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NONINVASIVE BIOMONITORING APPROACHES TO DETERMINE DOSIMETRY AND RISK FOLLOWING ACUTE CHEMICAL EXPOSURE: ANALYSIS OF LEAD OR ORGANOPHOSPHATE INSECTICIDE IN SALIVA

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There is a need to develop approaches for assessing risk associated with acute exposures to a broad range of metals and chemical agents and to rapidly determine the potential implications to human health. Noninvasive biomonitoring approaches are being developed using reliable portable analytical systems to quantitate dosimetry utilizing readily obtainable body fluids, such as saliva. Saliva has been used to evaluate a broad range of biomarkers, drugs, and environmental contaminants, including heavy metals and pesticides. To advance the application of noninvasive biomonitoring a microfluidic/electrochemical device has also been developed for the analysis of lead (Pb), using square-wave anodic stripping voltametry. The system demonstrates a linear response over a broad concentration range (1–2000 ppb) and is capable of quantitating saliva Pb in rats orally administered acute doses of Pb acetate. Appropriate pharmacokinetic analyses have been used to quantitate systemic dosimetry based on determination of saliva Pb concentrations. In addition, saliva has recently been used to quantitate dosimetry following exposure to the organophosphate insecticide chlorpyrifos in a rodent model system by measuring the major metabolite, trichloropyridinol, and saliva cholinesterase inhibition following acute exposures. These results suggest that technology developed for noninvasive biomonitoring can provide a sensitive and portable analytical tool capable of assessing exposure and risk in real-time. By coupling these noninvasive technologies with pharmacokinetic modeling it is feasible to rapidly quantitate acute exposure to a broad range of chemical agents. In summary, it is envisioned that once fully developed, these monitoring and modeling approaches will be useful for evaluating acute exposure and health risk.

In light of the potential for high-dose acute exposures to a broad range of chemical contaminants both from occupational accidents and from acts of terrorism there is a need to develop rapid approaches for assessing systemic

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dosimetry and the resulting health hazards. A strategy has been developed that effectively links noninvasive biomonitoring approaches that will utilize portable microanalytical systems for real-time analysis to pharmacokinetic models to predict dosimetry following acute exposures to xenobiotics (Timchalk et al., 2001).

Biomonitoring offers one of the best approaches for accurately assessing human dosimetry and for determining risk from both occupational and environmental exposure to xenobiotics (Friberg & Elinder, 1993; Christensen, 1995). Although biomonitoring has been conducted primarily by utilizing biological matrixes such as blood and urine, other matrixes such as saliva represent a simple and readily obtainable fluid. In this regard, saliva has been used to evaluate a broad range of biomarkers, drugs, and environmental contaminants, including drugs of abuse, hormones, chemotherapeutics, heavy metals, and pesticides (Joselow et al., 1968; Hayashi et al., 1989; Nigg & Wade, 1992; Schramm et al., 1992; Lu et al., 1997, 1998).

To facilitate biomonitoring there is a need to develop reliable, portable, and cost-effective analytical instrumentation for on-site monitoring of individuals. Microfabrication technology has been utilized to create a micro-scale total analytical system (μ-TAS). One potential application of μ-TAS is the development of microanalytical devices that can be easily integrated into microchemical or biological systems for real-time monitoring (Lin et al., 2001). Coupled to μ-TAS is the development of relevant sensor systems that can be based on a number of technologies including electrochemical detection, immunosensors, and immobilized enzyme-based detection (Lin et al., 2001; Andreescu et al., 2002; Mulchandani et al., 1998; Imato & Ishibashi; 1995; Penalva et al., 2000; Gaberlein et al., 2000). The general approach is illustrated in Figure 1

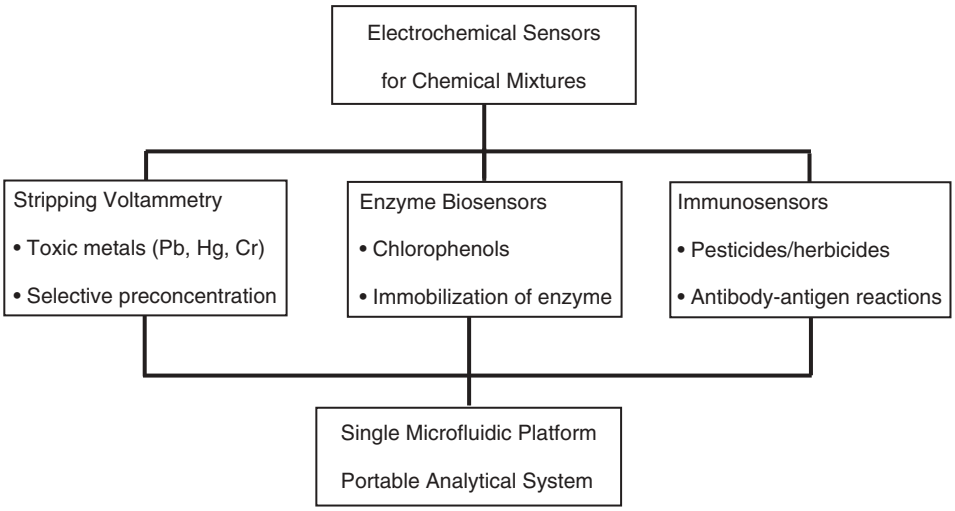

FIGURE 1. Schematic for integrating multiple sensors into a plug and play microfluidics platform for portable analytical systems.

and involves development of an array of sensors that are incorporated into a single microfluidics platform using a "plug-and-play" design where sensors are readily exchanged depending on the contaminants of interest.

To reliably estimate internal dosimetry from a "spot" saliva sample requires a good understanding of the pharmacokinetics of the chemical and the relationship between the chemical concentration in the saliva and blood. Nigg and Wade (1992) summarized a number of early studies that attempted to correlate saliva levels of drugs, hormones, and chemicals with plasma levels to accurately estimate internal dose. With increasing interest in the use of noninvasive monitoring for drug testing and for the evaluation of chemical exposures, it is anticipated that many more agents will be included in future lists. To facilitate understanding of the kinetics of drug and/or chemical clearance from blood into saliva, a fundamental understanding of salivary gland physiology and biochemistry is essential. Höld et al. (2000) provides a detailed review of human salivary gland anatomy, physiology, and the mechanisms associated with drug transfer from blood to saliva. Likewise, in rodents there are a number of studies evaluating the kinetics of saliva clearance for environmental contaminants (Borzelleca & Skalsky, 1980; Mobarak & P'an 1984; Bratt et al., 1995; Lu et al., 1997, 1998; Timchalk et al., 2002; Kousba et al., 2003). Although a fundamental understanding of saliva-gland physiology exists, comparative cross-species (i.e., rodent vs. human) pharmacokinetic studies are lacking and are needed to further develop and refine dosimetry models.

