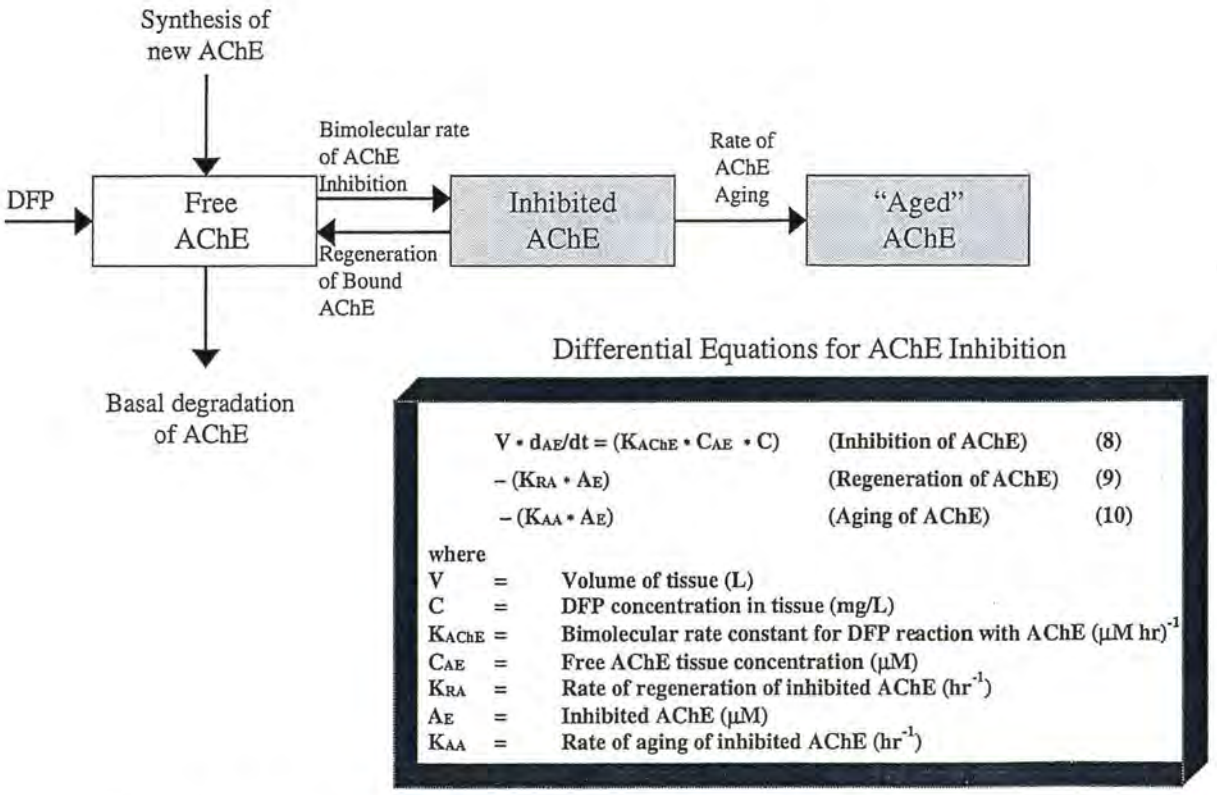


Figure 46.7 Pharmacodynamic (PD) model structure and mass balance differential equations describing the inhibition of acetylcholinesterase (AChE) by diisopropylfluorophosphate (DFP) in the rat. Figure adapted from Gearhart *et al.* (1990).

Gearhart *et al.* (1990) calculated basal AChE activity (μmol) based on a zero-order enzyme synthesis rate (μmol/h) and a first-order rate of enzyme degradation (h^{-1}). A balance between the bimolecular rate of inhibition and the rate of AChE regeneration and aging determined the amount of free AChE. Similar equations were utilized to quantify the impact of diisopropylfluorophosphate on tissue CaE and BChE activity. The capability of the diisopropylfluorophosphate model to simulate both diisopropylfluorophosphate tissue dosimetry and AChE inhibition is illustrated in Figs. 46.8 and 46.9 in mice that were administered a single intravenous (iv) dose of 1 mg diisopropylfluorophosphate/kg of body weight. The model does a reasonably good job of describing brain tissue dosimetry and AChE inhibition. In brain, the diisopropylfluorophosphate concentration rapidly falls to a fraction of its peak concentration within about 1 min, whereas AChE was rapidly inhibited to 20% of control activity. In both cases, the model simulations were consistent with the experimentally derived data.

The development and application of PBPK modeling for human health risk assessment are not without their challenges and limitations. Before a model can be used to assess risk, a determination must be made concerning the model's capability to accurately predict dosimetry and biological response (Frederick, 1995). Furthermore, PBPK/PD models are data intensive, so to adequately develop and validate a model generally requires extensive experimentation to support model parameterization and validation (Clewell, 1995). Nonetheless, a consensus opinion of an expert scientist panel concluded that biologically based risk assessments that include well-validated PBPK/PD models can provide the most accurate quantitative assessment of human health risk from exposure to environmental chemicals (Frederick, 1995).

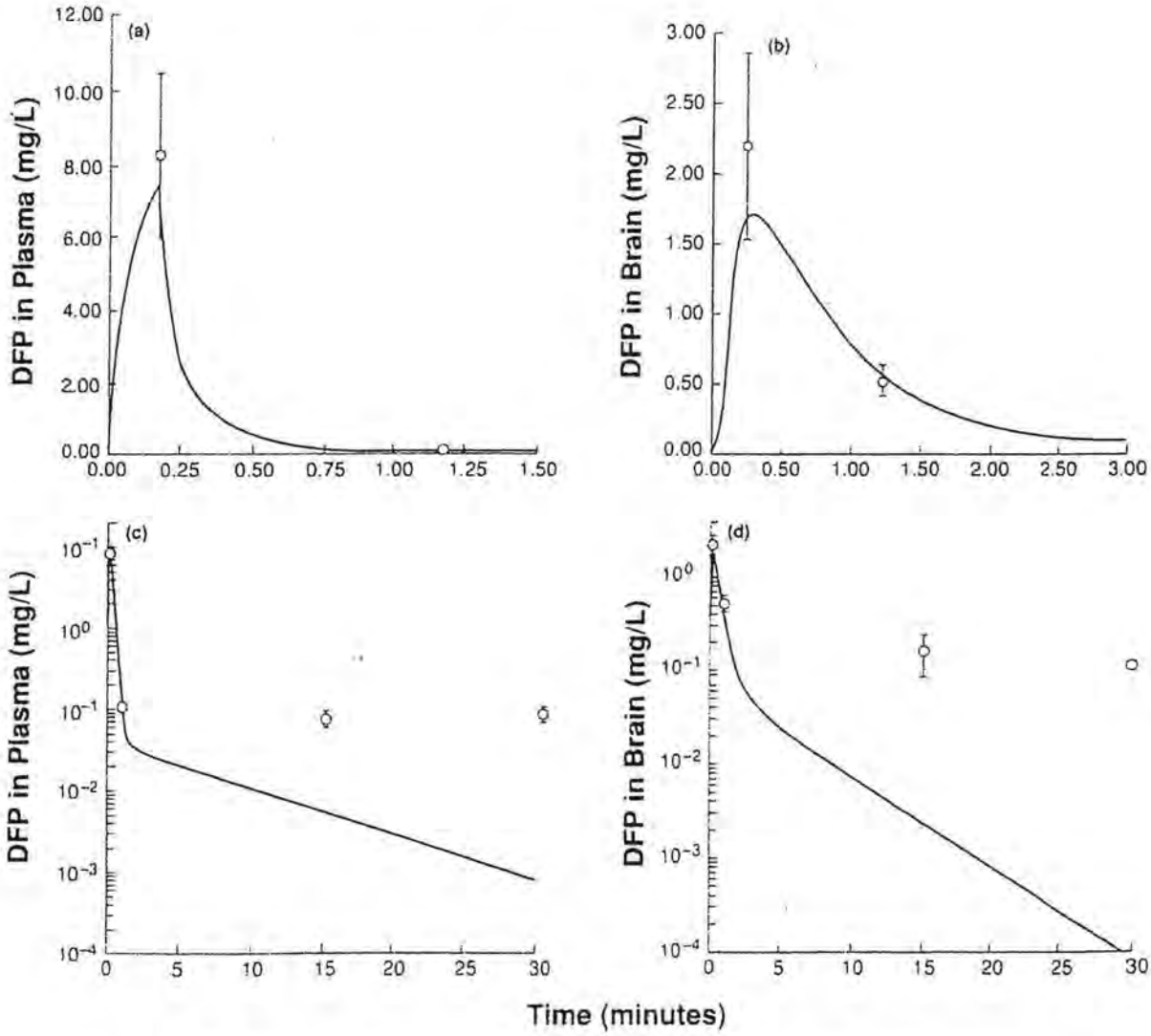


Figure 46.8 Time-course of free diisopropylfluorophosphate (DFP) concentration (mg/L) in plasma and brain in male mice after tail vein injection of 1 mg DFP/kg. Each datum represents the mean ± SD of 5 animals. Solid line depicts PBPK/PD model simulation. Figure used with permission from Gearhart *et al.* (1990).

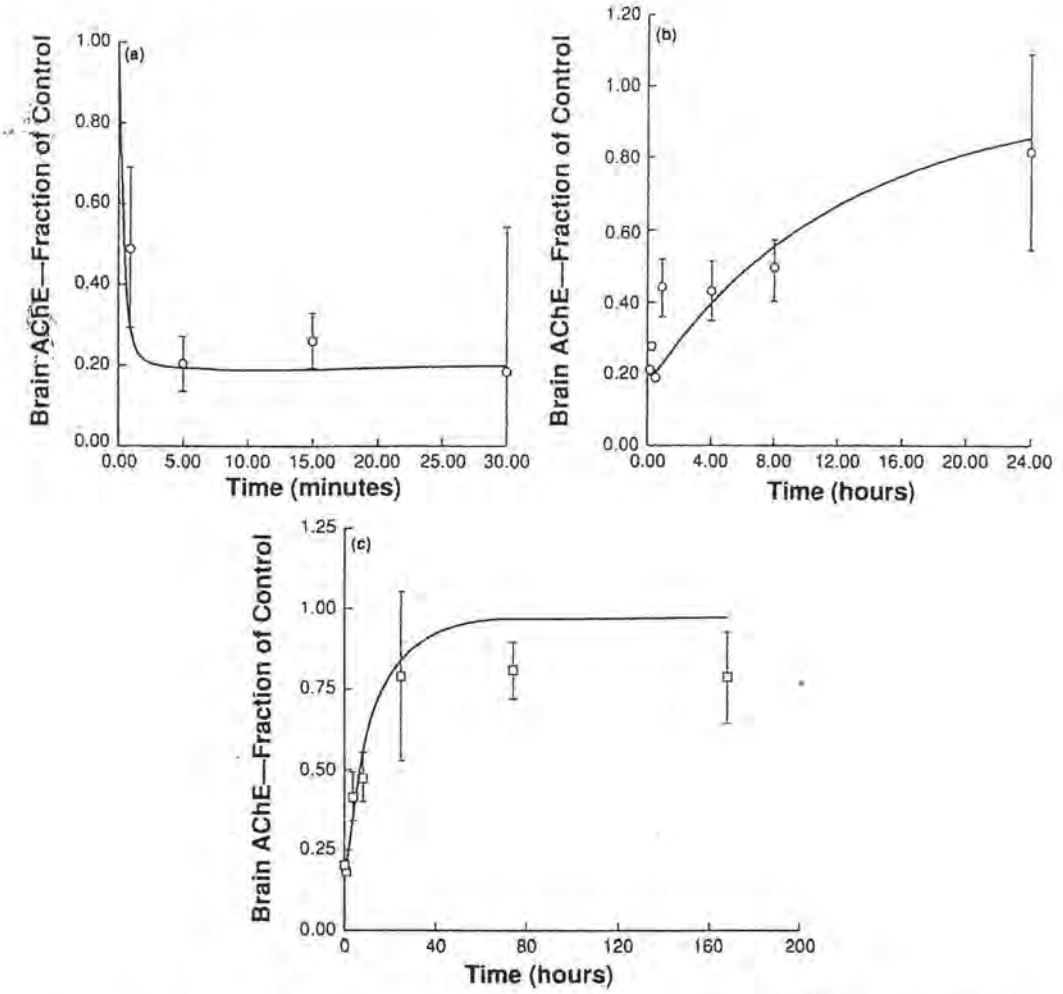


Figure 46.9 (a–c) Time-course of brain acetylcholinesterase (AChE) activity in male mice after tail vein injection of 1 mg diisopropylfluorophosphate/kg. Each datum represents the mean ± SD of 5 animals. Solid line depicts PBPK/PD model simulation. Figure used with permission from Gearhart *et al.* (1990).

