19

BIOMONITORING OF PESTICIDES: PHARMACOKINETICS OF ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

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19.1 INTRODUCTION

This chapter highlights the application of pharmacokinetics to facilitate biological monitoring for organophosphorus and carbamate insecticide exposures. The overarching goal of human biological monitoring (biomonitoring) is to assess exposure to specific chemical agents by quantifying specific biomarkers (Angerer et al., 2007; Gil and Pla, 2001; Jakubowski and Trzcinka-Ochocka, 2005). With regard to pesticides, biomonitoring is focused primarily on the measurement of the parent compound and key metabolites or reaction products in biological matrices such as blood and urine (Barr et al., 2006). A meaningful biomonitoring strategy is based on sound pharmacokinetic principles that enable quantification of chemical specific dosimetry and biological response, which can then be used to inform risk in humans who are exposed to these agents. Pharmacokinetics is associated with the absorption, distribution, metabolism, and excretion (ADME) of the insecticides, and these studies provide important 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 in both animals (relevant to toxicology) and humans (biomonitoring/risk assessment). These studies have been

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conducted with both organophosphorus and carbamate insecticides in multiple species, at various dose levels, and across different routes of exposure to understand *in vivo* pharmacokinetics which have important implications for the design of biomonitoring studies. For example, the determination of relevant biological matrices (urine, blood), needed detection limits, appropriate biomarker (i.e., parent vs. metabolite), and relevant timing for sample collection can be readily discerned from the pharmacokinetics. Although these two major classes of pesticides share a common toxicological mode of action associated with their ability to target and inhibit acetylcholinesterase (AChE), chemical/metabolite biomarkers are distinctly unique between these classes of agents. Within each class (organophosphate vs. carbamate) many insecticides share some common metabolites (e.g., dialkyl phosphates for organophosphates); however, chemical specific metabolites (i.e., α-naphthol for carbaryl or trichloropyridinol for chlorpyrifos) are available which can be directly linked to a specific insecticide. This chapter is focused on biomonitoring, and builds upon previously published works that focused on the pharmacokinetics of organophosphorus and carbamate agents and the development of pharmacokinetic and pharmacodynamic models for these insecticides (Timchalk, 2001, 2006).

19.2 PESTICIDE BIOMONITORING

Due to the ways in which pesticides are utilized human exposures involve a broad range of the population, including workers involved in manufacturing, applicators, field workers, and the general population, who may be exposed by domestic use and consumption of food and water containing low-level residues (Jakubowski and Trzcinka-Ochocka, 2005). As reviewed by Barr et al. (2006) biomonitoring has been successfully applied as a component of farm worker exposure assessments and is particularly relevant with regard to assessment of exposure for specific task (i.e., different application methods). Guidelines for the design and conduct of field biomonitoring studies have been developed (Fenske, 1997; Nigg and Stamper, 1989) and criteria have been established for deriving exposure from the analysis of biological samples (Honeycutt, 1986). These include: (1) adequate systemic absorption must be achieved, (2) pharmacokinetics associated with particular routes and rates of excretion must be known, (3) sensitive and quantitative analytical methods must be employed, and (4) the collection methods should be convenient for participants in the study to gain their cooperation. This framework clearly illustrates the central importance that pharmacokinetics play for designing and implementing a biomonitoring assessment for insecticides.

The primary focus of this chapter will be on the application of pharmacokinetic principles that have been applied to organophosphorus and carbamate insecticides for use in human biomonitoring. The approach will not entail a comprehensive review of the literature, but rather a focused presentation highlighting important principles using specific examples.

The organophosphorus and carbamate insecticides constitute two large families of pesticides that share a common mode of insecticidal and toxicological action associated with their ability to inhibit the enzyme acetylcholinesterase (AChE) within nerve tissue (Ecobichon, 2001a, 2001b; Sultatos, 1994). A major difference between these two insecticidal classes relates to the rate of AChE reactivation which is measured in minutes for carbamates versus days or even weeks for organophosphorus insecticides (Ecobichon, 2001b; Tobia et al., 2001).

Organophosphorus and carbamate insecticides have a high affinity for binding to and inhibiting the enzyme acetylcholinesterase (AChE), an enzyme specifically responsible for the destruction of the neurotransmitter acetylcholine (ACh) within nerve tissue (Ecobichon, 2001b; Wilson, 2001). As noted above, the modulation of the cholinergic system through AChE inhibition represents the primary toxicological mode of action for both these insecticides. Since the cholinergic system is widely distributed within both the central and peripheral nervous systems, chemicals that inhibit AChE are known to produce a broad range of well-characterized symptoms (Savolainen, 2001). A comparison of the AChE inhibition dynamics for the interaction of ACh, carbamate (carbaryl) or organophosphate (chlorpyrifos-oxon) with AChE is illustrated in Figure 19.1. Acetylcholine, carbaryl, and chlorpyrifos-oxon all have high affinities for AChE and readily complex with the enzyme; however, the rates of hydrolysis and reactivation of AChE following carbamylation or phosphorylation of the active site are dramatically slower than for the hydrolysis of the acetylated enzyme (Ecobichon, 2001b). Specifically, the turnover time for the neurotransmitter ACh is on the order of ~150 μsec; whereas $t_{1/2}$ for hydrolysis of the carbamylated and phosphorylated enzymes is substantially slower (~15 to 30 minutes and several days, respectively). Since the phosphorylated enzyme is highly stable ($t_{1/2} \sim$ days) depending upon the specific organophosphorus agent, further dealkylation of the phosphorylation group is possible. This produces an "aged" AChE enzyme which is irreversibly inhibited (Ecobichon, 2001b; Murphy, 1986; Sogorb and Vilanova, 2002; Taylor, 1980).

19.3 PHARMACOKINETICS: ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

Pharmacokinetics is the evaluation of those processes associated with the absorption, distribution, metabolism, and

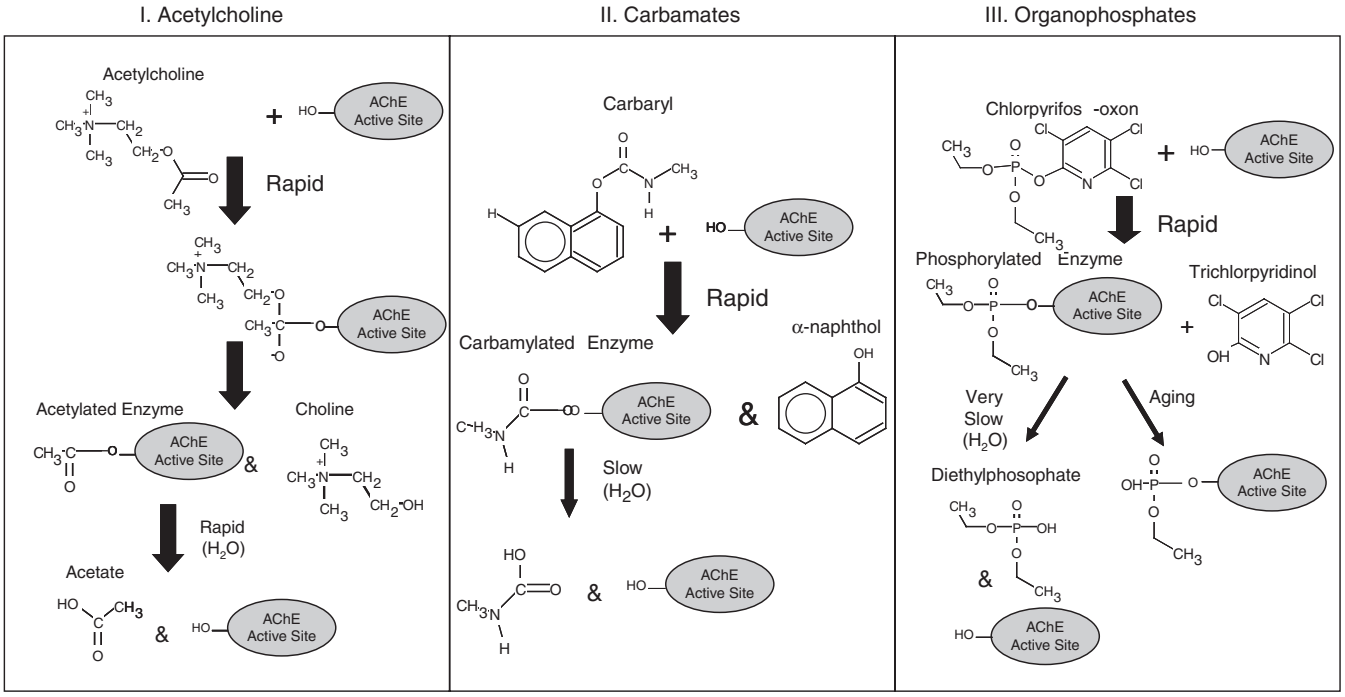

Figure 19.1 Schematic illustrating the interaction of acetylcholine (I), the carbamate insecticide carbaryl (II), and the organophosphate chlorpyrifos-oxon (III) with the active site of acetylcholinesterase (AChE). The general rate of bound AChE hydrolysis is: ACh $\gg$ carbaryl $>$ chlorpyrifos-oxon. [Figure reproduced with permission from Timchalk, C. (2006). In: Gupta, R. C. (Ed.), *Toxicology of Organophosphate and Carbamate Compounds*. San Diego, CA: Elsevier, pp. 103–125.].

