

## A new approach to the analysis of OP insecticides exposure.

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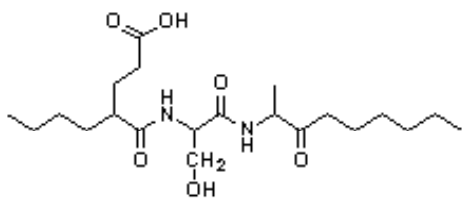
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A recent ban on organochlorines has created a sudden demand for their substitutes. Organophosphorus (OP) insecticides, due to their non-persistent properties has been found to be a suitable replacement. As a result of their rapid introduction to industry analytical support of monitoring of OP exposure has not been thoroughly developed.

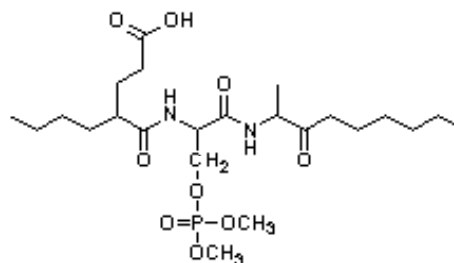
Traditionally, exposure to OP insecticides is assessed by the Ellman method which measures activity of the cholinesterase (ChE) enzyme in blood. If activity drops by 30% a person should be removed from work. This assay lacks sensitivity and necessitates the establishment of a ChE baseline level. Due to high natural variation of ChE levels in the body it is problematic to use the previously established baseline of an individual.

An alternative method for the assessment of an OP exposure is the analysis of dialkyl phosphate (DAP) metabolites in urine. The limitation of this method is that some OP compounds, significantly different in toxicity, produce identical metabolites, and therefore biological exposure indices cannot be set for the DAPs in urine.

The idea of measuring ChE phosphorylation for the biological monitoring of OP exposure was first suggested by S.W. Kennedy (1), using HPLC of FMOC derivatives. Phosphorylated ChE is a widely accepted biomarker for chemical warfare agents with phosphorylation being confirmed through phosphatase hydrolysis (2) or tryptic digestion (3) of ChE.



Fragment of AChE



Fragment of phosphorylated AChE

We propose mass spectrometric measuring of a ratio of phosphorylated/non-phosphorylated ChE tryptic digest in the blood ( $H_3N^+$ -LALQWVQENVAAFGGDPTSVTLFGES\*AGAASVGMHLLS PPSR-COO<sup>-</sup>) for the biological monitoring of OP compounds. This will allow the measurement of the true ChE inhibition and eliminate the necessity to establish a pre-exposure baseline and therefore improve biological monitoring of OP compounds.

### References.

1. Kennedy S.W. (1991). The mechanism of organophosphate inhibition of cholinesterase - proposal for a new approach to measuring inhibition. In: P. Mineau (ed.), *Chemicals in Agriculture*. vol. 2. Cholinesterase Inhibiting Insecticides. Their Impact on Wildlife and the Environment. pp.73-87. Amsterdam: Elsevier.
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3. Elhanany E, Ordentlich A, Dgany O, Kaplan D, Segall Y, Barak R, Velan B, Shafferman A. (2001). Resolving pathways of interaction of covalent inhibitors with the active site of acetylcholinesterases: MALDI-TOF/MS analysis of various nerve agent phosphyl adducts. *Chem Res Toxicol*. **14**:912-8.