46.3 PHARMACOKINETIC APPROACHES APPLIED TO ORGANOPHOSPHATE INSECTICIDES

46.3.1 APPLICATION OF PHARMACOKINETICS TO UNDERSTAND THE OVERALL DISPOSITION AND CLEARANCE OF ORGANOPHOSPHATE INSECTICIDES

Pharmacokinetic studies conducted in multiple species, at various dose levels, and across different routes of exposure can provide important insight into the *in vivo* behavior of a chemical agent and how it contributes to the observed toxicological response in a given species. To illustrate this point, a comparison is made of selected pharmacokinetic parameters obtained from a diverse group of studies conducted in animals exposed to either parathion or diazinon. As noted in Tables 46.2 and 46.3, no single study provides all the pertinent information; yet, collectively, they provide a consistent qualitative picture of the overall pharmacokinetics of these OP insecticides.

The bioavailability of OP insecticides, defined as the amount of systemically available dose, is a function of the extent of absorption and first-pass metabolism. Braeckman *et al.* (1983) conducted a pharmacokinetic study in the dog following both oral and iv administration of parathion. Comparisons of plasma parathion areas under the curve (AUCs) indicated that 1–29% of the orally administered parathion was bioavailable. The authors suggest that the low systemic oral bioavailability of parathion is primarily associated with a rapid hepatic first-pass metabolism based on the high hepatic extraction (82–97%) that was determined after iv administration. Wu *et al.* (1996) conducted similar bioavailability studies in the rat with diazinon. The blood time course of diazinon in the rat following iv and oral doses of 10 and 80 mg/kg, respectively, is presented in Fig. 46.10. The results suggest that, following oral administration, absorption is rapid (absorption $t_{1/2} = 2.6$ h) with peak plasma concentrations of diazinon being attained within 2 h postdosing; yet a comparison of AUCs, when corrected for administered dose, indicate

Table 46.2
Selected Model Parameters Describing Blood Concentration Pharmacokinetics of Parent Compounds in Various Species Following Exposure to the Organophosphate Insecticides Parathion and Diazinon

		Absorption/bioavailability kinetics			Distribution kinetics		Elimination kinetics: Two-compartment model			
Species	Dose (route)	Bioavailability percentage	Absorption $t_{1/2}$ (h)	Hepatic extraction percentage	Volume distribution $V_{d_{ss}}$ (l/kg)	Protein binding percentage	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	Elimination $k_e t_{1/2}$ (h)	Clearance Cl (l/h/kg)
Rabbit[a]	1.5 mg/kg (iv)	100	N/A	—	14.24 ± 6.34	—	—	5.08 ± 3.06	—	3.99 ± 1.13
Rabbit[a]	3 mg/kg (oral)	68[b]	0.021 ± 0.04	—	7.58 ± 6.45	—	0.13 ± 0.29	1.08 ± 0.27	2.54 ± 1.67	6.59 ± 3.36
Piglet[c]	0.5 mg/kg (iv)	100	N/A	—	2.6 ± 0.9	97 ± 1	—	—	3.0 ± 1.5	—
Pig[d]	1 mg/kg (iv)	100	N/A	—	9.76 ± 5.65	—	—	—	3.6 ± 1.08	4.42 ± 1.20
Pig[d]	50 mg/kg (dermal)	9.93 ± 5.28	—	—	—	—	—	—	—	—
Dog[e]	5 mg/kg (iv)	—	N/A	82–97	—	99	—	—	—	—
Dog[e]	10 mg/kg (oral)	1–29%	—	—	—	99	—	—	—	—
Rat[f]	5–10 mg/kg (iv)	100	N/A	48–55	20.01 ± 11.27	89.1	0.33 ± 0.10	4.70 ± 1.84	—	4.69 ± 0.8
Rat[f]	80 mg/kg (oral)	35.5	2.55	—	22.93 ± 4.82	89.1	—	—	2.86 ± 0.58	4.60 ± 1.05

[a] Pena-Egido *et al.* (1988).
[b] Estimated by comparing oral and iv AUC after adjusting for dose.
[c] Nielsen *et al.* (1991).
[d] Brimer *et al.* (1994).
[e]
[f] Wu *et al.* (1996).

Table 46.3
Tissue Concentration, Tissue Plasma Ratio and Partition Coefficients Following Exposure to the Organophosphate Insecticides Parathion and Diazinon

	^{14}C-Parathion[a] 0.5 mg/kg; iv; piglet 3 h postdosing		Parathion[b] partition coefficient (PC)	Paraoxon[b] partition coefficient (PC)	Diazinon[c] 10 mg/kg; iv; rat 4 h postdosing		Diazinon[d] 20 mg/kg; iv; mouse 5 h postdosing	
Tissue	ng/g	Tissue/plasma ratio	Tissue/blood	Tissue/blood	ng/g	Tissue/plasma ratio	ng/g	Tissue/plasma ratio
Blood/plasma	262 ± 145	—	—	—	~130	—	35	—
Liver	1254 ± 638	4.78	5.21	6.62	325 ± 25	2.50	120	3.42
Kidney	1360 ± 546	5.19	5.21	6.62	790 ± 60	6.08	3000	85.7
Lung	421 ± 92	1.60	5.21[e]	6.62[e]	—	—	—	—
Muscle	484 ± 92	1.85	2.55[f]	3.62[f]	—	—	—	—
Heart	302 ± 85	1.15	—	—	—	—	—	—
Fat	—	—	101.2	10.22	—	—	—	—
Brain	215 ± 76	0.82	4.56	2.31	280 ± 10	2.15	160	4.57

[a] Nielsen *et al.* (1991).
[b] Gearhart *et al.* (1994).
[c] Wu *et al.* (1996).
[d] Tomokuni *et al.* (1985).
[e] Well-perfused tissue.
[f] Poorly perfused tissue.

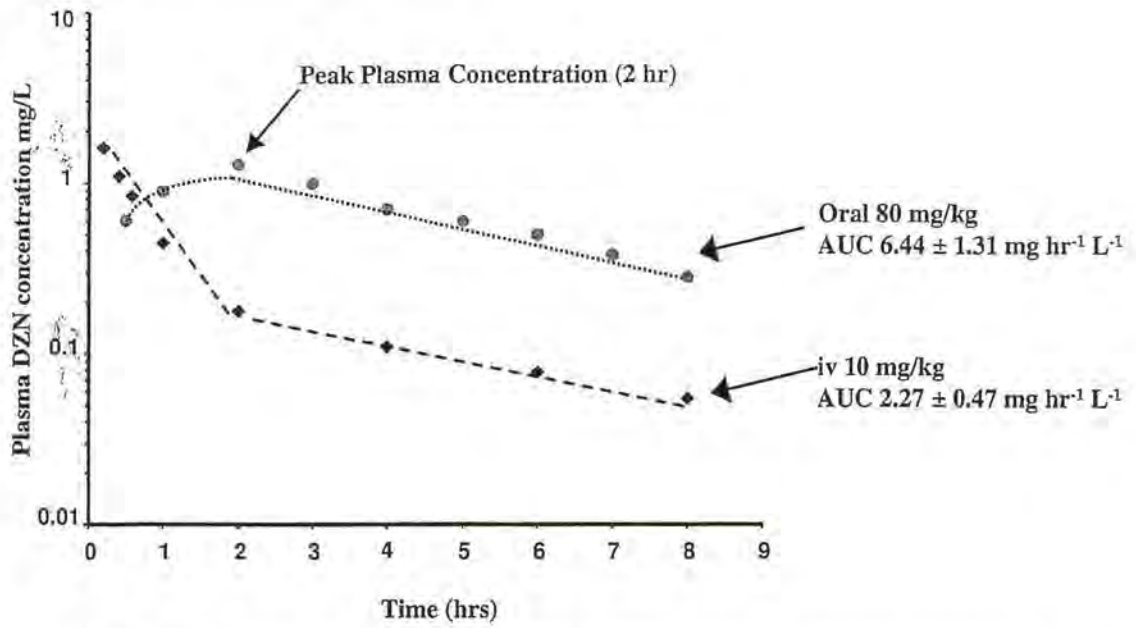


Figure 46.10 Plasma time-course of diazinon (DZN) in rats following intravenous (iv) and oral administration of 10 and 80 mg DZN/kg of body weight, respectively. Data extracted from Wu *et al.* (1996).

that only about 35% of the oral dose was systemically bioavailable. The hepatic extraction ratio for diazinon ranged from 48 to 55% and was qualitatively consistent with the findings of Braeckman *et al.* (1983) for parathion in the dog as well as chlorpyrifos in mice (hepatic extraction ratio ~46%) (Sultatos, 1988). Rapid oral absorption ($t_{1/2}$ = 0.02 h) and lower oral bioavailability (~68%) were also demonstrated in a study in which rabbits were administered iv and oral doses of parathion (Pena-Egido *et al.*, 1988). Likewise, *in vivo* animal models also suggest that dermal absorption and systemic bioavailability of OP insecticides will be quite low (Brimer *et al.*, 1994).

Once these OP compounds have been absorbed, systemic distribution throughout the body tissues is rapid (Vale, 1998). For example, a high volume of distribution was observed ranging from 3 to 14 and from 20 to 23 l/kg in several different species administered parathion or diazinon, respectively (see Table 46.2). A cross-species comparison of the tissue distribution data following parathion or diazinon exposure is consistent with the large volume of distribution, suggesting that the OP tissue concentration follows the order of kidney > liver > lung/muscle/heart > brain (see Table 46.3). Phosphorothioates such as diazinon and parathion are more lipophilic than their respective oxon metabolites and therefore can be sequestered in the fat compartment, which may account for prolonged intoxication and observed clinical relapses (Vale, 1998). Gearhart *et al.* (1994) determined the PCs for both parathion and the toxic metabolite paraoxon (see Table 46.3). In general, the PCs for parathion and paraoxon are comparable; however, parathion has an order of magnitude (101 vs. 10.11) greater affinity than paraoxon for fat.

The systemic distribution, elimination kinetics, metabolic transformation, and target site availability of a drug or chemical are often dependent on the extent of reversible plasma/serum protein binding (Renwick, 1994). For example, as shown in Table 46.2, parathion and diazinon are extensively bound to plasma protein (ranging from 89 to 99%) and the extent of binding is concentration independent. This response is likewise consistent with the findings of Sultatos *et al.* (1984), who reported that chlorpyrifos is approximately 97% bound to mouse plasma proteins over a broad concentration range. This high degree of protein binding in conjunction with the high volume of distribution also suggests that tissue binding may in fact be more important than plasma binding in determining the overall disposition and clearance of OPs (Braeckman *et al.*, 1983).

Although the OP insecticides parathion and diazinon are well distributed throughout the body and extensively bind to both plasma and tissue proteins, they are both rapidly cleared from the body primarily in the urine as degradation metabolites of the parent OPs [i.e., *p*-nitrophenol, 2-isopropyl-4-methyl-6-hydroxyprimidine (IMHP)] (Iverson *et al.*, 1975; Mücke *et al.*, 1970; Nielsen *et al.*, 1991; Vale, 1998). The overall systemic clearances for both parathion and diazinon are quite fast and comparable, ranging from 4 to 6.6 l/h/kg, and are consistent with the rapid blood/plasma terminal phase half-life (2.5–5 h; see Table 46.2).

As previously indicated, comparative species pharmacokinetic analysis is useful for understanding the *in vivo* behavior of OP insecticides. Although generalization to all OP agents is unwise, these types of comparative analyses do provide important insights. In summary, the oral absorption of both parathion and diazinon is rapid with peak plasma concentrations being obtained within a few hours of exposure. However, oral bioavailability is low and appears to be at least partially associated with a high rate of hepatic first-pass metabolism. Although these OP

insecticides are extensively bound to plasma proteins, they are equally well distributed throughout the body's tissues and the parent phosphorothioates can sequester within the fat compartment. Nonetheless, the overall clearance is quite fast and is most likely associated with the rapid metabolism and elimination of the OP metabolites.