excretion (ADME) of xenobiotics. The pharmacokinetics of organophosphorus and carbamate insecticides has been studied in a range of species, including humans (e.g., see Moody and Franklin, 1987; Nolan et al., 1984; Nong et al., 2008; Poet et al., 2004; Timchalk and Poet, 2008; Timchalk et al., 2002, 2006, 2007a, 2007b; Tomokuni et al., 1985; Tos-Luty et al., 2001; Wu et al., 1996). These insecticides are readily absorbed into the body and, based on the detection of metabolites in human urine, there is good evidence for widespread although low-level exposures (Aprea et al., 1999; Brouwer et al., 1993; Hill et al., 1995; Putnam et al., 2008; Shealy et al., 1997). These exposures can come from numerous sources, including ingestion of pesticide residues on foods or accidental as well as intentional ingestion of insecticides (Drevenkar et al., 1993). Dermal exposure represents an important exposure route particularly during the mixing, loading, and application of insecticides or from skin contact with contaminated surfaces (Knaak et al., 1993). Inhalation exposure of aerosolized insecticide associated with direct spray application or chemical drift has been reported (Vale and Scott, 1974). Once an individual is in contact with the pesticide, absorption is possible and based on the bioavailability for a given insecticide and exposure route, a systemic dose of the parent compound will enter the circulation. Although localized portal of entry metabolism (i.e., lung, intestines, skin) is feasible (Poet et al., 2003), the bulk of the metabolic activation as well as detoxification reactions occur within the liver (Sultatos, 1988; Sultatos et al., 1984).

It is likewise clear from both tissue distribution and partitioning studies that organophosphorus and carbamate insecticides are generally well distributed in tissue throughout the body (Tomokuni et al., 1985; Tos-Luty et al., 2001; Wu et al., 1996). Finally, due to the extensive metabolism, little if any parent insecticide is available for excretion; however, more stable degradation metabolites are readily excreted in the urine and are of potential utility as biomarkers of exposure (Colosio et al., 2002; Iverson et al., 1975; Mücke et al., 1970; Shealy et al., 1997).

19.3.1 Organophosphorus Insecticide Biotransformation

A more detailed overview of the metabolism of organophosphorus insecticides can be found in Calabrese (1991), Jokanovic (2001), Sogorb and Vilanova (2002), and Knaak et al. (2004). The three major classes of organophosphorus insecticides are the phosphoroamidothiolates, phosphorodithioates, and the phosphorothionates (Chambers, 1992; Chambers et al., 2001; Mileson et al., 1998). The common

insecticide chlorpyrifos is a phosphorothionate and the parent compound is a weak inhibitor of AChE, but once it undergoes metabolic activation (desulfation) to the corresponding oxygen analogs (chlorpyrofos-oxon) it becomes extremely potent. This enhanced toxicity is due to the oxon having a higher affinity and potency for phosphorylating the serine hydroxyl group within the active site of AChE as is illustrated in Figure 19.1 (Mileson et al., 1998; Sultatos, 1994). The toxic potency is dependent on the balance between a delivered dose to the target site and the rates of bioactivation and/or detoxification (Calabrese, 1991). Figure 19.2a illustrates the metabolic pathways for chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl]-phosphorothioate) and is being utilized for illustration purposes. Based on a common mode of action this scheme is readily extended to other structurally related organophosphorus insecticides.

As previously mentioned, phosphorothionates like chlorpyrifos do not directly inhibit AChE, but must first be metabolized to the active metabolite (chlorpyrifos-oxon; Iverson et al., 1975; Mücke et al., 1970; Murphy, 1986; Sultatos, 1994). Metabolic activation is mediated by cytochrome P450 mixed function oxidases (CYP450) primarily within the liver, although extra-hepatic metabolism has been reported (Chambers and Chambers, 1989; Guengerich, 1977). In the case of chlorpyrifos, oxidative dearylation produces both trichloropyridinol (TCP) and diethylthiophosphate (DETP), and represents a competing detoxification pathway that is 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 sensitivity (Ma and Chambers, 1994). Hepatic and extra-hepatic (i.e., blood and tissue) esterases, such as PON-1, effectively metabolize chlorpyrifos-oxon forming trichlorpyridinol and diethylphosphate (DEP). Likewise, B-esterases such as carboxylesterase (CaE) and butyrylcholinesterase (BuChE) also readily metabolize the oxon; however, these B-esterases become stoichiometrically bound (1 : 1 ratio) to the oxon and thereby become inactivated (Chanda et al., 1977; Clement, 1984). Human and animal studies indicate that the primary metabolite trichlorpyridinol can likewise undergo additional glucuronide or sulfate conjugation which is then readily excreted in the urine (Bakke et al., 1976; Nolan et al., 1984).

19.3.2 Carbamate Insecticide Biotransformation

The metabolism of carbamate insecticides involves oxidative metabolism, ester hydrolysis, and conjugation reactions (Ecobichon, 1994, 2001a; Sogorb and Vilanova, 2002) and the extent of metabolism is compound specific and highly dependent on the nature and position of substituent groups on either the oxygen or nitrogen (Ecobichon, 2001a). The metabolism of the carbamate insecticide carbaryl is presented in Figure 19.2b. The major pathway for detoxification of carbamates involves the hydrolysis by nonspecific esterases such as CaE (Sogorb and Vilanova, 2002); enzymatic hydrolysis results in the formation of α-naphthol and methyl-carbamic acid which rapidly decompose to CO_2 and mono-methylamine. The α-naphthol can be further conjugated with sulfate or glucuronide prior to excretion (Chin et al., 1979a, b, c). Additionally, oxidative metabolism forming either ring or side chain hydroxylation is anticipated for carbaryl, but is of potentially less importance than esterase-mediated metabolism (Hodgson and Levi, 2001).

19.4 PHARMACOKINETIC PRINCIPLES

Understanding the pharmacokinetics of an insecticide like chlopyrifos or carbaryl provides critically useful insights into the dosimetry of these insecticides following any given exposure. Pharmacokinetics provides quantitative data on the amount of toxicant delivered to a target site, and species-, age-, and gender-specific as well as dose-dependent differences in biological response. With regard to biomonitoring, an important application of pharmacokinetics has been to provide a realistic estimate of systemic exposure by providing a means to quantitatively estimate the absorbed dose of a chemical under realistic exposure conditions.

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 and carbamate 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 or 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 some organophosphorus and carbamate chemicals in multiple species (Brimer et al., 1994; Gearhart et al., 1990; Nong et al., 2008; Pena-Egido, 1988; Sultatos, 1990; Sultatos et al., 1990; Tomokuni et al., 1985; Wu et al., 1996).

19.4.1 Compartmental Pharmacokinetic Models

Compartmental pharmacokinetic analyses have been extensively utilized to assess bioavailability, tissue burden, and elimination kinetics in various species, including humans.

