Organophosphorus Insecticide Pharmacokinetics

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66.1 BACKGROUND

In this chapter an overview will be presented of the pharmacokinetic principles that are of major importance in understanding the toxicology of organophosphorus (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 organophosphorus 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 organophosphorus insecticides are the phosphorothionates, phosphorodithioates, and phosphoroamidothiolates (Chambers, 1992; Mileson *et al.*, 1998). As an example, phosphorothionate insecticides such as chlorpyrifos, parathion, and diazinon are weak inhibitors of AChE, but once they undergo metabolic activation (desulfation) to their corresponding oxygen analogues (oxon) they become extremely potent. 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.*, 1998; Sultatos, 1994). The toxic potency is dependent upon the balance between a delivered dose to the target site and the rates of bioactivation and/or detoxification (Calabrese, 1991). The pharmacokinetics and biochemical interactions between organophosphates and AChE and the toxicological implications of AChE inhibition are well understood. To further illustrate this point, a diagram relating insecticide toxicity with pharmacokinetic disposition and the formation of key metabolites is presented in Figures 66.1 and 66.2. 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 organophosphates.

Organophosphorus insecticides, like most chemical contaminants, are absorbed into the body, and, based on the detection of low levels of metabolites in urine within humans, there is good evidence for widespread although low-level exposures (Hill *et al.*, 1995; Aprea *et al.*, 1999). 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 organophosphorus insecticides is associated with acute poisoning resulting in significantly higher blood, tissue, and urine concentrations of relevant metabolites (Drevenkar *et al.*, 1993). Dermal represents a potential exposure route during the mixing, loading, and application of 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 exposures associated with chemical drift (Vale and Scott, 1974). Once the organophosphate arrives at a portal of entry it is available for absorption, and, based on the bioavailability for a given insecticide and exposure route, a systemic dose of the parent compound (Figure 66.2, #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 occurs within the liver (Sultatos *et al.*, 1984; Sultatos, 1988). As previously mentioned, phosphorothionates like diazinon do not directly inhibit AChE, but must first be metabolized to the corresponding oxygen analog (oxon; Figure 66.1, #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 (Chamber and Chambers, 1989; Guengerich, 1977). In addition, oxidative dearylation of the parent compound forming both 2-isopropyl-4-methyl-6-hydroxypyrimidine

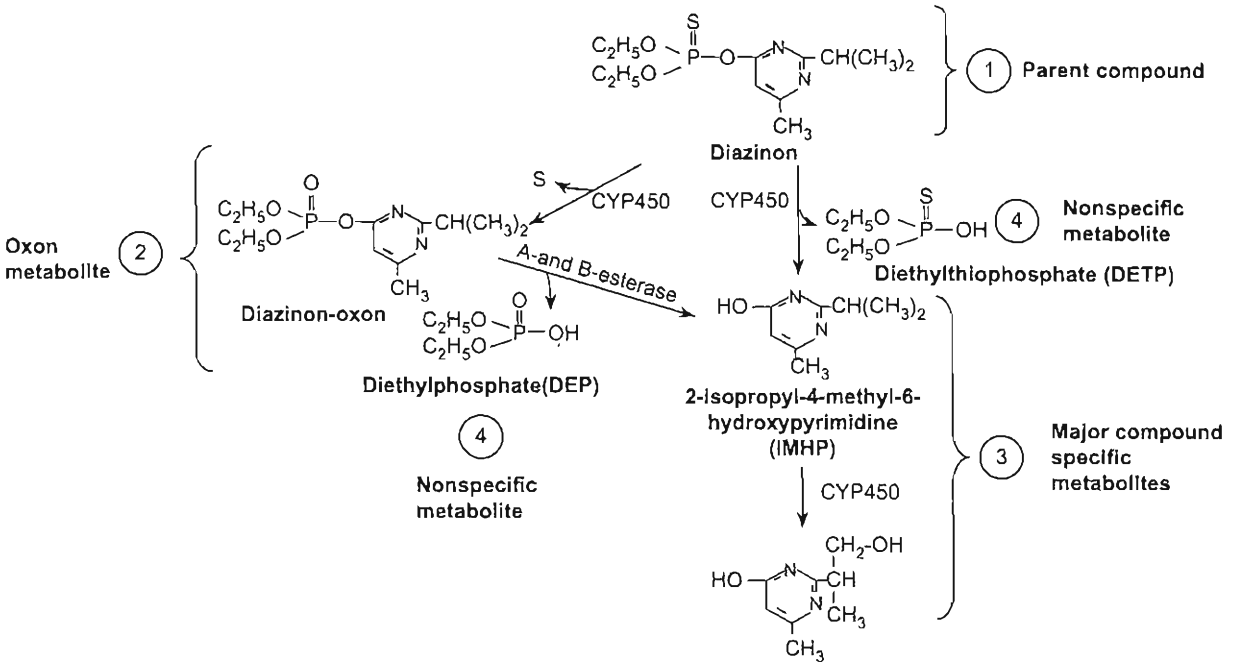


FIGURE 66.1 Metabolic scheme for the metabolism of the organophosphorus (OP) insecticide diazinon. CYP450, cytochrome P450.

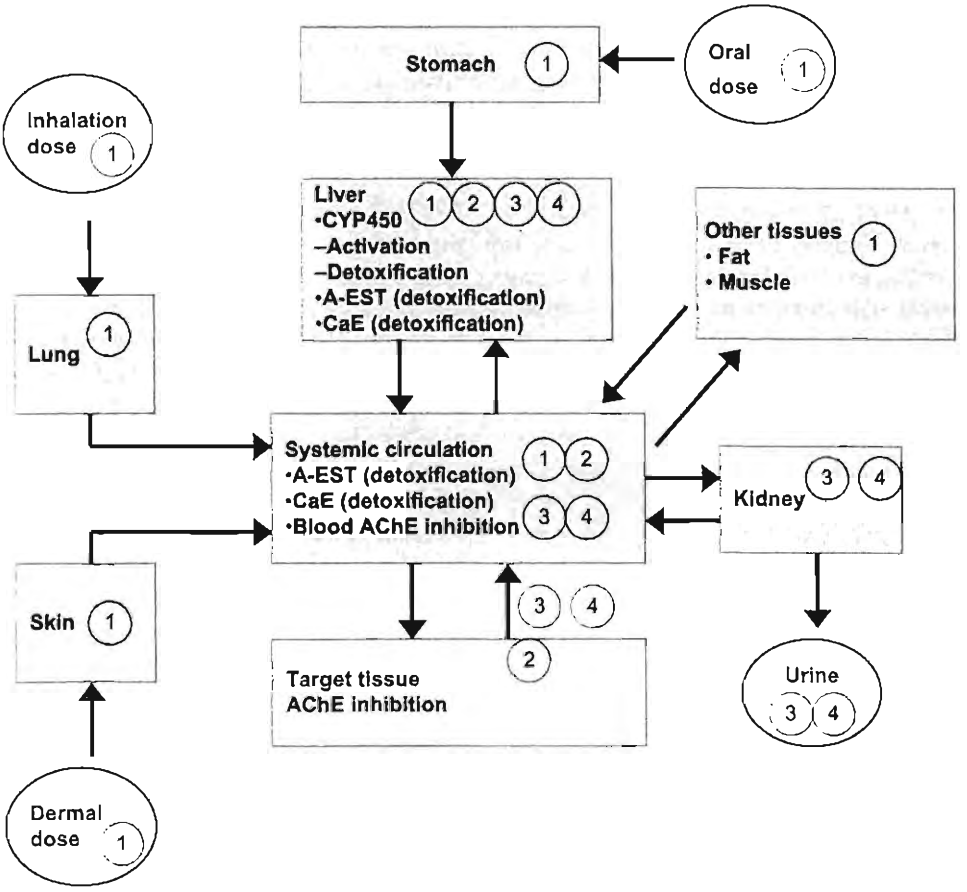


FIGURE 66.2 Compartmental flow diagram illustrating the critical tissue compartments associated with absorption, distribution, metabolism, and excretion of organophosphorus (OP) insecticides. The circled numbers (1–4) correspond to the parent compound and major metabolic products associated with metabolism of diazinon (see Figure 66.1) that are most likely found within each compartment. CYP450, cytochrome P450; A-EST, A-esterase; CaE, carboxylesterase; AChE, acetylcholinesterase.

(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 critical biotransformation steps required for toxicity (Neal, 1980). Differences in the ratio of activation to detoxification are associated with chemical-, species-, gender-, and age-dependent sensitivities to organophosphorus insecticides (Ma and Chambers, 1994). Hepatic and extrahepatic (i.e., blood and tissue) A-esterase (PON1) can effectively metabolize the oxon-metabolite (#2) forming IMHP (#3) and diethylphosphate (DEP, #4) metabolites. Likewise, B-esterases such as carboxylesterase (CaE) and butyrylcholinesterase (BuChE) 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 (Chandra *et al.*, 1997; Clement, 1984). It is likewise clear from both tissue distribution and partitioning studies that phosphothionate pesticides 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 organophosphorus insecticides, including:

1. Application of pharmacokinetics to understand the overall disposition and clearance
2. Development and application of pharmacokinetic models for quantitative biological monitoring to assess 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 to address health concerns associated with organophosphorus insecticides, several examples of these types of pharmacokinetic studies with these insecticides will be used to illustrate both their utility as well as their limitations.

66.2 PHARMACOKINETIC PRINCIPLES OF IMPORTANCE TO ORGANOPHOSPHORUS INSECTICIDES

Pharmacokinetics is 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 as well as 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).

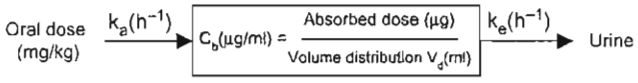
Toxicology studies are designed to provide a quantitative assessment of toxicity based on what the chemical agent does to the test animals. In contrast, pharmacokinetics focuses on what the animal does to the chemical. Clearly, toxicity and pharmacokinetics are integrally related since the extent of absorption, retention, metabolic activation, or detoxification is ultimately responsible for delivering a dose to a target tissue resulting in observed effects. Pharmacokinetics represents 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 organophosphorus insecticides since they share a common mode of action through their capability to inhibit AChE activity (Mileson *et al.*, 1998). Pharmacokinetic strategies for quantitating dosimetry can be developed to measure the parent compound and 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 organophosphorus chemicals in multiple species (Brimer *et al.*, 1994; Gearhart *et al.*, 1990; Pena-Egido, 1988; Poet *et al.*, 2004; Sultatos, 1990; Timchalk *et al.*, 2002a,b, 2005, 2006, 2007a,b; Timchalk and Poet, 2008; Tomokuni *et al.*, 1985; Wu *et al.*, 1996).

66.2.1 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 consider the organism as a single or multicompartment homogenous 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 a simple well-mixed single compartment 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 organophosphorus insecticides and their major metabolites (Braeckman *et al.*, 1983; Drevenkar *et al.*, 1993; Nolan *et al.*, 1984; Timchalk *et al.*, 2007b; 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 organophosphorus insecticide chlorpyrifos in human volunteers. A diagram of this single compartment model is illustrated in Figure 66.3. In this model the blood TCP concentration and urinary excretion data were simultaneously fit to a single compartment model using equations 1 and 2. Absorption (k_a) and elimination (k_e) are handled as first-order processes, and the blood TCP concentration is represented by C_b, while F and V_d represent fractional absorption and the volume of distribution, respectively. To develop this model, male volunteers were orally administered a 0.5 mg chlorpyrifos/kg of body weight dose 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 66.1 and Figure 66.4. The model provides an excellent fit of the experimental data, and based on the model parameters it was determined that ~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) has suggested that blood TCP concentration and/or urinary excretion rate could be utilized to quantify the amount of chlorpyrifos absorbed under actual use conditions.