46.3.2 DEVELOPMENT OF PHARMACOKINETIC MODELS FOR QUANTITATIVE BIOLOGICAL MONITORING TO ASSESS ORGANOPHOSPHATE INSECTICIDE EXPOSURE IN HUMANS

In assessing human exposure to chemical agents, biological monitoring (biomonitoring) is an important quantitative measure of the amount of chemical agent that is systemically absorbed. This approach entails the quantitation of the chemical or its metabolites in biological fluids (i.e., blood, urine, exhaled breath) and offers the best means of accurately assessing exposure because it measures actual, rather than potential, exposure (Woollen, 1993). However, to accurately predict human dosimetry from occupational and/or environmental exposure to xenobiotics, human volunteer pharmacokinetic studies conducted under controlled conditions are of vital importance (Wilks and Woollen, 1994; Woollen, 1993).

Both occupational and environmental exposures to OPs are primarily associated with dermal exposure; accounting for more than 90% of the absorbed dose (Aprea *et al.*, 1994). Therefore, an understanding of the percutaneous absorption of OPs is critical for quantitatively determining a systemic dose. The extent of dermal bioavailability for a number of ^{14}C-labeled OP insecticides has been determined in humans utilizing both *in vivo* studies in volunteers and *in vitro* dermal penetration with skin obtained from cadavers (Wester *et al.*, 1983, 1992, 1993). A summary of the percentage absorption following *in vivo* and *in vitro* dermal exposure to the OP insecticides diazinon, isofenphos, and malathion is illustrated in Fig. 46.11.

The general experimental design of these studies entailed three major components. First, human volunteers were administered a topical dose of a known concentration of ^{14}C-labeled OP for a specified exposure period. The extent of absorption was determined by quantitating the amount of ^{14}C excreted in the urine and remaining on the skin surface. Second, *in vitro* percutaneous absorption was determined using a glass flow-through penetration cell in which the percentage absorption through human cadaver skin was determined by the amount of radiotracer that transferred into the receptor fluid. Finally, to calculate the *in vivo* percentage absorption, rhesus monkeys were given a ^{14}C-labeled OP as an iv dose. The percentage dose absorbed in humans was calculated from the ratio of ^{14}C excreted in the urine after topical (humans) and iv (monkey) dosing. The *in vivo* absorption for the three OP insecticides diazinon, isofenphos, and malathion in human volunteers following a topical application is very low, ranging from 2.5 to 3.9% of the applied dose. The percentage absorption as determined *in vitro* was likewise comparable for isofenphos (3.64% $\pm$ 0.48), but slightly higher and considerably more variable for diazinon (14.1% $\pm$ 9.2). Percutaneous absorption studies conducted in humans are of particular importance because it is known that dermal absorption in animals, such as the rat, is often greater than in humans (Wester and Maibach, 1983). For example, Knaak *et al.* (1990) conducted a dermal absorption study in rats with isofenphos and reported that 47% of the applied dose was absorbed, which is 12-fold higher than the results seen in human volunteers. The major limitation associated with the experimental design of Wester *et al.* (1983, 1992, 1993) is that the quantitation of only ^{14}C provides no information on the specific form of the compound (i.e., parent or metabolite) that is systemically available. Nonetheless, these studies provide important quantitative information on the extent of dermal absorption.

To better understand the systemic pharmacokinetics of OPs and to develop pharmacokinetic models that can be utilized for biomonitoring, controlled human studies that quantitate the time course of parent chemical or metabolites in blood and urine are key. Nolan *et al.* (1984) conducted a controlled human pharmacokinetic study to follow the fate of a major metabolite, 3,5,6-trichloropyridinol (TCP), which is excreted in the urine following both oral and dermal administration of chlorpyrifos. Griffin *et al.* (1999) also conducted a controlled human study with chlorpyrifos in human volunteers, but quantitated the urinary excretion of the dialkylphosphate metabolite.

A selection of comparative pharmacokinetic parameters from the controlled human chlorpyrifos studies is presented in Table 46.4. Overall, the pharmacokinetic results obtained using TCP or dialkylphosphate in human volunteers are entirely consistent with each other. For example, following oral administration, chlorpyrifos is rapidly absorbed with maximum plasma concentration and excretion being obtained by 6 and 7 h postdosing for TCP and dialkylphosphate, respectively. The extent of absorption was quite good, based on the amount of metabolite (70–93%) recovered in the urine. In comparison, the dermal absorption was consistently slower with peak concentrations of metabolite being achieved by 17–24 h postdosing for both studies. Also, the amount recovered based on TCP and dialkylphosphate metabolites in the urine was 1.35 and 1%, respectively, suggesting limited dermal absorption of chlorpyrifos. Nolan *et al.* (1984) reported an elimination half-life of 26.9 h following oral administration, whereas Griffin *et al.* (1999) reported half-lives of 15.5 and 30 h for dialkylphosphate following oral and dermal exposure to chlorpyrifos, respectively. The increase in the urinary elimination half-life following dermal exposure is most likely associated with a delay in chlorpyrifos absorption through the skin. However, differences in the rates of TCP and dialkylphosphate kinetics are also a possible explanation (Griffin *et al.*, 1999). Nonetheless, the elimination half-life for chlorpyrifos based on either TCP or dialkylphosphate clearance is consistent.

These types of pharmacokinetic data are being used to develop models to biomonitor for OP exposure. Nolan *et al.* (1984) developed a one-compartment pharmacokinetic model having the same volume of distribution and elimination rate

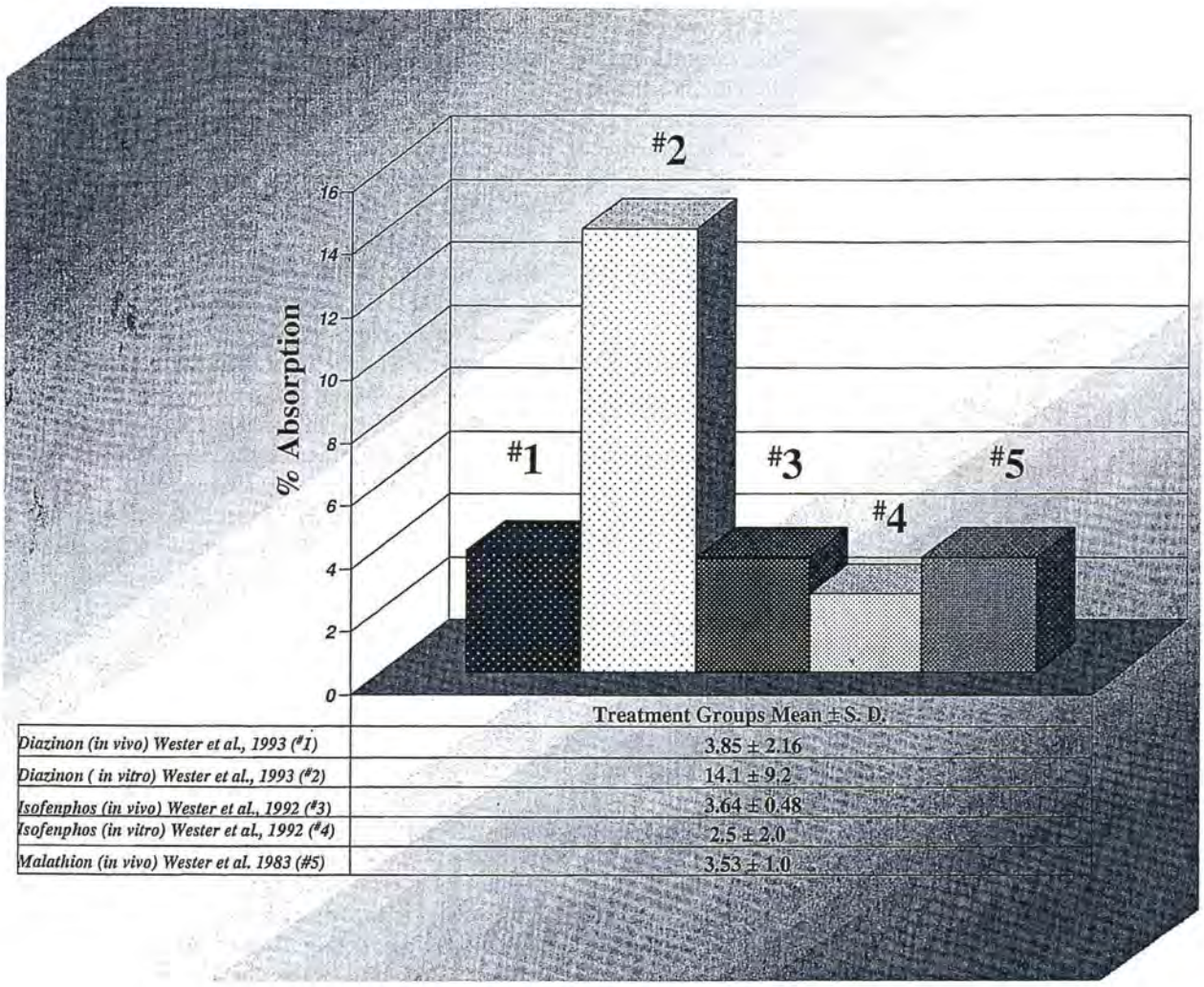


Figure 46.11 Summary of human dermal penetration (*in vivo/in vitro*) for the organophosphate (OP) insecticides diazinon, isofenphos and malathion.

Table 46.4
Comparison of Oral and Dermal Pharmacokinetic Parameters Describing the Blood Concentration and Urinary Excretion of 3,5,6-Trichloropyridinol (TCP) and Dialkylphosphate (DAP) by Volunteers Following Exposure to the Organophosphate Insecticide Chlorpyrifos

Exposure route/ metabolite	Dose (mg/kg)	Absorption rate (ng/cm^2/h)	Absorption rate constant k_a (h^{-1})	Absorption half-life (h)	Elimination rate constant k_e (h^{-1})	Elimination half-life (h)	Model predicted percentage dose absorbed	Percentage dose recovered in urine
Oral								
TCP[a]	0.5	—	1.5 ± 1.2	0.5	0.0258 ± 0.0051	26.9	72 ± 11	70 ± 11
DAP[b]	0.014[c]	—	—	—	—	15.5	—	93 (range 55–115)
Dermal								
TCP[a]	5	—	0.0308 ± 0.01	22.5	—	—	1.35 ± 1.02	1.28 ± 0.83
DAP[b]	0.41	456	—	—	—	30	—	1.00

[a] Nolan *et al.* (1984).
[b] Griffin *et al.* (1999).
[c] Estimated based on average body weight (71 kg).

constant to describe blood and urinary TCP kinetics following oral and dermal exposure to chlorpyrifos (see Fig. 46.4). Similarly, the quantitative measurement of urinary dialkylphosphate is increasingly being used for biomonitoring for OP exposures (Gargas *et al.*, 1989). The development of pharmacokinetic models that are capable of describing the uptake, distribution, and elimination of OP insecticides based on the quantitation of major degradation metabolites represents an extremely useful and simple approach for exposure biomonitoring.

46.3.3 THE APPLICATION OF PHARMACOKINETICS FOR QUANTIFYING EXPOSURE TO ORGANOPHOSPHATE INSECTICIDES

The ability to more accurately quantitate human exposure to OP insecticides has been enhanced by the use of biomonitoring approaches linked to pharmacokinetic analysis. This has successfully been used to estimate agricultural worker exposures during and after the application of insecticides, to evaluate secondary exposures within cross-sectional epidemiology studies, and to assess dosimetry in persons who have been acutely poisoned either accidentally or through intentional self-administration (Drevenkar *et al.*, 1993; Lavy *et al.*, 1993; Loewenherz *et al.*, 1997).

Historically, workplace exposure to chemicals has been controlled through environmental monitoring that has primarily focused on the measurement of the chemical contaminant in the ambient air. However, because airborne concentrations may not be linearly correlated with absorption, this approach does not provide an accurate assessment of the internal dose (Franklin *et al.*, 1986). In agricultural settings, worker exposure studies have incorporated personal external monitoring to estimate the amount of chemical available from inhalation (i.e., breathing-zone sampling pumps) and dermal absorption (i.e., patch method and hand washes). Where feasible, these studies have also incorporated biomonitoring (i.e., urinary metabolites) to quantitate the amount of absorbed dose (Chester, 1993; Franklin *et al.*, 1981, 1986). Franklin *et al.* (1981, 1986) estimated exposure of workers to the OP insecticide azinphos-methyl (guthion) utilizing both external personal monitoring and urinary biomonitoring of alkylphosphate metabolite. When patch data were utilized to calculate exposure and plotted against total urinary metabolite excretion, no correlation was observed (Franklin *et al.*, 1981). However, the authors did report a much better correlation when the amount of alkylphosphate metabolite excreted in the urine was compared against the amount of active ingredient sprayed. This relationship is illustrated in Fig. 46.12, where the amount of alkylphosphate metabolite excreted in the urine increases with increasing amounts of active ingredient.