The importance that physiologically based pharmacokinetic (PBPK) modeling can have in facilitating biomonitoring has been suggested (Mason & Wilson, 1999). These models are used to calculate target tissue dosimetry, facilitate extrapolation to low doses, and enable cross-species extrapolation (Andersen, 2003). These models integrate standard values for organ volumes and blood flows with chemical-specific parameters (e.g., tissue solubility, metabolic rate constants) to determine tissue dosimetry and pharmacodynamics. The PBPK models also provide a biologically based approach for assessing aggregate and cumulative risk associated with environmental exposures to a range of metals and chemical agents.

Physiologically based pharmacokinetic models for lead (Pb) and the organophosphate insecticide chlorpyrifos have recently been modified to incorporate a saliva compartment (O'Flaherty, 1991; Timchalk et al., 2001, 2002). An illustration of the PBPK model structure for Pb with the inclusion of a saliva compartment is presented in Figure 2. As previously noted, the application of these models has been recognized as an important tool for equating exposure to target tissue dosimetry (Andersen, 2003). To achieve a goal of employing saliva as a noninvasive quantitative biomonitoring matrix in humans, a number of improvements in the current understanding of xenobiotic salivary kinetics is warranted. Additional research is needed to provide a basic understanding of the mechanism by which chemicals are transported from the blood into the salivary glands, and subsequently secreted into the saliva. Finally, there is a

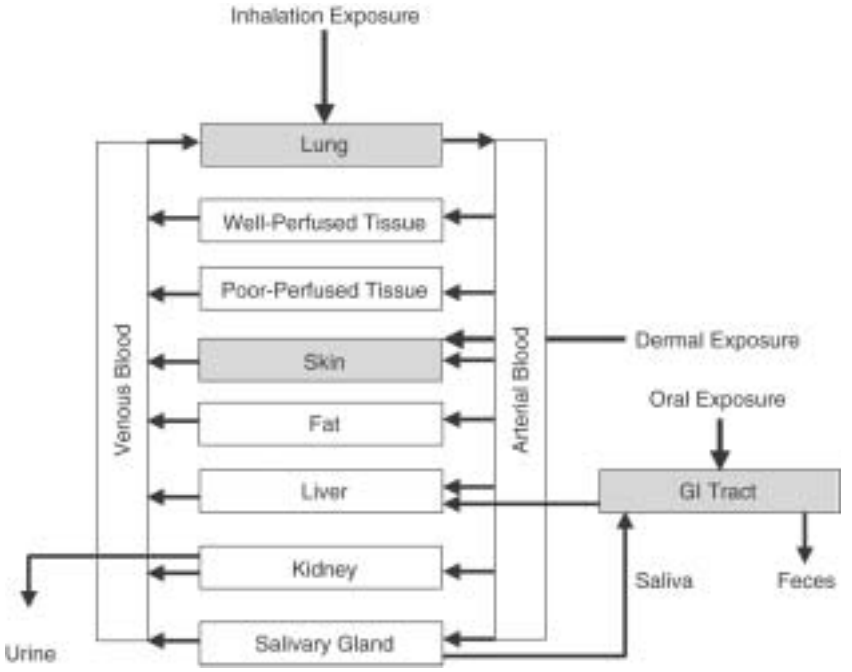

FIGURE 2. Physiologically based pharmacokinetic (PBPK) model for Pb that has been modified to incorporate a saliva gland compartment. PBPK model parameters were as previously described by O'Flaherty (1991) and modified by Timchalk et al. (2001) to incorporate saliva compartment parameters. Shaded compartments represent potential routes for exposure.

need to acquire a more extensive in vivo data set comparing the pharmacokinetics of xenobiotics in blood and saliva in both rodents and humans. Nonetheless, once fully validated these models hold promise to accurately predict systemic dosimetry based on a limited sampling of saliva, thereby providing an accurate predictor of "internal dose."

The objective of this article is to illustrate, using two examples, the potential utility of real-time saliva analysis coupled with pharmacokinetic modeling as a novel biomonitoring strategy. The first example focuses on the application of saliva biomonitoring for assessing dosimetry to the heavy metal Pb, while the second example highlights recent developments to extend this approach to biomonitoring pesticides and in particular organophosphate insecticides.

EXAMPLE 1: NONINVASIVE LEAD (Pb) BIOMONITORING

There has been a significant ongoing effort to reduce the utilization and subsequent release of Pb into the environment, resulting in a beneficial reduction in the blood Pb levels among the U.S. population and in particular children (Juberg et al., 1997). However, even though blood Pb levels have

decreased there are still significant numbers of children within susceptible socioeconomic groups where blood Pb levels are excessively high, representing a potential health threat. In the case of Pb, biomonitoring has primarily focused on the measurement of blood Pb concentration, although hair, urine, and saliva have likewise been utilized to assess exposure (P'an, 1981; Revich, 1994; Pirkle et al., 1995). Although blood biomonitoring represents the most common strategy for Pb, several studies suggest that saliva is a viable alternative matrix. The use of saliva is particularly enticing since this represents a simple noninvasive method that has distinct advantages in the evaluation of newborn infants and young children (Gorodischer & Koren, 1992). The potential utility of saliva as a biomonitoring medium for assessing human body burden of Pb from ambient environmental exposure has previously been demonstrated (P'an, 1981; Gonzalez et al., 1997). Gonzalez et al. (1997) utilized saliva to biomonitor for Pb and cadmium in a limited population of young adults living in Mexico City. Their results suggest that saliva is a good biomonitor for Pb and demonstrated that populations residing in Mexico City had elevated Pb levels. Mobarak and P'an (1984) conducted studies using rats to better understand the relationship between saliva Pb concentration and the distribution of Pb in whole blood, plasma, and plasma ultrafiltrate. A linear relationship was observed when comparing Pb concentrations in saliva with either whole blood or free plasma in rats administered multiple doses of Pb (100 mg/kg) by intraperitoneal injection and sacrificed up to 21 after the last injection. The data from this study are presented in Figure 3 and suggest a good linear correlation ($r^2 = .71$) for saliva and blood Pb concentration over the range tested.