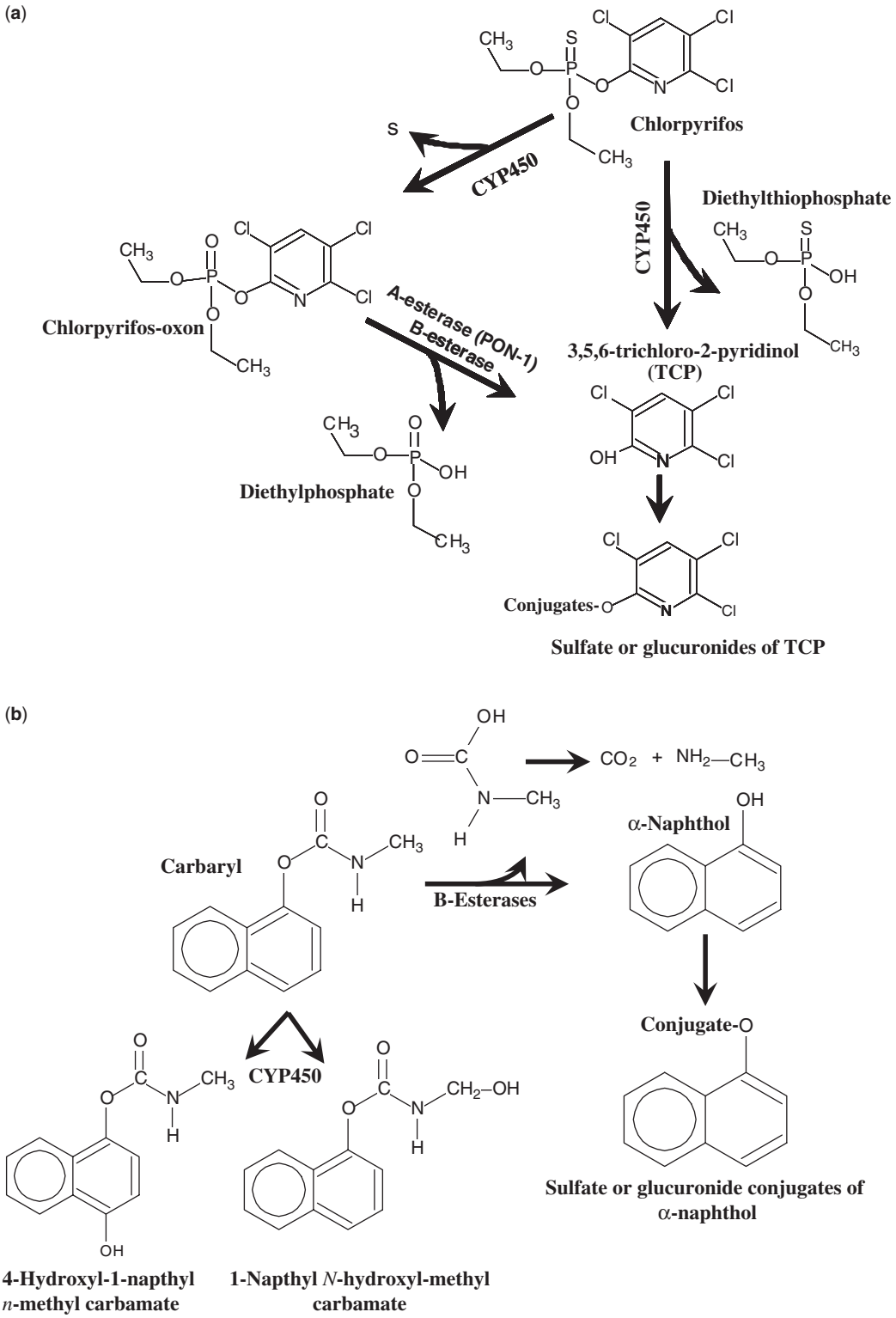

Figure 19.2 (a) Scheme for the metabolism of chlorpyrifos and the major metabolites chlorpyrifos-oxon, trichloropyridinol (and conjugates), diethylphosphate, and diethythiophosphate. (b) Scheme for the oxidative and B-esterase metabolism of carbaryl to the ring and side chain hydroxylated methyl-carbamates and the major metabolite α-naphthol. (Figure reproduced with permission from Timchalk, 2006.)

These modeling approaches have been exploited to quantify chemical absorption into the systemic circulation, distribution throughout the body, metabolic transformation, and clearance. Compartmental models are empirical and as such consider the organism as a single or multi-compartment homogeneous system. The number and behavior of the compartments are primarily determined by the equations chosen to describe the time-course data and not the physiological characteristics of the organism. In these models the net transfer between compartments is directly proportional to the difference in chemical concentration. However, the rate constants associated with the transfer between compartments cannot be experimentally determined (Srinivasan et al., 1994).

As suggested above, the models range from a simple, well-mixed single compartment to more complicated multicompartment models that are used to describe the blood and/or time-course of a chemical. 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; Wu et al., 1996). To illustrate their utility, Nolan et al. (1984) developed a one-compartment pharmacokinetic model that accurately describes the blood and urine time-course of trichloropyridinol (TCP) which is the major metabolite of chlorpyrifos. A diagram of this single-compartment model is illustrated in Figure 19.3; the blood trichlorpyridinol concentration and urinary excretion data were simultaneously fit to a single-compartment model structure based on Equations (1) and (2). Absorption (k_a) and elimination (k_e) are handled as first-order processes, and the blood trichlorpyridinol 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 trichlorpyridinol. The model parameters used to describe the time course and the model fit to the experimental data are presented in Table 19.1 and Figure 19.4. 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 as trichlorpyridinol with a half-life of 27 hours. Based on this model, Nolan et al. (1984) has suggested that blood trichlorpyridinol concentration and/or urinary excretion rate could be utilized to quantify the amount of chlorpyrifos absorbed under actual use conditions.

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, 2001). 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, 2001; Leung and Paustenbach, 1995; Mason and Wilson, 1999). Their potential utility as a modeling tool for biological monitoring has only recently been explored (Garabrant et al., 2008; Tan et al., 2007).

19.4.2 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

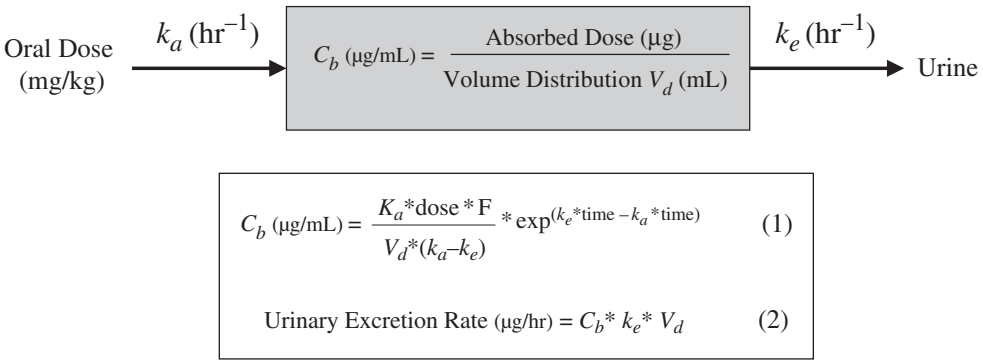

Figure 19.3 Compartment model structure used to describe the pharmacokinetics of the chlorpyrifos metabolite trichlorpyridinol in blood and urine. [Figure reproduced with permission from Timchalk, C. (2001). In: Krieger, R. (ed.), *Hayes' Handbook of Pesticide Toxicology*, 2nd ed. San Diego, CA: Academic Press, pp. 929–951.]

TABLE 19.1 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 (hr)	Absorption Rate Constant k_a (hr^{-1})	Absorption Half-life (hr)	Volume Distribution (V_d) (ml/kg)	Elimination Rate Constant (hr^{-1})	Elimination Half-life k_e (hr)	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

Source: Data obtained from 6 male volunteers.
Data adapted from Nolan *et al.*, 1980. (Table reproduced with permission from Timchalk, 2001).

the physiological characteristics of the species of interest (Krishnan and Andersen, 2001). PBPK models have been developed to describe target tissue dosimetry for a broad range of environmental contaminants, such as solvents, heavy metals, and pesticides, including organophosphate and carbamate insecticides (Andersen et al., 1987; Corley et al., 1990; Gearhart et al., 1990; Nong et al., 2008; Poet et al., 2004; Sultatos, 1990; Timchalk et al., 2002). A number of reviews have been published on the development, validation, application, and limitations of PBPK models in human health risk assessment (Andersen, 1995, 2003; Clewell, 1995; Clewell and Andersen, 1996; Frederick, 1995; Krishnan and Andersen, 2001; Leung and Paustenbach, 1995; Mason and Wilson, 1999).

19.4.3 PBPK/PD Model Structure for Cholinesterase-Inhibiting Pesticides (such as Chlorpyrifos)

For the organophosphate and carbamate insecticides, PBPK models have been extended to also incorporate a pharmacodynamic (PD) component to describe cholinesterase (ChE) inhibition (Nong et al., 2008; Poet et al., 2004; Timchalk and Poet, 2008; Timchalk et al., 2002, 2007a). These models are based on the conceptual structure developed by Gearhart et al. (1990) to describe AChE inhibition following an acute exposure to diisopropylfluorophosphate (DFP).

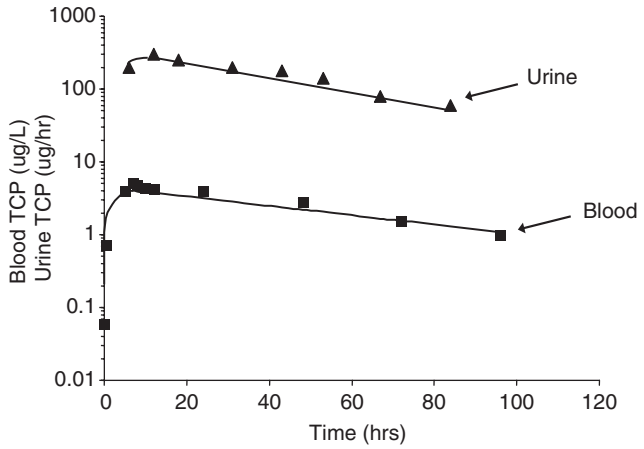

Figure 19.4 Time course of trichlorpyridinol (TCP) in the blood and urine of male volunteers following oral administration of 0.5 mg chlorpyrifos/kg of body weight. [Figure reproduced with permission from Timchalk, C. (2001). In: Krieger, R. (Ed.), *Hayes' Handbook of Pesticide Toxicology*, 2nd ed. San Diego, CA: Academic Press, pp. 929–951.]

