# *Rapid Neutralization of Organophosphate Nerve Gas Agents*

**Daqing Gao (PI), Anthony R. Arment**
**Department of Natural Sciences**
**Central State University**

Synthetic organophosphates are widely used as pesticides, herbicides and active components in chemical warfare agents (CWA). One of the most dangerous of the CWA is the compound designated VX. The abiotic hydrolysis of VX produces, depending on the pH of hydrolysis, results in a mixture of products including ethylmethylphosphonic acid (EMPA), diisopropylethylmercaptoamine (DESH or DIEM), EA-2192 and ethanol. Though less toxic than VX, these products are still both toxic and persistent. The enzymatic catalysis of VX, on the other hand, promises a better strategy over chemical hydrolysis, because degradation can be accomplished largely *in situ* and produces a set of products, both lower in toxicity and subject to further bioremediation. Because of the inherent dangers in handling VX, the chemical stimulant malathion is being used in initial screens and VX byproducts being used in subsequent analysis. However using either parent compound, under ideal conditions, will result in complete remediation, the final fate of the compound being utilized in normal metabolic processes.

Several enzymes are known to act on the C-P and P-S bonds in the malathion backbone, thereby, producing less toxic byproducts; the P-S-C (phosphonate) bond being the more difficult to catalyze. These enzymes, however, are not constitutively expressed and are regulated by conditions of phosphorus and sulfur starvation. Our labs propose to screen for degrading organisms, not using the traditional method of substituting malathion as the sole carbon source, but instead by identifying degraders based upon the ability to use malathion as sole sources of phosphorus and sulfur. In altering the screen, we hypothesize to also identify those organisms with the best activity towards phosphonate bonds, making these same degraders better candidates against VX and its byproducts. We have isolated over 100 clones from a screening of our microbiology culture collection, greenhouse soil sampling and activated wastewater sludge and are in the process of further characterizing them.

The specific aims of the project are to:

Isolate and identify microorganisms able to bioremediate malathion as a sole source of carbon, sulfur and phosphorus.

Study the kinetics of malathion bioremediation by comparative analysis of substrate disappearance and novel product appearance over time by GC-MS.

Screen the organisms (1) for the ability to metabolize the abiotic byproducts of VX degradation, e.g. EMPA, DESH/DIAM and EA-2192.

Study the kinetics of EMPA, DESH/DIAM and EA-2192 bioremediation by comparative analysis of substrate disappearance and novel product appearance over time by GC-MS.

Study the genetics of positive organisms (3) to identify and isolate specific genes and proteins involved in byproduct bioremediation.

Clone and express degrading enzymes for protein purification and further study.

***Corresponding Author:*** *Anthony R. Arment, Ph.D at aarment@centralstate.edu*

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## Main Menu

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- Welcome and Opening Remarks
- Keynote Speakers
- Podium Presentations
- Poster Presentations
- Video Montage of the 13th Annual PRP Symposium
- Participating Universities
- Steering Committee Members
- Acknowledgements
- Problems Viewing the Videos