Because agricultural workers routinely apply numerous pesticides and are often sequentially exposed to OP insecticides within a relatively short time span, a number of exposure studies have been conducted to evaluate mixed OP insecticide exposures. Hayes *et al.* (1980) evaluated the occupational exposure of pest control operators in which the bulk of the pesticide applications (~ 80%) involved the combined use of the OP insecticides vaponite, diazinon, and chlorpyrifos. Worker biomonitoring was based on blood AChE determination and the quantitation of dimethyl- and diethylphosphate and dimethyl- and diethylphosphothioate metabolites in the urine. The authors reported that external air monitoring did provide information regarding the levels and types of OP exposures, but did not provide adequate information on the degree to which these OPs were absorbed. The urinary alkylphosphate levels provided sensitive quantitative information on absorption and excretion of these pesticides. However, because the alkylphosphate metabolites are not specific to any one OP, this approach is indicative only of general OP exposures to these mixtures and cannot be used to quantitatively assess individual OP dosimetry.

More recently, Lavy *et al.* (1993) conducted a comprehensive year-long biomonitoring study of tree nursery workers, who are routinely exposed to multiple pesticides. In this study, it was recognized that as many as 28 pesticides are regularly used and 17 of the most common pesticides were selected for monitoring, including a number of OPs. Evaluation of the human and animal pharmacokinetic data suggested that adequate metabolism information was available on 8 of the selected pesticides to support biomonitoring. In this year-long study, 3134 urine specimens were analyzed, but only 42 of these contained measurable pesticide metabolites (1.3%) and were composed of only three pesticides (benomyl, bifenox, and carbaryl) (Lavy *et al.*, 1993). In addition, based on a calculated margin of safety, the exposure levels were clearly below a level that would be of concern to human health.

Biomonitoring strategies have also been successfully applied to quantitatively assess secondary exposures to OP insecticides resulting from both acute and chronic exposures. Richter *et al.* (1992) quantitated diethylphosphate in the urine of individuals who were symptomatic for OP exposure and resided in a house that had been sprayed with diazinon approximately 4.5 months earlier. In this particular study, very high levels of urinary diethylphosphate were observed in family members, whereas cholinesterase activity, although slightly depressed, was well within the range of "normal." The quantitation of urinary diethylphosphate was used to establish a persistent household exposure to diazinon residues as the most likely explanation. This study clearly illustrates the utility of urinary OP metabolites for quantitative biomonitoring of exposure.

Biomonitoring based on the measurement of OP metabolites has also been used to compare pesticide exposure in children who live in proximity to high spray areas (i.e., orchards) and whose parents/guardians are pesticide applicators (Loewenherz *et al.*, 1997). Based on known pesticide use patterns, it was determined that OP insecticide exposure would be primarily associated with azinphos-methyl, chlorpyrifos, and phosmet. Therefore, the study focused on the quantitation of the alkylphosphate metabolites (dimethylthiophosphate, dimethyldithiophosphate, dimethylphosphate) in the children's urine. Loewenherz *et al.* (1997) collected and evaluated 160 spot urine specimens from 88 children and reported detectable

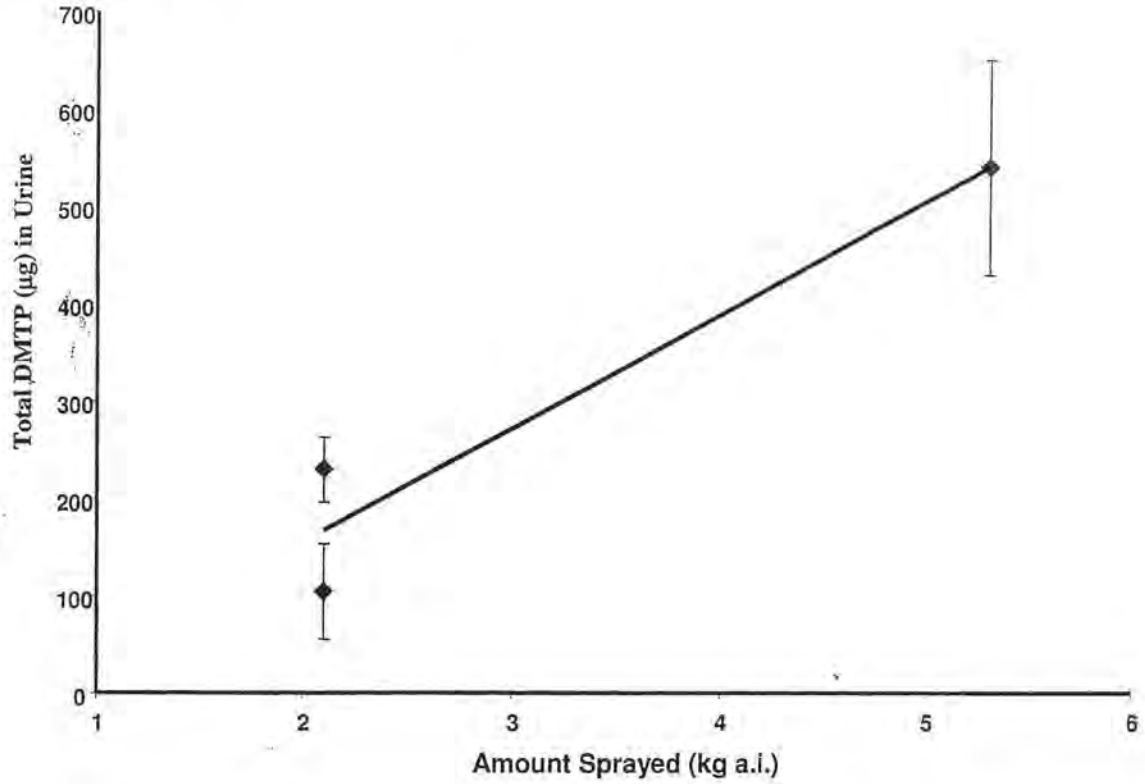


Figure 46.12 Relationship between the amount of alkyl phosphate (dimethylthiophosphate; DMTP) metabolite in urine of workers and the amount of active ingredient (a.i.) sprayed. Data obtained from Franklin *et al.* (1981).

levels of these metabolites in 27 and 47% of the reference children and applicator children, respectively. In addition, the biomonitoring data suggest that the children of applicators had a significantly higher dose than the reference children (0.021 vs. 0.005 µg/l, respectively).

Biomonitoring of parent OPs and metabolites in blood and urine has also been used to provide a quantitative assessment of dosimetry in human poisoning victims following acute high-dose exposures (Drevenkar *et al.*, 1993; Vasilic *et al.*, 1992). Although acute AChE depression (i.e., 50% of baseline) is used to substantiate OP poisoning, the analysis of intact pesticides or specific metabolites in body fluids (blood/urine) can be used to identify the specific causative chemical agent(s) (Ellenhorn and Barceloux, 1988; Lotti *et al.*, 1986). The utilization of pharmacokinetic models such as the one developed for chlorpyrifos (Nolan *et al.*, 1984) can be extremely useful for the estimation of dosimetry under these acute exposure scenarios.

To illustrate this point, a two-compartment pharmacokinetic model was used to fit pharmacokinetic data obtained from a poison victim who ingested a commercial insecticide formulation containing chlorpyrifos (Drevenkar *et al.*, 1993). These same data have been modeled utilizing a PBPK/PD model developed for the quantitation of chlorpyrifos, chlorpyrifos-oxon, and TCP in the rat and human (Timchalk and Nolan, 1998). The time course and PBPK/PD model–predicted TCP and chlorpyrifos concentration in the blood and serum of human volunteers and following oral ingestion for a single poison victim are presented in Fig. 46.13. The model adequately reflects the data from these limited human samples; more important, these examples illustrate the strength of using pharmacokinetic models for quantitating dosimetry under both controlled and noncontrolled conditions.

In summary, these examples have been presented to illustrate the practical application of pharmacokinetics to assess exposure to chemicals. Biomonitoring is clearly an integral component of the agricultural pesticide exposure assessment strategy. However, the successful application of biomonitoring for quantitative dosimetry is primarily limited by a lack of chemical-specific pharmacokinetic data in humans.

46.3.4 STUDIES THAT FACILITATE EXTRAPOLATION OF DOSIMETRY AND BIOLOGICAL RESPONSE FROM ANIMALS TO HUMANS AND THE ASSESSMENT OF HUMAN HEALTH RISK

Organophosphate insecticides constitute a large class of chemical pesticides that are widely used in the agricultural industry and in home applications. This suggests that there is significant potential for exposure and the health consequences of these exposures may be impacted by both interindividual and extrinsic variability (see Fig. 46.14). For example, extrinsic factors such as multiple exposure routes, chemical/drug interactions, and

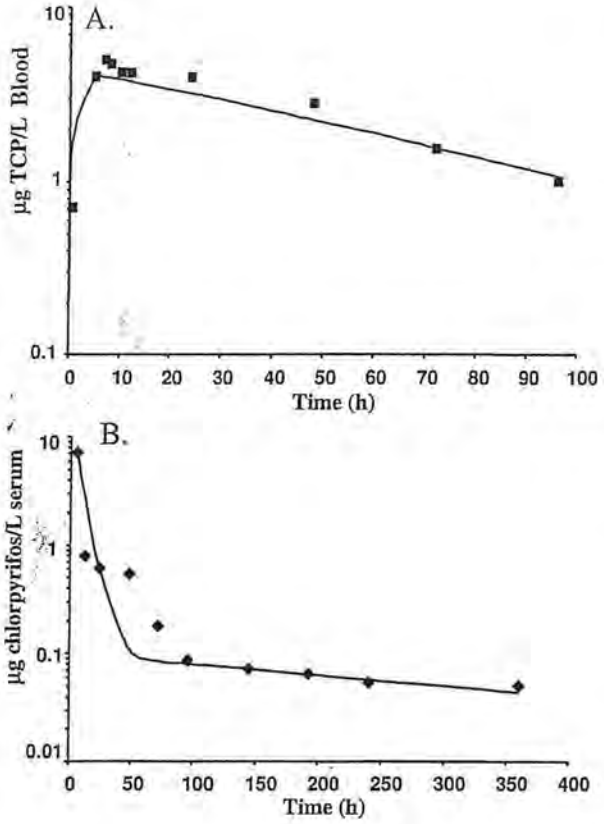


Figure 46.13 (A) Mean blood time-course of 3,5,6-trichloropyridinol (TCP) from six human volunteers administered a single oral dose of 0.5 mg chlorpyrifos (CPF)/kg of body weight. Data obtained from Nolan *et al.* (1984). (B) Time-course of CPF in the serum of a single poison victim that orally ingested a commercial insecticide product containing CPF. The symbols represent observed data while the line represents the model prediction. Data obtained from Drevenkar *et al.* (1993).

variable exposure rates may significantly modify the toxicological response to OPs. In addition, person-to-person differences in metabolism, genetic predisposition, physical environment, and age (infant, children, and elderly) are important determinants of pharmacokinetic and/or pharmacodynamic response. As previously discussed, Gearhart *et al.* (1990) developed a PBPK/PD model for quantitative OP dosimetry and AChE inhibition utilizing diisopropylfluorophosphate as a representative highly toxic OP (see Figs. 46.6 and 46.7). This PBPK/PD model was developed as a prototype that could readily be extended to the commercially important OPs. In this respect, the development and application of PBPK/PD modeling represents a logical approach for assessing risk and understanding the toxicological implications of known or suspected exposures to various OP insecticides.

To extend this initial modeling effort, Gearhart *et al.* (1994) modified their original model to incorporate the phosphorothionate insecticide parathion and its oxon, paraoxon. In addition, Timchalk and Nolan (1998) built a model to incorporate chemical-specific parameters for the OP insecticide chlorpyrifos that are based on the models of Gearhart *et al.* (1990, 1994). This model was developed to describe the time course of chlorpyrifos, the oxon metabolite, the elimination of TCP, and the inhibition of target esterases by the oxon. The chlorpyrifos PBPK/PD model incorporates CYP450-mediated activation of chlorpyrifos to the oxon and detoxification to TCP. In addition, hydrolysis of the oxon by B-esterases (AChE, BChE, and CaE) is modeled in the liver, blood, diaphragm, and brain. A diagram outlining the critical features of this model is presented in Fig. 46.15. Although this is a preliminary PBPK/PD model requiring further validation and refinement, it qualitatively behaves consistent with the general understanding of OP toxicity, pharmacokinetics, and pharmacodynamic responses. To illustrate this point, the model has been used to simulate the serum time course of chlorpyrifos and TCP in poisoned humans (see Fig. 46.13). In addition, a simulation of the dynamics of tissue esterase inhibition following a single acute exposure to two different doses of chlorpyrifos in the rat is qualitatively consistent with observed AChE inhibition kinetics and is illustrated in Fig. 46.16. It is anticipated that this basic model structure can be readily modified to accommodate other important phosphorothioates. Likewise, once validated, these models can be used as a foundation for understanding complex-mixture interactions, sensitive subpopulations, and the role of metabolic polymorphisms in altering dosimetry and biological response.

