Measurement of chlorpyrifos adducts to plasma cholinesterase: A new tool for monitoring exposures to organophosphate pesticides

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Outline

- Background on organophosphorus (OP) pesticides chemistry & toxicology
- Measuring human exposure to OP pesticides via cholinesterase monitoring
- Enhancements to cholinesterase monitoring
  - In vitro studies (human blood)
  - Application to monitoring occupational exposures in farm workers

Exposure to OP pesticides and Health

- OP pesticides are still widely used in agriculture
- The abundant use of OP pesticides world wide causes several hundred thousand poisonings per year[1]
- The primary acute toxicological effect of OP exposure is related to inhibition of cholinesterase enzymes.
- Chronic low-level (non-occupational) exposure to OP pesticides is associated with neurological deficits and behavioral impairment.[2] The mechanism behind these long term health effects is unclear

1 Worek et al, 1999, Hum. Exp. Toxicol. 16(8): 466-72
2 Marks et al, 2010, Environ Health Perspect. 118(12):1768-74

Cholinesterase testing for monitoring occupational exposures to OP pesticides

- Advantages
  - Relatively fast and inexpensive
  - Test-kits available for use in the field
- Disadvantages
  - Need baseline activity measure for each worker
  - Lack of specificity
    - Does not identify specific pesticide
    - High frequency of false positives
  - Lack of sensitivity
    - Does not provide reliable evidence for exposures at inhibition levels < ~20%

Project Aims

- Develop/validate a sensitive, accurate and robust analytical procedure based on HPLC/MS/MS for the measurement of OP-adducts to plasma ChE (butyryl ChE, BChE).
- Evaluate the relationships between OP-adduct levels, and ChE activity *in vitro,* and in humans exposed to OP pesticides.

Measurement of OP-adducts to plasma ChE by HPLC/MS/MS.

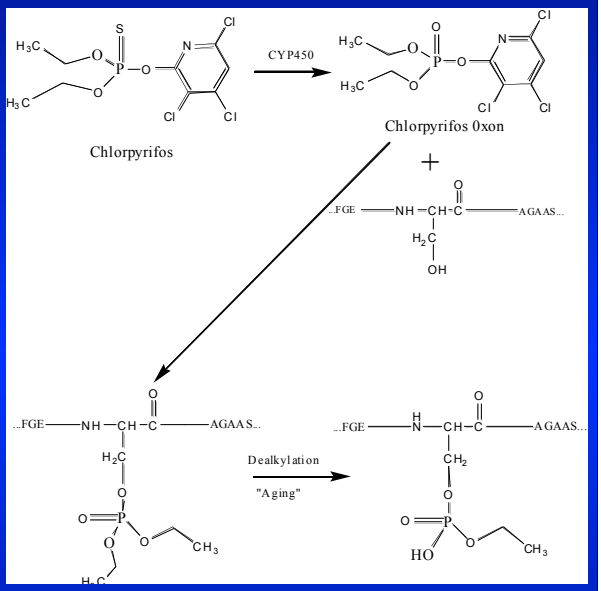
- A "protein adduct" is the compound formed when a chemical binds (irreversibly) to a protein.
- Potential advantages:
  - Specific
  - Sensitive
- Assay initially developed for plasma cholinesterase; could subsequently be expanded to quantify adducts to other proteins

Toxicological Mechanism

Metabolism →

Adduct formation →

Aging →



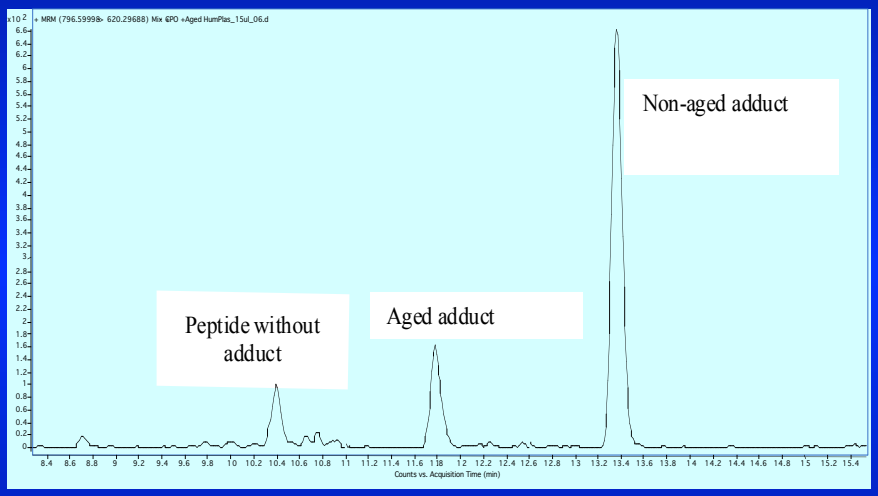
Measurement of OP-adducts to plasma ChE by HPLC/MS/MS.