To illustrate the application of PBPK/PD models for assessing dosimetry and biological response in humans, the organophosphorus insecticide chlorpyrifos will be utilized as an example, and is illustrated in Figure 19.5. The conceptual representation of the PBPK/PD model for this insecticide is based on the anatomical and physiological characteristics of humans. The major determinants of insecticide disposition include absorption rates, tissue partitioning, plasma protein-binding, CYP450 metabolism, and esterase binding and hydrolysis (Poet et al., 2004; Timchalk et al., 2002).

The PBPK/PD model allows for the simulation of differing exposure scenarios such as acute oral "gavage," chronic dietary, dermal, and inhalation. In these models, physiological and metabolic parameters are scaled as a function of body weight according to the methods of Ramsey and Andersen (1984). The CYP450-mediated activation and detoxification is limited to the liver, and is linked to the oxon model that incorporated equations to describe A-esterase (PON-1) metabolism in both liver and blood. The CYP450 activation/detoxification and PON-1 detoxification are all described as Michaelis–Menten processes. Oxon metabolites bind with and inhibit B-esterases including AChE, BuChE, and CaE. Although binding to AChE is associated with acute neurotoxicity, the binding to BuChE and to CaE are suggested to be without adverse physiological effect and as such represent detoxification pathways (Clement, 1984; Fonnum et al., 1985; Pond et al., 1995). Interactions of the oxon with B-esterases are modeled as second-order processes occurring in the liver, blood (plasma and red blood cells), diaphragm, and brain. The B-esterase enzyme levels (μmol) are calculated based on the enzyme turnover rates and enzyme activities (Maxwell et al., 1987). A balance between the bimolecular rate of inhibition and the rate of cholinesterase regeneration and aging determined the

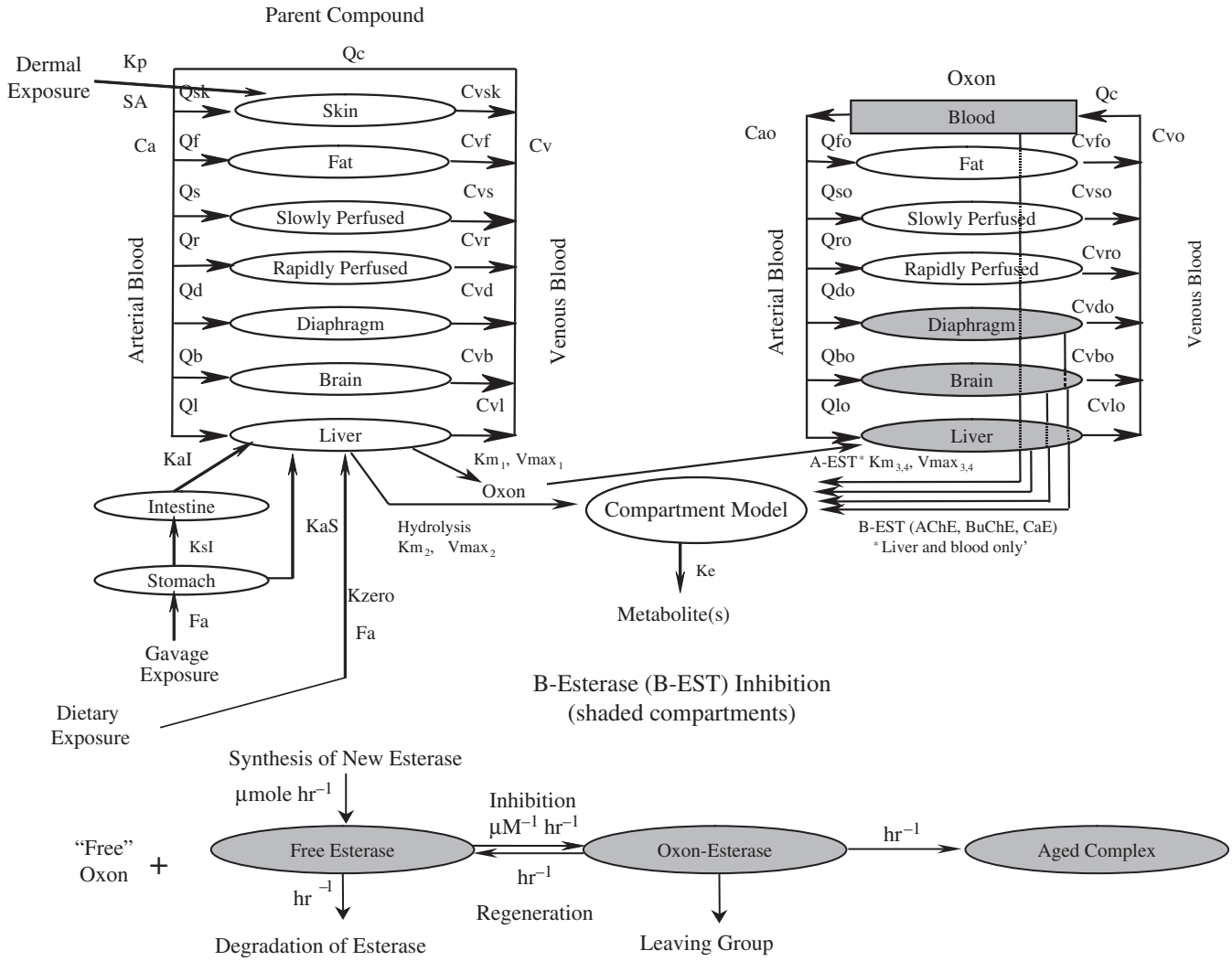

Figure 19.5 Physiologically based pharmacokinetic and pharmacodynamic model used to describe the disposition for a parent phosphorothionate insecticide, its oxon metabolite, associated leaving groups, and B-esterase inhibition in rats and humans following oral (gavage, dietary) and dermal exposures. The shaded tissues compartments indicate organs in which B-esterase (AChE, BuChE, and CarbE) enzyme activity is described. Model parameter definitions: QC cardiac output (L/hour); Q_i, blood flow to "*i*" tissue (L/hour); 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); $Cv_i o$, venous blood concentration of oxon draining "*i*" tissue (μmol/L); SA surface area of skin exposed (cm^2); KP, skin permeability coefficient (cm/hour); K_{zero}, zero (μmol/hour) rate of absorption from diet; Fa fractional absorption (%); KaS and KaI, first-order rate constants for absorption from compartments 1 and 2 (per hour); KsI, first-order rate constant for transfer from compartment 1 and 2 (per hour); Ke, first-order rate constant for elimination of metabolite from compartment 3; $K_{m(1\text{-}4)}$, Michaelis constant for saturable processes ((μmol/L); $V_{max(1\text{-}4)}$, maximum velocity for saturable process (μmol/hour). [Figure adapted with permission from Timchalk et al. (2002)].

amount of free cholinesterase. In this model the major metabolite trichloropyridinol (TCP) was formed by CYP450 metabolism of the parent compound, or PON-1 and B-esterase hydrolysis of oxon. The pharmacokinetics of the trichlorpyridinol metabolite was fit using a simple one-compartment model as previously described by Nolan et al. (1984). This PBPK/PD model developed for chlorpyrifos and related organophosphorus insecticides are fairly complex and are data intensive, so to adequately develop and validate these models generally requires extensive experimentation to support model parameterization and validation.

The primary objective associated with the development of a PBPK/PD model is to predict with some confidence dosimetry and dynamic response in humans. Confidence in the predictive capability of these models is enhanced by validating the model against carefully conducted controlled human pharmacokinetic/pharmacodynamic studies or against available exposure and dosimetry data obtained

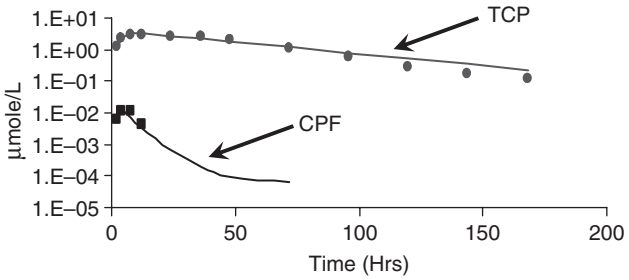

Figure 19.6 Experimental data (symbol) and model simulations (lines) for the plasma concentration of trichloropyridinol (TCP) and chlorpyrifos (CPF) in a volunteer administered CPF as an oral dose of 2 mg CPF/kg of body weight. [Figure adapted with permission from Timchalk, C. et al. (2002). *Toxicol. Sci.*, 66: 34–53.]

