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***Project Title:*** Non-Invasive Biomonitoring of Pesticides

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**List of Terms and Abbreviations**

| | |
|---|---|
| AChE | Acetylcholine esterase |
| BuChE | Butyrylcholine esterase |
| CPF | Chlorpyrifos |
| CPF-oxon | Chlorpyrifos-oxon |
| ChE | Cholinesterase |
| ELISA | Enzyme linked immunoassay |
| OP | Organophosphorus Insecticide |
| PK | Pharmacokinetic |
| PD | Pharmacodynamic |
| PBPK/PD | Physiologically based pharmacokinetic/pharmacodynamic |
| TCPy | Trichloropyridinol |

**Abstract.**

This research project established a non-invasive biomonitoring capability to evaluate exposure to organophosphorus (OP) insecticides utilizing a sensitive, non-invasive, micro-analytical instrument for real-time analysis of biomarkers of exposure and response in saliva. This project created a miniaturized nanobioelectronic biosensor that is highly selective and sensitive for the target analyte(s). In addition, a physiologically based pharmacokinetic and pharmacodynamic model (PBPK/PD) for the OP insecticide chlorpyrifos (CPF) was modified to incorporate a salivary gland compartment to quantitatively predict blood CPF concentration and saliva cholinesterase (ChE) inhibition to estimate exposure based on a saliva specimen. The utilization of saliva for biomonitoring, coupled to real-time monitoring and modeling is a novel approach with broad application for evaluating occupational exposures to insecticides. An OP sensor was developed based on a new biosensing principle of antigen-induced formation of nanoparticle-immuno complex nanostructure. A ChE sensor was also developed based on the electrodetection of the ChE hydrolyzed reaction products. In addition, immunoassays capable of sensitive detection of phosphorylated acetylcholinesterase (AChE) and butyrlcholinesterase (BuChE) in biological samples were also developed into a sensor platform. Hence, the sensor systems developed within this project enabled detection of chemical metabolites, enzymatic activity and protein modifications within the same sample matrix. Subsequently, the sensors were transformed to a “lab-on-a-chip”, and the sensors performance was characterized, optimized and validated. To validate this approach the *in vivo* pharmacokinetics of CPF and ChE inhibition in rat saliva under various physiological conditions and dose levels was assessed to ensure that these endpoints are accurate predictors of internal dose. Finally, the sensor platform has undergone further evaluation initially utilizing human samples (blood & saliva) that were spiked with known concentrations of the target analyte and more recently in saliva samples obtained from chemical plant workers who were exposed to low concentrations of chlorpyrifos during their normal work routines. These data in both animal models and humans provides strong support for the use of saliva as a non-invasive biomonitoring matrix and demonstrates the potential utility of a novel sensor platform that could be broadly utilized for human biomonitoring. The development of a real-time saliva analysis coupled to a predictive pharmacokinetic model represents a significant advancement over current biomonitoring strategies. This model system represents the next generation of biomonitoring tools and approaches that can be utilized to assess worker exposure to insecticides under a wide range of occupational situations.

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**Section 1.**

**Executive Highlight**

In articulating a vision for Toxicity Testing in the 21st Century, the National Research Council (NRC) noted that exposure science will play a critical role in a new risk-based framework. In this context, biomonitoring is a key component that quantitatively associates an internal dose with a measurable effect. It has also been suggested that epidemiology studies which accurately assess chemical exposures along with biological effects will have the most meaningful interpretation and thus maximal impact. However, a major impediment in conducting quantitative biomonitoring within epidemiology studies is the lack of rapid, field deployable, quantitative technologies that measure chemical exposure/response biomarkers using minimally invasive biological fluids (i.e. saliva). Due to the complex nature of environmental exposures and biological systems, measurement of a single biomarker may not provide adequate quantitative assessment of exposure. Hence, this project has undertaken development, validation, and refinement of novel sensor platforms capable of quantifying multiple biomarkers associated with pesticide exposures (dose), utilizing saliva as a readily obtainable biological fluid. In addition, pharmacokinetic evaluations have been exploited to facilitate quantifying dosimetry. Our ongoing research has focused on the development of a number of organophosphorus specific pesticide sensor platforms. These include a quantum dot-based immunochromatographic fluorescent biosensor for quantifying the chlorpyrifos metabolite trichloropyridinol (TCPy), and a carbon nanotube-enhanced flow-injection amperometric detection system for quantifying cholinesterase (ChE) activity. This technology innovation makes the proposed multiplex sensor strategy substantially more compelling for use in detecting anticipated low level biomarker concentrations. A key focus of the current project has been to develop and validate the sensors (*in vitro* & *in vivo*) in experimental animals and human systems. This novel platform will enable a more accurate prediction of exposures and can readily be employed to assess dosimetry to pesticides in support of biomonitoring and epidemiology studies.

**Significant (Key) Findings.** The primary objective(s) of this project has focused on three key objectives: **(A)** to develop a sensor platform for measuring OP metabolites, and cholinesterase (ChE) enzyme activity in saliva; **(B)** to establish an animal model of *in vivo* saliva clearance rates in rats that can be exploited to understand how chemicals are cleared from blood into saliva and to assess saliva based biomarkers; and **(C)** to develop and utilize computational dosimetry biological response models to enable prediction of systemic dose and biological response based upon a saliva measurement.

**(A)**. The sensor platform development conducted under these objectives resulted in the development of a number of different sensor platforms that enable the quantification of low concentrations of chemical metabolite (TCPy), measurement of enzyme activity (ChE) or a combination of enzyme inhibition of modified (i.e. phosphorylated) ChE concentrations. These results have gone far beyond initial project objectives and suggest that multi-analyte quantitation can be achieved with excellent analytical performance (i.e. sensitivity, specificity and reproducibility).

(**B**). A major focus of this project was the establishment of a fundamental understanding of saliva as a biological matrix for quantification of exposure by measuring parent pesticide (CPF) and metabolite (TCPy), or ChE enzyme activity. To achieve this goal, a rat *in vivo* model capable of characterizing metabolite clearance or enzyme activity in saliva was developed. This animal model was utilized to establish the pharmacokinetic relationships between saliva measurements and blood concentrations or enzyme activity and results were exploited for physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model refinement (see **C**). The rat *in vivo* data were also critical for validation of the sensor platforms to detect and quantify dosimetry and ChE response. Hence, extensive *in vivo* dose- and route-dependent pharmacokinetic studies were conducted in rats and blood, saliva and urine were analyzed for parent pesticide and metabolite as well as ChE activity. Biological samples from these experiments were also utilized to test the performance of the sensor platforms. This research was also extended to include human *in vitro* plasma and saliva assessment for analyte detection and enzyme activity measurements and most recently the analysis of human saliva samples collected from chemical plant workers (collaboration with Dow Chemical) who were exposed to low concentrations of

chlorpyrifos (CPF) during their normal work routines. These data in both animal models and humans provides strong support for the use of saliva as a non-invasive biomonitoring matrix and demonstrates the potential utility of a novel sensor platform that could be broadly utilized for human biomonitoring.

(**C**). A chlorpyrifos (CPF) physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model that was developed as part of this project was refined to incorporate a saliva compartment. The model was capable of predicting blood CPF and TCPy concentrations based upon the analysis of analytes in saliva utilizing a simple compartmental modeling strategy that incorporated saliva:blood partitioning, and saliva flow. Computational model simulations were consistent with the experimental results.

**Translation of Findings.**

The findings of this study do demonstrate that saliva can be exploited as a biological matrix for biomonitoring and that sensors can be developed that enable quantification of key metabolites and other relevant biomarkers. In the current project a computational model was also utilized to quantitatively evaluate the relationship between saliva and blood analyte concentrations to enable a quantitative prediction of exposure based upon saliva. It is anticipated that the technology developed under this project will be exploited to develop a new biomonitoring strategy that will enable risk assessors to evaluate the potential impact of occupational exposures on worker health. The sensor platforms and computational tool that has been developed under this project can be readily modified/adapted to accommodate other important occupational chemical exposures and can form the foundation for evaluation of worker exposures, dosimetry and by extrapolation risk.