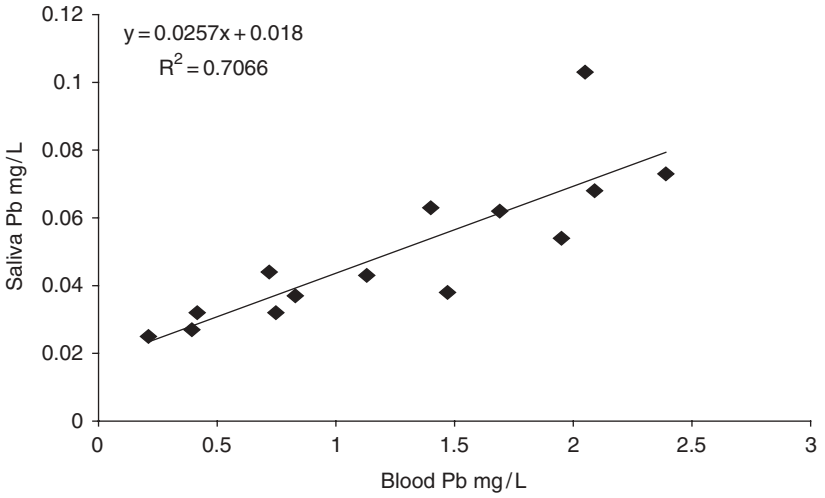

FIGURE 3. Correlation between the amount of Pb detected in saliva and blood in rats. The data was obtained from a study of Mobarak and P'an (1984) and reanalyzed by Timchalk et al. (2001). Adapted with permission from Timchalk et al. (2001).

Integrated Microfluidics/Electrochemical Sensor System for Pb

Details concerning the fabrication of a microfluidics/electrochemical device have been previously published (Lin et al., 2001), and a schematic diagram illustrating the components of the integrated microfluidic device is presented in Figure 4. The microelectrochemical flow cell is based on a wall-jet (flow-onto) design that integrates three electrodes (Bohs et al., 1994; Zhou et al., 1997), a mercury-film (Hg) working electrode and a platinum (Pt) and silver (Ag/AgCl) auxiliary and reference electrode, respectively. The sample solution is loaded into the sample reservoir and delivered by an integrated micropump, and square-wave anodic stripping voltametry (SWASV) is used for quantitation of Pb in the specimens (Timchalk et al., 2001; Lin et al., 2001).

As is illustrated in Figure 5, the response of the sensor system is linear up to 2000 ppb with a detection limit of 0.2 ppb (Figure 5 illustrates the low end of the curve, 0–10 ppb). Pirkle et al. (1998) reported that the overall mean blood Pb for the U.S. population was 2.3 μg Pb/dl (23 ppb), with 2.2% of the population having blood Pb >10 μg Pb/dl (100 ppb) (i.e., above a health concern level). Based on these blood Pb levels and the levels of Pb detected in human saliva (Gonzalez et al., 1997), detection capability in the low ppb range is reasonable for a portable sensor system. In addition, the Hg-film electrode was shown to have good stability when repeatedly used in the flow stream with a reported 4–5% standard deviation for up to 16 consecutive runs (Lin et al., 2001).

In Vivo Saliva Validation and PBPK Modeling of Blood and Saliva Pb in Rats

Details describing the animal exposures, analytical methods, and PBPK model development have been previously reported (Timchalk et al., 2001). In

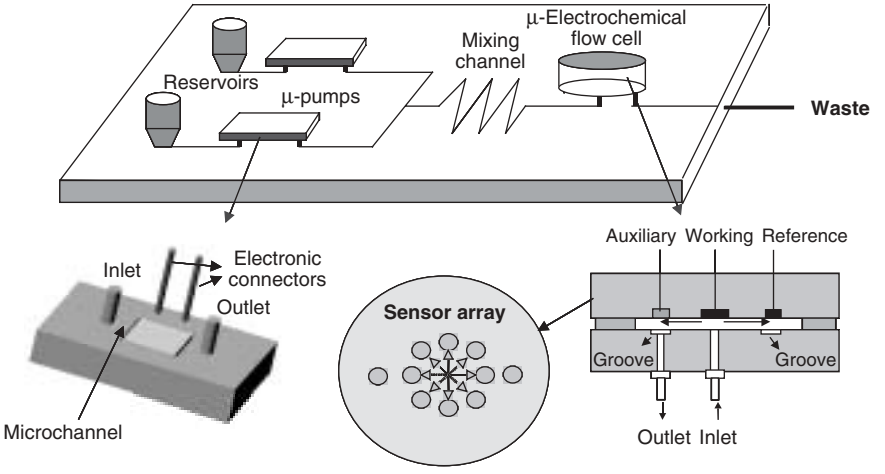

FIGURE 4. Schematic diagram of the integrated micro-fluidic system with plug-in micropump and microelectrochemical detector.

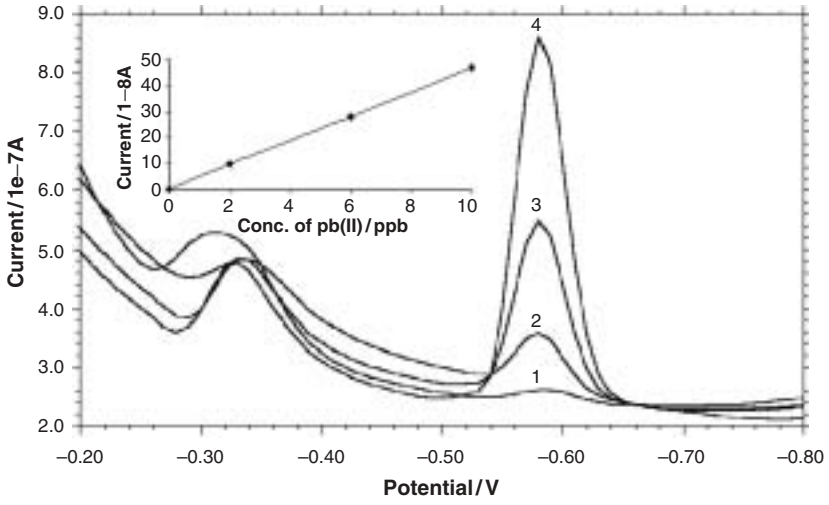

FIGURE 5. Representative SWASV voltammogram for Pb^{2+} concentrations of 0, 2, 6, and 10 ppb for peaks 1–4, respectively; limit of detection 0.2 ppb with 3 min pre-concentration period. Adapted with permission from Timchalk et al. (2001).