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

$$\text{Urinary excretion rate } (\mu g/h) = C_b \times k_e \times V_d \times \text{body weight} \quad (2)$$

FIGURE 66.3 Single compartment model used to describe the blood and urine time course of 3,5,6-trichloropyridinol (TCP), a major metabolite of the organophosphorus (OP) insecticide chlorpyrifos (CPF) (equations 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 models are limited in their capability to extrapolate across dose, 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).

66.2.2 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

TABLE 66.1 Selected Model Parameters Describing Blood Concentrations and Urinary Excretion of 3,5,6-Trichloropyridinol (TCP) by Individual Volunteers following Oral Administration of the Organophosphate (OP) 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 dose absorbed (%)	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 ± S.D.	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., *1980).*

metals, and pesticides, including organophosphorus insecticides (Andersen *et al.*, 1987; Corley *et al.*, 1990; Gearhart *et al.*, 1990; O'Flaherty, 1995; Sultatos, 1990; Timchalk *et al.*, 2002a). 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).

With regards to the application of PBPK modeling to organophosphorus insecticides, 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 organophosphorus insecticides. A diagram of the PBPK and PD model for diisopropylfluorophosphate in rats is illustrated in Figures 66.5 and 66.6. 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). Since this organophosphorus-ester does not require metabolic activation, like thionophosphate insecticides, the hydrolysis of diisopropylfluorophosphate by blood and tissue A-esterase (PON1) is a major factor in determining the protection against AChE inhibition.

Diisopropylfluorophosphate binds to and inhibits B-esterases including AChE, BuChE, and CaE. Although binding to AChE is associated with acute neurotoxicity, the binding to BuChE 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, diaphragm), those containing high A-esterase (PON1) activity (i.e., liver, kidney, and blood), and a fat compartment having the highest tissue/blood partitioning, and the remaining tissues were 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). The generalized mass balance differential equations for calculating diisopropylfluorophosphate tissue concentration and AChE tissue inhibition are also presented in Figures 66.5 and 66.6. 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 (PON1) and inhibition of B-esterases (AChE, BuChE and CaE). 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 BuChE activity.

The PBPK/PD model developed by Gearhart *et al.* (1990) was also used as a framework for development of a model for the organophosphorus insecticide chlorpyrifos and diazinon (Poet *et al.*, 2004; Timchalk *et al.*, 2002a). The

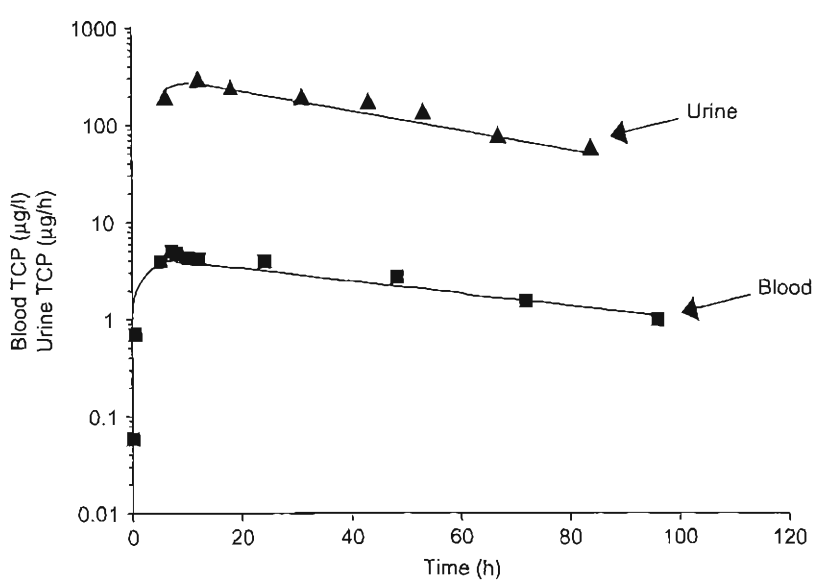


FIGURE 66.4 Time course of 3,5,6-trichloropyridinol (TCP) in the blood and urine of male volunteers orally administered 0.5 mg chlorpyrifos (CPF)/kg of body weight (adapted from Nolan *et al.*, 1984).

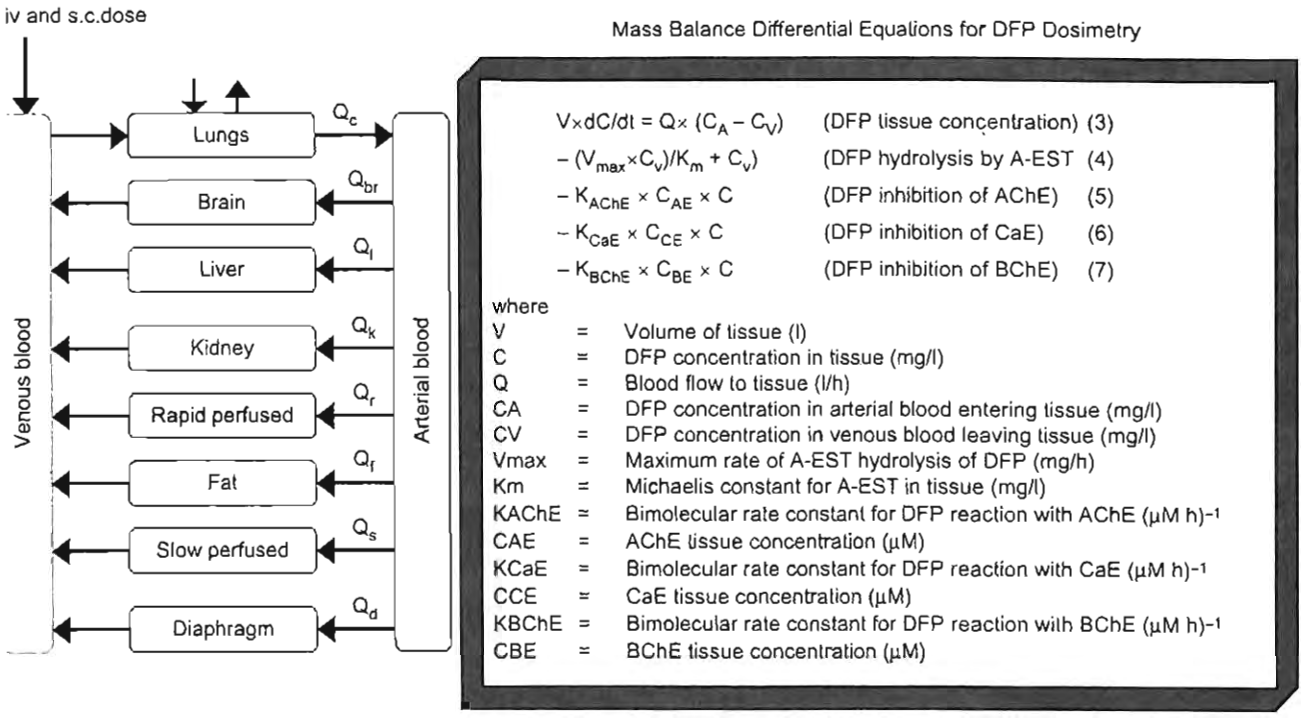


FIGURE 66.5 Physiologically-based pharmacokinetic (PBPK) model structure and mass balance differential equations describing the distribution of diisopropylfluorophosphate (DFP) in the rat (adapted from Gearhart *et al.*, 1990).

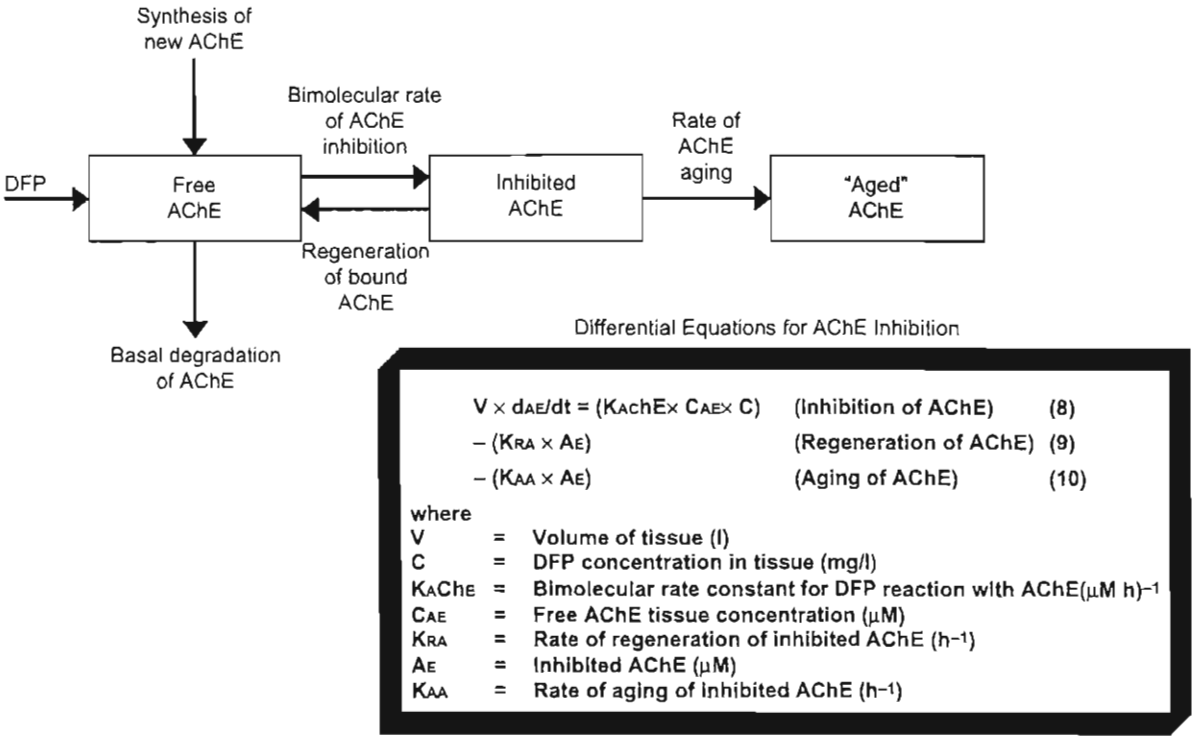


FIGURE 66.6 Pharmacodynamic (PD) model structure and mass balance differential equations describing the inhibition of acetylcholinesterase (AChE) by diisopropylfluorophosphate (DFP) in the rat (adapted from Gearhart *et al.*, 1990).

key metabolites of chlorpyrifos are illustrated in Figure 66.7. Chlorpyrifos is a phosphorothionate insecticide like diazinon; therefore, they have similar metabolic activation and detoxification reactions (see Figure 66.1). Specifically, chlorpyrifos undergoes metabolic desulfuration (CYP450) to form the neurotoxic metabolite chlorpyrifos-oxon or dearylation to form 3,5,6-trichloro-2-pyridinol (TCP). A diagram of the PBPK/PD model structure for chlorpyrifos