**Outcomes/Impact.** There are a number of important outcomes and impacts of this research on occupational safety and health. This project has resulted in the development and laboratory based validation of a point-of-care sensor platform that is designed to quantify occupational exposure to commonly utilized OP insecticides. Once field validated, this technology can revolutionize occupational biomonitoring and epidemiology assessment by provide rapid, real-time, quantitative assessment of exposure utilizing non-invasive techniques (i.e. saliva assessment). Historically biomonitoring for these pesticides has focused on the measurement of urinary metabolites and blood ChE inhibition using laboratory based analysis; hence, the development of a field based sensor system that provides rapid and sensitive quantitative assessment of exposure is highly innovative. Of particular importance is the ability to exploit sensor platforms with increased detection limits using quantum dot-based biosensors over more conventional laboratory based or ELISA methods. This technology innovation makes this sensor strategy substantially more compelling for use in detecting anticipated low level biomarker concentrations. This novel platform enables a more accurate prediction of exposures and once field validated, can readily be employed to assess dosimetry to pesticides in support of biomonitoring and epidemiology studies. Secondly, the findings also demonstrate the utility of quantitative computational modeling approaches such as PBPK/PD modeling to assess the impact of exposures on occupational risk. This strategy has not historically been the approach that has been utilized by risk assessors, but does offer a more biologically based approach that can be utilized to evaluate worker exposure and risk. Although more research is warranted, it is clear from the finding of this project that a risk assessment approach that incorporates biomonitoring coupled with PBPK/PD modeling represents a cost-effective quantitative approach for evaluating occupational risk associated chemical exposures.

**Section 2.**

**Scientific Report.**

**A. SPECIFIC AIMS:** This research project is focused on the development of a real-time saliva analysis coupled to a predictive pharmacokinetic model which represents a significant advancement over current biomonitoring strategies. It is anticipated that this model system, once fully validated, can be employed to assess worker exposures to insecticides under a wide range of occupational situations. The following Specific Aims have been evaluated:

1. To develop a sensor/platform for measuring organophosphorus (OP) metabolites in saliva.

2. To develop a sensor/platform for measuring cholinesterase (ChE) activity in saliva.
3. To develop gas chromatography-mass spectrometry methods for quantitation of chlorpyrifos (CPF) and trichloropyridinol (TCPy) at low environmentally relevant concentrations.
4. To determine the *in vivo* salivary clearance rate for the OP metabolite TCPy in rats.
5. To experimentally validate the micro-analytical system to quantitate TCPy and ChE inhibition in saliva, blood and urine obtained from rats following *in vivo* exposure to CPF.
6. To modify and validate the current phyiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model to incorporate a saliva compartment.

**B. STUDIES AND RESULTS:** The primary objective(s) of this project has focused on three key objectives: **Objective (A)** to develop a sensor platform for measuring OP metabolites, and cholinesterase (ChE) enzyme activity in saliva; **(B)** to establish an animal model of *in vivo* saliva clearance rates in rats that can be exploited to understand how chemicals are cleared from blood into saliva and to assess saliva based biomarkers; and **(C)** to develop and utilize computational dosimetry biological response models to enable prediction of systemic dose and biological response based upon a saliva measurement.

***Objective (A)*:** The most recent sensor development has focused on a portable fluorescence sensor platform which combines the quantum dot label-based test strip with a fluorescence test strip reader (**Fig. A-1**). The sensor has good sensitivity, selectivity, wide linear range (1 ~ 50 ng / mL), and good reproducibility (RSD, %). The detection limit of the sensor is about 1 ng/mL. This sensor platform has been successfully used to detect TCPy in rat and human plasma and saliva specimens. **Fig. A-2** shows the fluorescence response of the sensor to different concentration of TCP spiked in rat plasma samples. It was found that the method has good recovery as shown in **Table A-1**. In parallel, a colorimetric sensor that integrates gold nanoparticle label-based test strip with a colorimetric test strip reader has been developed for sensitive detection of TCPy. **Fig. A-3** shows analytical performance of the sensor for detecting TCPy in buffer. This sensor is sensitive and the detection limit is about 1 ng/mL, its dynamic rang is from 1 to 100 ng/mL, and the sensor has good reproducibility. Total analysis time is about 15 min. The sensor was also subject to detecting TCPy in spiked rat plasma and likewise demonstrated good recovery.

For quantifying ChE inhibition we have developed a portable sensing platform that integrates a flow-injection system with an electrochemical sensor for a simple, rapid, and sensitive characterization of ChE enzyme activities (**Fig. A-4**). The sensor has been tested in different biological samples (i.e. rat saliva, plasma). **Fig. A-5** demonstrated the typical enzyme activity curves obtained with the sensor using spiked rat plasma samples.

Finally, we developed a multiplexed immunosensing platform for detecting the combination of enzyme inhibition and phosphorylated ChE. In such an assay, the sensor platform can simultaneously detect ChE concentration-based enzyme activity and total amount of ChE. As a result, we can detect both enzyme inhibitions as well as phosphorylated ChE concentration. Results suggest that this method is sensitive for detection of low doses of OP exposure **(Fig. A-6).**

***Objective (B)*:** The saliva pharmacokinetics of TCPy from TCPy or CPF dosing and saliva cholinesterase (ChE) activity were characterized. A general description of the experimental design is presented in **Fig. B-1**. A major focus was to validate the utility of saliva as a biological monitoring (biomonitoring) matrix for quantifying systemic dosimetry and ChE inhibition kinetics. Saliva flow rate increased with increasing pilocarpine infusion (**Fig. B-2**). TCPy pharmacokinetics were very similar in blood and saliva and saliva/blood TCPy concentration ratios were not affected by CPF or TCPy concentration in blood (**Fig. B-3 A**) or saliva flow rate (**Fig. B-3 B**). The TCPy concentration in saliva was highly correlated to the amount of unbound TCPy in plasma, and the amount TCPy protein-binding in plasma was substantial (98.5%) (**Fig. B-4**). Most recent efforts have focused on the evaluation of blood and saliva pharmacokinetics following a CPF dose. The CPF was rapidly metabolized to TCPy which was readily quantified in both blood and saliva (**Fig. B-5 A**). The extent of TCPy saliva excretion following the CPF dose was very consistent with the results seen following the TCPy only dosing. ChE in both plasma and saliva were likewise inhibited following CPF exposure, consistent with the metabolism of CPF to CPF-oxon (**Fig. B-5 B**).

A major focus of our sensor development efforts has been to create a saliva ChE activity sensor as a non-invasive biomarker for OP exposure; however since little is known about baseline saliva ChE activity and associated variability, studies were conducted to characterize baseline saliva enzyme activity. Comparison of total ChE activity (**Fig. B-6 A-C**) `indicated that rat salivary ChE is variable among individual rats at each time point of collection (CV ~33-65%) and over the entire duration of collection for each rat (CV ~16-104%). Overall CV for all rats over the entire collection time was 67%. Variation of total protein in saliva (**Fig. B-6 C**) was also observed among individuals (CV ~36-57%) and over the duration of collection (CV ~15-105%) as well. Protein content and total ChE activity were correlated (**Figure B-7 A**), and normalization of ChE activity by total protein resulted in a CV reduction to 37% (**Fig. B-7 B**). These results demonstrate the variability of salivary ChE activity and suggest that salivary protein may normalize ChE activity in saliva.