brief, adult male rats were orally administered acute doses (0–500 mg/kg of body weight) of Pb acetate; 24 h postdosing they were anesthetized, and saliva and blood were collected and analyzed for Pb using the microanalytical system and compared against inductively coupled plasma mass spectrometry (ICP-MS) analysis. The predictive capability of the PBPK model was evaluated against both previously published data (Mobarak & P'an, 1984) and experimental data generated in our laboratory (Timchalk et al., 2001). As illustrated in Figure 6, the concentration of Pb in saliva was consistently less than the

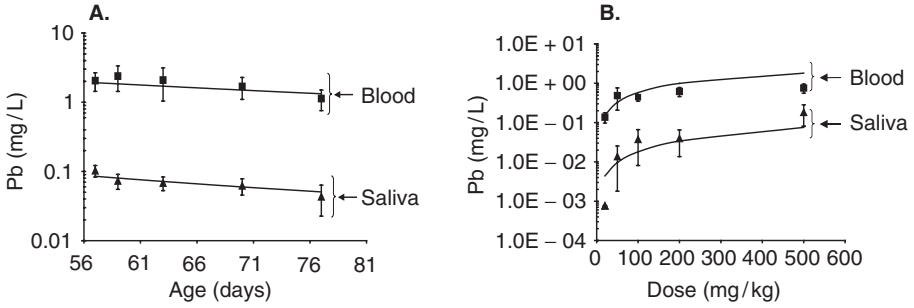

FIGURE 6. (A) Time course of Pb in blood and saliva of rats following 3 intraperitoneal (ip) doses of 100 mg Pb/kg body weight. The data were obtained from Mobarak and P'an (1984) and the symbols represent mean ± SD for four animals per group. (B) concentration of Pb in blood and saliva of rats ~24 h following oral gavage doses of 20, 50, 100, and 200 mg Pb/kg of body weight. The symbols represent the mean ± SD for three to five animals per group, and the lines depict the PBPK predicted blood and saliva concentrations. Adapted with permission from Timchalk et al. (2001).

blood concentration (~1–2 orders of magnitude); however, the kinetics in saliva reasonably paralleled the response seen in blood. In addition, the goodness of model fit as demonstrated by the model prediction (line) versus. experimental results (data points) suggests that the PBPK model can be used to describe the kinetics of Pb in both blood and saliva. Although the PBPK saliva Pb model was developed with a limited data set, the results are encouraging and suggest that with further model development, refinement, and validation the model will be a useful tool to assess Pb dosimetry using saliva.

Saliva Pb Summary

A portable microanalytical system has been developed to quantitate Pb in saliva with analytical sensitivity in the low ppb range and has been evaluated using saliva and blood specimens obtained from rats pretreated with Pb acetate. The results suggest that the microanalytical system can be used for real-time monitoring of saliva Pb; however, further validation in animal model systems and with human samples is needed.

EXAMPLE 2: NONINVASIVE ORGANOPHOSPHATE INSECTICIDE BIOMONITORING

Organophosphates, like chlorpyrifos, constitute a large class of chemical insecticides that are widely used in the agricultural industry and in home applications (Aspelin, 1992, 1994). As a result, they are involved in more occupational poisoning cases than any other single class of insecticide, and in-home use has resulted in both intentional (suicide) and accidental human exposure (Al-Saleh, 1994).

The toxic effects of organophosphate insecticides are associated with the capacity of the parent chemical, or an active metabolite, to inhibit acetylcholinesterase (AChE) enzyme activity within nerve tissue (Murphy, 1986; Sultatos, 1994). The biochemical interactions between organophosphates and AChE and the toxicological implications of AChE inhibition are well understood. In general, phosphorothionates like chlorpyrifos lack the capacity to directly inhibit AChE and must first be metabolized to the corresponding oxygen analog (chlorpyrifos-oxon). The metabolic scheme for the thionophosphate insecticide chlorpyrifos is presented in Figure 7. Activation to the oxon is mediated by cytochrome P-450 (CYP) mixed function oxidases primarily in the liver. In addition, oxidative dearylation of chlorpyrifos to trichloropyridinol (TCP) and diethylthiophosphate represents a competing detoxification pathway, which is likewise mediated by hepatic CYP (Ma & Chambers, 1994). Studies in humans and rodents indicate that trichloropyridinol represents the primary urinary metabolite of chlorpyrifos, although glucuronide and sulfate conjugates of trichloropyridinol have also been observed (Nolan et al., 1984; Bakke et al., 1976).

Since the mode of action for organophosphate insecticide toxicity primarily involves the chemical inhibition of AChE in nerve tissue, it is feasible to extend the dosimetry models to incorporate this pharmacodynamic (PD) response.

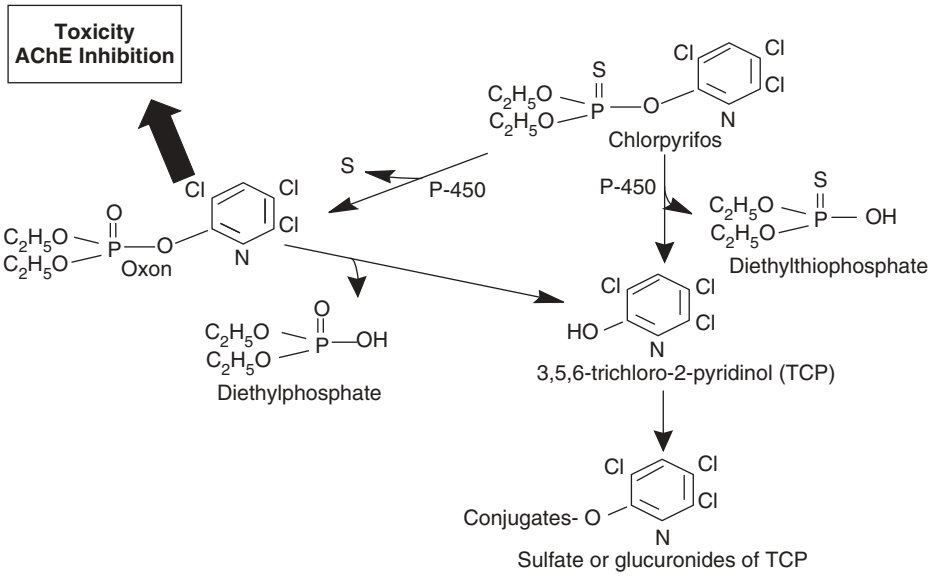

FIGURE 7. Metabolic scheme for chlorpyrifos and the major metabolites chlorpyrifos-oxon, trichloropyridinol, diethylphosphate, and diethylthiophosphate.