- A peptide from the active site of ChE, containing the OP adduct, is separated and quantified using HPLC/MS/MS
- Different peptides corresponding to unadducted enzyme, dialkyl-adducts and aged (monoalkyl)-adducts can be detected.
- Ratios of these different adducts provide a measure of the extent of enzyme inhibition and the proportion of aged enzyme

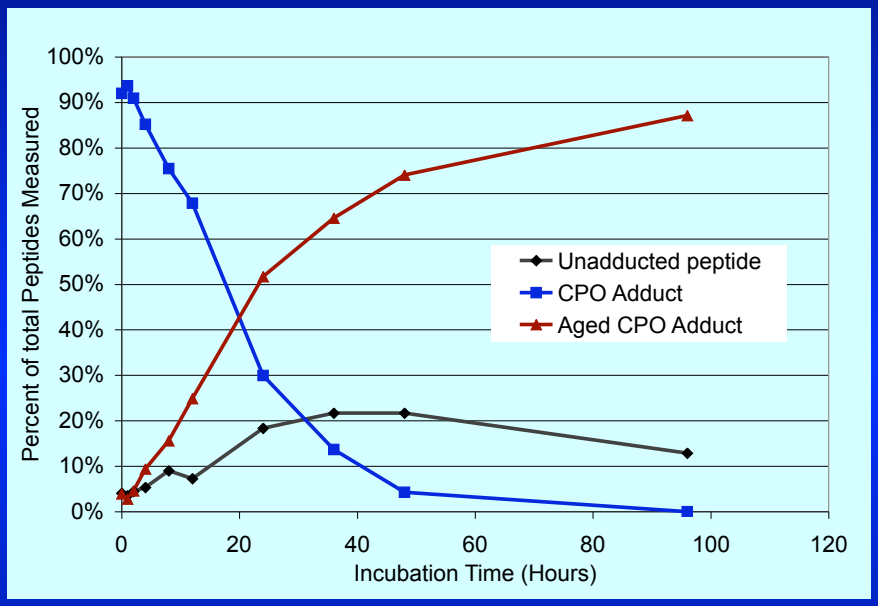
In vitro Study

- Human plasma was dosed with chlorpyrifos-oxon.
- Aliquots were collected after 1, 2, 4, 8, 24, 36, and 48 hours following treatment
- Samples analyzed for cholinesterase activity and were also analyzed by LC/MS/MS to measure adducts

HPLC/MS/MS Analysis of Human Plasma following *In Vitro* Exposure to Chlorpyrifos oxon

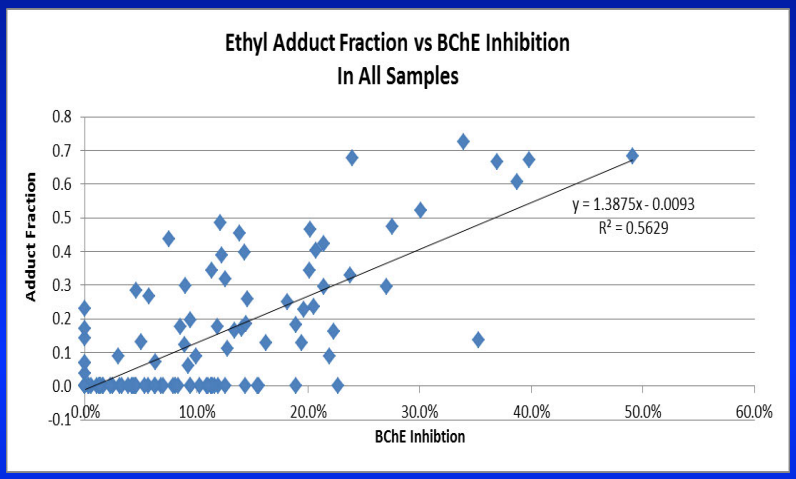


HPLC/MS/MS Analysis of Human Plasma following *In Vitro* treatment with Chlorpyrifos oxon



Farmworker Study

- Study population: handlers & applicators participating in the WA State cholinesterase monitoring program
- Participants had blood drawn prior to the spray season (baseline sample)
- Follow-up blood samples were drawn after working with OP/carbamate pesticides for 30 hrs within a 30 day period
- 128 of these follow up samples were tested for OP adducts. Adduct levels were compared with plasma ChE depression.



Conclusions

- In the in vitro study, the HPLC/MS procedure was able to detect peptides from unadducted, adducted and aged ChE.
  - Aging of the OP-adduct was rapid - largely complete within 40 hours
- In samples collected from OP exposed farm workers, a robust relationship between mono-ethyl OP adduct levels and inhibition of plasma cholinesterase was observed.
  - Greater sensitivity compared to ChE monitoring
  - Greater specificity also; eliminates false positives, but may fail to detect pesticides that don't form stable adducts with BChE
- This technique appears promising for use in human studies.

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