***Objective (C):*** Over the course of this project, the PBPK/PD modeling efforts have focused on further refining the model (**Fig. C-1 & C-2**) and improving fits to *in vivo* salivary clearance studies (**Objective B**). The saliva/blood CPF and TCPy partitioning coefficients were determined experimentally. Since the physiological conditions of rats in this study were different than normal physiological values in humans (lower saliva pH), saliva/blood CPF and TCPy partitioning coefficients were estimated using a modified algorithm based upon the composition of tissues. This algorithm assumes ionized compounds are able to transverse membranes at a much lower rate (usually 3 orders of magnitude) than the non-ionized form. Inputs into this algorithm included various physiochemical properties of CPF and TCPy as well as physiological properties of rat and human plasma and mixed saliva (**Table D-1**). The model accurately predicts TCPy concentrations in saliva (**Fig.C-8 A-D**) and these studies suggest that saliva TCPy concentration can be utilized to ascertain CPF exposure. Assuming that the sole source of plasma ChE inhibition is due to CPF exposure, the PBPK/PD model predicts that a 70 kg human would require a daily oral CPF dose of 12 µg/kg to achieve 40% inhibition of plasma ChE after 30 days (Action level) (**Fig. C-3 A**). Model simulations of that same dose predict saliva TCPy levels to approximate 0.01 µmol/L (**Fig. C-3 B**) for humans. The developed QD TCPy sensor has a detection limit of 0.005 µmol/L TCPy in plasma. This detection limit is below the PBPK/PD predictions of TCPy concentrations in saliva after 30 days.

**C. SIGNIFICANCE:** This project has focused on the development and validation of new biosensor technologies in experimental animals and human systems for the quantification of OP insecticide exposures based upon the non-invasive measurement in saliva. This novel platform will enable a more accurate prediction of exposures and can readily be employed to assess dosimetry to pesticides in support of biomonitoring and epidemiology studies.

**D. PLANS:** The results of this project are now being used to extend the current research by focusing on other chemical agents that could be important candidates for saliva biomonitoring. The in vivo saliva clearance rat model and the computational dosimetry model that have been developed can now be exploited to evaluate a broader range of chemicals that have different physical and chemical properties to help identify candidate agents that would have a high probability of being detected in saliva. Secondly, the sensor platforms that have now been developed represent the technical foundation for further sensor development, with a particular focus on multiplex sensors development that can readily accommodate multiple analytes within a single system.

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**Peer-reviewed publications (credited to this project).**


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21. Smith, J. N., Wang, J. Lin, Y., Timchalk, C. (2010). Pharmacokinetics of 3,5,6-trichloro-2-pyridinol (TCPy), a chlorpyrifos metabolite, in rat saliva. *Society of Toxicology Annual Meeting*, March 7-11th, Salt Lake City, UT.
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**Relevant Figures and Tables (in order of discussion):**

**Objective A:**

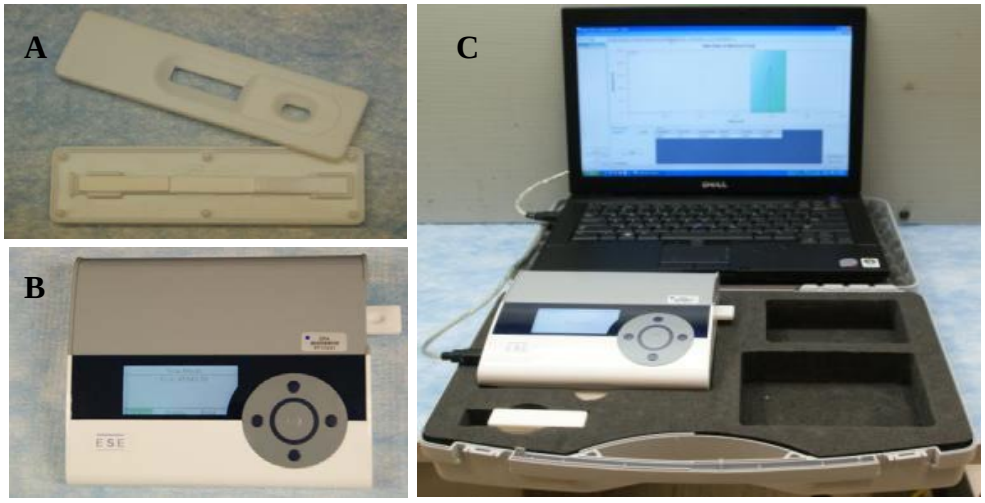


**Figure A-1.** Photograph of **(A)** the test strip in the cassette **(B)** portable test strip reader integrated with test strip in the cassette shown in (A), **(C)** the entire immunosensor system including a connected laptop.

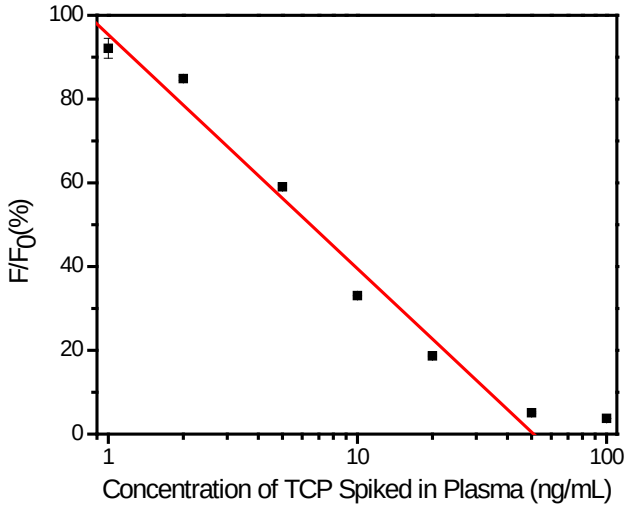


**Figure A-2.** Normalized signals with increasing concentration of TCPy spiked in plasma. The plasma was 10-fold diluted, then different amount of TCPy standard were spiked into this diluted plasma to final concentrations of 1, 2, 5, 10, 20, 50, 100 ng/mL.

**Table A-1.** Recovery of TCPy spiked in plasma samples obtained by the quantum dot-based fluorescent ITSA.

| TCPy spiked in plasma (ng/mL) | TCPy found by ITSA (ng/mL) | Recovery (%) |
|---|---|---|
| 1 | 1.14 | 114.0 |
| 2 | 1.54 | 77.0 |
| 5 | 4.47 | 89.4 |
| 10 | 13.04 | 130.4 |
| 20 | 23.61 | 118.1 |
| 50 | 41.31 | 82.6 |

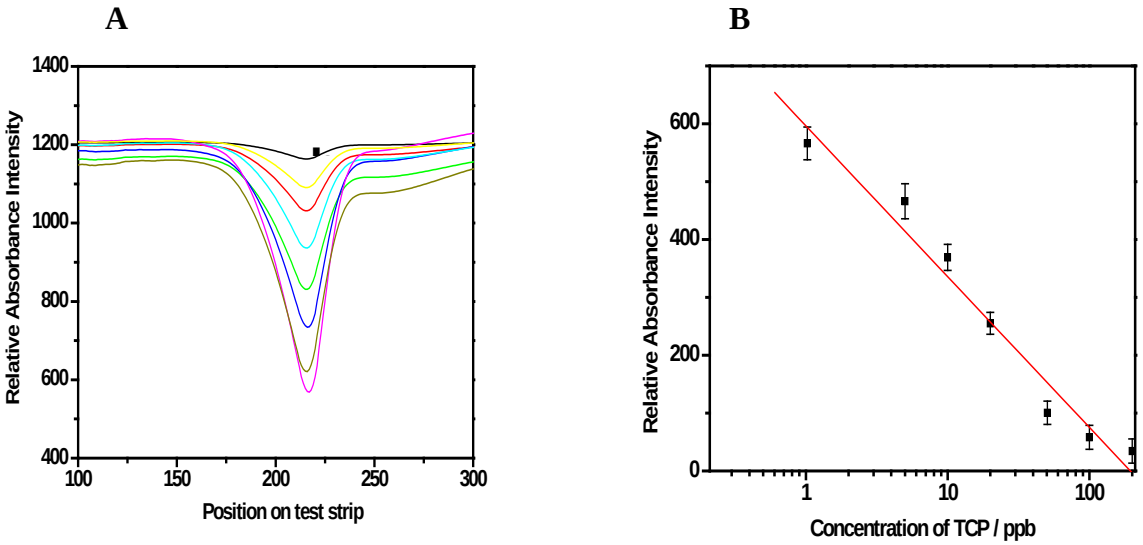


**Figure A-3**. **(A)** The responses of the sensor with increasing TCPy concentrations in PBS buffer (0, 1, 5, 10, 25, 50, 100 and 200 ppb, respectively). **(B)** Calibration curve.