Andersen (1995) noted that a linkage of dosimetry and PD models provides a strong biologically based framework to establish dose-response extrapolations. With regard to organophosphate insecticides, the formation of the oxon metabolite (pharmacokinetics) can be directly coupled to the inhibition of AChE (pharmacodynamics), forming an integrated PBPK/PD dosimetry and response model as illustrated in Figure 8. In this regard a limited number of PBPK/PD models for organophosphate insecticides and nerve agents have been published in the literature (Maxwell et al., 1988; Gearhart et al., 1990; Sultatos, 1990; Abbas & Hayton, 1997; Timchalk et al., 2002). Most recently, a PBPK/PD model has been developed for chlorpyrifos, and the pharmacokinetic and pharmacodynamic response has been validated in both rats and humans; the model structure has been previously described (Timchalk et al.,

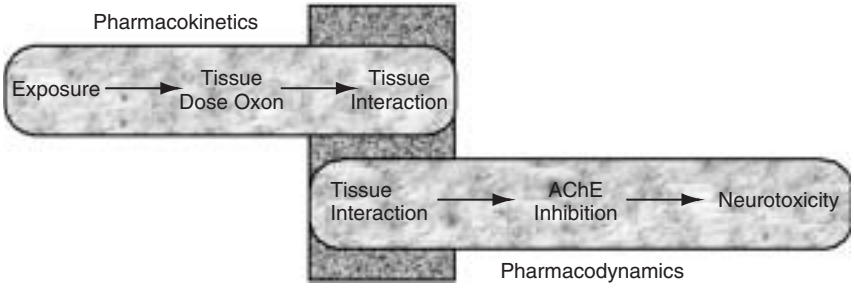

FIGURE 8. Relationship between pharmacokinetic and pharmacodynamic interactions for organophosphate insecticides.

2002). An illustration of the model fit to human serum dosimetry and ChE inhibition data is presented in Figure 9. As illustrated, the model is capable of accurately describing the overall pharmacokinetics of chlorpyrifos in the blood of a poison victim (Drevenkar et al., 1993) who ingested 30–60 ml of a commercially available insecticide (see Figure 9A). Likewise, the time course of plasma cholinesterase (ChE) inhibition following a single oral (0.5 mg/kg) or dermal (5 mg/kg) dose of chlorpyrifos in human volunteers (Nolan et al., 1984) was well described with the model. This model is now being extended to incorporate a salivary-gland compartment similar to the model structure previously described for Pb (see Figure 2).

Organophosphate insecticide biomonitoring has been primarily focused on the assessment of ChE activity in blood or the quantification of metabolites in

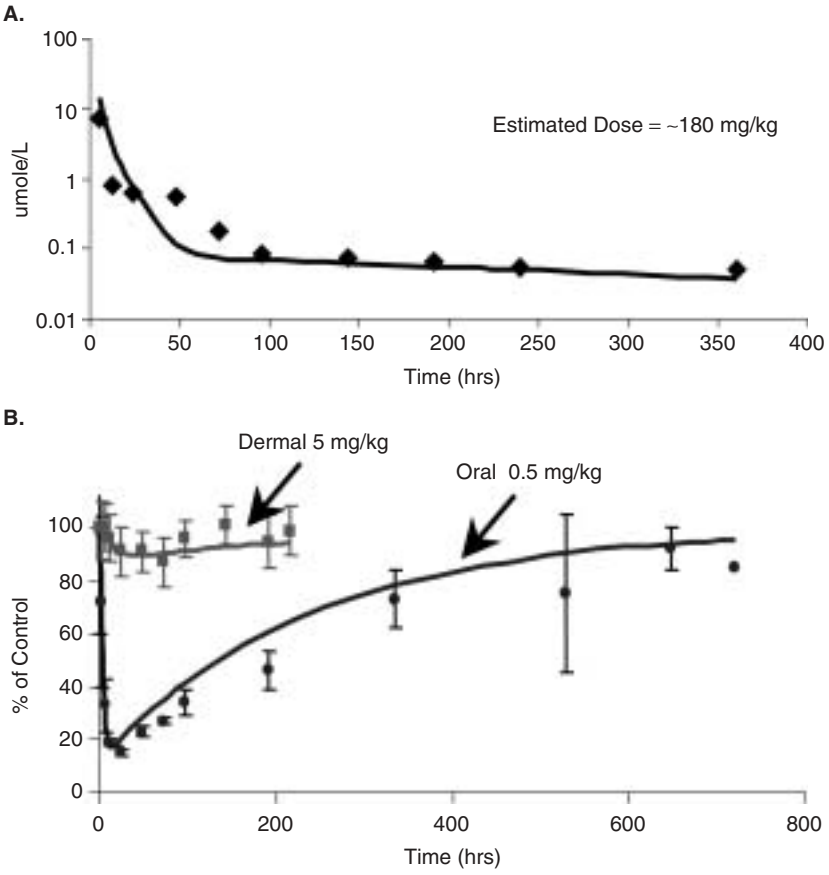

FIGURE 9. Examples of PBPK/PD model fit of experimental human data for chlorpyrifos exposure. (A) Time course of chlorpyrifos in serum of a single poison victim who orally ingested a commercial insecticide product containing chlorpyrifos (data extracted from Drevenkar et al., 1993). (B) Plasma ChE inhibition in human volunteers administered chlorpyrifos orally or dermally (data from Nolan et al., 1984). The line represents the model simulation of the experimental data. Adapted with permission from Timchalk et al.

urine (Peoples & Knaak, 1982; Chester, 1993). As such, trichloropyridinol is an important candidate for biomonitoring (Nolan et al., 1984). Borzelleca and Skalsky (1980) noted that in rats exposed to the carbamate insecticide carbaryl, saliva ChE inhibition was consistent with the plasma and red blood cell ChE inhibition and the saliva carbaryl metabolite concentrations correlated with the metabolite blood concentrations. Overall, this suggests that both the saliva chemical concentration and ChE inhibition may be particularly useful noninvasive markers to predict the chemical concentration and ChE activity in the blood.