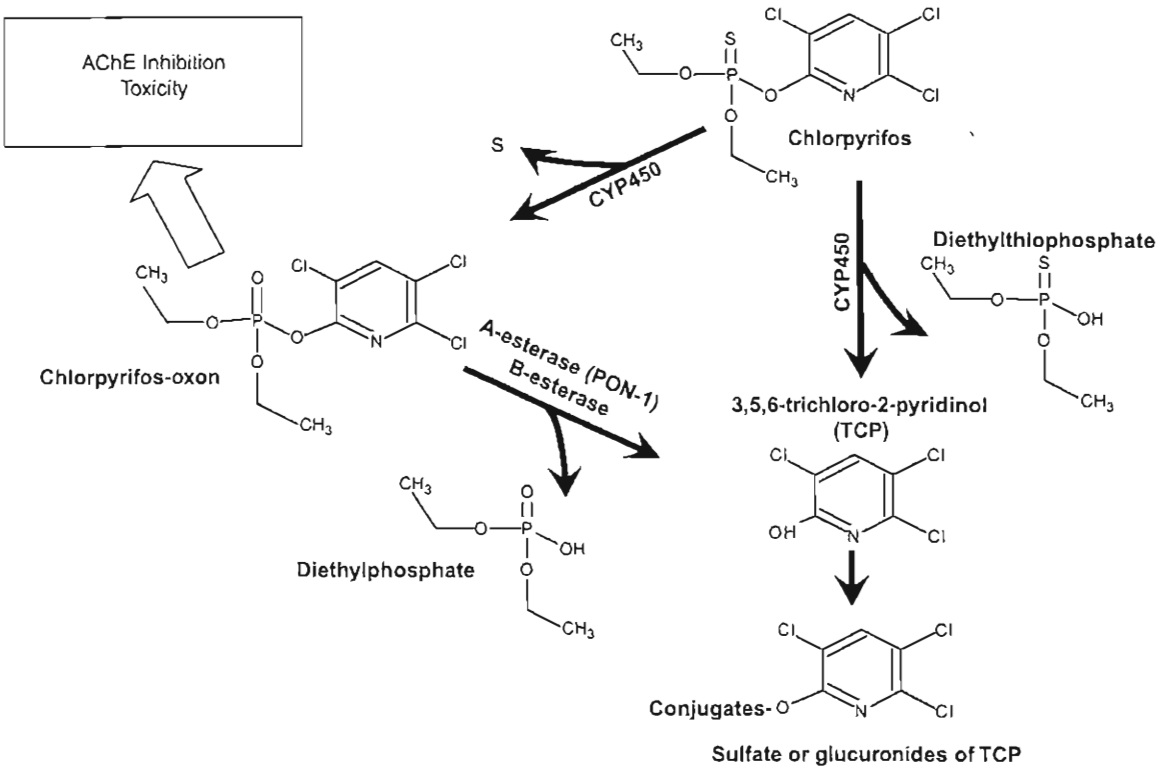


FIGURE 66.7 Metabolic scheme for the organophosphorus insecticide chlorpyrifos (CPF).

and chlorpyrifos-oxon is illustrated in Figure 66.8. The major differences between the DFP and chlorpyrifos models is that for chlorpyrifos the model included a PBPK and PD model code for chlorpyrifos-oxon and a compartment model to account for TCP pharmacokinetics. Likewise, metabolic parameters, partition coefficients, and inhibition constants were specifically determined for chlorpyrifos and chlorpyrifos-oxon and used in the model to simulate the pharmacokinetic and pharmacodynamic response in both rats and humans. The capability of the model to simulate both chlorpyrifos and chlorpyrifos-oxon tissue dosimetry and cholinesterase inhibition is illustrated in Figures 66.9 and 66.10, in rats that were administered a range of single oral doses of chlorpyrifos (0.5–100 mg/kg of body weight).

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). Secondly, 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 a panel of expert scientist 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).

66.3 PHARMACOKINETIC APPROACHES APPLIED TO ORGANOPHOSPHORUS INSECTICIDES

66.3.1 Application of Pharmacokinetics to Understand the Overall Disposition and Clearance of Organophosphorus 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 is noted in Tables 66.2 and 66.3, no single study provides all the pertinent information; yet collectively they provide a consistent qualitative picture of the overall pharmacokinetics of these insecticides.

The bioavailability of organophosphorus 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 area under the curve (AUC) indicated that 1–29% of the orally administered parathion was bioavailable. The authors suggest that the low systemic oral bioavailability of parathion is primarily

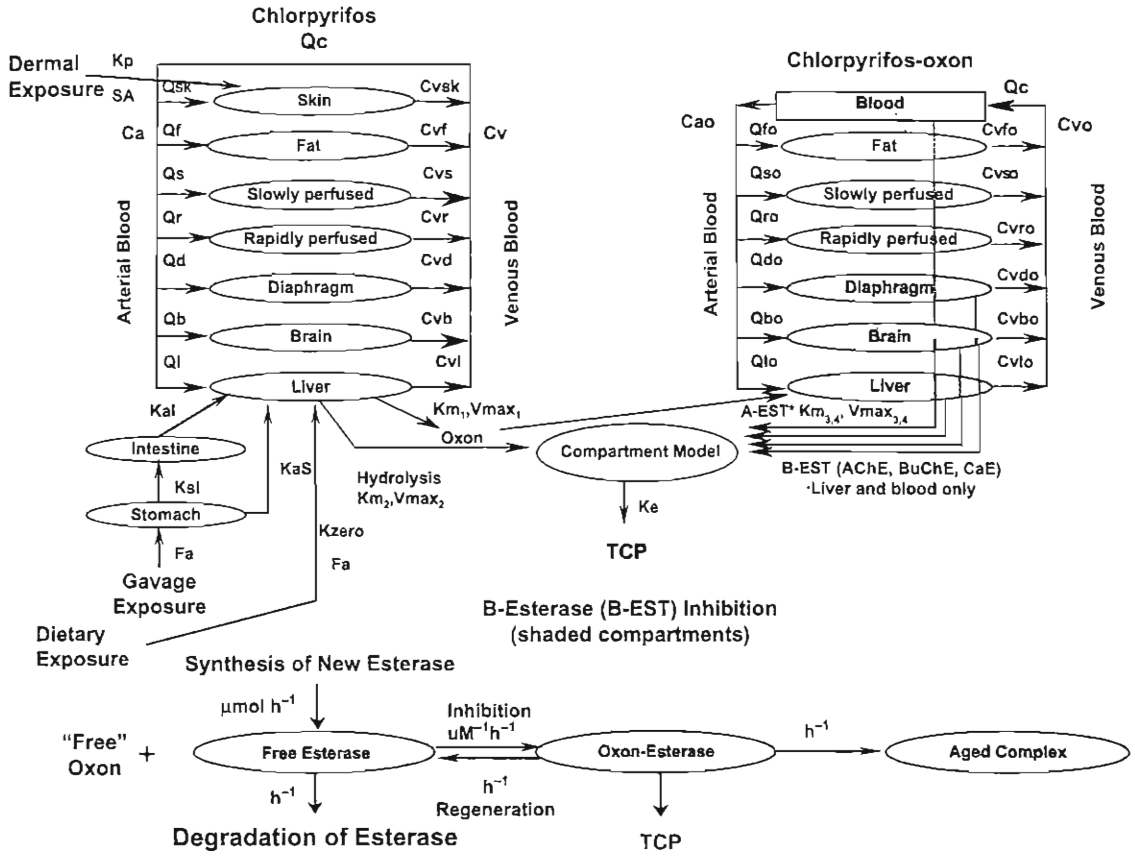


FIGURE 66.8 Physiologically-based pharmacokinetic and pharmacodynamic model used to describe the disposition of the parent insecticide chlorpyrifos (CPF), its oxon metabolite (CPF-oxon), trichloropyridinol (TCP), and *B-est* inhibition in rats and humans following oral (gavage, dietary) and dermal exposures. The shaded tissue compartments indicate organs in which *B-est* (AChE, BuChE, and CaE) enzyme activity is described. Model parameter definitions: Qc, cardiac output (l/h); Q_i, blood flow to "i" tissue (l/h), Ca, arterial blood concentration (μmol/l); Cao, arterial blood concentration of oxon (μmol/l); Cv, pooled venous blood concentration (μmol/l); Cv_i, venous blood concentration draining "i" tissue (μmol/l); Cv_io, venous blood concentration of oxon draining "i" tissue (μmol/l); SA, surface area of skin exposed (cm^2); K_p, skin permeability coefficient (cm/h); K_{zero}, zero order (μmol/h) rate of absorption from diet; Fa, fractional absorption (%); KaS and Kal, first-order rate constants for absorption from compartments 1 and 2 (per h); KsI, first-order rate constant for transfer from compartments 1 and 2 (per h); Ke, first-order rate constant for elimination of metabolite from compartment 3; $K_{m(1-4)}$, Michaelis constant for saturable processes (μmol/l); $V_{max(1-4)}$, maximum velocity for saturable process (μmol/h).

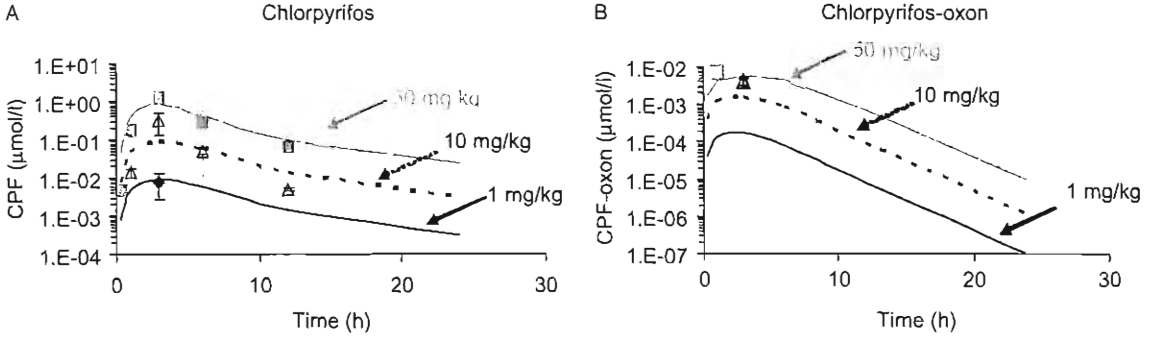


FIGURE 66.9 PBPK/PD model simulations of (A) chlorpyrifos (CPF) and (B) CPF-oxon blood concentrations in adult rats following single oral gavage doses of 1, 10, or 50 mg CPF/kg of body weight. The lines represent the model simulations utilizing the age-dependent PBPK/PD model (experimental data were obtained and adapted from Timchalk *et al.*, 2002 and 2007, respectively, with permission).

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 Figure 66.11. The results suggest that following oral administration absorption is rapid (absorption $t_{1/2}$ = 2.6 h), with

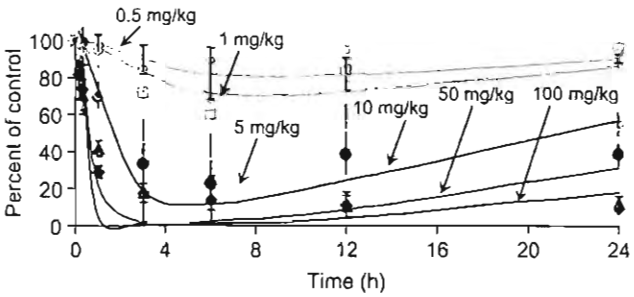


FIGURE 66.10 Experimental data (symbols) and simulations (lines) from the inhibition of plasma cholinesterase in rats administered chlorpyrifos (CPF) by oral gavage at dose levels of 0.5 (open circle), 1 (open square), 5 (open diamond), 10 (filled circle), 50 (filled triangle), and 100 mg/kg (filled diamond). The data represent the mean ± SD of 4 animals per treatment (from Timchalk *et al.*, 2002, with permission).

peak plasma concentrations of diazinon being attained within 2 h postdosing, yet a comparison of AUCs, when corrected for administered dose, indicates that only ~35% of the oral dose was 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). A rapid oral absorption ($t_{1/2}$ = 0.02 h) and lower oral bioavailability (~68%) were also demonstrated in a study where 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 organophosphorus insecticides will be quite low (Brimer *et al.*, 1994).

Once these pesticides have been absorbed, systemic distribution throughout the body tissues is rapid (Vale, 1998). For example, a high volume of distribution was observed ranging from 3–14 and 20–23 l/kg, in several different species administered parathion or diazinon, respectively (see Table 66.2). A cross-species comparison of the tissue distribution data following parathion or diazinon exposure is consistent with the large volume of distribution and suggests that the pesticide tissue concentration follows the order of kidney > liver > lung/muscle/heart > brain (see Table 66.3). Phosphorothioates like 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 partitioning coefficients (PCs) for both parathion and the toxic metabolite paraoxon (see Table 66.3). 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 is seen in Table 66.2, 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 ~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 that plasma binding in determining the overall disposition and clearance of organophosphorus insecticides (Braeckman *et al.*, 1983).

