# New Methods for Measuring Exposure to Organophosphorus Pesticides

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## Introduction

We developed two new methods for improved measurement of exposure to organophosphorus pesticides. The existing method for assessing occupational exposure to organophosphorus pesticides involves measuring activity of cholinesterase in blood. This method requires a baseline sample from the worker collected before the exposure occurred. Sensitivity of the current method is limited because of natural variability of cholinesterase activity within and between workers.

The toxicological mechanism of organophosphorus pesticides (OP) involves covalent binding of the pesticide to cholinesterase enzymes, resulting in inactivation of the enzyme. The enzyme-OP molecule is referred to as an adduct and the two new methods described here both involve measuring the adduct.

The first method utilizes HPLC/MS/MS for directly measuring cholinesterase-OP adducts in plasma samples. Cholinesterase is first extracted from plasma, then digested into small peptide fragments. HPLC/MS/MS was successfully used to separate different peptide adducts from the unadducted peptide. This method is expected to provide a sensitive tool for exposure measurement and can also provide information regarding the specific pesticide the individual was exposed to.

The second method for measuring OP exposure involves treating plasma samples with an "oxime" type of chemical. We demonstrated the ability of oximes to reactivate cholinesterase following exposure to OP pesticides. Experiments were carried out *in vivo* in rats and *in vitro* with human blood and plasma. Increased cholinesterase activity following oxime treatment provides evidence of OP exposure.

## Project Aims

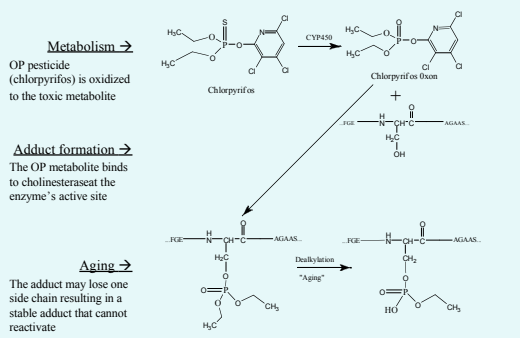
•Develop and validate a sensitive, accurate and robust analytical procedure based on LC/MS/MS for measuring OP-adducts to plasma cholinesterase

•Evaluate the potential of oxime reactivation followed by measurement of cholinesterase activity for confirmation of depressed cholinesterase

•Evaluate the relationships between OP-adduct levels, reactivatability of cholinesterase, and cholinesterase activity in vitro, in vivo in rats, and in humans exposed to OP pesticides

•Research to practice: Incorporate the assays with the practice of OP pesticide exposure monitoring in Washington State

## Organophosphorus Pesticides and Cholinesterase Adducts




## Procedure for LC/MS/MS Assay

**Sample preparation**

Cholinesterase is extracted from 1 ml plasma samples using procainamide affinity gel

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Gel is washed with buffer to remove plasma salts and unbound proteins

↓

Cholinesterase is eluted from gel with high salt solution

↓

Cholinesterase is digested with pepsin generating many peptide fragments

↓

A nine amino acid peptide ("nonapeptide") containing the cholinesterase active site is partially purified by centrifugal ultrafiltration

**Instrumental analysis by HPLC with tandem mass spectrometry**

HPLC is used to separate nonapeptides with different adducts from the unadducted nonapeptide

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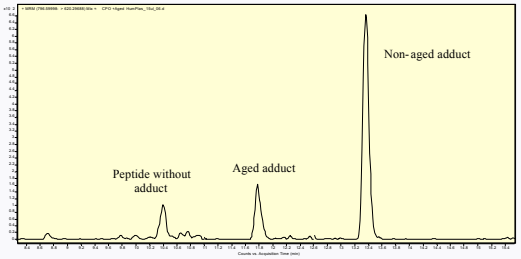
Tandem mass spectrometry ("MS/MS") is used to measure the nonapeptides of interest

## LC/MS/MS Results

Chromatogram from *in vitro* sample of human plasma treated with chlorpyrifos oxon




Good separation was observed for aged and non-aged adducts and for the nonapeptide without the OP adduct.

Other OP pesticides (e.g., methylparathion) form adducts with methyl groups on the phosphonate moiety. Mono and di-methyl adducts can be measured separately from the mono- and di-ethyl adducts from chlorpyrifos as shown in the above chromatogram. Therefore, measurement of only five analytes will allow exposure measurement for all common OP pesticides.

## Procedure for Oxime Reactivation Assay

**Sample preparation**

Plasma samples are mixed with 2-PAM (oxime) and incubated 1 hour at 37°C

Controls include plasma mixed with water and incubated 1 hour at 37°C

**Cholinesterase analysis**

Cholinesterase activity in plasma was measured in-house using a 96-well microplate assay developed for this project. Assay is based on the method by Ellman

Higher cholinesterase activity after treatment with 2-PAM is suggestive of OP exposure

## Animal Study

•Rats were exposed to chlorpyrifos at 10 mg/kg (control rats were not exposed)

•Blood samples were collected after 1, 2, 4, 8, 24, 36, and 48 hours following exposure

•Samples analyzed for cholinesterase with and without 2-PAM treatment

•Future work may include LC/MS/MS analysis of these samples to measure adducts

## Results

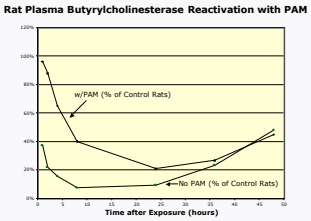
•Cholinesterase inhibition reached a maximum around 8 hours after exposure

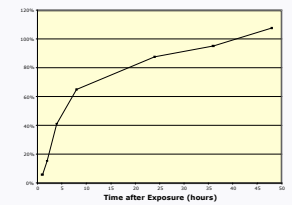
•1 hour after exposure, all of the cholinesterase activity could be restored with PAM treatment

•36 hours after exposure, it appears the adduct had aged and could not be restored with PAM treatment

•Increased cholinesterase activity after 24 hours may be due to spontaneous reactivation and/or production of new cholinesterase enzyme

•*In vivo* aging of rat plasma butyrylcholinesterase was complete within 24 to 48 hours after exposure




## Conclusions

•We are developing new analytical tools with the goal of improving measurement of personal exposures to OP pesticides. Using affinity chromatography and LC/MS/MS, we measured cholinesterase in human plasma with and without the OP bound to it. This should provide improved sensitivity and specificity compared with the existing method.

•The second method involves treating plasma samples with an oxime to reactivate inhibited cholinesterase. Samples with increased cholinesterase activity following oxime treatment are suspected to have come from OP-exposed individuals. A pilot test conducted with rats exposed to chlorpyrifos showed a timecourse for plasma cholinesterase inhibition and recovery. Oxime reactivation of inhibited cholinesterase was successful in plasma samples collected within around 8 hours after exposure. However, after around 36 hours, oxime reactivation did not occur, presumably due to aging of the OP adduct.

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