Saliva ChE Activity in Rats

To determine the feasibility of utilizing salivary ChE activity as a biomonitor for ChE inhibiting agents there is a need to adequately characterize the ChE enzyme activity within the saliva matrix. In this regard, Kousba et al. (2003) recently characterized the saliva ChE activity in rats using specific enzyme substrates (acetylthiocholine and butyrylthiocholine) and inhibitors of AChE (BW284C51) and butyrl cholinesterase (BuChE) (iso-OMPA) enzyme activity and compared the response in saliva against brain (pure AChE) and plasma (50:50 AChE:BuChE) enzyme activity. In summary, these experiments demonstrated that rat saliva ChE is primarily associated with BuChE activity (>95%). A time course illustrating the in vitro activity of saliva BuChE is presented in Figure 10. The maximum inhibition of BuChE activity was achieved by ~5 h postincubation, and a PD model was used to determine the BuChE active-site concentration,

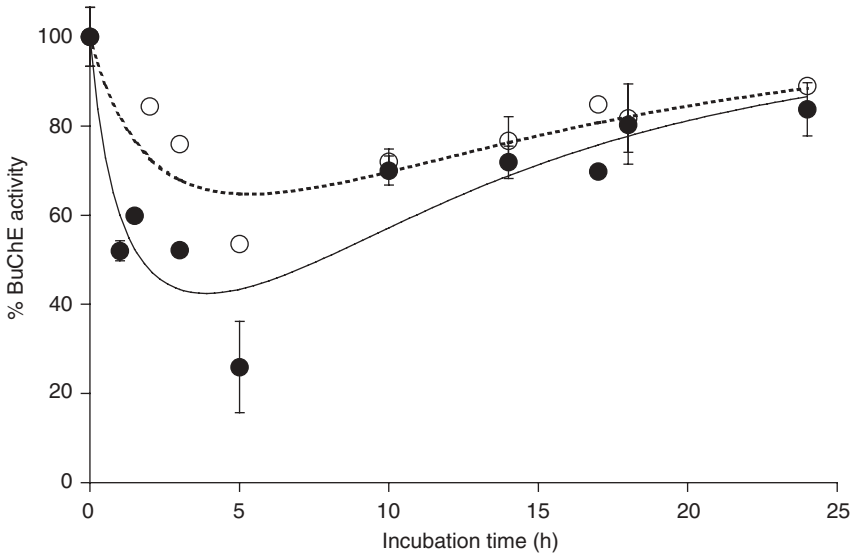

FIGURE 10. Pharmacodynamic model simulation output against experimental data expressed as per cent of BuChE activity for chlorpyrifos-oxon concentration of 0.1 p*M* (open circle and dashed line) and 0.2 p*M* (filled circle and solid line) incubated with saliva from naive adult rats. The maximum enzyme inhibition was achieved at 5 h. Adapted with permission from Kousba et al. (2003).

yielding an actual BuChE concentration of ~1.20±0.13 fmol ml^{-1} saliva. This concentration of BuChE enzyme activity is ~12,000- and 1000-fold less than that of the brain and plasma, respectively (Kousba et al., 2003), but nonetheless could be easily measured. These results clearly indicate that the ChE activity is present in saliva, and it is possible to follow the kinetics of inhibition over time.

To further establish the potential utility of saliva for organophosphate insecticide biomonitoring, adult male rats were orally exposed to single chlorpyrifos doses (1–50 mg/kg), and the time course of the major metabolite trichloropyridinol and the extent of ChE inhibition were determined in both blood and saliva. The procedures for saliva collection were as previously described (Timchalk et al., 2001). The results from these experiments are presented in Figure 11. The time course of trichloropyridinol in the blood of rats demonstrated a linear response with increasing dose and was comparable to previously observed kinetics in humans (Timchalk et al., 2002). Trichloropyridinol was readily detected in the saliva although the concentrations were ~2 orders of magnitude less than that observed in blood; however, the kinetic profile in saliva clearly paralleled the response seen in the blood. The ChE response in both plasma and saliva likewise demonstrated a dose-dependent inhibition, with maximum inhibition being achieved 3–6 h post-dosing, which is consistent with the plasma ChE inhibition kinetics previously observed in the rat (Timchalk et al., 2002). The degree of saliva ChE inhibition is fairly comparable to the response observed in the plasma and may be related to the fact that saliva is nearly all BuChE, which is particularly sensitive to chlorpyrifos inhibition (Kousba et al., 2003; Timchalk et al., 2002), whereas, plasma ChE in rats is an equal mixture of AChE and BuChE enzyme.

Saliva Organophosphate Insecticide Summary

These initial in vitro and in vivo experimental results indicate that both the major metabolite of chlorpyrifos and the degree of saliva ChE inhibition can be measured following in vivo exposure to a range of chlorpyrifos doses in rats. Although additional studies are needed in both rats and humans to further validate the observed results, these are promising and suggest that the utility of saliva as a biomonitoring matrix for organophosphate insecticides is plausible.

UTILITY AND CHALLENGES OF SALIVA AS A BIOMONITORING MATRIX

Although saliva as a biomonitoring matrix holds great promise, a clear understanding of its limitations is needed before it can be used quantitatively to assess chemical exposure (Niggs & Wade, 1992). In general, the ease of collection makes saliva ideally suited as a noninvasive approach and should result in excellent compliance, which is particularly critical in biomonitoring potentially sensitive subpopulations such as children and newborns. However, to utilize saliva for chemical biomonitoring, the relationship between the chemical concentrations in blood and saliva needs to be established. If a correlation