Although the 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 compounds [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 clearance for both parathion and diazinon is quite fast and comparable, ranging from 4 to 6.6 l/h/kg, and is consistent with the rapid blood/plasma terminal phase half-life (2.5–5 h) (see Table 66.2).

As previously indicated, comparative species pharmacokinetic analysis is useful for understanding the *in vivo* behavior of insecticides. Although generalization to all organophosphorus 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 insecticides are extensively bound to plasma proteins, they are equally well distributed throughout the body's tissues, and the parent phosphothioates 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 metabolites.

66.3.2 Development of Pharmacokinetic Models for Quantitative Biological Monitoring to Assess Organophosphorus 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. The 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 since 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

TABLE 66.2 Selected Model Parameters Describing Blood Concentration Pharmacokinetics of Parent Compounds in Various Species Following Exposure to the Organophosphate (OP) Insecticides Parathion and Diazinon

		Absorption/bioavailability kinetics			Distribution kinetics		Elimination kinetics			
							Two-compartment model			
Species	Dose (mg/kg) Route	Bioavailability (%)	Absorption $t_{1/2}$ (h)	Hepatic extraction (%)	Volume distribution Vd_{ss} (l/kg)	Protein binding (%)	$t_{1/2}\ \alpha$ (h)	$t_{1/2}\ \beta$ (h)	Elimination k_e $t_{1/2}$ (h)	Clearance Cl (l/h/kg)
Rabbit[b]	1.5 mg/kg iv	100	N/A	—	14.24 ± 6.34	—	—	5.08 ± 3.06	—	3.99 ± 1.13
Rabbit[b]	3 mg/kg oral	68[a]	.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

(1) *Estimated by comparing oral and iv AUC after adjusting for dose.*
(2) *Data were extracted from the following sources:* [b]*Pena-Egido* et al., *1988;* [c]*Nielsen* et al., *1991;* [d]*Brimer* et al., *1994;* [e]*Iverson* et al., *1975;* [f]*Wu* et al., *1996.*
(3) *N/A, not applicable.*

TABLE 66.3 Tissue Concentration, Tissue Plasma Ratio, and Partition (PC) Coefficients Following Exposure to the Organophosphate (OP) 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	
Tissues	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.*

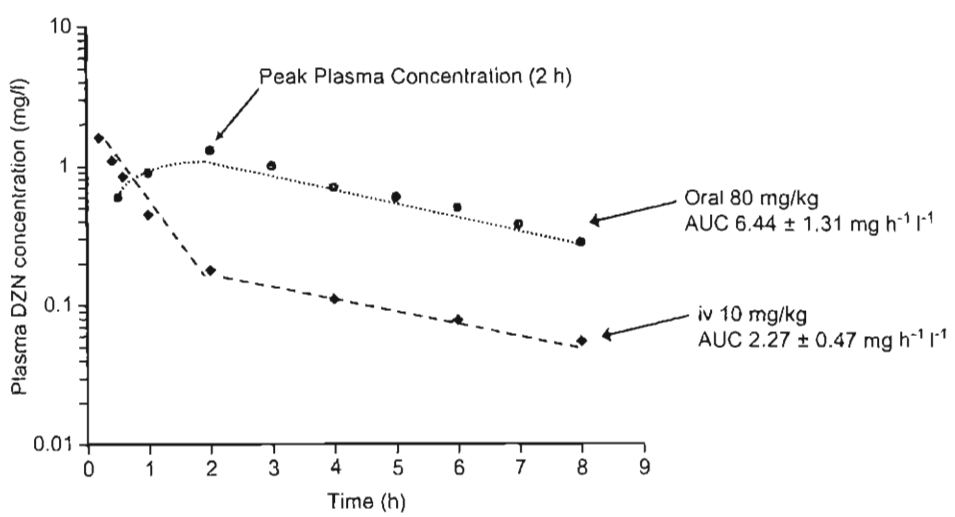
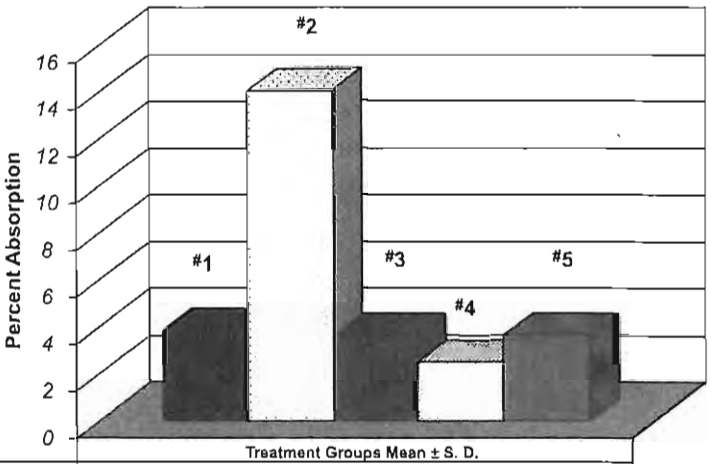


FIGURE 66.11 Plasma time course of diazinon (DZN) in rats following intravenous (iv) and oral administration of 10 and 80 mg DZN/kg of body weight, respectively (data extracted from Wu *et al.*, 1996).

pharmacokinetic studies conducted under controlled conditions are of vital importance (Wilks and Woollen, 1994; Woollen, 1993).

Both occupational and environmental exposure to organphosphorus insecticides is 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 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



Diazinon (in vivo) Wester et al., 1993 (#1)	3.85 ± 2.16
Diazinon (in vitro) Wester et al., 1993 (#2)	14.1 ± 9.2
Isofenphos (in vivo). Wester et al., 1992 (#3)	3.64 ± 0.48
Isofenphos (in vitro). Wester et al., 1992 (#4)	2.5 ± 2.0
Malathion (in vivo) Wester et al., 1983 (#5)	3.53 ± 1.0

FIGURE 66.12 Summary of human dermal penetration (*in vivo/in vitro*) for the organophosphate (OP) insecticides diazinon, isofenphos, and malathion.

obtained from cadavers (Wester *et al.*, 1983a, 1992, 1993). A summary of the percent absorption following *in vivo* and *in vitro* dermal exposure to the insecticides diazinon, isofenphos, and malathion is illustrated in Figure 66.12.

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 insecticide 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. Secondly, *in vitro* percutaneous absorption was determined using a glass flow-through penetration cell in which the percent absorption though human cadaver skin was determined by the amount of radiotracer that transferred into the receptor fluid. Finally, to calculate the *in vivo* percent absorption, rhesus monkeys were given a ^{14}C-labeled pesticide as an iv dose. The percent 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 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 percent absorption as determined *in vitro* was likewise comparable for isofenphos (3.64% ± 0.48), but slightly higher and considerably more variable for diazinon (14.1% ± 9.2). Percutaneous absorption studies conducted in humans are of particular importance since it is known that dermal absorption in animals, such as the rat, is often greater than in humans (Wester and Maibach, 1983b). 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 that the results seen in human volunteers. The major limitation associated with the experimental design of Wester *et al.* (1983a, 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 organophosphorus insecticides 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 66.4. 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, respectively, for TCP and dialkylphosphate. 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%, suggesting limited dermal absorption of chlorpyrifos. Nolan *et al.* (1984) reported an elimination half-life of

TABLE 66.4 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 dose absorbed (%)	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

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

26.9 h following oral administration, whereas Griffin *et al.* (1999) reported half-lives of 15.5 and 30 h for dialkylphosphate following an 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 organophosphate insecticide exposure. Nolan *et al.* (1984) developed a one-compartment pharmacokinetic model having the same volume of distribution and elimination rate constant to describe blood and urinary TCP kinetics following oral and dermal exposure to chlorpyrifos (see Figure 66.3). Similarly, the quantitative measurement of urinary dialkylphosphate is increasingly being used as a nonspecific biomarker for organophosphorus pesticide exposures (Griffin *et al.*, 1999). Although dialkyl phosphate and TCP have been routinely utilized as biomarkers for insecticide exposure, it is important to acknowledge that organophosphorus pesticides can undergo environmental degradation to form these same chemicals. In this regard, Lu *et al.* (2005) reported the detection of the breakdown product dialkylphosphate in fruit juices, and Morgan *et al.* (2005) noted higher concentrations (12–29×) of the chlorpyrifos metabolite TCP relative to chlorpyrifos in solid food samples obtained from homes and day care centers; higher dietary exposures to TCP may be a confounding factor when attempting to assess dietary exposure to chlorpyrifos. Hence, due to the environmental stability of the dialkylphosphate and TCP, recent research has questioned whether total urinary metabolite levels may be reflective of not only an individual's contact with the parent pesticide, but also exposure with intact metabolites present in the environment (Barr *et al.*, 2004; Bradman *et al.*, 2005; Duggan *et al.*, 2003; Lu *et al.*, 2005). Thus, measured urinary organophosphate metabolite levels may represent an exaggerated indicator of an individual's exposure to the parent insecticide (Duggan *et al.*, 2003). Nonetheless, the development of pharmacokinetic models that are capable of describing the uptake, distribution, and elimination of insecticides based on the quantitation of major degradation metabolites represents an extremely useful and simple approach for exposure biomonitoring.

66.3.3 The Application of Pharmacokinetics for Quantifying Exposure to Organophosphorus Insecticides

The ability to more accurately quantitate human exposure to 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, as an integral component within cross-sectional epidemiology studies to evaluate secondary exposures, 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

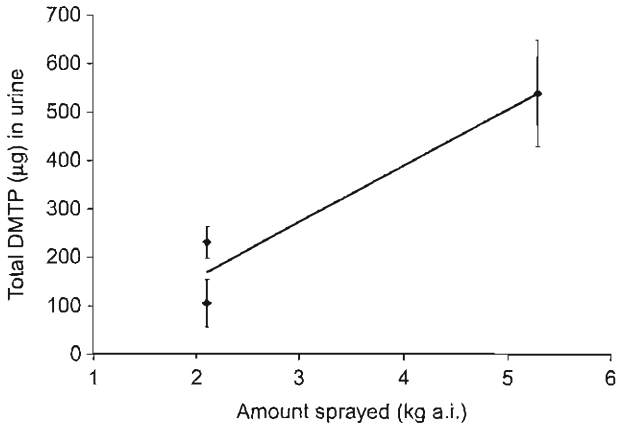


FIGURE 66.13 Relationship between the amount of alkyl phosphate (dimethylthiophosphate; DMTP) metabolite in urine of workers and the amount of active ingredient (a.i.) sprayed (data obtained from Franklin *et al.*, 1981).

in the ambient air. However, since airborne concentrations may not be linearly correlated with absorption, this approach does not provide an accurate assessment of 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 insecticide azinphos-methyl (guthion) utilizing both external personal monitoring and urinary biomonitoring of alkylphosphate metabolites. 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 with the amount of active ingredient sprayed. This relationship is illustrated in Figure 66.13, showing that the amount of alkylphosphate metabolite excreted in the urine increases with increasing amounts of active ingredient.

Since agricultural workers routinely apply numerous pesticides and are often sequentially exposed to insecticides within a relatively short time span, a number of exposure studies have been conducted to evaluate mixed 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 insecticides vaponite, diazinon, and chlorpyrifos. Worker biomonitoring was based on blood cholinesterase 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 exposures, but did not provide adequate information on the degree to which these insecticides were absorbed. The urinary alkyl phosphate levels provided sensitive quantitative information on absorption and excretion of these pesticides. However, since the alkyl phosphate metabolites are not specific to any one organophosphate insecticide, this approach is indicative only of general exposures to these mixtures and can not be used to quantitatively assess individual insecticide dosimetry.