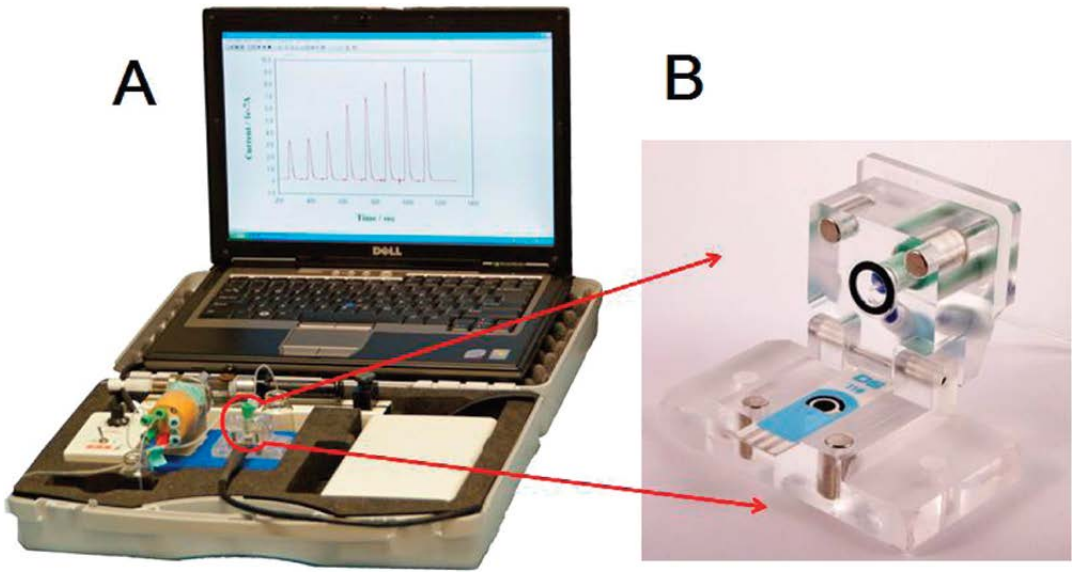


**Figure A-4. (A)** The entire analysis system and **(B)** the part of the sensing area.

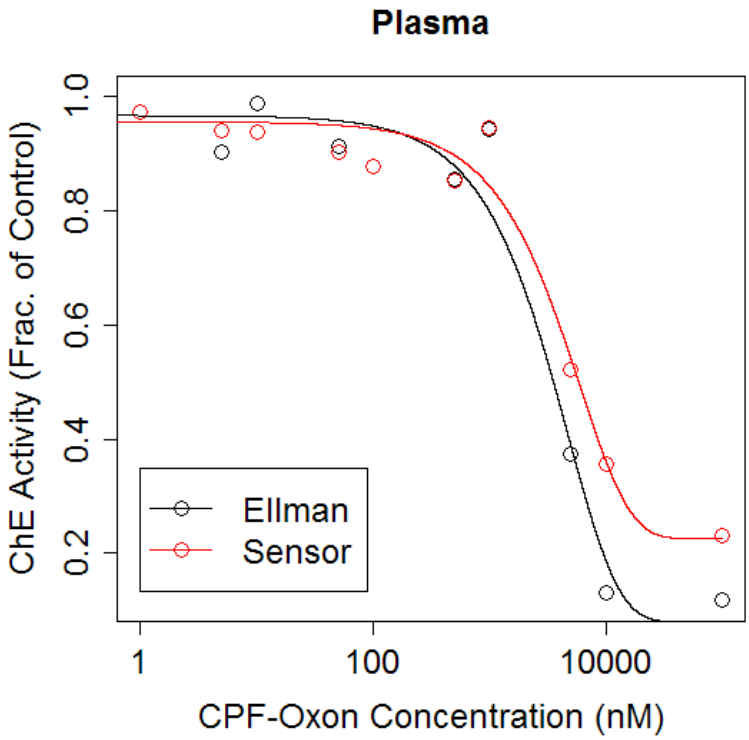


**Figure A-5.** A typical response of enzyme activity obtained from the sensor with rat plasma samples dosed with different concentration of chlorpyrifos-oxon (CPF-oxon).

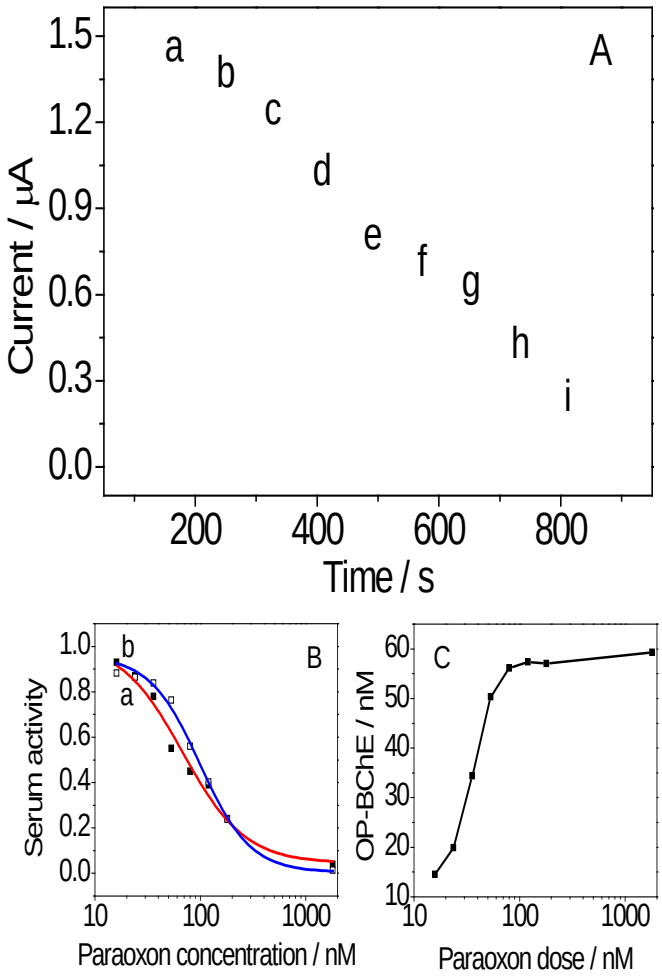


**Figure A-6.** Analytical performance of the multiplexed sensor for detecting enzyme inhibition and phosphorylated butyrylcholinesterase. **(A)** Amperometric responses for MBs-anti-BChE incubated with 10-fold diluted serum dosed with different concentrations of paraoxon. (a) 0 nM; (b) 15.8 nM; (c) 23.7 nM; (d) 35.6 nM; (e) 53.3 nM; (f) 80 nM; (g) 120 nM; (h) 180 nM; (i) 1800 nM. **(B)** Human serum activity curves determined by (a) the proposed method and (b) Ellman assay method. **(C)** Relationship between paraoxon-BChE adducts and dosed paraoxon in the human serum samples.