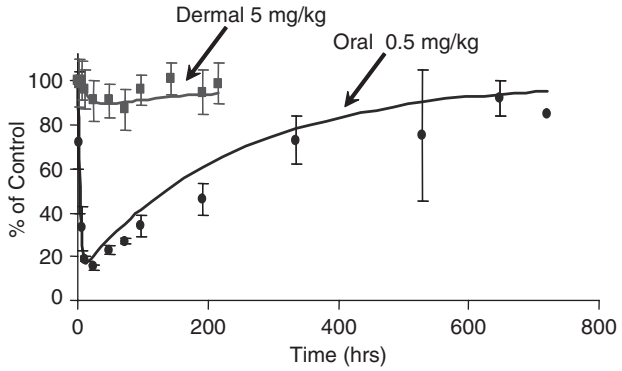

Figure 19.7 Experimental data (symbol) from Nolan et al. (1984) and model simulations (lines) of the plasma ChE inhibition in human volunteers administered an oral or dermal dose of chlorpyrifos. The time-course data represents the mean ± SD for 5 male volunteers. [Figure adapted with permission from Timchalk, C. et al. (2002). *Toxicol. Sci.*, 66: 34–53.]

from biomonitoring or clinical evaluations (Wilks and Woollen, 1994; Woollen, 1993). For chlorpyrifos, controlled human pharmacokinetic studies have been conducted to facilitate biomonitoring and have also been utilized to validate the PBPK/PD model (Nolan et al., 1984; Timchalk et al., 2002). The time course of chlorpyrifos and the major metabolite trichloropyridinol in blood of a volunteer who ingested a capsule containing chlorpyrifos at a dosage of 2 mg/kg is presented in Figure 19.6. Although both chlorpyrifos and the major metabolite trichloropyridinol were readily detected in the blood, the blood levels for chlorpyrifos were approximately two orders of magnitude less than for the metabolite, consistent with the model simulations and the known rapid metabolism of chlorpyrifos to trichloropyridinol (Timchalk et al., 2002). Likewise the pharmacodynamics have also been evaluated in human volunteer studies, where the time course of plasma BuChE inhibition dynamics following a single oral (0.5 mg/kg) or dermal (5 mg/kg) dose of chlorpyrifos were evaluated (Nolan et al., 1984) and are presented in Figure 19.7. In this example, the amount of available plasma cholinesterase enzyme and the rate of enzyme recovery were optimized to fit the plasma cholinesterase inhibition time-course (oral). The model parameters were then held constant and the model was used to predict the plasma cholinesterase inhibition for the dermal exposure. In this case the model predicted a maximum inhibition of ∼90% of control (i.e., 10% inhibition), which was comparable to the observed 87% seen with the experimental data (Timchalk et al., 2002).

To further validate the capability of the model to reasonably describe the chlorpyrifos blood pharmacokinetics, the time course of chlorpyrifos in serum obtained from an individual who ingested a concentrated formulation of chlorpyrifos (Drevenkar et al., 1993) was simulated and the results are presented in Figure 19.8. In this particular case the subject was a young man who had consumed an unknown quantity of a commercial insecticide that contained chlorpyrifos. The subject was admitted into the hospital within ∼5 hours of the ingestion and blood samples were repeatedly taken and analyzed for both chlorpyrifos and the oxon metabolite. Although Drevenkar et al. (1993) reported that oxon was not detectable in any of the samples, chlorpyrifos was readily measured (1 to 10 µmol/L blood), as late as 15 days post-exposure. As is noted in Figure 19.8, the PBPK model reasonably simulated the serum chlorpyrifos time course, and the predicted dosage is well within the range (>150 mg/kg) to elicit acute toxicity.

Since organophosphorus and carbamate 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. The exploitation of PBPK/PD modeling to inform about biological monitoring has only recently been explored (Garabrant et al., 2008; Tan et al., 2007), but offers a potential opportunity to enable quantitation of key dosimetry (i.e., metabolites) and

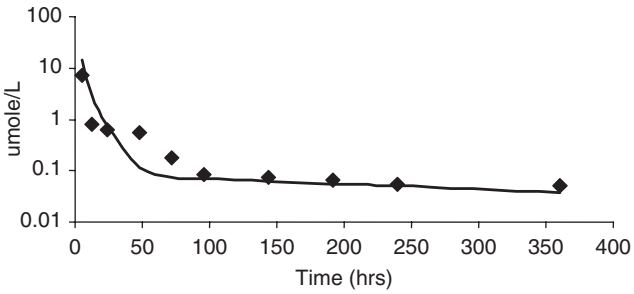

Figure 19.8 Time course of chlorpyrifos (CPF) in the serum of a single poison victim who orally ingested a commercial insecticide product containing CPF (data from Drevenkar et al. 1993). The symbols represent observed data while the line represents the model prediction. [Figure adapted with permission from Timchalk, C. et al. (2002). *Toxicol. Sci.*, 66: 34–53.]

pharamacodynamic (i.e., ChE) biomarkers into a unified and quantifiable computational approach.

19.5 PK APPROACHES FOR ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

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

Both occupational and environmental exposure to organophosphorus insecticides is primarily associated with dermal exposure, which has been suggested to account for more than 90% of the absorbed dose (Aprea et al., 1999). Therefore, an understanding of the percutaneous absorption of these pesticides is critical to quantitatively determining a systemic dose. The extent of dermal bioavailability for a number of ^{14}C-labeled organophosphorus insecticides has been determined in humans utilizing both *in vivo* studies in volunteers and *in vitro* dermal penetration with skin obtained from cadavers (Wester et al., 1983, 1992, 1993).

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 organophosphorus pesticide for a specified exposure period. The extent of absorption was determined by quantitating the amount of radioactivity excreted in the urine and remaining on the skin surface. Second, *in vitro* percutaneous absorption was estimated based on the percent absorption though human cadaver skin. Finally, to calculate the *in vivo* percent absorption, rhesus monkeys were utilized as human surrogates and were given a ^{14}C-labeled insecticide as an intravenous dose. The extent of absorption in humans was calculated from the ratio of radioactivity excreted in the urine after topical (humans) and intravenous (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 *in vitro* percent absorption was comparable for isofenphos (3.64% $\pm$ 0.48%), but slightly higher and considerably more variable for diazinon (14.1% $\pm$ 9.2%). Percutaneous absorption studies conducted in humans are of particular importance since it is known that dermal absorption in animals, such as the rat, is often greater than in humans (Wester and Maibach, 1983). For example, Knaak et al. (1990) conducted a dermal absorption study in rats with isofenphos and reported that 47% of the applied dose was absorbed, which is 12-fold higher that the results seen in human volunteers. The major limitation associated with the experimental design of Wester et al. (1983, 1992, 1993) is that the quantitation of only radioactivity provides no information on the specific form of the absorbed insecticide (i.e., parent or metabolite). Nonetheless, these studies do provide important quantitative information on the extent of dermal absorption, which can help inform us about biomonitoring.

To better understand the systemic pharmacokinetics of organophosphorus insecticides and to develop pharmacokinetic models that can be utilized for biomonitoring, controlled human studies that quantify the time course of parent chemical or metabolites in blood and urine are key. As has been previously discussed, Nolan et al. (1984) conducted a controlled human pharmacokinetic study to follow the fate of the major urinary metabolite (trichlorpyridinol) after administration of chlorpyrifos. A similar study was conducted by Griffin et al. (1999) in which they quantified the urinary excretion of the dialkylphosphate (DAP) metabolite (see Fig. 19.2a).

A selection of comparative pharmacokinetic parameters from the controlled human chlorpyrifos studies is presented in Table 19.2. The pharmacokinetic results measured the amount of trichloropyridinol or dialkylphosphate in human volunteers and the results are entirely consistent with each other. For example, following oral administration, chlorpyrifos is rapidly absorbed, with maximum blood concentration and excretion being obtained by 6 and 7 hours post-dosing, respectively, for trichloropyridinol and dialkylphosphate. The extent of absorption was initially estimated based on the amount of metabolite (70% to 93%) recovered in the urine. In comparison, the dermal absorption was limited and consistently slower, with peak concentrations of metabolite being achieved by 17 to 24 hours post-dosing for both studies, and the amount recovered in the urine was substantially less (1.35% and 1% for trichloropyridinol and dialkylphosphate metabolites, respectively). Nolan et al. (1984) reported an elimination half-life of $\sim$27 hours following oral administration, whereas Griffin et al. (1999) reported half-lives of $\sim$16 and 30 hours for dialkylphosphate following oral and dermal exposure to chlorpyrifos, respectively. The longer urinary clearance following dermal exposure is most likely associated with a delay in chlorpyrifos absorption through the skin. However, differences in the rates of trichloropyridinol and dialkylphosphate kinetics are also a possible explanation (Griffin et al., 1999). Nonetheless, the

TABLE 19.2 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/hr	Absorption Rate Constant k_a (hr^{-1})	Absorption Half-Life (hr)	Elimination Rate Constant k_e (hr^{-1})	Elimination Half-life (hr)	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

Abbreviations: TCP, trichloropyridinol; DAP, dialkylphosphate.
Data extracted from: [a]Nolan et al.,1984; [b]Griffin et al., 1999.
[c]Estimated based on average body weight (71 kg).
Source: Table reproduced with permission from Timchalk, 2001.

elimination half-life for chlorpyrifos based on either trichloropyridinol or dialkylphosphate clearance is consistent.