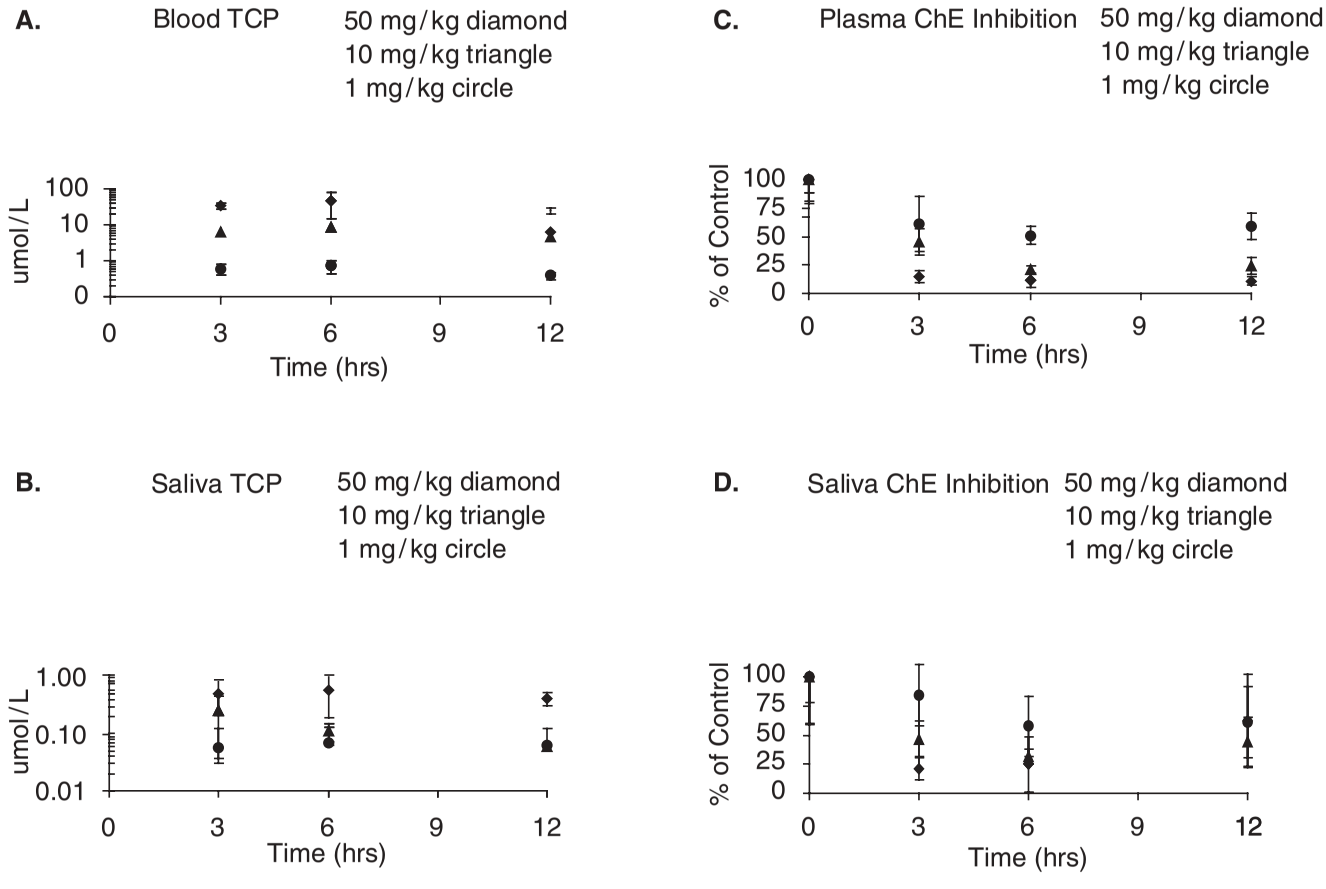

FIGURE 11. Time course of trichloropyridinol (TCP) in blood (A), saliva (B), and ChE inhibition in plasma (C) and saliva (D) following oral administration of chlorpyrifos to rats at doses ranging from 1–50 mg/kg body weight.

exists, then it will be feasible to directly predict a blood concentration for a given chemical based on a "spot" saliva analysis. For example, in the case of Pb the biological exposure index (BEI) is based on blood Pb concentration and is independent of time, so a spot saliva sample that correlates with blood Pb can be directly compared to the current BEI. For organophosphate insecticides, the BEI is based on the degree of inhibition of red blood cell ChE activity (70% of baseline). In this case it is anticipated that the PBPK/PD model can be used to correlate the dynamics of saliva ChE inhibition directly with the red blood cell response, thereby enabling us to determine the extent of systemic ChE inhibition from a "spot" saliva specimen. Again, the key to the utility of saliva for biomonitoring will be to conduct the appropriate validation studies to establish a fundamental understanding of what a saliva measurement means with regard to systemic dosimetry for a xenobiotic.

CONCLUSIONS

There is a growing need to develop reliable and portable analytical instruments for on-site monitoring of xenobiotic contaminants that use readily obtainable biological fluids like saliva for real-time analysis. To facilitate quantitating systemic dosimetry using a "spot" saliva sample, pharmacokinetic models are being developed. The results presented are encouraging and suggest that once fully developed the microanalytical system coupled to PBPK modeling will be an important tool for real-time biomonitoring for both Pb and organophosphate insecticides, for both occupational and environmental exposures.

REFERENCES

Abbas, R. and Hayton, W. L. 1997. A physiologically based pharmacokinetic and pharmacodynamic model for paraoxon in rainbow trout. *Toxicol. Appl. Pharmacol*. 145:192–201.

Al-Saleh, I. A. 1994. Pesticides: A review article. *J. Environ. Pathol. Toxicol. Oncol*. 13:151–161.

Andersen, M. E. 1995. Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment. *Toxicol. Lett*. 79:35–44.

Andersen, M. E. 2003. Toxicokinetic modeling and its applications in chemical risk assessment. *Toxicol. Lett*. 138:9–27.

Andreescu, S., Avaramescu, A., Bala, C., Magearu, V., and Marty, J. L. 2002. Detection of organophosphorus insecticide with immobilized acetylcholinesterase—Comparative study of two enzyme sensors. *Anal. Bioanal. Chem*. 374:39–45.

Aspelin, A. L. 1992. *Pesticide industry sales and usage: 1990 and 1991 Market estimates*. EPA 733-K-92–001. Washington, DC: Office of Pesticide Programs, U.S. Environmental Protection Agency.

Aspelin, A. L. 1994. *Pesticide industry sales and usage: 1992 and 1993 Market estimates*. EPA 733-K-94–001. Washington, DC: Office of Pesticide Programs, U.S. Environmental Protection Agency.

Bakke, J. E., Feil, V. J., and Price, C. E. 1976. Rat urinary metabolites of *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate. *J. Environ. Sci. Health Bull*. 3:225–230.

Bohs, C. E., Linhares, M. C., Kissinger, P. T. 1994. Stripping voltametry microflow systems. *Current Separations*. 13:6–8.