**Objective B:**

$$\text{Saliva TCP Clearance } Cl_s \text{ (mL min}^{-1}\text{)} = \frac{\text{Saliva TCP Conc. (µg mL}^{-1}\text{) x Saliva Flow (mL min}^{-1}\text{)}}{\text{Blood TCP Conc. (µg mL}^{-1}\text{)}}$$

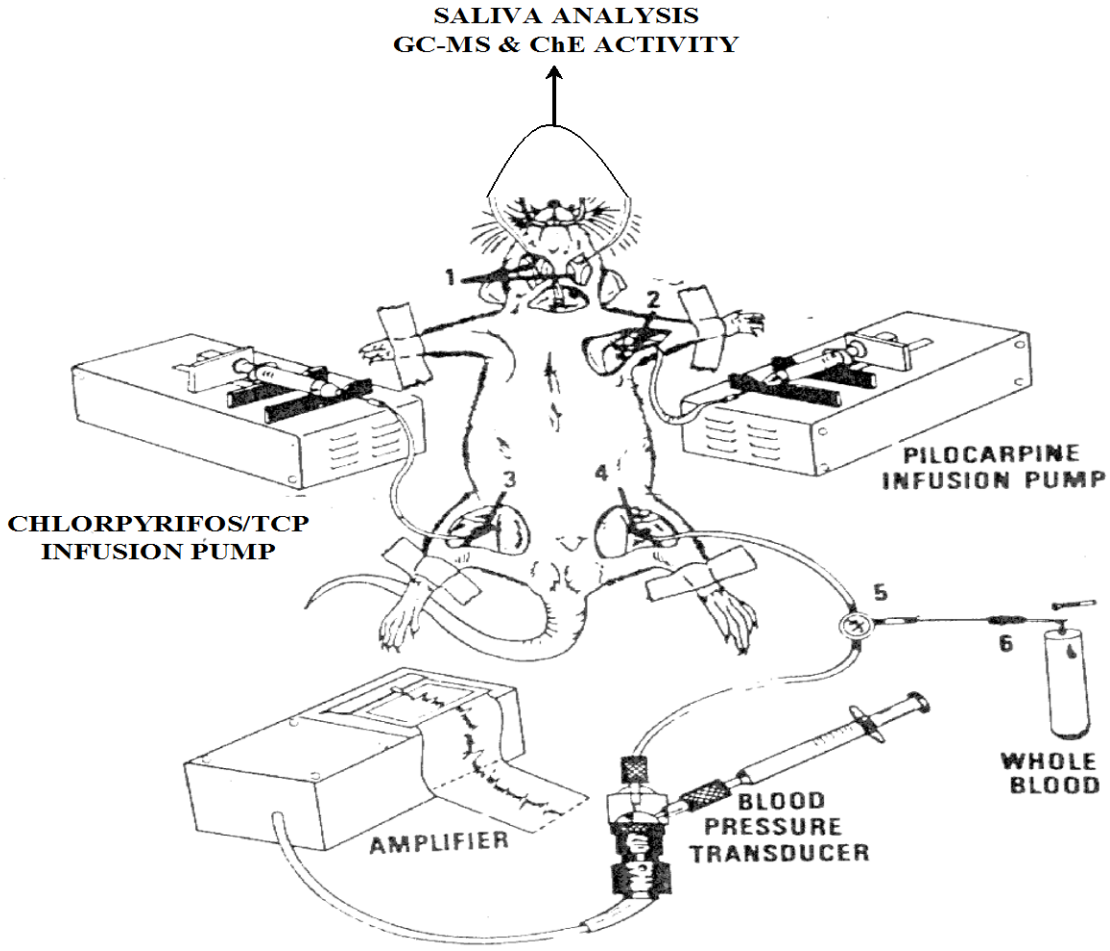


**Figure B-1.** Illustration of experimental approach for quantifying salivary clearance. Rats were anesthetized with isoflurane gas (Baxter Healthcare Corp., Deerfield, IL, USA). Once under anesthesia, femoral and jugular veins were cannulated using PE50 polyethlyene tubing. The trachea was then intubated using PE 240 polyethlyene tubing. The tracheal tube was connected to the aesthesia machine to maintain anesthesia. Once all microsurgical techniques had been achieved, rats were infused with 0.1-1 mg/mL pilocarpine in physiological saline via the femoral vein cannula at a rate of 3 mL/min using an infusion pump. Pilocarpine induced salivation, and total saliva was collected gravimetrically from the oral cavity using a 9.52 cm x 2.49 mm o.d. x 1.42 i.d. glass capillary tube draining into a collection vial. Once salivation had been stimulated and maintained for five minutes, rats were dosed with 1 mg/kg body weight of TCPy or 5 mg/kg CPF in saline with 5% tween 20 via the femoral vein cannula (t=0). Total saliva was collected continuously, changing collection vials at 15 minute intervals. Blood (~200 µL) was collected from the jugular vein cannula at the midpoint of the saliva collection intervals. Blood was analyzed for TCPy, CPF and ChE activity (CPF-only dose), while saliva was analyzed for TCPy and ChE activity (CPF-only dose).

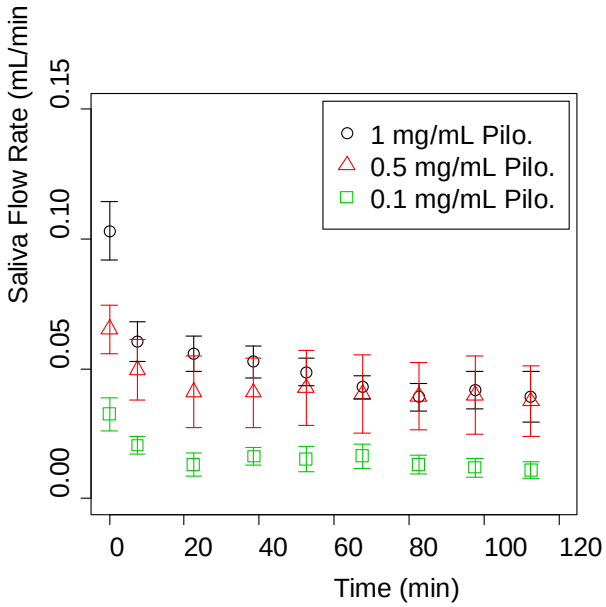


**Figure B-2.** Mean saliva flow rates (mL/min) with SEM error bars of rats intravenously infused at 3 mL/h with varying concentrations of pilocarpine. The solid line is a non-linear regression fit to the saliva flow rate of rats receiving 1 mg/mL of pilocarpine. Rats were handled as described in Figure B-1