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 organophosphates. Evaluation of the human and animal pharmacokinetic data suggested that adequate metabolism information was available on eight 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 insecticides resulting from both acute and chronic exposures. Richter *et al.* (1992) quantitated diethyl phosphate in the urine of individuals who were symptomatic for organophosphate 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 diethyl phosphate were observed in family members, whereas cholinesterase activity, although slightly depressed, was well within the range of "normal." The quantitation of urinary diethyl phosphate was used to establish a persistent household exposure to diazinon residues as the most likely explanation. This study clearly illustrates the utility of urinary metabolites for quantitative biomonitoring of exposure.

Biomonitoring based on the measurement of pesticide metabolites has also been used to compare pesticide exposure in children who live in proximity to high spray areas (e.g., orchards) and whose parents/guardians are pesticide applicators (Loewenherz *et al.*, 1997). Based on known pesticide use patterns, it was determined that insecticide exposure would be primarily associated with azinphos-methyl, chlorpyrifos, and phosmet. Therefore, the study focused on the quantitation of the alkyl phosphate metabolites (dimethylthiophosphate, dimethyldithiophospate, dimethylphosphate) in the children's urine. Loewenherz *et al.* (1997) collected and evaluated 160 spot urine specimens from 88 children and reported detectable 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 that the reference children (0.021 vs. 0005 μg/l, respectively).

Biomonitoring of parent pesticide 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 cholinesterase depression (i.e., 50% of baseline) is used to substantiate pesticide 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). In this regard, the utilization of pharmacokinetic models like 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 *et al.*, 2002a). 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 is presented in Figure 66.14. The model adequately reflects the data from these limited human samples, but, more importantly, 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 and, more specifically, organophosphorus insecticides. Biomonitoring is clearly an integral component of the agricultural pesticide exposure assessment strategy. However, the successful application of biomonitoring for quantitating dosimetry is primarily limited by a lack of chemical-specific pharmacokinetic data in humans.

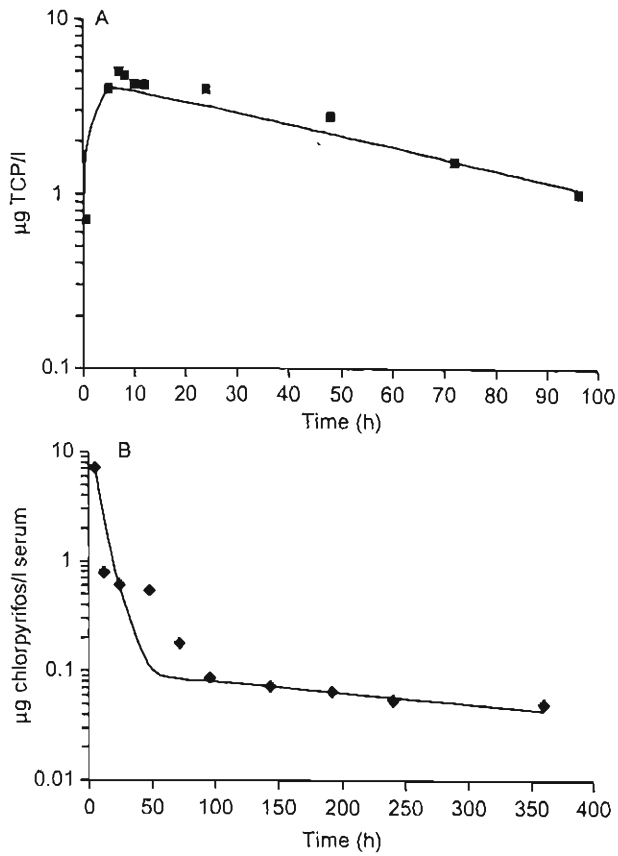


FIGURE 66.14 (A) Mean blood time course of 3,5,6-trichloropyridinol (TCP) from six human volunteers administered a single oral dose of 0.5 mg chlorpyrifos (CPF)/kg of body weight (data obtained from Nolan *et al.*, 1984). (B) Time course of CPF in the serum of a single poison victim who orally ingested 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).

66.3.4 Studies that Facilitate Extrapolation of Dosimetry and Biological Response from Animals to Humans and the Assessment of Human Health Risk

Organophosphorus 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. For example, extrinsic factors such as multiple exposure routes, chemical/drug interactions, and variable exposure rates may significantly modify the toxicological response to organophosphates. 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.

The development and application of PBPK/PD modeling represent a logical approach to assessing risk and understanding the toxicological implications of known or suspected exposures to various insecticides. The capability of these models to accurately simulate dosimetry and cholinesterase inhibition has been demonstrated in both rodents and humans (Timchalk *et al.*, 2002; Poet *et al.*, 2004). As previously noted, the PBPK/PD model accurately simulates both the pharmacokinetics of chlorpyrifos (Figure 66.9) and the dynamics of cholinesterase inhibition (Figure 66.10) in the rat. Likewise, the model has also been used to simulate human dosimetry and cholinesterase dynamics utilizing pharmacokinetic and pharmacodynamic data obtained from control human studies (Nolan *et al.*, 1984; Timchalk *et al.*, 2002). For example, the time course of plasma BuChE inhibition kinetics

following a single oral (0.5 mg/kg) or dermal (5 mg/kg) dose of chlorpyrifos in human volunteers is presented in Figure 66.15. These results clearly illustrate the route-dependent (oral vs. dermal) differences in the extent of plasma cholinesterase response. The PBPK/PD model also accurately simulated the time course of chlorpyrifos and TCP in blood of human volunteers following oral exposure (1 and 2 mg/kg) to chlorpyrifos (see Figure 66.16). These model simulations are consistent with the rapid metabolic clearance of chlorpyrifos resulting in the formation of TCP, which has a considerably slower elimination rate and is readily detected in the blood of volunteers through 160 h postdosing.

With the development and validation of the PBPK/PD model in both rats and humans, the model can now be exploited to quantitatively assess dosimetry and dynamic response over a range of relevant occupational and environmental chlorpyrifos exposures. Hence, this model is a strong framework for refining a biologically based risk assessment for exposure to chlorpyrifos under a variety of scenarios (Timchalk *et al.*, 2002).

66.3.4.1 Insecticide Mixtures

Both occupational and secondary exposures to insecticides often entail simultaneous or sequential contact with mixtures (Hayes *et al.*, 1980; Lavy *et al.*, 1993; Loewenherz, *et al.*, 1997). The potential for organophosphorus insecticide interactions has been well understood for some time. Early studies demonstrated the acute, synergistic, and toxicological interactions between malathion and EPN (ethyl-*p*-nitrophenyl phenylphosphonothionate) (Frawley *et al.*,

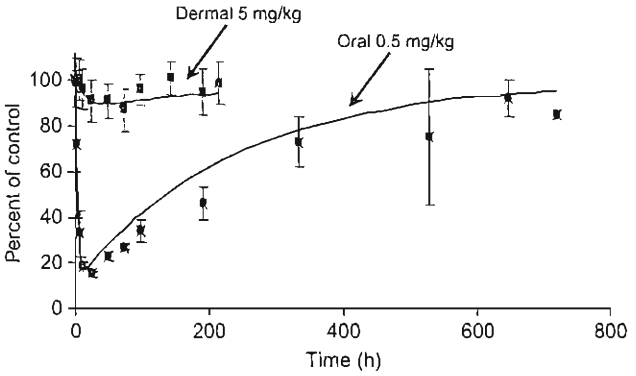


FIGURE 66.15 Experimental data (symbols) from Nolan *et al.* (1984) and model simulations (lines) of the plasma cholinesterase (ChE) inhibition in human volunteers administered an oral dose (filled circles) of 0.5 mg chlorpyrifos (CPF)/kg or a dermal dose (filled squares) of 5 mg CPF/kg. The time course data represent the mean ± SD for five male volunteers (adapted from Timchalk *et al.*, 2002, with permission).

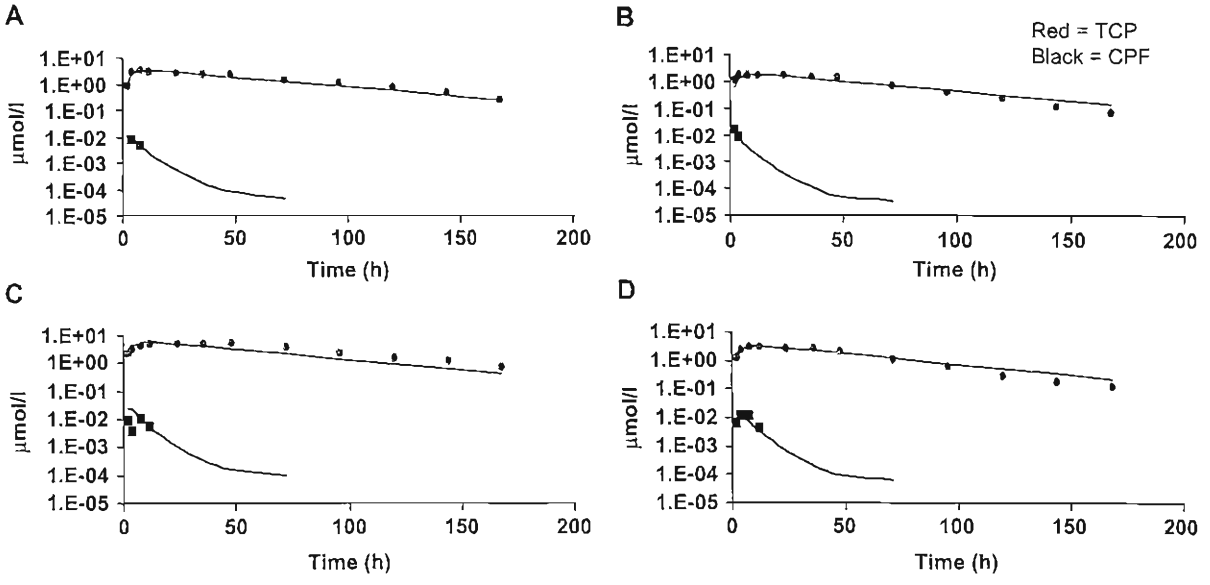


FIGURE 66.16 Experimental data (symbols) and model simulations (lines) for the plasma concentration of trichloropyridinol (TCP) (filled circles) and chlorpyrifos (CPF) (filled squares) in five volunteers administered CPF as an oral dose of 1 mg/kg (A and B) or 2 mg/kg (C and D) (adapted from Timchalk *et al.*, 2002, with permission).

1957). In addition, coexposures to noninsecticides have been reported to influence the pharmacokinetic and toxicological response of organophosphates. For example, phenobarbital or alcohol pretreatment of mice protects against the acute toxicity of chlorpyrifos and parathion, respectively (O'Shaughnessy and Sultatos, 1995; Sultatos *et al.*, 1984). 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, coadministration 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).

Organophosphorus 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, a potential for interactions between mixtures of these insecticides is hypothesized. Organophosphates can interact at a number of important metabolic steps (see Table 66.5) including: (1) CYP450-mediated activation/detoxification; (2) plasma protein binding; (3) PON-1 (A-esterase) detoxification; and (4) AChE binding/inhibition. The net effect of these interactions (additivity, synergy, or antagonism) will be dependent upon the specific mixture, dose ranges of exposures, and sensitivity of the individual.