These types of pharmacokinetic data are being used to develop models to biomonitor for organophosphate exposure. As previously noted, 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 trichloropyridinol kinetics following oral and dermal exposure to chlorpyrifos (see Fig. 19.3). Similarly, the quantitative measurement of urinary dialkylphosphate is increasingly being used as a nonspecific metabolite biomarker for organophosphorus insecticide exposures (Griffin et al., 1999). The development of pharmacokinetic models that are capable of describing the uptake, distribution, and elimination of organophosphorus insecticides based on the quantitation of major degradation metabolites represents an extremely useful and simple approach for exposure biomonitoring.

19.5.2 Metabolite Biomarker Considerations

As noted above, dialkylphosphates (DAP) such as diethylphosphate (DEP) and diethylthiophosphate (DETP), which are two metabolic products of diethylphosphorothionate insecticides, have long been used as general urinary biomarkers for this class of organophosphorus insecticides (Bradway and Shafik, 1977; CDC, 2005; Hardt and Angerer, 2000). For assessing human exposure to many of these insecticides, the metabolite containing the major leaving group, such as trichloropyridinol in the case of chlorpyrifos, has been used as a more specific urinary metabolite biomarker (Berkowitz et al., 2004; CDC, 2005; Eskenazi et al., 2004; Nolan et al., 1984).

Although dialkylphosphate and trichloropyridinol have been routinely utilized as biomarkers for organophosphorus insecticide exposure, organophosphates can undergo environmental degradation to form these same chemicals, which have been detected in fruit products (juice) or in solid foods (Lu et al., 2005; Morgan et al., 2005). Hence, due to the environmental stability of the dialkylphosphate and trichloropyridinol, recent research has questioned whether total urinary organophosphate metabolite levels may be reflective not only of an individual's contact with the parent organophosphorus pesticide but, in addition, exposure with intact metabolites present in the environment (Barr et al., 2005; Bradman et al., 2005; Duggan et al., 2003; Lu et al., 2005). Thus, measured urinary metabolite levels may represent an exaggerated indicator of an individual's exposure to the parent compound (Duggan et al., 2003). As illustrated in Figure 19.9, recent comparative metabolism studies have evaluated the individual metabolite pharmacokinetics of diethylphosphate, diethylthiophospate, and trichlorpyridinol with their kinetics following oral dosing with the parent insecticide chlorpyrifos in the rat (Timchalk et al., 2007a). These data support the hypothesis that diethylphosphate, diethylthiophospate, and trichloropyridinol present in the environment can be readily absorbed and eliminated in the rat. In this regard, it would not then be unreasonable to assume that the pharmacokinetics of these individual pesticide metabolites would show a similar pharmacokinetic response in humans. Hence, the total organophosphorus insecticide metabolite levels obtained in human urine may be reflective not only of an individual's contact with the parent pesticide, but also exposure to intact metabolites present in the environment (Timchalk et al., 2007a). Hence, where feasible it is important to obtain a base-line measurement of

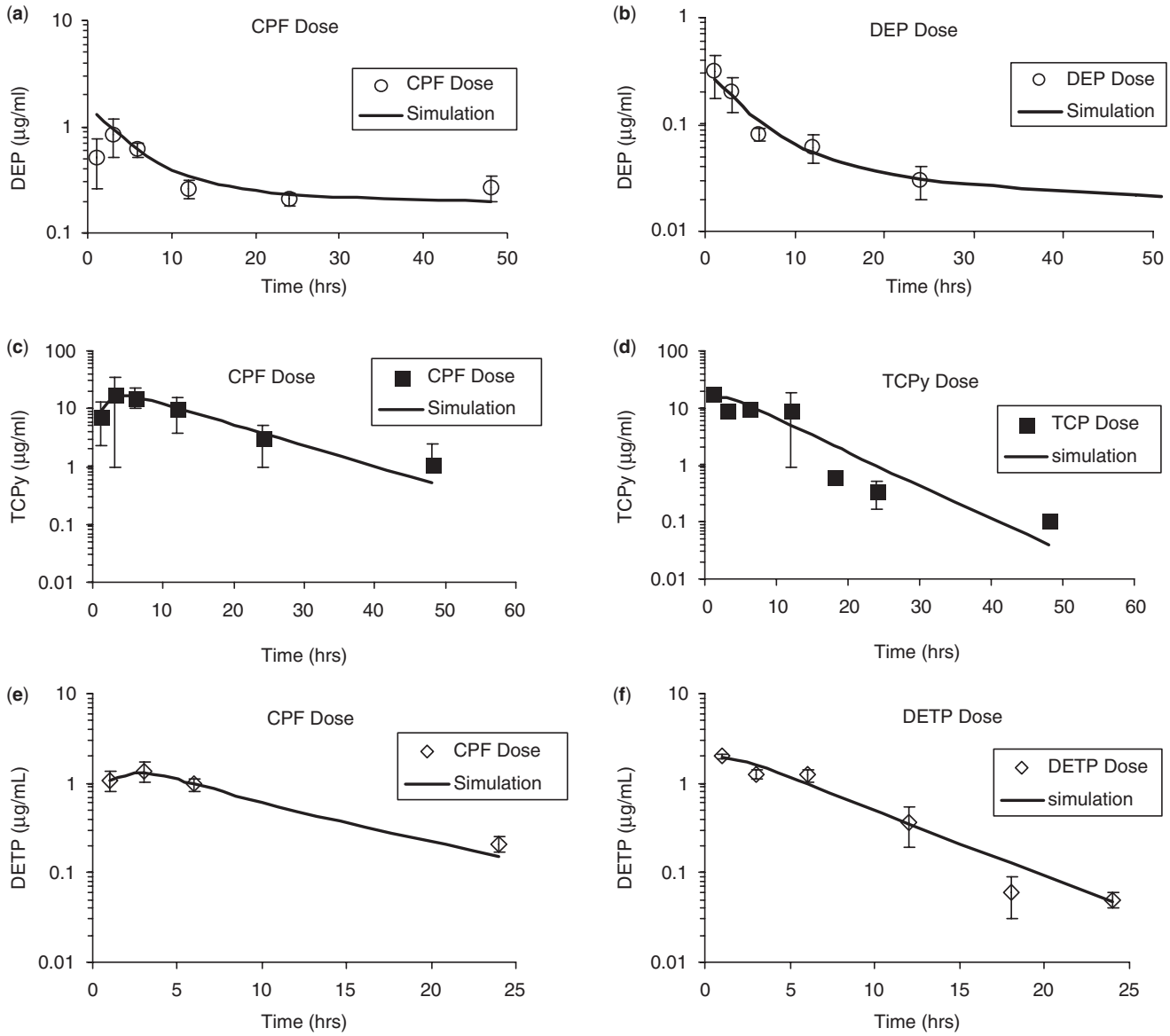

Figure 19.9 Time course of chlorpyrifos (CPF) metabolites [diethylphosphate (DEP), trichlorpyridinol (TCPy) and diethylthiophosphate (DETP)] in groups of rats orally administered CPF (a, c, e) or DEP (b), TCPy (d), and DETP (f). [Figure adapted with permission from Timchalk et al., 2007a).

alkyl phosphate and trichloropyridinol levels prior to conducting a pesticide exposure study. A potentially more useful biomonitoring strategy could include an approach that measures the insecticides as well as their urinary metabolites not only in urine samples, but also in relevant environmental media and in a person's diet (Lu et al., 2005).

19.5.3 Cholinesterase (ChE) Biomonitoring

As previously noted, the organophosphorus and carbamate insecticides share a common mode of insecticidal and toxicological action associated with their ability to inhibit the enzyme acetylcholinesterase (AChE) within nerve tissue (Ecobichon, 2001a, b; Sultatos, 1994). Although brain AChE is a key toxicological target, other B-esterases such as red blood cell (RBC) AChE and plasma butyrylcholinesterase (BuChE) are utilized as relevant biomarkers (Garabrandt et al., 2008). The extent and rate of B-esterase inhibition and recovery is dependent on the amount of available enzyme, differences in the inhibition rates, and the amount of time the enzyme is exposed to the toxicant (Timchalk et al., 2002; Vale, 1998). Hence, the *in vivo* sensitivity based on delivered target tissue dose and B-esterase sensitivity specifically for the organophosphorus insecticide chlorpyrifos follows the order: plasma ChE (BuChE) > RBC ChE (AChE) > brain ChE (AChE).