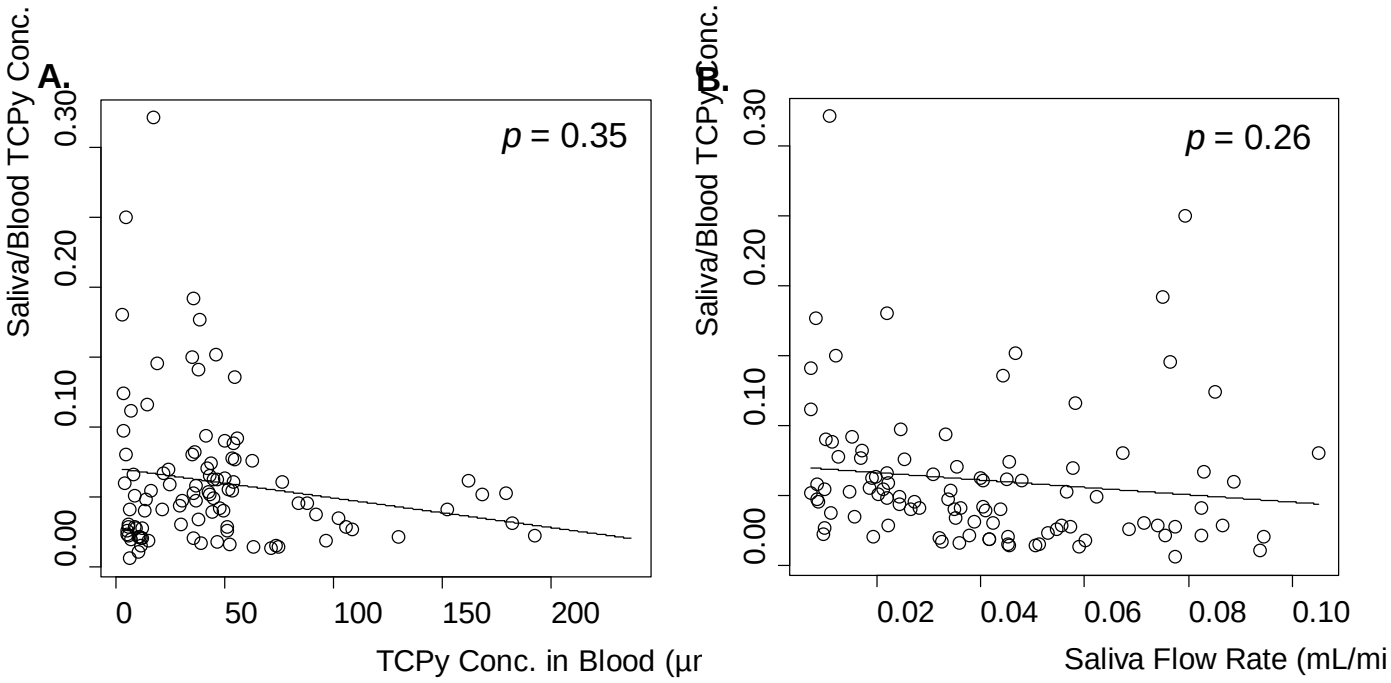


**Figure B-3. (A)** Relationship of 3,5,6-trichloro-2-pyridinol (TCPy) concentrations in blood and saliva from rats dosed with TCPy. The correlation coefficient of TCPy concentration in saliva to the TCPy concentration in blood was 0.68 ($p < 0.001$). The line is a linear regression model fit to the data. **(B)** Saliva/blood 3,5,6-trichloro-2-pyridinol (TCPy) concentration ratios over varying TCPy concentrations in blood from rats dosed with TCPy. The line is a linear regression model fit to the data. There was no significant relationship when analyzed using a mixed linear regression model ($p = 0.35$). Rats were handled as described in Figure B-1

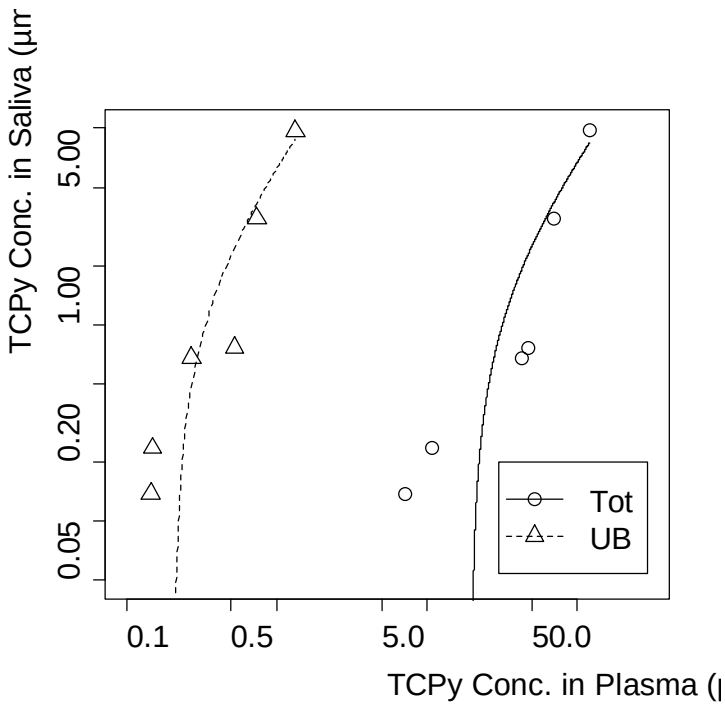


**Figure B-4.** Concentrations of 3,5,6-trichloro-2-pyridinol (TCPy) in saliva and plasma (total TCPy (Tot) in plasma and TCPy unbound to plasma proteins (UB)) from rats dosed intravenously (iv) with TCPy and infused with pilocarpine. Lines are a linear regression model fits to the data. The correlation coefficient of TCPy concentration in saliva to the unbound TCPy concentration in plasma was 0.96 ($p$ = 0.002). Rats were handled as described in Figure B-1.

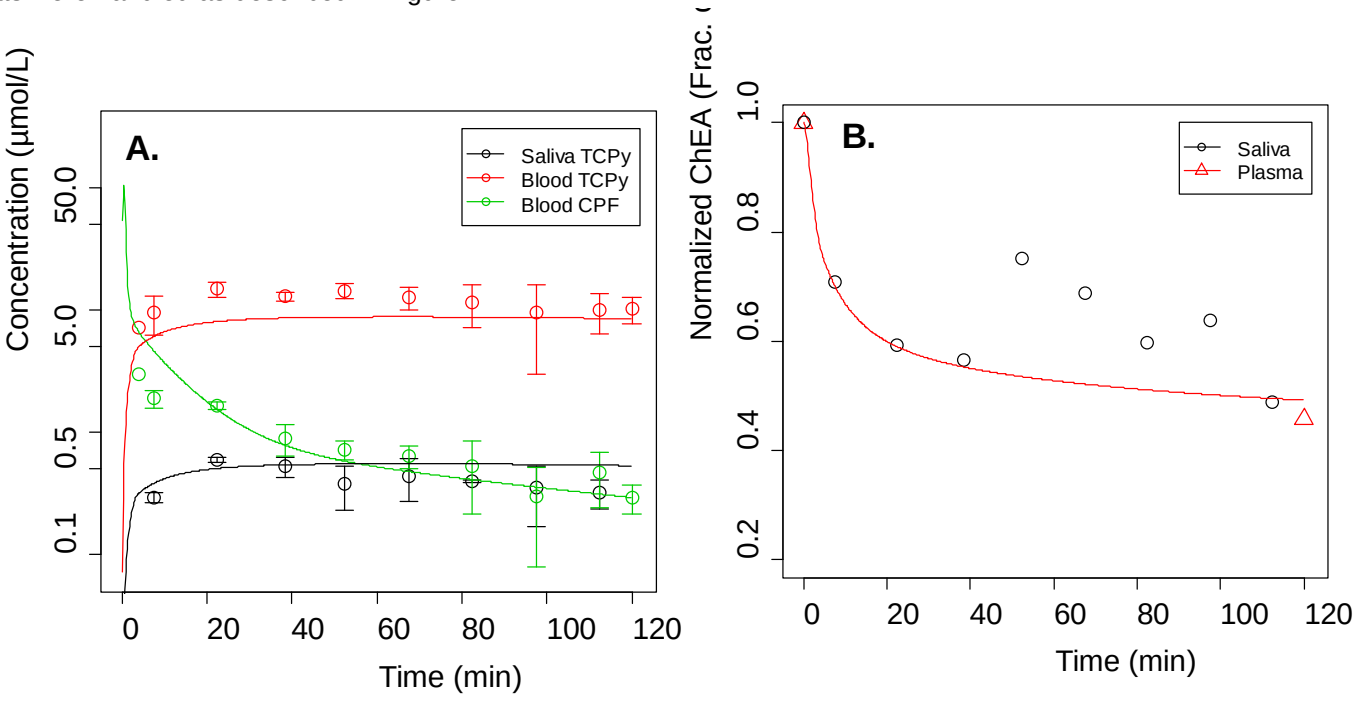


**Figure B-5. (A)** Concentration of chlorpyrifos (CPF) and 3,5,6-trichloro-2-pyridinol (TCPy) in blood and TCPy in saliva following intravenous (iv) administration of 5 mg/kg CPF. (B) Normalized cholinesterase (ChEA) activity in saliva and plasma following the CPF exposure. Rats were handled as described in Figure B-1.