46.3.4.1 Organophosphate Mixtures

Both occupational and residential exposures to OP insecticides often entail simultaneous or sequential contact with OP mixtures (Hayes *et al.*, 1980; Lavy *et al.*, 1993; Loewenherz *et al.*, 1997). The potential for OP interactions has been well understood for some time. Early studies demonstrated the acute, synergistic, and toxicological interactions between the OPs malathion and EPN (ethyl-*p*-nitrophenyl phenylphosphonothionate) (Frawley *et al.*, 1957). In addition, non-OPs have been reported to influence the pharmacokinetic and toxicological response of OPs. For example, phenobarbital or alcohol pretreatment of mice protects against the acute toxicity of chlorpyrifos and parathion, respectively (O'Shaughnessy and Sultatos, 1995; Sultatos, 1988). Wu *et al.* (1996) reported that pretreatment of rats with cimetidine potentiated the acute toxicity of diazinon as a result of reducing diazinon total body clearance. Likewise, co-administration of diazinon with cocaine significantly increased the concentration of cocaine and norcocaine in the blood and tissues of mice apparently due to competition for esterase enzyme detoxification (Benuck *et al.*, 1989; Kump *et al.*, 1994). A combination of malathion and the carbamate pesticide carbaryl alters the fundamental pharmacokinetic properties of the individual compounds and it has been suggested that this may explain some of the observed toxicity seen from exposure to this chemical mixture (Waldron-Lechner and Abdel-Rahman, 1986).

OP pesticides as a class of compounds share common metabolic processes for activation and detoxification as well as a common mechanism for toxicological response through the inhibition of AChE (Murphy, 1986; Sultatos, 1994). Based on similar pharmacokinetic and mode-of-action properties,

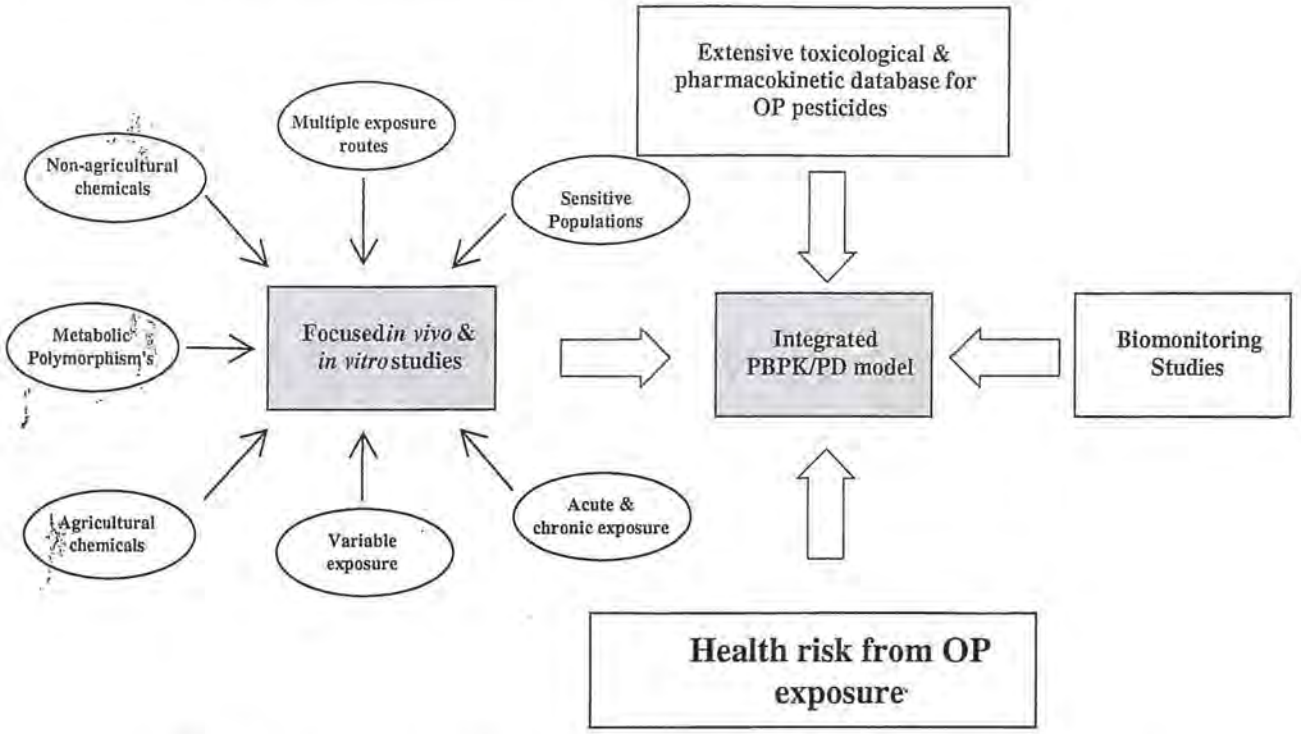


Figure 46.14 A conceptual model for evaluating the impact of intrinsic/extrinsic factors on human health risk from exposure to organophosphate (OP) insecticides.

the potential for interactions between mixtures of OPs has been hypothesized. Organophosphates can interact at a number of important metabolic steps (see Table 46.5), including (1) CYP450-mediated activation/detoxification, (2) plasma protein binding, (3) A-esterase detoxification, and (4) AChE binding/inhibition. The net effect of these interactions (additivity, synergy, or antagonism) will be dependent on the specific OP mixture, dose ranges of exposures, and sensitivity of the individual.

Several integrated approaches have been proposed to investigate the potential for toxicological interactions of chemical mixtures (El-Masri *et al.*, 1997). The proposed strategies all emphasize the utilization of PBPK modeling. Until recently, the majority of these models have focused on a single chemical exposure. However, exposure to low-dose chemical mixtures represents a more realistic exposure scenario. Although several PBPK models have been developed for binary and ternary mixtures of organic solvents, little is known about the potential metabolic and toxicological interactions of these mixtures on biological systems (Andersen *et al.*, 1987b; Pelekis and Krishnan, 1997; Purcell *et al.*, 1990; Tardif *et al.*, 1993, 1995, 1997). The evaluation of mixtures is complicated by the myriad of chemicals, doses, exposure routes, and dynamic responses observed, making it impractical to effectively test all possible permutations. In this regard, the application of PBPK models for mixtures provides a limited means to quantitatively describe the disposition of chemicals as a result of exposure to various combinations, doses, and routes of administration.

46.3.4.2 Sensitive Subpopulations (Children and Polymorphisms)

There is currently a significant focus and concern over the potential increased sensitivity of infants and children to the toxic effects of chemicals. The importance of this issue is highlighted by the National Research Council's report, *Pesticides in the Diets of Infants and Children*, and the passage of the Food Quality Protection Act. It is recognized that children are not just "small adults," but rather a unique subpopulation that may be particularly vulnerable to chemical insult. Age-dependent changes in a child's physiology (i.e., body size, blood flow, organ functions) and metabolic capacity (i.e., phase I and II metabolism) may significantly impact their response to a chemical insult, resulting in either beneficial or detrimental effects (Miller *et al.*, 1997). Clear variability in the capacity to detoxify environmental chemicals has been established in both animals and humans. However, the current risk assessment paradigms do not adequately consider the implications of these differences on the risk to infants and children. Numerous studies have demonstrated that juvenile animals are more susceptible to the acute effects of OP insecticides than adults (Benke and Murphy, 1975; Brodeur and DuBois, 1963; Gaines and Linder, 1986; Harbison, 1975; Moser and Padilla, 1998; Pope and Liu, 1997; Pope *et al.*, 1991). This greater sensitivity has primarily been attributed to the lack of complete metabolic competence during neonatal and postnatal development (Benke and Murphy, 1975).

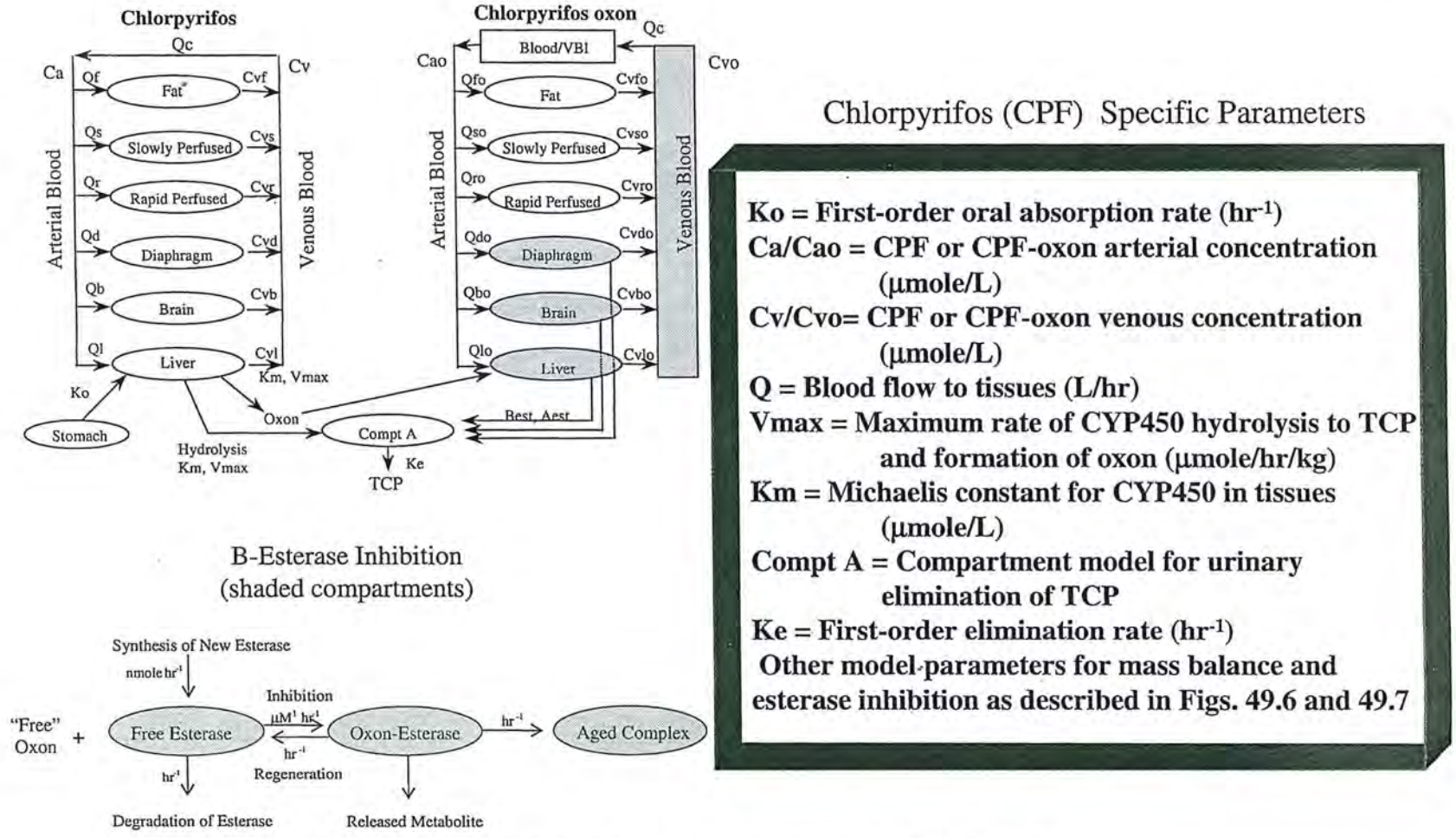


Figure 46.15 Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model structure describing the distribution of chlorpyrifos (CPF), CPF-oxon and 3,5,6-trichloropyridinol (TCP) in the rat.