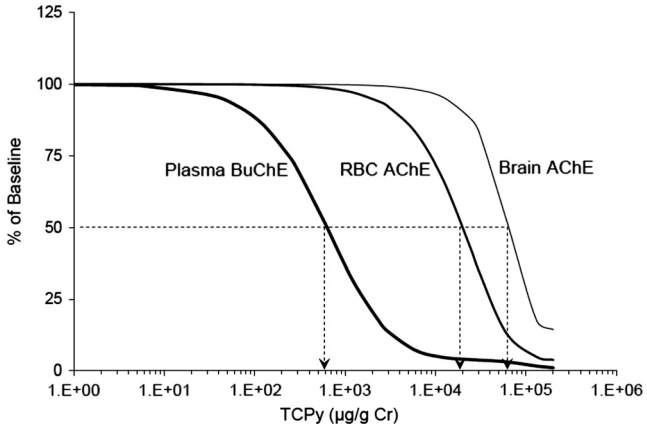

Figure 19.10 PBPK/PD model simulation of dose-response for inhibition of plasma BuChE, RBC AChE, and brain AChE under steady-state conditions as a function of urinary trichlorpyridinol (TCPy). [Figure reproduced with permission from Garabrant, D. H. et al. (2008). *J. Exp. Sci. Environ. Epidemiol*. (E-Pub) 1–9.]

Figure 19.10 further illustrates the quantitative differences between plasma, RBC, and brain ChE response in humans (Garabrandt et al., 2008). For these simulations, the PBPK/PD model (Timchalk et al., 2002) was utilized to predict ChE inhibition as a function of urinary trichlorpyridinol excretion at a steady-state exposure in humans. These simulations clearly illustrate the *in vivo* dose-dependent and relative sensitivity of the key blood ChE enzyme responses relative to brain AChE inhibition. A similar although quantitatively different response would likewise be observed for carbamate insecticides.

19.5.4 The Application of Pharmacokinetics for Quantifying Exposure to Organophosphorus Insecticides

The ability to more accurately quantify human exposure to insecticides has been enhanced by the use of biomonitoring approaches linked to pharmacokinetic analysis (Barr et al., 2006). This has been used successfully 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 (Lavy et al., 1993; Loewenherz et al., 1997; Richter et al., 1992).

Historically, workplace exposure to chemicals has been controlled through environmental monitoring that has primarily focused on the measurement of the chemical contaminant in the ambient air. However, 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 to quantify the amount of absorbed dose (Chester, 1993; Franklin et al., 1981, 1986; Garabrant et al., 2008; Heudorf and Angerer, 2001; Putnam et al., 2008).

For example, Franklin et al. (1981, 1986) estimated exposure of workers to the organophosphorus 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 alkyl phosphate metabolite excreted in the urine was compared against the amount of active ingredient sprayed.

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 exposures (Hayes et al., 1980; Lavy et al., 1993; Loewenherz et al., 1997). Hayes et al. (1980) evaluated the occupational exposure of pest control operators in which there was combined use of insecticides (vaponite, diazinon, and chlorpyrifos). Biomonitoring was based on blood ChE determination and the quantitation of alkyl phosphate metabolites in the urine. The authors reported that external air monitoring did provide information regarding the levels and types of pesticide exposures, but did not provide adequate information on the degree to which these insecticides were absorbed. However, by quantifying the urinary alkyl phosphate levels, quantitative information on absorption and excretion of these pesticides was obtained. Biomonitoring based on the measurement of alkyl phosphate metabolites has also been used to compare pesticide exposure in children living in proximity to high spray areas (i.e., orchards) and whose parents or guardians are pesticide applicators (Loewenherz et al., 1997). Based on known pesticide use patterns, it was determined that insecticide exposures would be primarily associated with azinphos-methyl, chlorpyrifos, and phosmet. Loewenherz et al. (1997) collected and evaluated 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 than the reference children (0.021 vs. 0005 µg/L, respectively). As noted by Maroni et al. (2000), a major limitation of using nonspecific metabolites, such as alkyl phosphates, for organophosphate biomonitoring is that they provide no specificity and therefore are only indicative of a general exposure and cannot be used to quantitatively assess individual insecticide dosimetry.

An alternative strategy is to biomonitor for parent organophosphates and pesticide-specific metabolites in blood and

urine to provide a quantitative assessment of dosimetry (Byrne et al., 1998; Drevenkar et al., 1993; Hryhorczuk et al., 2002; Maroni et al., 2000). For example, Byrne et al. (1998) conducted a multipathway residence exposure study to evaluate resident exposure to chlorpyrifos. Biomonitoring of urinary trichlorpyridinol was utilized to directly estimate total post-application chlorpyrifos absorption. In this study each house had crack and crevice treatment for control of cockroach infestations by a licensed applicator, and six human volunteers participated by following their normal routine. The study design involved the collection of 24-hour urine specimens beginning one day prior to application and for 10 consecutive days following the initial exposure. By converting the amount of trichlorpyridinol quantified in the urine based on the known pharmacokinetics of chlorpyrifos and trichlorpyridinol, it was possible to calculate both the pre- and post-exposure chlorpyrifos doses. A similar biomonitoring strategy was utilized to evaluate chlorpyrifos exposure in golfers following turf grass application (Putnam et al., 2008). This exposure assessment design involved collection of a pre- and post-exposure urine sample (27 hours). The calculation of absorbed dose was based on the amount of trichlorpyridinol collected in the urine over a 27-hour collection period, which was then adjusted to account for the amount of trichlorpyridinol excreted in the urine (~71%) based on the previous pharmacokinetic analysis in humans (Nolan et al., 1984). The results of this study indicate that the estimated exposure to golfers was 19 to 68 times below the U.S. Environmental Protection Agency (EPA) reference dose (RfD) values, and dermal exposure was determined to be the predominant exposure pathway (Putnam et al., 2008).

A recent study was conducted to evaluate the relationship between RBC AChE and plasma BuChE activity relative to the amount of urinary trichlorpyridinol in chemical manufacturing workers occupationally exposed (over one year) to chlorpyrifos (Garabrant et al., 2008). This study was particularly unique in that it compared biomarkers of dose (trichlorpyridinol) and biological response (ChE) in a cohort of workers with known occupational exposure to the insecticide. The focus of this study was to quantitatively ascertain the relationship between biomarkers of response (RBC AChE), and biomarkers of exposure (plasma BuChE and urinary trichlorpyridinol) in occupationally exposed populations.

In this study design, each participant had a series of plasma BuChE and trichlorpyridinol urinary excretion measurements taken over time; in addition, RBC AChE activity was measured at two time points directly in coincidence with the measurement of urinary trichlorpyridinol. Figure 19.11 illustrates the relationship between RBC AChE or plasma BuChE and the amount of urinary trichloropyridinol per gram of urinary creatinine (μg/g Cr). There were no statistically significant differences in RBC AChE activity between chlorpyrifos workers and nonworkers (referent group; Albers et al., 2004). As illustrated in Figure 19.11a, over the entire range of trichloropyridinol urinary concentrations measured there was no evidence of a decline in RBC AChE activity; in addition, the observed RBC AChE response was predicted (dashed line) by the chlorpyrifos PBPK/PD model (Garabrandt et al., 2008). For the plasma BuChE response the no-effect level (cut point) based on urinary trichlopyridinol was 110 μg/g Cr. This urinary trichlopyridinol concentration was comparable to a steady-state absorbed chlopyrifos dose of ~5 μg/kg/day. These data demonstrate the association between ChE activity (RBC AChE or plasma BuChE) and a specific urinary metabolite biomarker for chlorpyrifos exposure in humans. Most importantly these dose-response data obtained under realistic exposure scenarios provides strong additional validation of the utility of the chlorpyrifos PBPK/PD model as a biomonitoring tool (Timchalk et al., 2002).

19.5.5 The Application of Pharmacokinetics for Quantifying Exposure to Carbamate Insecticides

For carbamate insecticides, biomonitoring of acute exposure has focused primarily on the assessment of ChE activity (RBC AChE or plasma BuChE). Although both organophosphorus and carbamate insecticides inhibit AChE in nervous tissue, for carbamates the inhibition is very labile, of a shorter duration, highly reversible, and repeated exposure results in no cumulative inhibition as is seen with organophosphorus agents (Maroni et al., 2000). Again, although the measurement of ChE activity can be used to assess carbamate exposure, it is not possible to ascertain a specific insecticide exposure based only on a determination of enzyme activity. However, as with organophosphate insecticides, there are a number of chemical specific biomarkers (parent insecticide and/or metabolite) that have been exploited as markers for individual carbamate insecticides (see Table 19.3).