Borzelleca, J. F., and Skalsky, H. L. 1980. The excretion of pesticides in saliva and its value in assessing exposure. *J. Environ. Sci. Health*. 15:843–866.

Bratt, P., Johansson, I., Linder, J., Ericson, T. 1995. Function of the rat salivary glands after exposure to inorganic mercury. *The Science of Total Environment*, 172:47–55.

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

Christensen, J. M. 1995. Human exposure to toxic metals: Factors influencing interpretation of biomonitoring results. *Sci. Total Environ.* 166:89–135.

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

Friberg, L. and Elinder, C. G. 1993. Biological monitoring of toxic metals. *Scand. J. Work Environ. Health* 19(suppl. 1):7–13.

Gaberlein, S., Knoll, M., Spner, F., and Zaborosch, C. 2000. Disposable potentiometric enzyme sensor for direct determination of organophosphorus insecticides. *Analyst* 125:2274–2279.

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.

Gonzalez, M., Banderas, J. A., Baez, A., and Belmont, R. 1997. Salivary lead and cadmium in a young population residing in Mexico city. *Toxicol. Lett.* 93:55–64.

Gorodischer, R., and Koren, G. 1992. Salivary excretion of drugs in children: Theoretical and practical issues in therapeutic drug monitoring. *Dev. Pharmacol. Ther.* 19:161–177.

Hayashi, Y., Watanabe, J., and Ozeki, S. 1989. Salivary excretion of 4-fluorouracil (5-FU). IV. Dependency of saliva/plasma concentration ratio and salivary clearance on plasma concentration of 5-FU during constant-rate intravenous infusion in rats. *J. Pharmacobiodyn.* 12:137–144.

Höld, K. M., De Boer, D., Zuidema, J., and Maes, R. A. A. 2000. Saliva as an analytical tool in toxicology. *Int. J. Drug Testing* 1:1–33.

Imato, T., and Ishibashi, N. 1995. Potentiometric butyrylcholine sensor for organophosphate pesticides. *Biosens. Bioelectron.* 10:435–441.

Joselow, M. M., Ruiz, R., and Goldwater, L. J. 1968. Absorption and excretion of mercury in man. XIV. Salivary excretion of mercury and its relationship to blood and urine mercury. *Arch. Environ. Health* 17:39–43.

Juberg, D. R., Kleiman, C. F., and Kwon, S. C. 1997. Position paper of the American Council on Science and Health: Lead and human health. *Ecotoxicol. Environ. Safety* 38:162–180.

Kousba, A. A., Poet, T. S., Timchalk, C. 2003. Characterization of the in vitro kinetic interaction of chlorpyrifos-oxon with rat salivary cholinesterase: a potential biomonitoring matrix. *Toxicology*, 188:219–232.

Lin, Y., Timchalk, C., Matson, D. W., Wu, H., and Thrall, K. D., 2001. Integrated microfluidics/electrochemical sensor system for monitoring of environmental exposures to lead and chlorophenols. *Biomed. Microdevices* 3:331–338.

Lu, C., Anderson, L. C., Morgan, M. S., and Fenske, R. A. 1997. Correspondence of salivary and plasma concentrations of atrazine in rats under variable salivary flow rate and plasma concentration. *J. Toxicol. Environ. Health* 52:317–329.

Lu, C., Anderson, L. C., Morgan, M. S., and Fenske, R. A. 1998. Salivary concentrations of atrazine reflect free atrazine plasma levels in rats *J. Toxicol. Environ. Health* 53:283–292.

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

Maxwell, D. M., Vlahacos, C. P., and Lenz, D. E. 1988. A pharmacodynamic model for soman in rats. *Toxicol. Lett.* 43:175–188.

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

Mobarak, N., and P'an, A. Y. S. 1984. Lead distribution in the saliva and blood fractions of rats after intraperitoneal injections. *Toxicology* 32:67–74.

Mulchandani, A., Mulchandani, P., Kaneva, I., and Chen, W. 1998. Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase.1. Potentiometric microbial electrode. *Anal. Chem.* 70:4140–4145.

Murphy, S. D. 1986. Toxic Effects of Pesticides. In: *Casarett and Doull's toxicology, The basic science of poison*, 3rd. ed., eds. C. D. Klaassen, M. O. Amdur, and J. Doull, pp. 519–581. New York: Macmillan.

Nigg, H. N., and Wade, S. E. 1992. Saliva as a monitoring medium for chemicals. *Rev. Environ. Contam. Toxicol*. 129:95–119.

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. 1991. Physiologically based models for bone-seeking elements. II. Kinetics of lead disposition in rats. *Toxicol. Appl. Pharmacol.* 111:313–331.

P'an, A. Y. S. 1981. Lead levels in saliva and blood. *J. Toxicol. Environ. Health* 7:273–280.

Penalva, J., Puchades, R., Maquieira, A., Gee, S., and Hammock, B. D. 2000. Development of immunosensors for the analysis of 1-naphthol in organic media. *Biosens. Bioelectron*. 15:99–106.

Peoples, S. A., and Knaak, J. 1982. Monitoring pesticide blood cholinesterase and analyzing blood and urine for pesticides and their metabolites. In *Pesticide residues and exposure*, ed. J. R. Plimmer, pp. 41–57. Am. Chem. Soc. Symp. Series No. 182. Washington, DC: American Chemical Society.

Pirkle, J. L., Kaufmann, R. B., Brody, D. J., Hickman, T., Gunter, E. W., Paschal, D. C. 1998. Exposure of the U.S. population to lead 1991–1994. *Environmental Health Perspective*, 106(11):745–750.

Revich, B. A. 1994. Lead in hair and urine of children and adults from industrialized areas. *Arch. Environ. Health* 49:59–62.

Schramm, W., Smith, R. H., Craig, P.A. and Kidwell, D. A. 1992. Drugs of abuse in saliva: A review. *J. Anal. Toxicol*. 16:1–9.

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

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

Timchalk, C., Poet, T. S., Lin, Y., Weitz, K. K., Zhao, R., and Thrall, K. D. 2001. Development of an integrated microanalytical system for analysis of lead in saliva and linkage to a physiologically based pharmacokinetic model describing lead saliva secretion. *Am. Ind. Hyg. Assoc. J.* 62:295–302.

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

Zhou, F., Aronson, J. T., Ruegnitz, M. W. 1997. High throughput fast-scan anodic stripping voltametry in a microflow system. *Analytical Chemistry*, 69:728–733.