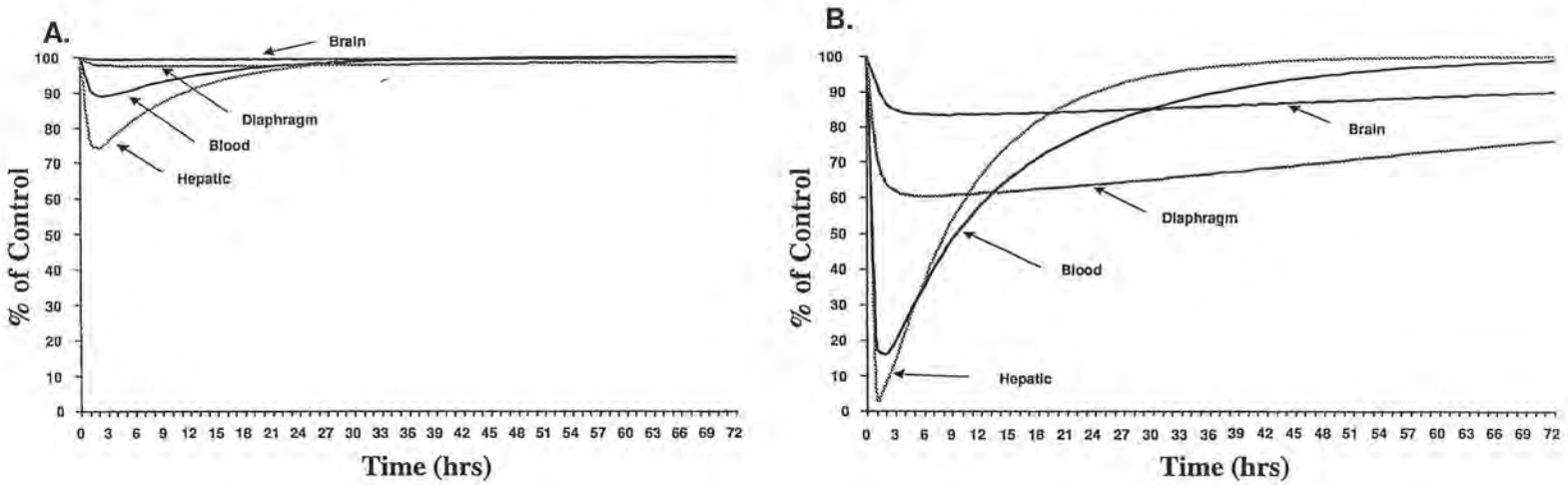


Figure 46.16 Simulated AChE inhibition in selected tissues (brain, diaphragm, blood and liver) following a single oral dose of (A) 0.5 mg and (B) 5 mg chlorpyrifos (CPF)/kg of body weight.

Table 46.5
Important Metabolic and Response Interactions for Mixtures of Organophosphate Insecticides

Parameter	Importance	Type of chemical interaction	Implication
CYP450 mixed-function oxidase metabolism	Metabolic activation/ detoxification of parent compound	Substrate (parent compound) competition for enzyme	Changes in oxon concentrations
Reversible plasma protein binding	Systemic transport of parent compound	Substrate (parent compound) competition for available protein binding sites	Increased levels of "free" parent chemical available for metabolism
A-esterase metabolism	Important metabolic step responsible for detoxification	Substrate (oxon) competition for enzyme	Changes in oxon concentrations
AChE binding/inhibition	Toxicological response	Substrates (oxon) combine to increase inhibition of AChE	Increased toxicity due to additive response

Several recent studies provide important perspective on this age-dependent sensitivity and the selected results are illustrated in Fig. 46.17. Atterberry *et al.* (1997) evaluated the developmental changes in brain AChE levels and hepatic CaE- and CYP450-mediated metabolism of chlorpyrifos in the juvenile rat. They correlated age-dependent chlorpyrifos toxicity (Fig. 46.17a) with the capacity to activate and detoxify chlorpyrifos (Fig. 46.17b and c). Their results suggest that the age-dependent sensitivity of young animals is associated with a decreased CaE-mediated hydrolysis and CYP450-mediated dearylation capacity in young animals relative to adults. In addition, it has likewise been suggested that the sensitivity of young animals is associated with a lower A-esterase activity than is found in adults (Mortensen *et al.*, 1996). The importance of A-esterase in protecting against OP toxicity has been demonstrated in several studies in which exogenous administration of A-esterase can protect against OP poisoning in rodents (Costa *et al.*, 1990; Li *et al.*, 1993, 1995; Main, 1956).

Mortensen *et al.* (1996) compared both plasma and hepatic A-esterase enzyme activity in adult and neonatal animals and reported that neonatal plasma and liver A-esterase activity were 9 and 50% of adult activity, respectively. This is consistent with the results of Li *et al.* (1997), who reported that serum A-esterase activity toward paraoxon, chlorpyrifos-oxon, and diazoxon were very low at birth, but reached adult levels of activity by about 25 days of age in rodents. This time span in rodents corresponds to *in utero* humans to approximately in 6 months old. These findings in animals are in agreement with observations in which newborns and children less than 6 months old have lower plasma A-esterase activity than adults (Augustinsson and Barr, 1963; Mueller *et al.*, 1983). In addition to age-dependent differences in A-esterase activity, a human and animal genetic polymorphism has been well established (Eckerson *et al.*, 1983; Furlong *et al.*, 1988; Geldmacher-von Mallinckrodt *et al.*, 1983). This polymorphism is known to result in the expression of a broad range of A-esterase enzyme activity within a large segment of the human population.

Although young rats appear to be more sensitive (based on LD_{50} and AChE inhibition) to the acute effects of OP insecticides relative to adults, AChE activity is reported to recover faster in young animals (Moser and Padilla, 1998; Pope *et al.*, 1991). This more rapid recovery of AChE (Lajtha and Dunlop, 1981; Moser and Padilla, 1998) is associated with a faster synthesis rate as well as higher steady-state enzyme levels. The capacity of young rats to recover AChE activity faster than adults may be of greater importance in dealing with intermittent low-dose exposure to OPs. In summary, these data suggest that the sensitivity of juvenile animals and humans to the toxic effects of OP insecticides may be a function of the maturational stage of development for a number of critical metabolic steps. These include CYP450-mediated activation/detoxification, hepatic CaE hydrolysis binding, and plasma and hepatic A-esterase detoxification.

The application of PBPK/PD modeling offers a unique opportunity to integrate age-dependent changes in OP metabolic activation and detoxification pathways into a comprehensive model that is capable of quantitating dose and response across all ages. In this context, PBPK models are being extended to the modeling of chemical exposure in developing neonatal animals. A number of these models have focused on the incorporation of xenobiotic lactational transfer to nursing pups (Byczkowski *et al.*, 1994; Fisher *et al.*, 1990; Sundberg *et al.*, 1998). Based on the potential sensitivity of children to OP insecticides, there is a need to develop quantitative models that can be used to assess the risk associated with OP exposure in infants and children. However, there are currently no published models available for OPs that are readily applicable for quantitating age-dependent changes in dose and response. For toxicants that have long residence times within the body, there is a need to develop PBPK models that appropriately incorporate growth and maturational development of physiological and metabolic function (O'Flaherty, 1991a).

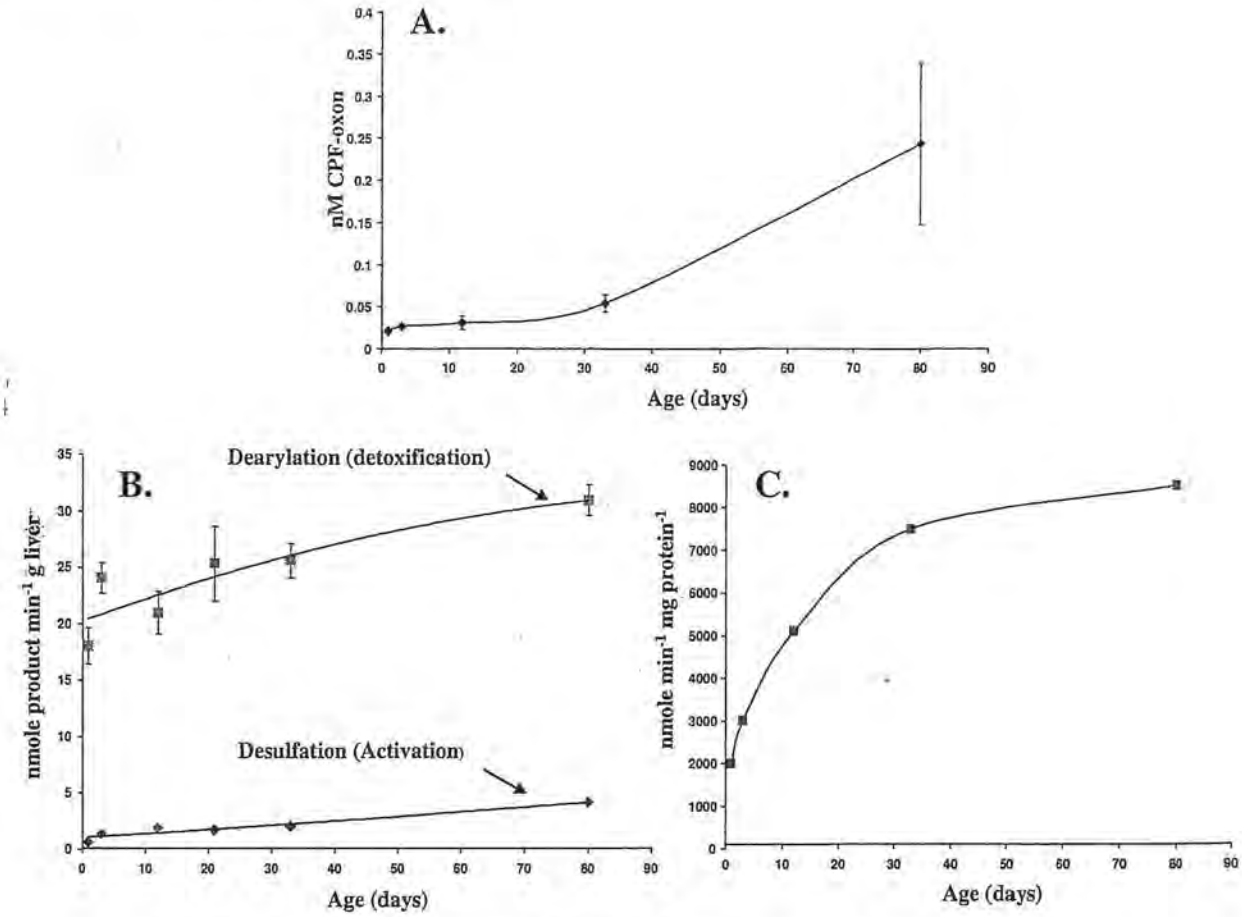


Figure 46.17 (A) Inhibitory concentration 50% (IC_{50}) of chlorpyrifos-oxon (CPF-oxon) (nM) to CaE activity in the liver of rats with increasing age, (B) CPF (50 μM) CYP450 desulfation and dearylation by rat hepatic microsomes with increasing age, (C) Developmental pattern of liver CaE activity with increasing age in the rat. All data was adapted from Atterberry *et al.* (1997).

O'Flaherty (1991a, b, 1993) has developed a series of PBPK models that begin to describe lead kinetics from birth to adulthood. Ultimately these models will enable quantitation of lead dosimetry over an entire lifetime. It is envisioned that the framework of the PBPK/PD model that has been developed for diisopropylfluorophosphate, parathion and chlorpyrifos (Gearhart *et al.*, 1990, 1994; Sultatos, 1990; Timchalk and Nolan, 1998) can readily be extended to incorporate age-dependent changes in CYP450, CaE, A-esterase and AchE activity.

In summary, pharmacokinetics and in particular the application of PBPK/PD modeling have been shown to be extremely useful approaches for dosimetry and biological response extrapolation for the assessment of human health risk from chemical exposures. The utilization of PBPK/PD modeling to address OP insecticide toxicity issues is particularly intriguing since these models can be used to assess the health consequences of both inter-individual (i.e., age, gender) and extrinsic factors (i.e., multiple exposure routes, chemical/drug interactions and variable exposure rates) that may significantly modify the toxicological response to OPs.

46.4 SUMMARY AND CONCLUSIONS

This chapter has illustrated a number of current and future applications of pharmacokinetics to assess OP dosimetry, biological response and risk in humans exposed to these insecticides. Pharmacokinetics is concerned with the quantitative integration of absorption, distribution, metabolism and excretion and can be used to provide useful insight into the toxicological responses associated with OP insecticides. Since OP insecticides share a common mode of action through their capability to inhibited AChE activity, it is feasible to develop pharmacokinetic strategies that link quantitative dosimetry with biologically based pharmacodynamic (PD) response modeling. Pharmacokinetic studies that have been conducted with OP insecticides in multiple species, at various dose levels, and across different routes of exposure have provided important insight into *in vivo* behavior of these OPs. The development and application of pharmacokinetic models capable of describing uptake, distribution, metabolism, and elimination of OP insecticides in humans represent a crucial research element needed for quantitative biomonitoring. In this regard, the successful application of biomonitoring for quantitating OP dose is primarily

limited by the lack of this chemical-specific pharmacokinetic data in humans. The development and application of PBPK/PD modeling for OP insecticides represent a unique opportunity to quantitatively assess human health risk and to understand the toxicological implications of known or suspected exposures OPs. Validated PBPK/PD models for OPs can be used to consider the potential variability in human response associated with both interindividual (i.e., age, gender, polymorphism) and extrinsic variability (i.e., exposure routes and rates, single vs. multiple exposures).