To provided needed perspective on organophosphorus insecticide mixture interactions, a binary PBPK/PD model for chlorpyrifos, diazinon, and their metabolites was developed (Timchalk and Poet, 2008) based on previously published models for the individual insecticides (Poet *et al.*, 2004; Timchalk *et al.*, 2002, 2007a). This model was designed to quantitatively integrate both tissue dosimetry and dynamic response (ChE inhibition) in blood and tissues. In addition, the metabolic interactions (CYP450) between chlorpyrifos and diazinon were evaluated *in vitro* to characterize the binary mixture enzymatic kinetic interactions for the mixture. Based on the *in vitro* metabolism results, the PBPK model codes used to describe the CYP450 metabolism of chlorpyrifos and diazinon were appropriately modified to reflect the type of inhibition kinetics (i.e., competitive vs. noncompetitive), while *B-est* metabolism was described as dose additive, and no PON-1 interactions were assumed between chlorpyrifos- and diazinon-oxon with the enzyme. The binary model was evaluated against previously published rodent dosimetry and cholinesterase inhibition data for the mixture (Timchalk *et al.*, 2005).

The metabolic interaction (CYP450) between chlorpyrifos and diazinon was evaluated *in vitro* and the results indicated that chlorpyrifos was more substantially metabolized to its oxon metabolite than diazinon, which is consistent with the *in vivo* potency (chlorpyrifos > diazinon). Each insecticide inhibited the other's *in vitro* metabolism in a concentration-dependent manner. Based on the differences in oxon formation rates, the most dramatic difference is associated with the extent of cholinesterase inhibition for single insecticides versus. the binary mixtures. To illustrate this point, the time- and mixture-dependent inhibition of brain AChE following single or binary exposures to chlorpyrifos and diazinon is presented in Figure 66.17. The general response for brain AChE and model simulations is also consistent with the pharmacodynamics observed for plasma cholinesterase and RBC AChE inhibition. In the brain there is a potential shift toward shorter times to achieve maximum inhibition with increasing doses of insecticides (Timchalk *et al.*, 2005), which is reasonably simulated by

TABLE 66.5 Important Metabolic and Response Interactions for Mixtures of Organophosphate (OP) Insecticides

Parameters	Importance	Type of Chemical Interaction	Implications
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

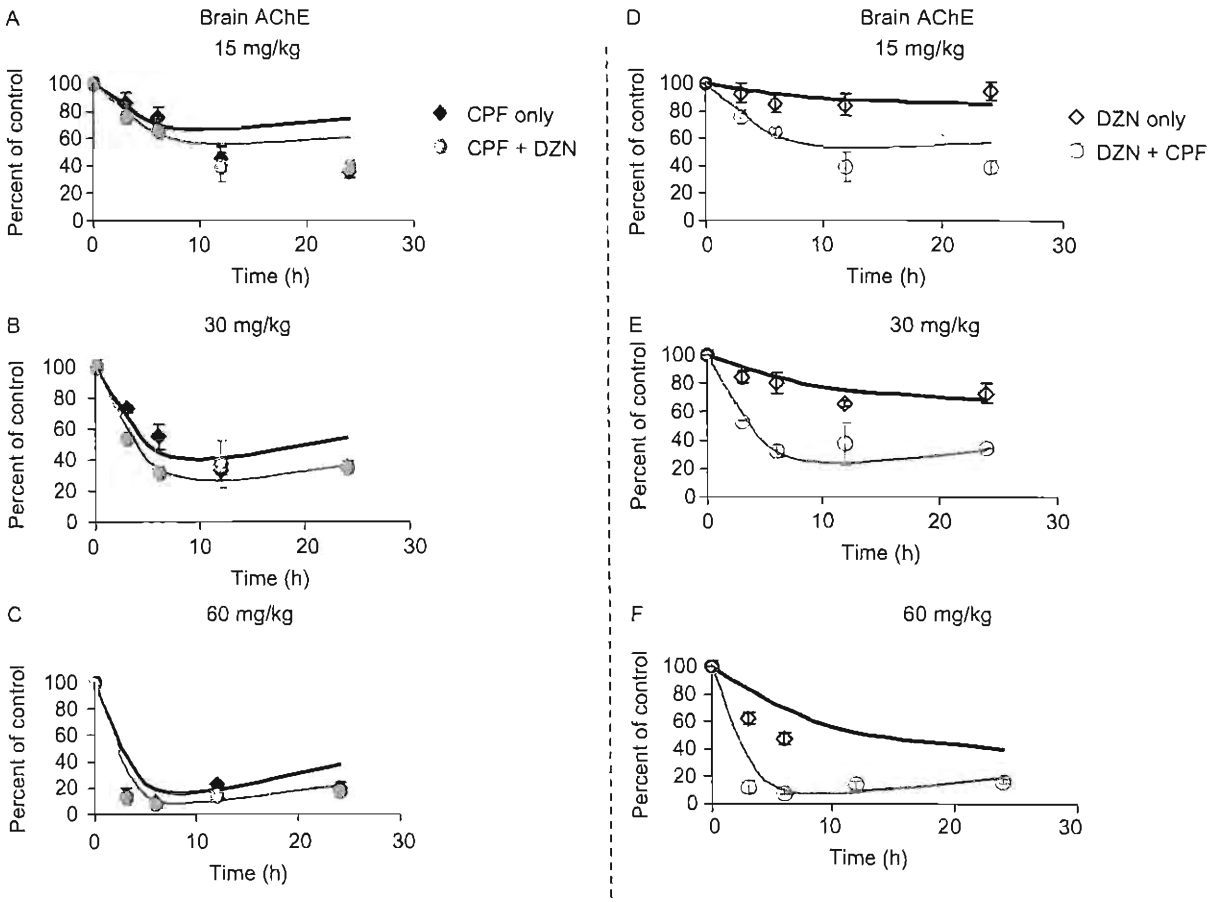


FIGURE 66.17 Brain acetylcholinesterase (AChE) activity in rats following 15, 30, or 60 mg/kg oral gavage doses of chlorpyrifos (CPF) (solid black diamonds), diazinon (DZN) (open black diamonds), and their binary mixtures (solid or open gray circles). The data are expressed as percent of total ChE activity as a function of time (h) and represent the mean ± SD for four animals per time point. The lines represent the PBPK/PD model simulations (adapted from Timchalk and Poet, 2008, with permission).

the PBPK/PD model. Consistent with the experimental finding, model simulations suggest that chlorpyrifos has a substantially greater inhibitory impact on brain AChE activity than diazinon at all dose levels.

The PBPK/PD model was also utilized to further evaluate theoretical mixture interactions over a very broad range of mixture doses. In these simulations (see Figure 66.18) single acute doses ranging from 1 to 200 mg/kg were evaluated, recognizing that the high end of the dose range (50–200 mg/kg) would result in substantial adverse acute toxicity, including lethality. The rationale for simulating these very high acute doses was to try and establish at what dosage the model would predict a deviation between the single and binary exposures. These simulations are very consistent with the experimental findings and suggest that binary interactions between chlorpyrifos and diazinon at environmentally relevant exposures levels will most likely be negligible and that chlorpyrifos has a greater impact than diazinon as a binary mixture (Timchalk *et al.*, 2008).

Based on the results with this PBPK/PD model, it is anticipated that at low binary doses most likely to be encountered in both occupational- and environmental-related exposures, the pharmacokinetics are expected to be linear, and cholinesterase inhibition kinetics are well described using a dose-additive model. Hence, this binary model provides a mechanistic framework for understanding the lack of important synergistic interactions at occupationally and environmentally realistic exposures, even for pesticides that are as similar as chlorpyrifos and diazinon (Timchalk *et al.*, 2008).

66.3.4.2 Sensitive Subpopulations (Children)

There is currently a significant focus on 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 *On Pesticides in the Diets of Infants and Children* and 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 the

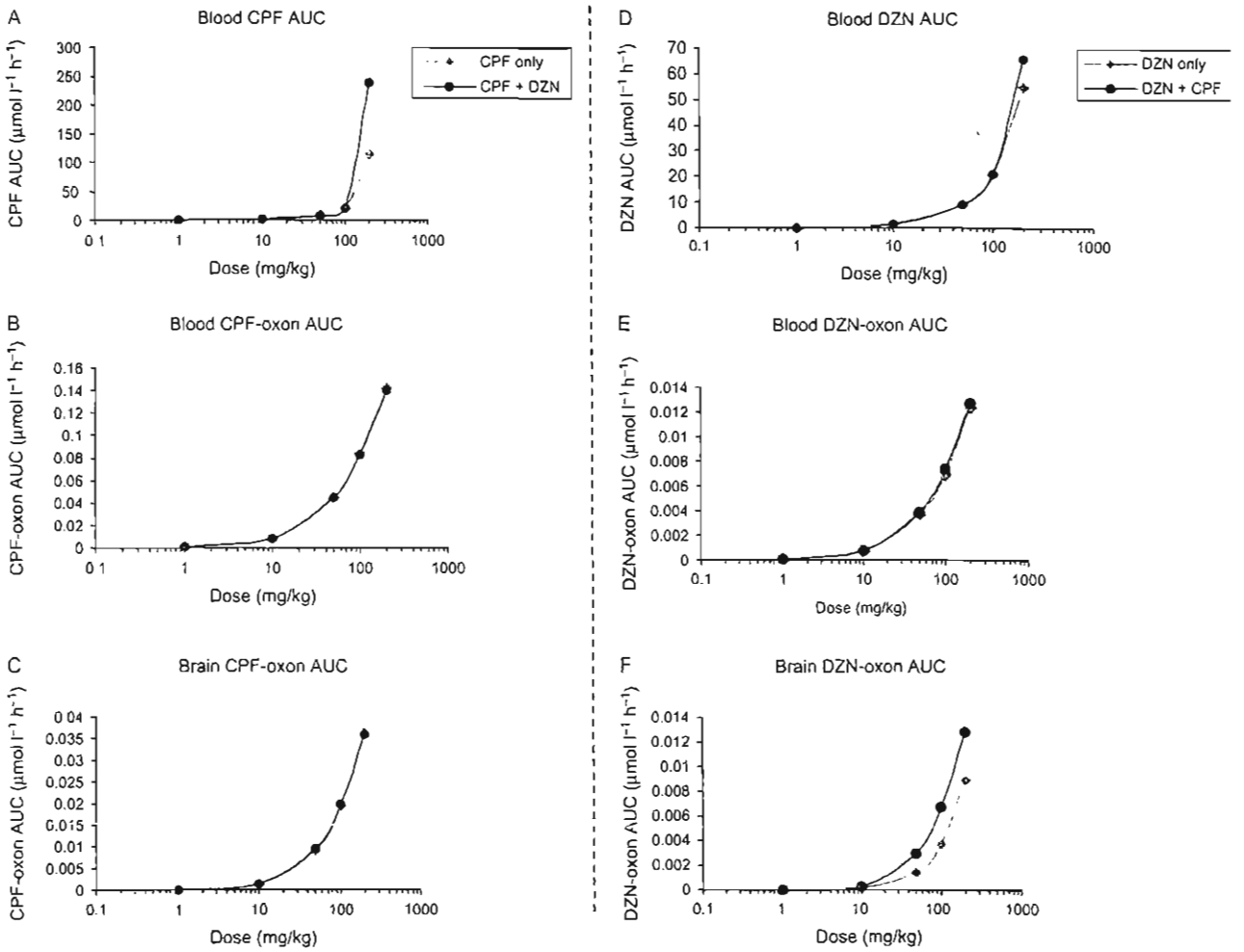


FIGURE 66.18 Model simulation of chlorpyrifos (CPF), diazinon (DZN), CPF-oxon, and DZN-oxon area under the concentration curve (AUC) for single and binary exposures to CPF and DZN over a broad range of doses (1–200 mg/kg). The solid gray diamonds represent CPF and DZN as single doses, while the solid black circles represent the binary mixture (adapted from Timchalk and Poet, 2008, with permission).

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 organophosphorus insecticides than adults (Benke and Murphy, 1975; Brodeur and DuBois, 1963; Gaines and Linder, 1986; Harbison, 1975; Moser and Padilla, 1998; Pope *et al.*, 1991; Pope and Liu, 1997). This greater sensitivity has primarily been attributed to the lack of complete metabolic competence during neonatal and postnatal development (Benke and Murphy, 1975).