Relative to organophosphorus insecticides there are fewer pharmacokinetic studies available for the carbamates (Maroni et al., 2000). Nonetheless, as a chemical class these insecticides have relatively short systemic half-lives, so that biomonitoring would need to be conducted relatively quickly (~24 hours) after the exposure. May et al. (1992) conducted a controlled human pharmacokinetic study with carbaryl in which male volunteers were given a single oral dose of 1 mg carbaryl/kg of body weight. This study reported that following oral administration blood carbaryl concentration rapidly (~30 minutes) reached peak levels then quickly decreased in blood with a half-life of 0.8 ± 0.5 hours; overall blood clearance was likewise rapid (5.5 ± 2.0 L/min). This study also reported a clear correlation ($r = 0.92$) between blood carbaryl concentration and changes in RBC AChE inhibition following *in vivo* carbaryl exposure. In earlier human studies, Knaak et al. (1968) noted that carbaryl is rapidly metabolized

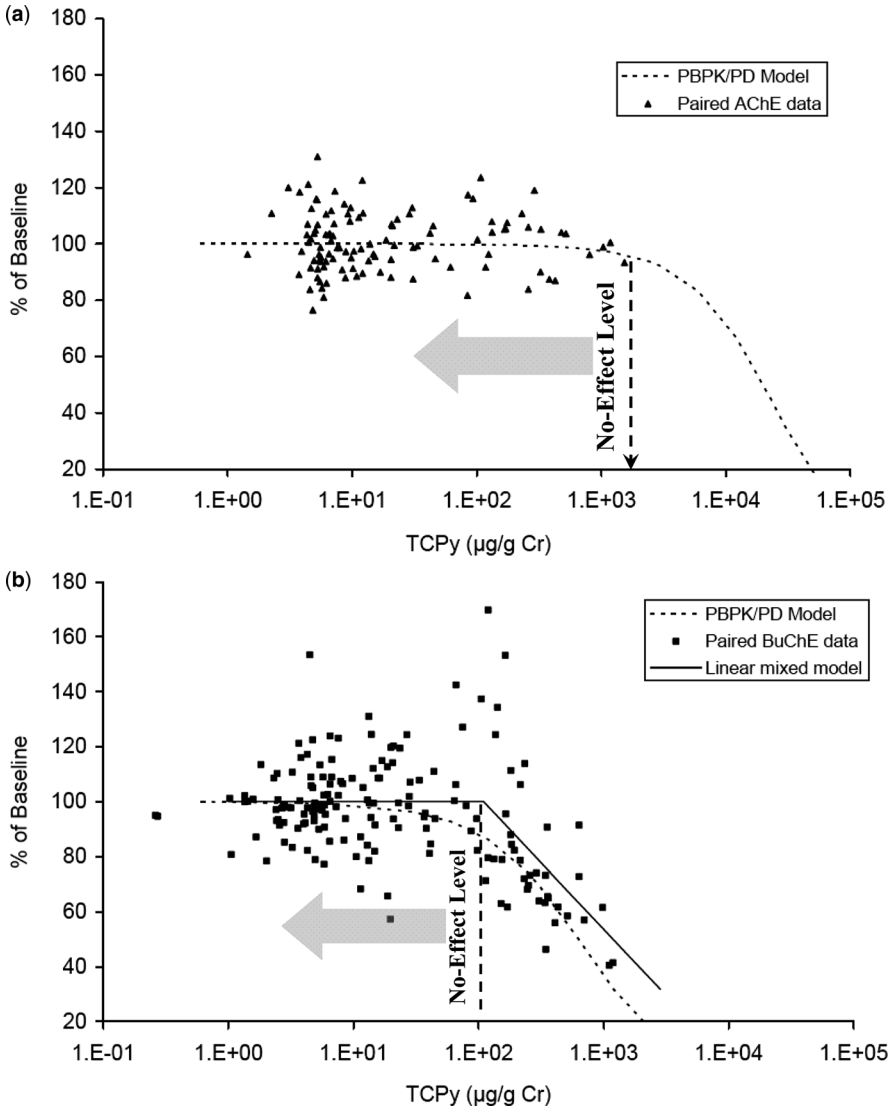

Figure 19.11 (A) RBC AChE scatter plot with predicted (dashed line) model simulation under steady-state exposure conditions. (B) Plasma BuChE scatter plot with PBPK/PD predicted dose response (dashed line) and linear mixed model results (solid line) results. [Figures adapted with permission from Garabrant, D. H. et al. (2008). *J. Exp. Sci. Environ. Epidemiol.* (E-Pub) 1–9.]

to 1-naphthol and associated glucuronide and sulfate conjugates, which are then readily excreted in the urine (see Fig. 19.2b). These pharmacokinetics studies have subsequently been utilized to design field biomonitoring studies to evaluate human exposures primarily for agricultural applications.

Biomonitoring studies have been utilized to quantify carbaryl exposure in farmers and their families by quantifying the parent insecticide and the major metabolite, 1-naphthol, in blood and/or urine. The biomonitoring study was a component of a broader exposure assessment evaluation that also quantified pesticide exposure by the measurement of air, dermal, and hand-wipe samples (Shealy et al., 1997). Following application of the insecticide, serum levels of carbaryl and 1-naphthol were measured; at peak blood levels the 1-naphthol exceeded the carbaryl concentration by a factor of 5 (500 μg/L vs. 100 μg/L) in the farm workers. 1-Naphthol was also rapidly measured in the urine of workers (peak levels $\sim$9300 μg/g creatinine); likewise the applicator's spouse and children showed the presence of 1-naphthol in the urine, but their urine levels were quite low (12 to 27 μg/g creatinine), suggesting that exposure for the nonapplicators, although present, was substantially less. This study demonstrated a strong correlation between serum and urinary 1-naphthol and also suggested that there was good agreement between the environmental and biological measurements (Shealy et al., 1997). These findings provide some confidence in the ability of biomonitoring results to reflect actual

TABLE 19.3 Primary Biomonitoring Markers for Carbamate Insectides

Carbamates*	Insecticide Specific Biomarker	Matrix
Aldicarb	Aldicarb-sulfone	Urine
Carbaryl	Carbaryl	Blood
	1-Naphthol	Urine
Methomyl	Methomyl	Blood
Pirimicarb	2-Dimethyl-4-hydroxy-5,6-dimethylpyrimidine	Urine
	2-Methyl-4-hydroxy-5,6-dimethylpyrimidine	Urine
Propoxur	Propoxur	Blood
	2-Isopropoxyphenol	Urine

*For all carbamates RBC AChE or plasma BuChE are used as exposure biomarkers

Source: Table adapted from Maroni et al., 2000.

exposures resulting from both occupational and nonoccupational exposure to carbaryl. Putnam et al. (2008) recently conducted an exposure study to evaluate golfers' exposure to chlorpyrifos and carbaryl following turf grass application. In this study the total carbaryl dose was determined by urinary biomonitoring of 1-naphthol. The glucuronide and sulfate conjugates of 1-naphthol were enzymatically hydrolyzed, such that total urinary 1-naphthol could be determined. In all cases the post-exposure metabolite concentrations were higher than pre-exposure and are indicative of exposure. Based on the use of whole body dosimeters, it was determined that dermal exposure was the dominant exposure route for golfers; however, based on the biomonitoring results the absorbed dose of carbaryl was substantially lower than the U.S. EPA hazard quotient criteria (Putnam et al., 2008).

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

19.5.6 Next-Generation ChE Biomarkers

As has been previously discussed, biomarkers for organophosphorus and carbamate insecticides have focused primarily on the measurement of ChE activity in blood and/or the detection of pesticide metabolites in blood or urine. However, the chemical reactivity and covalent binding of organophosphorus insecticides with blood and tissue proteins also produce novel chemical adducts (known as alkylphosphorylation and referred to as simply phosphorylation) on specific matrix proteins that have the potential to be exploited as biomarkers of exposure (Barry et al., 2008). The most common modification is phosphorylation of cholinesterases (AChE and BuChE) and their subsequent inactivation, leading to cholinergic system failure in the case of tissue and brain AChE. Phosphorylated adducts have also been detected on other proteins, including CaE, neuropathy target esterase (NTE), trypsin, chymotrypsin, and human serum albumin (Black et al., 1999; Boter and Ooms, 1967; Ecobichon and Comeau, 1973; Elhanany et al., 2001; Fonnum et al., 1985; Johnson, 1975; Johnson and Glynn, 1995; Li et al., 2007; Ooms and van Dijk, 1966; Peeples et al., 2005). Biomonitoring of protein adducts has the potential to extend the time interval between exposure and sampling and may be a suitable approach to detect low-level exposure. In this regard, Polhuijs et al. (1997) developed a procedure for the analysis of phosphorylated binding sites, which is based on reactivation of the phosphorylated enzyme with fluoride ions. Based on these methods, it was suggested that detection levels in the range of ~0.01% inhibited BChE should be quantifiable. This represents a detection level that is several orders of magnitude greater than what is currently possible on the basis of measuring ChE activity.

19.6 CONCLUSIONS

This chapter has discussed the application of pharmacokinetics and pharmacodynamics to facilitate a biomonitoring strategy for orgranophosphate and carbamate insecticides. Pharmacokinetics is concerned with the quantitative integration of absorption, distribution, metabolism, and excretion and can be used to provide useful insight into the dosimetry of these insecticides. Since organophosphorus and carbamate insecticides share a common mode of action through their capability to inhibited AChE activity, it is feasible to develop pharmacokinetic strategies that also link quantitative dosimetry with biologically based response modeling. Pharmacokinetic studies that have been conducted in multiple species, at various dose levels and across different routes of exposure have provided important insight into the *in vivo* behavior of these insecticides. The development and application of pharmacokinetic modeling for these insecticides represents a unique opportunity to quantitatively assess human health risk and to understand the toxicological implications of known or suspected exposures.

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