In conclusion, pharmacokinetics have been successfully utilized to better understand the toxicological implications of human exposure to OP insecticides. Nonetheless, there is still a significant need to further develop and refine pharmacokinetic models that can be used to accurately assess the risk associated with OP insecticide exposures.

REFERENCES

Andersen, M. E. (1995). Physiologically based pharmacokinetic (PB-PK) models in the study of the disposition and biological effects of xenobiotics and drugs. *Toxicol. Lett.* **82/83**, 341–348.

Andersen, M. E., Clewell, H. J., III, Gargas, M. L., Smith, F. A., and Reitz, R. H. (1987a). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* **87**, 185–205.

Andersen, M. E., Gargas, M. L., Clewell, H. J., III, and Severyn, K. M. (1987b). Quantitative evaluation of the metabolic interactions between trichloroethylene and 1,1-dichloroethylene *in vivo* using gas uptake methods. *Toxicol. Appl. Pharmacol.* **89**, 149–157.

Aprea, C., Betta, A., Catenacci, G., Lotti, A., Magnaghi, S., Barisano, A., Passini, V., Pavan, I., Sciarra, G., Vitalone, V., and Minoia, C. (1999). Reference values of urinary 3,5,6-trichloro-2-pyridinol in the Italian population—Validation of analytical method and preliminary results (multicentric study). *J. AOAC Int.* **82**, 305–312.

Aprea, C., Sciarra, G., Sartorelli, P., Desideri, E., Amati, R., and Sartorelli, E. (1994). Biological monitoring of exposure to organophosphate insecticides by assay of urinary alkylphosphates: Influence of protective measures during manual operations with treated plants. *Int. Arch. Occup. Environ. Health* **66**, 333–338.

Atterberry, T. T., Burnett, W. T., and Chambers, J. E. (1997). Age-related differences in parathion and chlorpyrifos toxicity in male rats: Target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. *Toxicol. Appl. Pharmacol.* **147**, 411–418.

Augustinsson, K.-B., and Barr, M. (1963). Age variation in plasma arylesterase activity in children. *Clin. Chem. Acta* **8**, 568–573.

Benke, G. M., and Murphy, S. D. (1975). The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. *Toxicol. Appl. Pharmacol.* **31**, 254–269.

Benuck, M., Reith, M. E., Sershen, H., Wiener, H. L., and Lajtha, A. (1989). Oxidative metabolism of cocaine: Comparison of brain and liver. *Proc. Soc. Exp. Biol. Med.* **190**, 7–13.

Braeckman, R. A., Audenaert, F., Willems, J. L., Belpaire, F. M., and Bogaert, M. G. (1983). Toxicokinetics and methyl parathion and parathion in the dog after intravenous and oral administration. *Arch. Toxicol.* **54**, 71–82.

Brimer, L., Gyrd-Hansen, N., and Rasmussen, F. (1994). Disposition of parathion after dermal application in pigs. *J. Vet. Pharmacol. Ther.* **17**, 304–308.

Brodeur, J., and DuBois, K. P. (1963). Comparison of acute toxicity of anticholinesterase insecticides to weanling and adult male rats. *Proc. Soc. Exp. Biol. Med.* **114**, 509–511.

Byczkowski, J. Z., Kinkead, E. R., Leahy, H. F., Randall, G. M., and Fisher, J. W. (1994). Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model. *Toxicol. Appl. Pharmacol.* **125**, 228–236.

Calabrese, E. J. (1991). Comparative metabolism: The principal cause of differential susceptibility to toxic and carcinogenic agents. *In* "Principles of Animal Extrapolation" (E. J. Calabrese, ed.), pp. 203–276. Lewis Publishers, Chelsea, MI.

Chambers, H. W. (1992). Organophosphorus compounds: An overview. *In* "Organophosphates—Chemistry, Fate and Effects" (J. E. Chambers and P. E. Levi, eds.), pp. 3–17. Academic Press, San Diego.

Chambers, J. E., and Chambers, H. W. (1989). Oxidative desulfation of chlorpyrifos, chlorpyrifos-methyl, and leptophos by rat brain and liver. *J. Biochem. Toxicol.* **4**, 201–203.

Chanda, S. M., Mortensen, S. R., Moser, V. C., and Padilla, S. (1997). Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: An *in vitro* and *in vivo* comparison. *Fundam. Appl. Toxicol.* **38**, 148–157.

Chandra *et al.* (1977).

Chester, G. (1993). Evaluation of agricultural worker exposure to and absorption of pesticides. *Occup. Hyg.* **37**, 509–523.

Clement, J. G. (1984). Role of aliesterase in organophosphate poisoning. *Fundam. Appl. Toxicol.* **4**, S96–S105.

Clewell, H. J., III (1995). The application of physiologically based pharmacokinetic modeling in human health risk assessment of hazardous substances. *Toxicol. Lett.* **79**, 207–217.

Clewell, H. J., III, and Andersen, M. E. (1996). Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk. *Toxicology* **111**, 315–329.

Corley, R. A., Mendrala, A. L., Smith, F. A., Staats, D. A., Gargas, M. L., Conolly, R. B., Andersen, M. E., and Reitz, R. H. (1990). Development of a physiologically based pharmacokinetic model for chloroform. *Toxicol. Appl. Pharmacol.* **103**, 512–527.

Costa, L. G., McDonald, B. E., Murphy, S. D., Omenn, G. S., Richter, R. J., Motulsky, A. G., and Furlong, C. E. (1990). Serum paraoxonase and its influence on paraoxon and chlorpyrifos-oxon toxicity in rats. *Toxicol. Appl. Pharmacol.* **103**, 66–76.

Drevenkar, V., Vasilic, Z., Stengl, B., Frobe, Z., and Rumenjak, V. (1993). Chlorpyrifos metabolites in serum and urine of poisoned persons. *Chem.-Biol. Interact.* **87**, 315–322.

Eckerson, H. W., Wyte, C. M., and La Du, B. N. (1983). The human serum paraoxonase/arylesterase polymorphism. *Am. J. Hum. Genet.* **35**, 1126–1138.

Ellenhorn, M. J., and Barceloux, D. G. (1988). "Medical Toxicology—Diagnosis and Treatment of Human Poisoning," pp. 1074–1075. Elsevier, New York.

Ellman, G. L., Courtney, K. D., Andres, V., Jr., and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**, 88–95.

El-Masri, H. A., Reardon, K. F., and Yang, R. S. H. (1997). Integrated approaches for the analysis of toxicologic interactions of chemical mixtures. *Crit. Rev. Toxicol.* **27**, 175–197.

Fisher, J. W., Whittaker, T. A., Taylor, D. H., Clewell, H. J., III, and Andersen, M. E. (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol. Appl. Pharmacol.* **102**, 497–513.

Fonnum, F., Sterri, S. H., Aas, P., and Johnsen, H. (1985). Carboxyesterases, importance for detoxification of organophosphorus anticholinesterases and trichothecenes. *Fundam. Appl. Toxicol.* **5**, S29–S38.

Franklin, C. A., Fenske, R. A., Greenhalgh, R., Mathieu, L., Denley, H. V., Leffingwell, J. T., and Spear, R. C. (1981). Correlation of urinary pesticide metabolite excretion with estimated dermal contact in the course of occupational exposure to guthion. *J. Toxicol. Environ. Health* **7**, 715–731.

Franklin, C. A., Muir, N. I., and Moody, R. P. (1986). The use of biological monitoring in the estimation of exposure during the application of pesticides. *Toxicol. Lett.* **33**, 127–136.

Frawley, J. P., Fuyat, H. N., Hagen, E. C., Blake, J. R., and Fitzhugh, O. G. (1957). Marked potentiation in mammalian toxicity from simultaneous ad-

ministration of two anticholinesterase compounds. *J. Pharmacol. Exp. Ther.* **121**, 96–106.

Frederick, C. B. (1995). Summary of panel discussion on the "Advantages/limitations/uncertainties in the use of physiologically based pharmacokinetic and pharmacodynamic models in hazard identification and risk assessment of toxic substances." *Toxicol. Lett.* **79**, 201–206.

Furlong, C. E., Richter, R. J., Seidel, S. L., and Motulsky, A. G. (1988). Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. *Am. J. Hum. Genet.* **43**, 230–238.

Gaines, T. B., and Linder, R. E. (1986). Acute toxicity of pesticides in adult and weanling rats. *Fundam. Appl. Toxicol.* **7**, 172–178.

Gargas, M. L., Burgess, R. J., Voisard, D. E., Cason, G. H., and Andersen, M. E. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* **97**, 87–99.

Gearhart, J. M., Jepson, G. W., Clewell, H. J., III, Andersen, M. E., and Conolly, R. B. (1990). Physiologically based pharmacokinetic and pharmacodynamic model for the inhibition of acetylcholinesterase by diisopropylfluorophosphate. *Toxicol. Appl. Pharmacol.* **106**, 295–310.

Gearhart, J. M., Jepson, G. W., Clewell, H. J., Andersen, M. E., and Conolly, R. (1994). Physiologically based pharmacokinetic model for the inhibition of acetylcholinesterase by organophosphate esters. *Environ. Health Perspect.* **102**, 51–59.

Geldmacher-von Mallinckrodt, M., Diepgen, T. L., Duhme, C., Hommel, G. (1983). A study of the polymorphism and ethnic distribution differences of human serum paraoxonase. *Am. J. Phys. Anthropol.* **62**, 235–241.

Griffin, P., Mason, H., Heywood, K., and Cocker, J. (1999). Oral and dermal absorption of chlorpyrifos: A human volunteer study. *Occup. Environ. Med.* **56**, 10–13.

Guengerich, F. P. (1977). Separation and purification of multiple forms of microsomal cytochrome P450. *J. Biol. Chem.* **252**, 3970–3979.

Harbison, R. D. (1975). Comparative toxicity of some selected pesticides in neonatal and adult rats. *Toxicol. Appl. Pharmacol.* **32**, 443–446.

Hayes, A. L., Wise, R. A., and Weir, F. W. (1980). Assessment of occupational exposure to organophosphates in pest control operators. *Am. Ind. Hyg. Assoc. J.* **41**, 568–575.

Hill, R. H., Jr., Head, S. L., Baker, S., Gregg, M., Shealy, D. B., Bailey, S. L., Williams, C. C., Sampson, E. J., and Needham, L. L. (1995). Pesticide residues in urine of adults living in the United States: Reference range concentrations. *Environ. Res.* **71**, 99–108.

Iverson, F., Grant, D. L., and Lacroix, J. (1975). Diazinon metabolism in the dog. *Bull. Environ. Contam. Toxicol.* **13**, 611–618.

Knaak, J. B., Al-Bayati, M. A., and Raabe, O. G. (1993). Physiologically based pharmacokinetic modeling to predict tissue dose and cholinesterase inhibition in workers exposed to organophosphorus and carbamate pesticides. *In* "Health Risk Assessment: Dermal and Inhalation Exposure and Absorption of Toxicants" (R. G. M. Wang, J. B. Knaak, and H. I. Maibach, eds.), pp. 3–29. CRC Press, Boca Raton, Fl.

Knaak, J. B., Al-Bayati, M., Raabe, O. G., and Blancato, J. N. (1990). *In vivo* percutaneous absorption studies in the rat: Pharmacokinetics and modeling of isofenphos absorption. *In* "Perdictions of Percutaneous Penetration" (R. Scott, R. Guy, and J. Hadgraft, eds.). IBC Technical Services, London.

Krishnan, K., and Andersen, M. E. (1994). Physiologically based pharmacokinetic modeling in toxicology. *In* "Principles and Methods of Toxicology" (A. Wallace Hayes, ed.), 3rd ed., pp. 149–188. Raven Press, New York.

Kump, D. F., Matulka, R. A., Edinboro, L. E., Poklis, A., and Holsapple, M. P. (1994). Disposition of cocaine and norcocaine in blood and tissues of $B_6C_3F_1$ mice. *J. Anal. Toxicol.* **18**, 342–345.