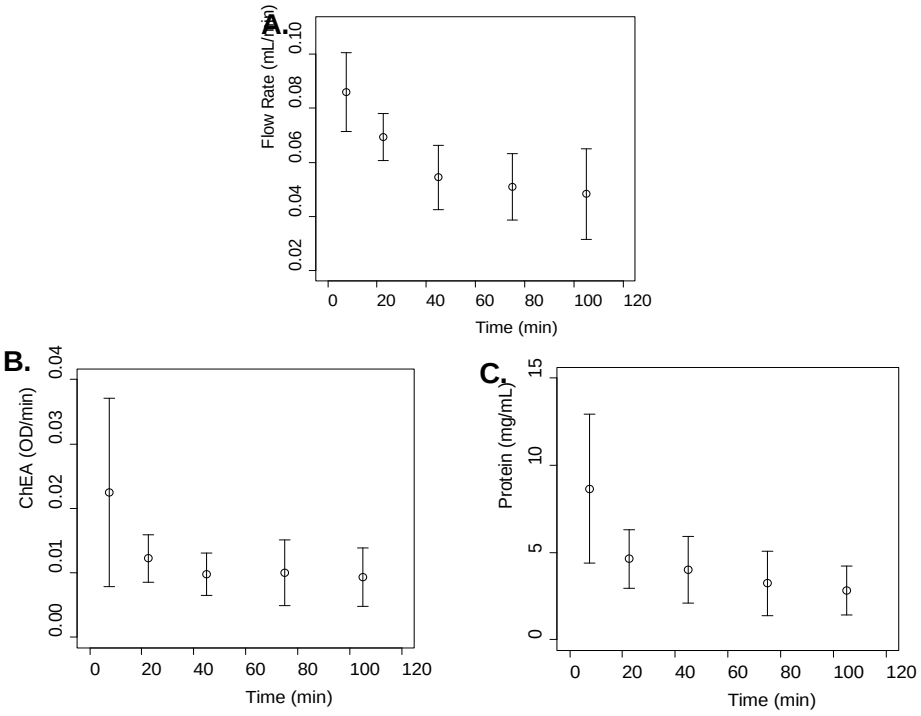


**Figure B-6. (A)** Mean saliva flow rates (mL/min) with SD error bars of rats intravenously infused at 3 mL/h of 1 mg/mL pilocarpine in saline. **(B)** Mean saliva cholinesterase activity (ChEA) with SD error bars of rats intravenously infused with pilocapine. Overall CV is 67%. **(C)** Mean saliva protein content with SD error bars of rats intravenously infused with pilocapine. Rats were handled as described in Figure B-1.

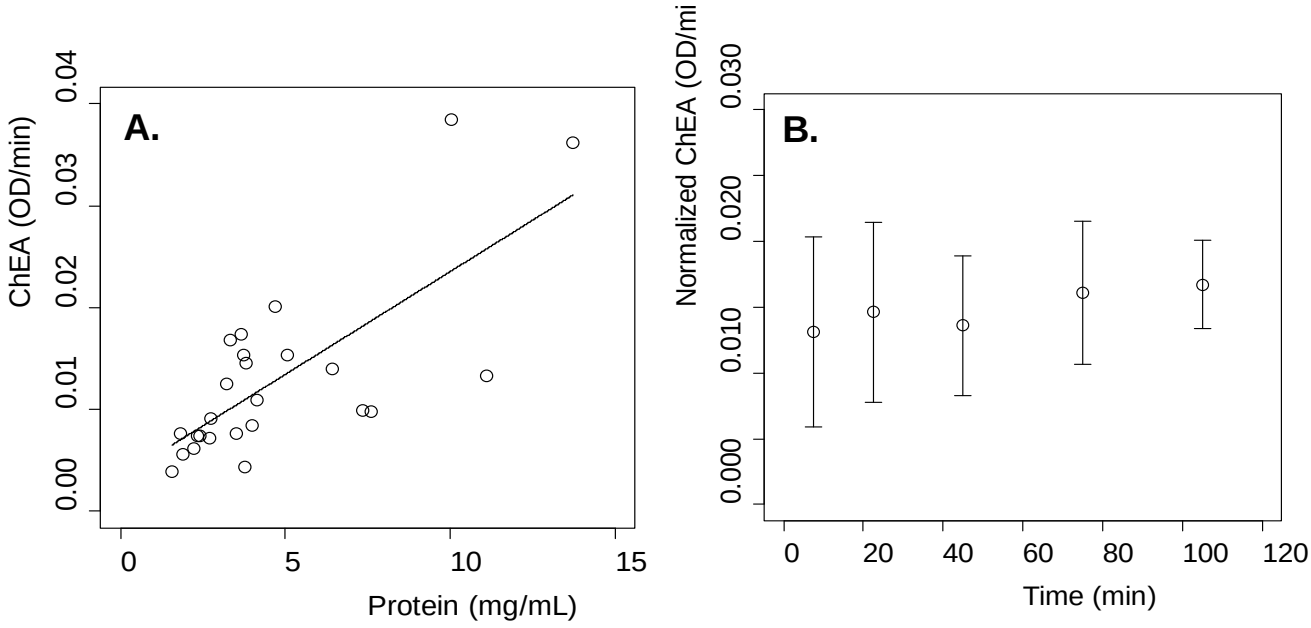


**Figure B-7. (A)** Cholinesterase activity (ChEA) over protein content of saliva from rats intravenously infused with pilocapine. The line is a linear regression model fit to the data. The correlation coefficient is 0.73, $p < 0.001$. **(B)** Cholinesterase activity (ChEA) nomalized by protein content from rats intravenously infused with pilocapine. Overall CV is 37%.

**Objective C:**

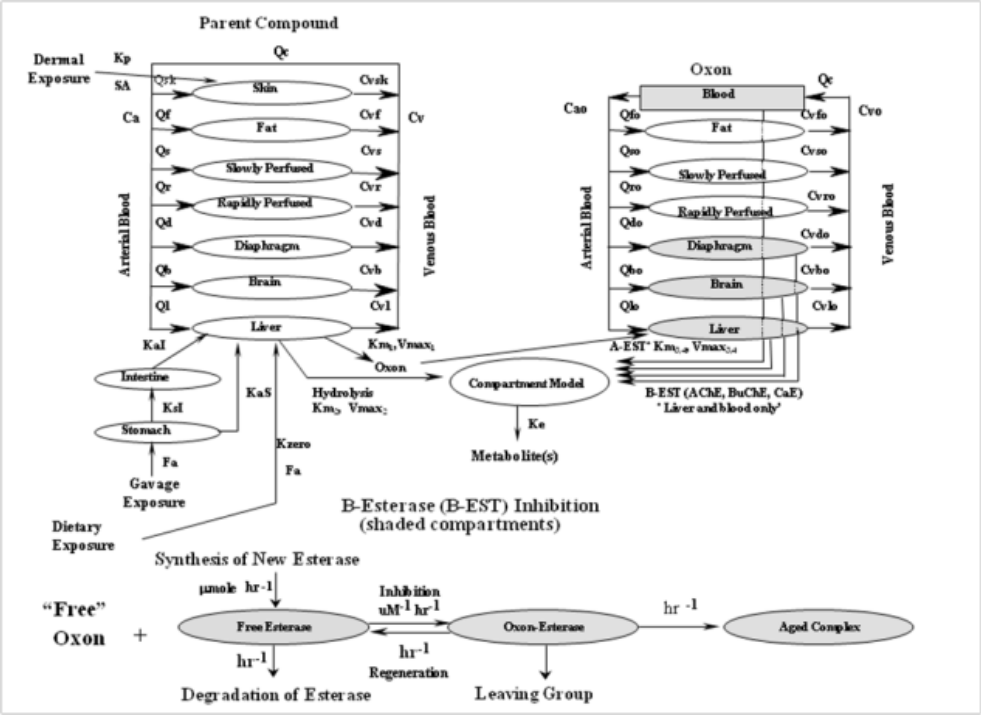


**Figure C-1.** Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model used to describe the disposition of chlorpyrifos (CPF), chlorpyrifos-oxon (CPF-oxon), trichloropyridinol (TCPy) and B-esterase inhibition in rats and humans following oral (gavage and dietary), and dermal exposures.