The application of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling offers a unique opportunity to integrate age-dependent changes in metabolic activation and detoxification pathways into a comprehensive model that is capable of quantifying dosimetry and response across all ages (for review, see Corley *et al.*, 2003). In this context, PBPK models are being extended to the modeling of chemical exposure in developing/juvenile animals and in children (Byczkowski *et al.*, 1994; Clewell *et al.*, 2002, 2003, 2004; Fisher *et al.*, 1990; Sundberg *et al.*, 1998; Price *et al.*, 2003).

To address this issue for the organophosphorus insecticides, the PBPK/PD model that was developed for chlorpyrifos in adult rats and humans (Timchalk *et al.*, 2002) was modified by incorporating age-dependent scaling to adjust physiology, organ volumes and blood flows, metabolism rates, *B-est* tissue levels, and bimolecular inhibition rates for chlorpyrifos-oxon and cholinesterase as a function of age (Timchalk *et al.*, 2007a). The model was then used to predict tissue dosimetry and pharmacodynamic (PD) response (i.e., esterase inhibition) in preweanling and adult rats exposed to chlorpyrifos (Timchalk *et al.*, 2006). To simulate the kinetics of chorpyrifos dosimetry and cholinesterase inhibition in preweanling rats, the PBPK/PD model was modified to scale metabolism, cholinesterase

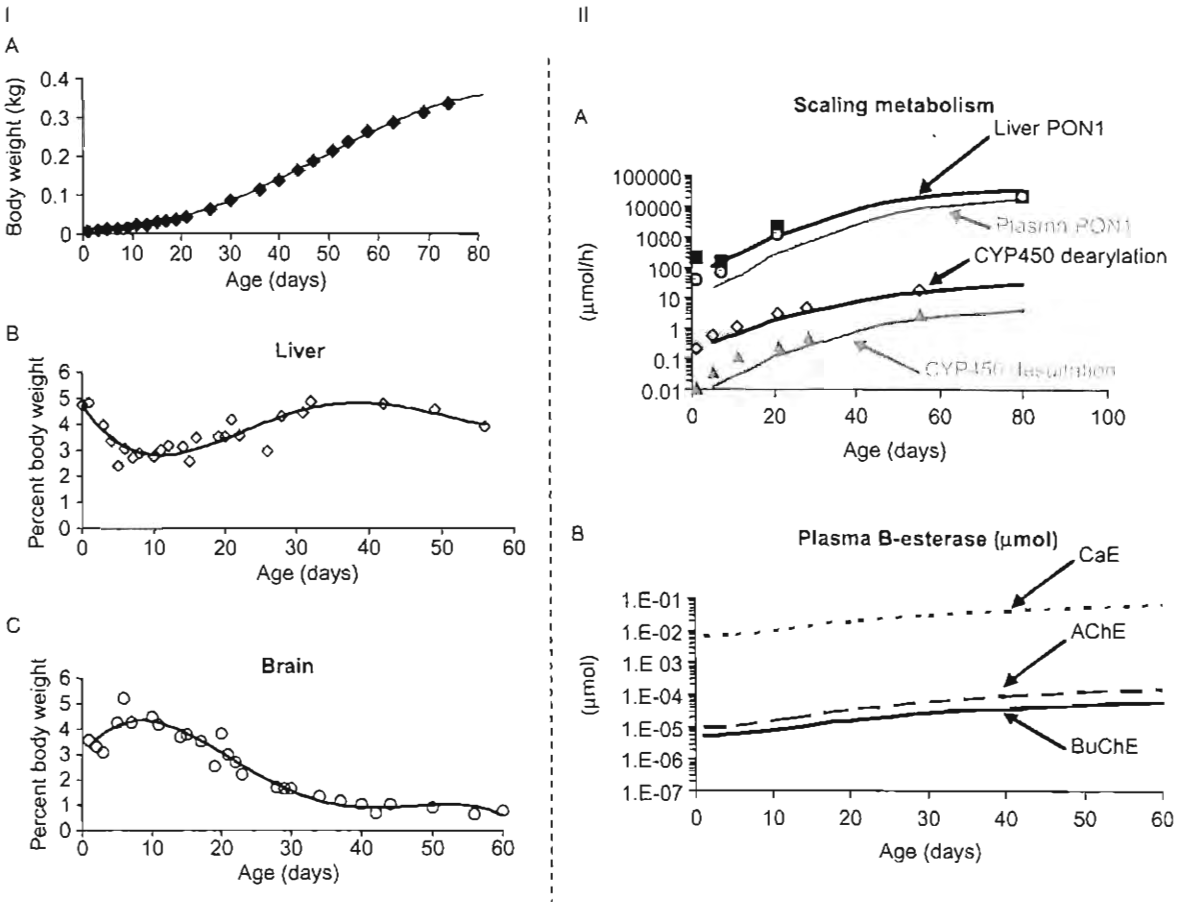


FIGURE 66.19 (I) Age-dependent scaling of (A) body weight (kg), (B) liver volume (percent of body weight), and (C) brain volume (percent of body weight) as a function of age in Sprague-Dawley rats. (II) (A) Scaling of metabolic rates as a function of age for hepatic and plasma PON-1 and hepatic CYP450-mediated dearylation and desulfation of chlorpyrifos (CPF) and CPF-oxon in the rat. Symbols represent experimentally determined enzyme activity (Atterberry *et al.*, 1997), whereas lines represent model prediction. (B) Scaling of the amount of plasma *B-est* (CaE, AChE, and BuChE) as a function of age in the rat; similar scaling was done for hepatic, diaphragm, and brain *B-est* levels (from Timchalk *et al.*, 2007, with permission).

activity, and most relevant organ volumes and body weight as a function of age (Timchalk *et al.*, 2007a). Figure 66.19 illustrates the dynamic changes in physical parameters, key metabolism pathways, and cholinesterase levels for the developing preweanling rat. It should be noted that a number of other model parameters were modified to accommodate age-dependent developmental changes; for a more detailed discussion see Timchalk *et al.* (2007a).

Figure 66.20 illustrates the capability of the model to simulate blood concentrations of both chlorpyrifos and TCP in very young preweanling rats following a single bolus or fractioned dose. The overall pharmacokinetic profile was very comparable and the PBPK/PD model reasonably simulated both the chlorpyrifos and TCP blood time course. As anticipated, the C_{max} for chlorpyrifos and TCP following the fractionated doses was lower (2.8- to 7.4-fold) than following the single bolus administration, and the PBPK/PD model reasonably simulated this response (Timchalk *et al.*, 2007a).

The model was also utilized to simulate the time courses of plasma cholinesterase, RBC AChE, and brain AChE inhibition in adult and preweanling rats (Timchalk *et al.*, 2002, 2006) following single oral gavage administration of chlorpyrifos (Timchalk *et al.*, 2007a). In the brain, the AChE inhibition demonstrated both an age and dose dependency in the preweanling rats (Timchalk *et al.*, 2006), and at all dose levels and ages the model reasonably simulated the dynamics of brain AChE inhibition (Figure 66.21). Of particular importance was the observation that even in rats as young as postnatal day 5 (PND-5), the CYP450 metabolic capacity was adequate to metabolize chlorpyrifos to both TCP and oxon based on the detection of TCP in blood and extensive cholinesterase inhibition.

A comparison of the simulated oxon AUC ratios (PND-5 vs. adult) in both blood and brain over a broad range of doses is illustrated in Figure 66.22. At doses ranging from 0.001 to 1 mg/kg the preweanling to adult oxon ratio (PND − 5(AUC)/Adult(AUC)) for blood and brain was ~1.3; however, at chlorpyrifos doses >1 mg/kg the ratio rapidly increased in both blood and brain and approached 2 at ~10 mg/kg. This suggests that age-dependent difference in brain oxon concentration may be an important contributing factor associated with the increased sensitivity of

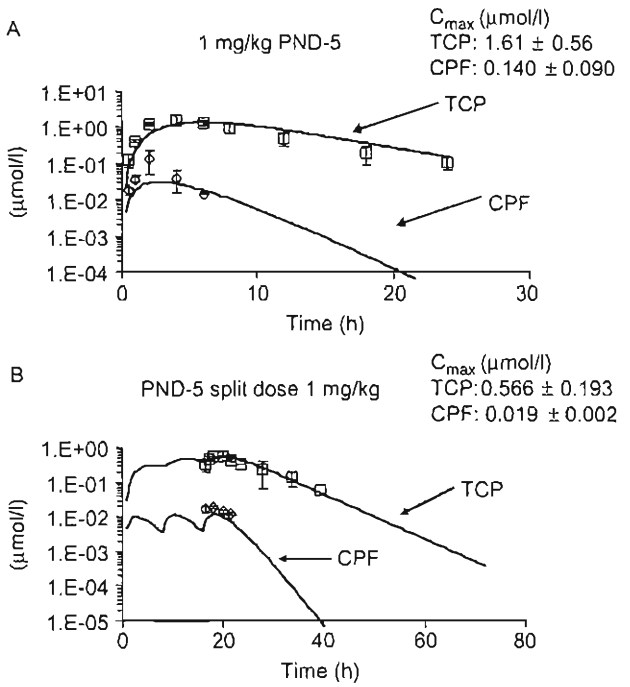


FIGURE 66.20 Observed data and model prediction (lines) for blood concentrations of chlorpyrifos (CPF) and trichloropyridinol (TCP) in postnatal day 5 (PND-5) rats given (A) a single oral gavage dose of 1 mg/kg or (B) three split doses of 0.33 mg/kg, each administered at 0, 8, and 16 h. The observed data are a mean ± s.d. of four to five animals per time point. The C_{max} is the maximally measured blood concentration (adapted from Timchalk *et al.*, 2007, with permission).

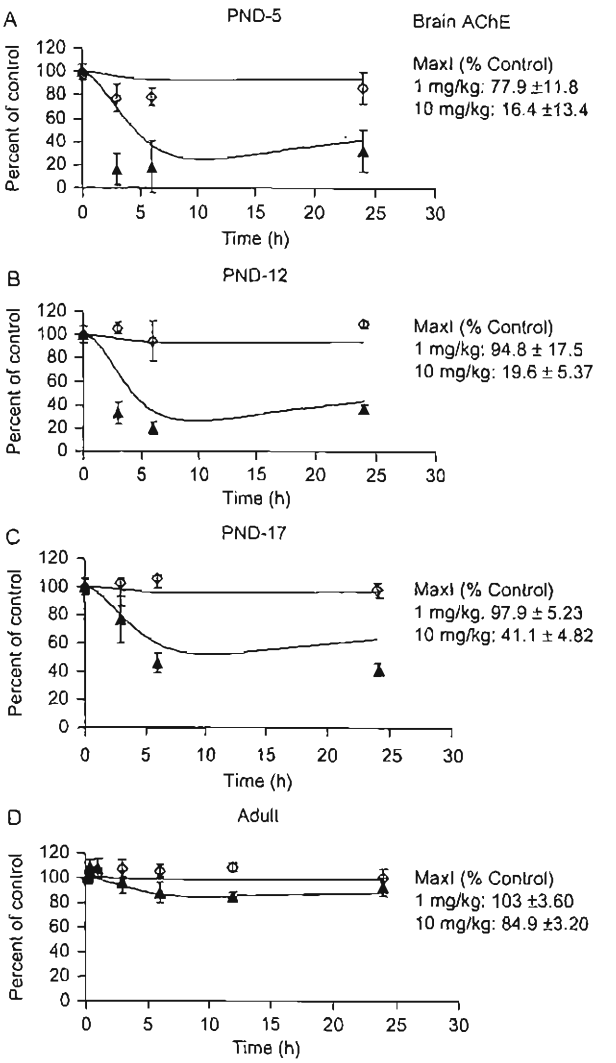


FIGURE 66.21 Observed data and model prediction (lines) for the brain AChE inhibition time course in (A) postnatal day 5 (PND-5), (B) PND-12, (C) PND-17, and (D) adult rats following a single acute oral gavage dose of 1 (open diamonds) or 10 (closed triangles) mg CPF/kg of body weight. The observed data are presented as a mean ± s.d. of four to five animals per time point. The maximum inhibition (MaxI) is expressed as percent of control activity for each of the dose levels (adapted from Timchalk *et al.*, 2007, with permission).

preweanling rats relative to adults, particularly at the higher doses utilized in toxicology studies.