Lajtha, A., and Dunlop, D. (1981). Turnover of protein in nervous system. *Life Sci.* **28**, 755–767.

Lavy, T. L., Mattice, J. D., Massey, J. H., and Skulman, B. W. (1993). Measurements of year-long exposure to tree nursery workers using multiple pesticides. *Arch. Environ. Contam. Toxicol.* **24**, 123–144.

Leung, H. W., and Paustenbach, D. J. (1995). Physiologically based pharmacokinetic and pharmacodynamic modeling in health risk assessment and characterization of hazardous substances. *Toxicol. Lett.* **79**, 55–65.

Li, W. F., Costa, L. G., and Furlong, C. E. (1993). Serum paraoxonase status: A major factor in determining resistance to organophosphates. *J. Toxicol. Environ. Health* **40**, 337–346.

Li, W. F., Furlong, C. E., and Costa, L. G. (1995). Paraoxonase protects against chlorpyrifos toxicity in mice. *Toxicol. Lett.* **76**, 219–226.

Li, W.-F., Matthews, C., Disteche, C. M., Costa, L. G., and Furlong, C. E. (1997). Paraoxonase (PON1) gene in mice: Sequencing, chromosomal localization and developmental expression. *Pharmacogenetics* **7**, 137–144.

Loewenherz, C., Fenske, R. A., Simcox, N. J., Bellamy, B., and Kalman, D. (1997). Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington state. *Environ. Health Perspect.* **105**, 1344–1353.

Lotti, M., Moretto, A., Zoppellari, R., Dainese, R., Rizzuto, N., and Barusco, G. (1986). Inhibition of lymphocytic neropathy target esterase predicts the development of organophosphate-induced delayed neuropathy. *Arch. Toxicol.* **59**, 176–179.

Ma, T., and Chambers, J. E. (1994). Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. *Food Chem. Toxicol.* **32**, 763–767.

Main, A. R. (1956). The role of A-esteraseease in the acute toxicity of paraoxon, TEPP and parathion. *Can. J. Biochem.* **34**, 197–216.

Mason, H., and Wilson, K. (1999). Biological monitoring: The role of toxicokinetics and physiologically based pharmacokinetic modeling. *Am. Ind. Hyg. Assoc. J.* **60**, 237–242.

Mileson, B. E., Chambers, J. E., Chen, W. L., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., Gaylor, D. W., Kamernick, K., Hodgson, E., Karczmar, A. G., Padilla, S., Pope, C. N., Richardson, R. J., Saunders, D. R., Sheets, L. P., Sultatos, L. G., and Wallace, K. B. (1998). Common mechanism of toxicity: A case study of organophosphate pesticides. *Toxicol. Sci.* **41**, 8–20.

Miller, M. S., McCarver, D. G., Bell, D. A., Eaton, D. L., and Goldstein, J. A. (1997). Genetic polymorphisms in human drug metabolic enzymes. *Fundam. Appl. Toxicol.* **40**, 1–14.

Mortensen, S. R., Chanda, S. M., Hooper, M. J., and Padilla, S. (1996). Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. *J. Biochem. Toxicol.* **11**, 279–287.

Moser, V. C., and Padilla, S. (1998). Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. *Toxicol. Appl. Pharmacol.* **149**, 107–119.

Mücke, W., Alt, K. O., and Esser, H. O. (1970). Degradation of ^{14}C-labeled diazinon in the rat. *J. Agric. Food Chem.* **18**, 208–212.

Mueller, R. F., Homung, S., Furlong, C. E., Anderson, J., Giblett, E. R., and Motulsky, A. G. (1983). Plasma paraoxonase polymorphism: A new enzyme assay, population, family, biochemical and linkage studies. *Am. J. Hum. Genet.* **35**, 393–408.

Murphy, S. D. (1986). Toxic effects of pesticides. *In* "Casarett and Doull's Toxicology: The Basic Science of Poison" (C. D. Klaassen, M. O. Amdur, and J. Doull, eds.), 3rd ed., pp. 519–581. MacMillam Co., New York.

Neal, R. A. (1980). Microsomal metabolism of thiono-sulfur compounds, mechanisms and toxicological significance. *In* "Reviews in Biochemical Toxicology" (E. Hodgson, J. R. Bend, and R. M. Philpot, eds.), Vol. 2, pp. 131–172. Elsevier–North-Holland, New York.

Nielsen, P., Friis, C., Gyrd-Hansen, N., and Kraul, I. (1991). Disposition of parathion in neonatal and young pigs. *Pharmacol. Toxicol.* **68**, 233–237.

Nolan, R. J., Rick, D. L., Freshour, N. L., and Saunders, J. H. (1984). Chlorpyrifos: Pharmacokinetics in human volunteers. *Toxicol. Appl. Pharmacol.* **73**, 8–15.

O'Flaherty, E. J. (1991a). Physiologically based models for bone-seeking elements. I. Rat skeletal and bone growth. *Toxicol. Appl. Pharmacol.* **111**, 299–312.

O'Flaherty, E. J. (1991b). Physiologically based models for bone-seeking elements. II. Kinetics of lead disposition in rats. *Toxicol. Appl. Pharmacol.* **111**, 313–331.

O'Flaherty, E. J. (1993). Physiologically based models for bone-seeking elements. II. Kinetics of lead disposition in humans. *Toxicol. Appl. Pharmacol.* **118**, 16–29.

O'Flaherty, E. J. (1995). PBK modeling for metals: Examples with lead, uranium and chromium. *Toxicol. Lett.* **82/83**, 367–372.

O'Shaughnessy, J. A., and Sultatos, L. G. (1995). Interaction of ethanol and the organophosphorus insecticide parathion. *Biochem. Pharmacol.* **50**, 1925–1932.

Pelekis, M., and Krishnan, K. (1997). Assessing the relevance of rodent data on chemical interactions for health risk assessment purposes: A case study with dichloromethane–toluene mixture. *Regul. Toxicol. Pharmacol.* **25**, 79–86.

Pena-Egido, M. J., Rivas-Gonzalo, J. C., and Marino-Hernandez, E. L. (1988). Toxicokinetics of parathion in the rabbit. *Arch. Toxicol.* **61**, 196–200.

Pond, A. L., Chambers, H. W., and Chambers, J. E. (1995). Organophosphate detoxification potential of various rat tissues via A-esterase and aliesterase activity. *Toxicol. Lett.* **78**, 245–252.

Pope, C. N., and Liu, J. (1997). Age-related differences in sensitivity to organophosphorus pesticides. *Environ. Toxicol. Pharmacol.* **4**, 309–314.

Pope, C. N., Chakraborti, T. K., Chapman, M. L., Farrar, J. D., and Arthun, D. (1991). Comparison of the *in vivo* cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. *Toxicology* **68**, 51–61.

Purcell, K. J., Cason, G. H., Gargas, M. L., Andersen, M. E., and Travis, C. C. (1990). *In vivo* metabolic interactions of benzene and toluene. *Toxicol. Lett.* **52**, 141–152.

Renwick, A. G. (1994). Toxicokinetics—Pharmacokinetics in toxicology. *In* "Principles and Methods of Toxicology" (A. Wallace Hayes, ed.), 3rd ed., pp. 101–147. Raven Press, New York.

Richter, E. D., Kowalski, M., Leventhal, A., Grauer, F., Marzouk, J., Brenner, S., Shkolnik, I., Lerman, S., Zahavi, H., Bashari, A., Peretz, A., Kaplanski, H., Gruener, N., and Ben Ishai, P. (1992). Illness and excretion of organophosphate metabolites four months after household pest extermination. *Arch. Environ. Health* **47**, 135–138.

Sato, A., and Nakajima, T. (1979). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. *Br. J. Ind. Med.* **36**, 231–234.

Slob, W., Janssen, P. H., and van den Hof, J. M. (1997). Structural identifiability of PBPK models: Practical consequences for modeling strategies and study design. *Crit. Rev. Toxicol.* **27**, 261–272.

Srinivasan, R. S., Bourne, D. W. A., and Putcha, L. (1994). Application of physiologically based pharmacokinetic models for assessing drug disposition in space. *J. Clin. Pharmacol.* **34**, 692–698.

Sultatos, L. G. (1988). Factors affecting the hepatic biotransformation of the phosphorothioate pesticide chlorpyrifos. *Toxicology* **51**, 191–200.

Sultatos, L. G. (1990). A physiologically based pharmacokinetic model of parathion based on chemical-specific parameters determined *in vitro*. *J. Am. Coll. Toxicol.* **9**, 611–619.

Sultatos, L. G. (1994). Mammalian toxicology of organophosphorus pesticides. *J. Toxicol. Environ. Health* **43**, 271–289.

Sultatos, L. G., Basker, K. M., Shao, M., and Murphy, S. D. (1984). The interaction of the phosphorothioate insecticides chlorpyrifos and parathion and their oxygen analogues with bovine serum albumin. *Mol. Pharmacol.* **26**, 99–104.

Sultatos, L. G., Shao, M., and Murphy, S. D. (1984). The role of hepatic biotransformation in mediating the acute toxicity of the phosphorothionate insecticide chlorpyrifos. *Toxicol. Appl. Pharmacol.* **73**, 60–68.

Sundberg, J., Jonsson, S., Karlsson, M. O., Palminger Hallen, I., and Oskarson, A. (1998). Kinetics of methylmercury and inorganic mercury in lactating and nonlactating mice. *Toxicol. Appl. Pharmacol.* **151**, 319–329.

Tardif, R., Charest-Tardif, G., Brodeur, J. and Krishnan, K. (1997). Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans. *Toxicol. Appl. Pharmacol.* **144**, 120–134.

Tardif, R., Lapare, S., Charest-Tardif, G., Brodeur, J., and Krishnan, K. (1995). Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. *Risk Anal.* **15**, 335–342.

Tardif, R., Lapare, S., Krishnan, K., and Brodeur, J. (1993). Physiologically based modeling of the toxicokinetic interaction between toluene and *m*-xylene in the rat. *Toxicol. Appl. Pharmacol.* **120**, 266–273.

Timchalk, C., and Nolan, R. J. (1998). Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of chlorpyrifos and its oxon metabolite in the rat. *Toxicol. Sci.* **42**, 693.

Tomokuni, K., Hasegawa, T., Hirai, Y., and Koga, N. (1985). The tissue distribution of diazinon and the inhibition of blood cholinesterase activities in rats and mice receiving a single intraperitoneal dose of diazinon. *Toxicology* **37**, 91–98.

Vale, J. A. (1998). Toxicokinetic and toxicodynamic aspects of organophosphate (OP) insecticide poisoning. *Toxicol. Lett.* **102–103**, 649–652.

Vale, J. A., and Scott, G. W. (1974). Organophosphate poisoning. *Guy's Hosp. Gazette* **123**, 12–25.

Vasilic, Z., Drevenkar, V., Rumenjak, V., Stengl, B., and Frobe, Z. (1992). Urinary elimination of diethylphosphorus metabolites in persons poisoned by quinalphos or chlorpyrifos. *Arch. Environ. Contam. Toxicol.* **22**, 351–357.

Waldron-Lechner, D., and Abdel-Rahman, M. S. (1986). Kinetics of carbaryl and malathion in combination in the rat. *J. Toxicol. Environ. Health* **18**, 241–256.

Wester, R. C., and Maibach, H. I. (1983). Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. *Drug Metab. Rev.* **14**, 169–205.

Wester, R. C., Maibach, H. I., Bucks, D. A. W., and Guy, R. H. (1983). Malathion percutaneous absorption after repeated administration to man. *Toxicol. Appl. Pharmacol.* **68**, 116–119.

Wester, R. C., Maibach, H. I., Melendres, J., Sedik, L., Knaak, J., and Wang, R. (1992). *In vivo* and *in vitro* percutaneous absorption and skin evaporation of isofenphos in man. *Fundam. Appl. Pharmacol.* **19**, 521–526.

Wester, R. C., Sedik, L., Melendres, J., Logan, F., Maibach, H. I., and Russell, I. (1993). Percutaneous absorption of diazinon in humans. *Food Chem. Toxicol.* **31**, 569–572.

Wilks, M. F., and Woollen, B. H. (1994). Human volunteer studies with non-pharmaceutical chemicals: Metabolism and pharmacokinetic studies. *Hum. Exp. Toxicol.* **13**, 383–392.

Woollen, B. H. (1993). Biological monitoring for pesticide absorption. *Occup. Hyg.* **37**, 525–540.

Wu, H. X., Evreux-Gros, C., Descottes, J. (1996). Diazinon toxicokinetics, tissue distribution and anticholinesterase activity in the rat. *Biomed. Environ. Sci.* **9**, 359–369.