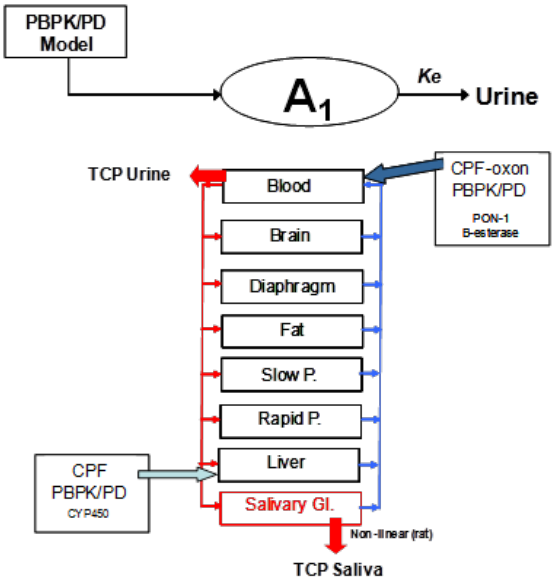


**Figure C-2.** Initial compartmental model structure and more detailed PBPK model structure for the chlorpyrifos (CPF) metabolite trichlorpyridinol (TCPy) in rats and humans.

**Table C-1.** Input parameters for a modified algorithm for calculating the saliva/blood 3,5,6-trichloro-2-pyridinol (TCPy) partitioning coefficient for humans (Schmitt, (2008) *Toxico. In vitro*, **22:** 457-467.)

| Parameter | Value | Source |
|---|---|---|
| TCPy Physiochemical Properties | | |
| Frac. Unbound in Plasma | 0.015 | Experimentally |
| $pK_a$ | 4.55 | Fixed[a] |
| Log $K_{ow}$ at a pH of 7 | 1.3 | Fixed[a] |
| Log $K_{ow}$ at a pH of 3 | 3.2 | Fixed[a] |
| α | 0.0126 | Estimated[b] |
| Tissue Properties | | |
| Plasma | | |
| Frac. Plasma Protein | 0.073 | Fixed[c] |
| Frac. Plasma Water | 0.915 | Fixed[c] |
| pH plasma | 7.4 | Fixed |
| Interstitial Space | | |
| Frac. Tissue | 1 | Estimated |
| Frac. Inter. Protein | 0.003 | Fixed[c] |
| Frac. Inter. Water | 0.98 | Fixed[c] |
| pH interstitial | 6.7 | Fixed[c] |

[a]Racke (1993, *Rev. Environ. Contam. Toxicol*., **131:** 1-150), Shemer *et al.* (2005, *Water, Air Soil Poll*., **168:** 145-155).

[b]α is defined as the ratio of the partitioning coefficient ($K_{ow}$) of the ionized form of TCPy and the partitioning coefficient of the non-ionized form of TCPy (Scmitt, 2008). Using the Henderson-Hasselbalch Equation, at a pH of 7, 99.6% of TCPy is ionized; and at a pH of 3, 97.2% of TCPy is non-ionized. Thus, α was estimated as a ratio of the $K_{ow}$ values at those respective pH values.

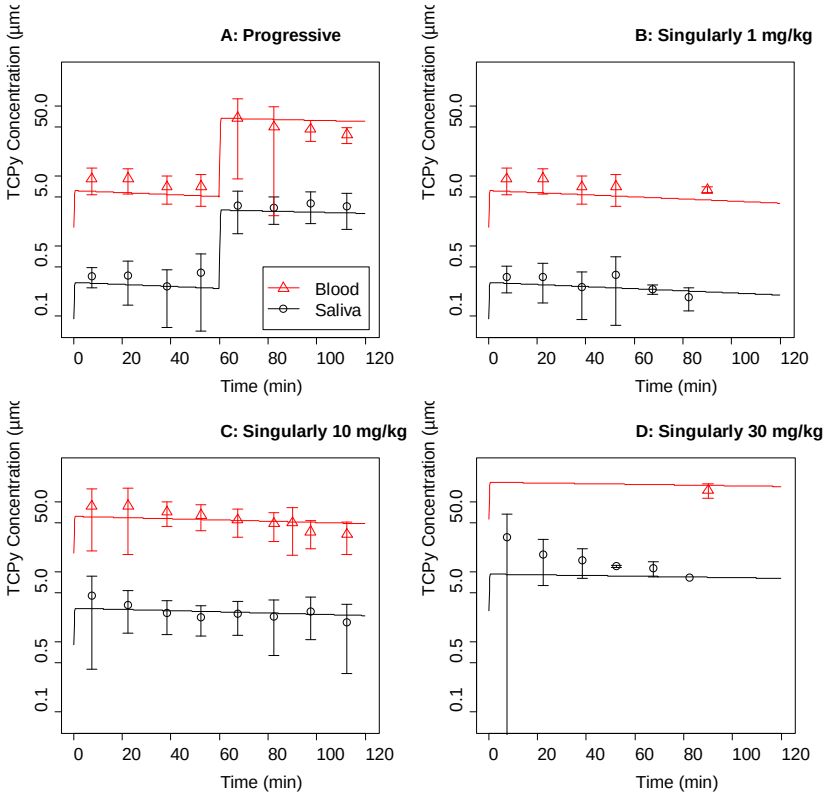


**Figure C-3.** Mean concentration with SD error bars of 3,5,6-trichloro-2-pyridinol (TCPy) in blood and saliva from rats being progressively dosed with 1 mg/kg at 0 min. and 10 mg/kg at 60 min. **(A)** or singularly dosed at 0 min with 1 mg/kg **(B)**, 10 mg/ kg **(C)**, or 30 mg/kg **(D)** with TCPy. Lines are physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model fits to the data.

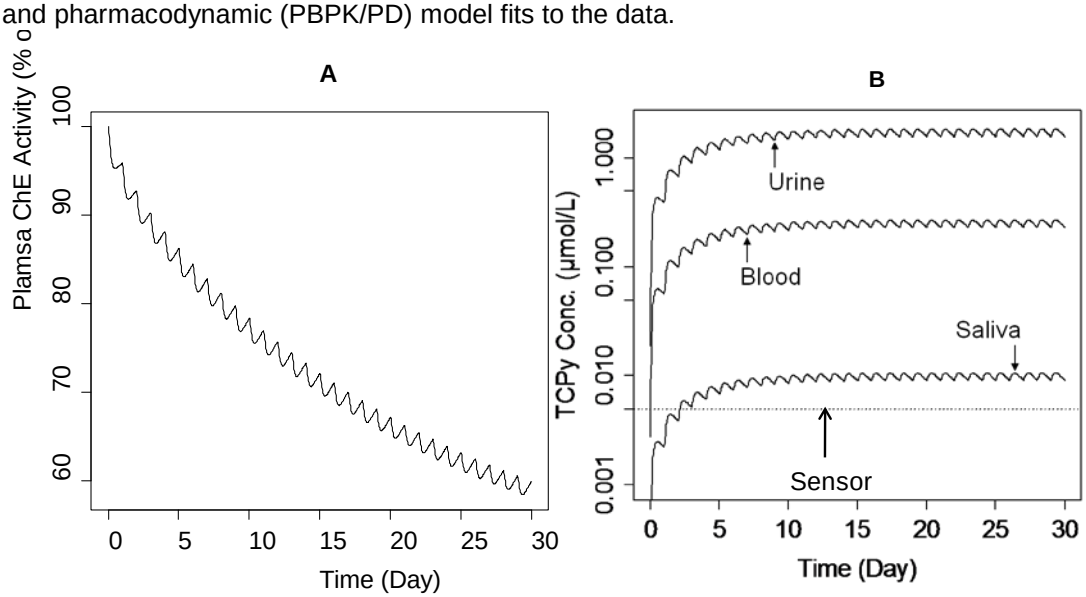


**Figure C-4.** Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model of chlorpyrifos (CPF) simulations of a 70 kg human exposed to 12 µg/kg CPF daily for 30 days, the approximate dose to reduce plasma cholinesterase (ChE) activity to 60% of control **(A)**. In **(B)**, the dashed line indicates TCPy concentrations in blood, the solid line indicates TCPy concentrations in saliva using a saliva pH of 6.7, the shaded area indicates TCPy concentrations in

saliva over a normal range of salivary pH values (6.2 to 7.4), and the dotted lines indicate the limit of TCPy detection for a sensor in plasma currently in development (Zou *et al*., 2010).