As these results indicate, an age-dependent PBPK/PD model for the organophosphorus insecticide chlorpyrifos behaves consistently with the general understanding of toxicity, pharmacokinetics, and tissue cholinesterase inhibition in preweanling and adult rats. Future model development must entail further development and validation with the ultimate goal of developing a model that is capable of predicting biological response in infants and children. Nonetheless, the utilization of PBPK/PD modeling to address organophosphorus insecticide toxicity issues is particularly intriguing since these models can be used to assess the health consequences of both interindividual (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.

CONCLUSION

This chapter has illustrated a number of current and future applications of pharmacokinetics to assess organophosphorus insecticide 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 these insecticides. Since organophosphorus insecticides share a common mode of action through their capability to inhibit 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 organophosphorus insecticides in multiple species, at various dose levels, and across different routes of exposure have provided important insights into the *in vivo* behavior of these insecticides. The development and application of pharmacokinetic models capable of describing uptake, distribution, metabolism, and elimination of insecticides in humans represent a crucial

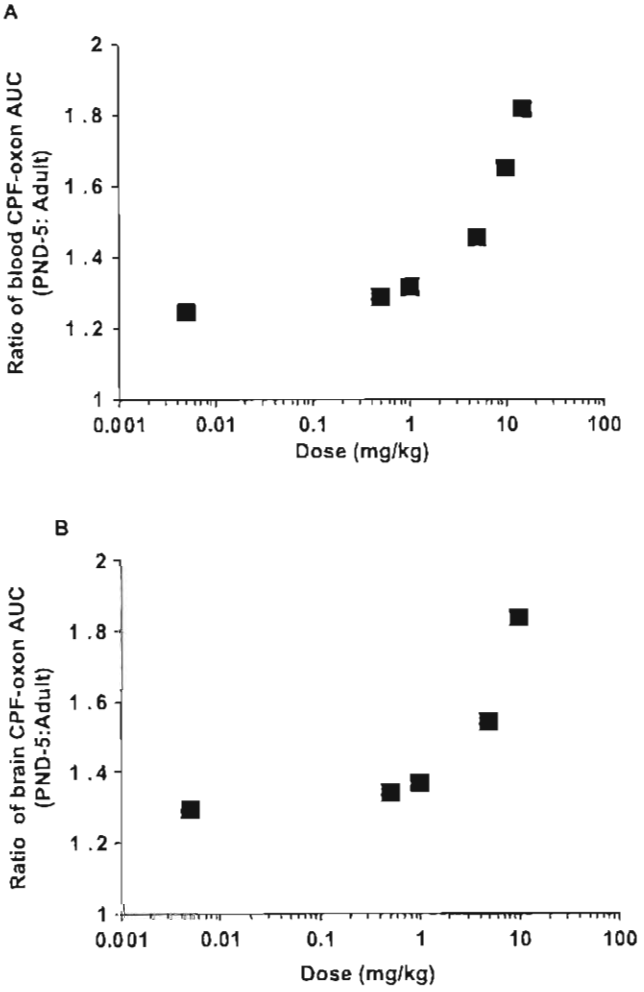


FIGURE 66.22 The ratio of (A) blood and (B) brain chlorpyrifos-oxon (CPF-oxon) area under the concentration curves ($AUC_{0-\infty}$) comparing neonatal (PND-5) and adult rats (PND − 5(AUC)/Adult(AUC)) following simulation of an acute oral exposure to a broad range of CPF doses (adapted from Timchalk *et al.*, 2007, with permission).

research element needed for quantitative biomonitoring. In this regard, the successful application of biomonitoring for quantitating dosimetry is primarily limited by the lack of this chemical-specific pharmacokinetic data in humans. The development and application of PBPK/PD modeling for organophosphorus insecticides represent a unique opportunity to quantitatively assess human health risk and to understand the toxicological implications of known or suspected exposures to various insecticides. Validated PBPK/PD models for these insecticides 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 has been successfully utilized to better understand the toxicological implications of human exposure to organophosphorus 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 insecticide exposures.

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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. Letters* **82/83**, 341–348.

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

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**(2), 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.

Barr, D. B., Bravo, R., Weerasekera, G., Caltabiano, L. M., Whitehead, R. D. Jr., Olsson, A. O., Caudill, S. P., Schober, S. E., Pirkle, J. L., Sampson, E. J., Jackson, R. J., and Needham, L. L. (2004). Concentrations of dialkyl phosphate metabolites of organophosphorus pesticides in the U. S. population. *Environ. Health Perspect.* **112**, 186–200.

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**(1), 7–13.

Bradman, A., Eskenazi, B., Barr, D., Bravo, R., Castorina, R., Chevrier, J., Kogut, K., Harnly, M., and McKone, T. (2005). Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. *Environ. Health Persp.* **12**(113), 1802–1807.

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., Therap.* **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 Eds.), 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, CA.

Chambers, J. E., and Chamber, H. W. (1989). Oxidative desulfation of chlorpyrifos, chlorpyrifos-methyl, and leptophos by rat brain and liver. *J. Biochem. Tox.* **4**(1), 201–203.

Chandra, 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.

Chester, G. (1993). Evaluation of agricultural worker exposure to and absorption of pesticides. *Occup. Hyg.* **37**(5), 509–523.

Clement, J. G. (1984). Role of aliesterase in organophosphate poisoning. *Fund. 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**(1–3), 207–217.

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

Clewell, H. J., Gentry, P. R., Covington, T. R., Sarangapani, R., and Teeguarden, J. G. (2004). Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol. Sci.* **79**, 381–393.

Clewell, H. J., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Covington, T., Gentry, R., and Shipp, A. (2002). Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Crit. Rev. Toxicol.* **32**, 329–389.

Clewell, R. A., Merrill, E. A., Yu, K. O., Mahle, D. A., Sterner, T. R., Fisher, J. W., and Gearhart, J. M. (2003). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicol. Sci.* **74**(2), 416–436.

Corley, R. A., Mast, T. J., Carney, E. W., Rogers, J. M., and Daston, G. P. (2003). Evaluation of physiologically based models of pregnancy and lactation for their application in children's health risk assessment. *Crit. Rev. Toxicol.* **33**(2), 137–211.

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.

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

Duggan, A., Charnley, G., Chen, W., Chukwudebe, A., Hawk, R., Kreiger, R. I., Ross, J., and Yarborough, C. (2003). Di-alkyl phosphate biomonitoring data: assessing cumulative exposure to organophosphate pesticides. *Reg. Toxicol. Pharmacol.* **37**, 382–395.

Ellenhorn, M. J., and Barceloux, D. G. (1988). "Medical toxicology—diagnosis and treatment of human poisoning," pp. 1074–1075. Elsevier, New York.

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. *Fund. 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 administration 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**(1-3), 201–206.

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 Persp.* **102**(11), 51–59.

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**(2), 99–108.

Iverson, F., Grant, D. L., and Lacroix, J. (1975). Diazinon metabolism in the dog. *Bull. Environ. Contamin. Toxicol.* 13(5), 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 Ltd., London, U.K.

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, Ltd., 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**(6), 342–345.

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**(1-3), 55–65.

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 Persp.* **105**(2). 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.

Lu, C., Bravo, R., Caltabiano, L. M., Irish, R. M., Weerasekera, G., and Barr, D. B. (2005). The presence of dialkylphosphates in fresh fruit juices: implication for organophosphorus pesticide exposure and risk assessments. *J. Toxicol. Environ. Health, Part A* **68**, 209–227.

Ma, T., and Chambers, J. E. (1994). Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. *Fd. Chem. Tox.* **32**(8), 763–767.

Mason, H., and Wilson, K. (1999). Biological monitoring: the role of toxicokinetics and physiologically based pharmacokinetic modeling. *AIHA Journal*(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. *Fund. Appl. Toxicol.* **40**, 1–14.

Morgan, M. K., Sheldon, L. S., Croghan, C. W., Jones, P. A., Robertson, G. L., Chuang, J. C., Wilson, N. K., and Lyu, C. W. (2005). Exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environment. *J. Exp. Analy. Environ. Epidemiol.* **15**, 297–309.

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. Agr. Food Chem.* **18**(2), 208–212.

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 Publishers, New York, N.Y.

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. and 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. (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**(11), 1925–1932.

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.

Poet, T. S., Kousba, A. A., Dennison, S. L., and Timchalk, C. (2004). Physiologically based pharmacokinetic/pharmacodynamic model for the organophosphorus pesticide diazinon. *NeuroTox.* **25**, 1013–1030.

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., 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. *Toxicol.* **68**, 51–61.

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

Price, K., Haddad, S., and Krishnan, K. (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J. Toxicol. Environ. Health Part A*, **66**, 417–433.

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, Ltd., 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**(2), 135–138.

Sato, A., and Nakajima, T. (1979). Partition coefficients of some aromatic hydrocarbons and ketones in water, blood, and oil. *Brit. 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**(3), 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. *Toxicol.* **51**, 191–200.

Sultatos, L. G. (1990). A physiologically based pharmacokinetic model of parathion based on chemical-specific parameters determined *in vitro*. *J. Amer. Coll. Toxicol.* **9**(6), 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 phsphorothioate insecticides chlorpyrifos and parathion and their oxygen analogues with bovine serum albumin. *Mol. Pharmacol.* **26**(1), 99–104.

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

Timchalk, C., Busby, A., Campbell, J. A., Needham, L. L., and Barr, D. B. (2007b). Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3,5,6-trichloro-2-pyridinol in the rat. *Toxicol.* **237**, 145–157.

Timchalk, C., Kousba, A. A., and Poet, T. S. (2007a). An age-dependent physiologically based pharmacokinetic/pharmacodynamic model for the organophosphorus insecticide chlorpyrifos in the preweanling rat. *Toxicol. Sci.* **98**(2), 348–365.

Timchalk, C., Kousba, A., and Poet, T. S. (2002b). Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. *Toxicol. Lett.* **135**, 51–59.

Timchalk, C., Nolan, R. J., Mendrala, A. L., Dittenber, D. A., Brzak, K. A., and Mattsson, J. L. (2002a). A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicol. Sci.* **66**, 34–53.

Timchalk, C., and Poet, T. S. (2008). Development of a physiologically based pharmacokinetic and pharmacodynamic model to determine dosimetry and cholinesterase inhibition for a binary mixture of chlorpyrifos and diazinon in the rat. *NeuroTox.* **29**, 428–443.

Timchalk, C., Poet, T. S., Hinman, M. N., Busby, A. L., and Kousba, A. A. (2005). Pharmacokinetic and pharmacodynamic interaction for a binary mixture of chlorpyrifos and diazinon in the rat. *Toxicol. Appl. Pharmacol.* **205**, 31–42.

Timchalk, C., Poet, T. S., and Kousba, A. A. (2006). Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. *Toxicol.* **220**, 13–25.

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. *Toxicol.* **37**, 91–98.

Vale, J. A. (1998). Toxicokinetic and toxicodynamic aspects of organophospate (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**(2), 241–256.

Wester, R. C., and Maibach, H. I. (1983b). 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. (1983a). 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. *Fund. 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. *Fd.Chem. Toxic.* **31**(8), 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**(6), 383–392.

Woollen, B. H. (1993). Biological monitoring for pesticide absorption. *Occup. Hyg.* **37**(5), 525–540.

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

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