Utilization of a New Toxicity Testing System As a Water Security Monitoring Tool

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By

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There is a need for increased surveillance of our water supply systems.

- Al-Qaida interest in water treatment and distribution systems
- Incident in Rome with Ferricyanide
- Near panic caused by hoax in city in the Midwest.

The large number and diversity of possible threat agents precludes monitoring on an individual basis.

- Chemical warfare agents. VX, Sarin, etc.
- Herbicides, Pesticides, and Rodenticides
- Heavy metals. Mercury, Thallium., etc.
- Radionuclides
- Industrial Chemicals. Cyanide, etc.

This leaves Toxicity testing.

- Toxicity is the ability of a chemical or mixture of chemicals to cause a living organism to undergo adverse effects upon exposure. These effects,can include negative impacts on survival, growth, reproduction, etc. Toxicity tests are laboratory experiments which attempt to detect or quantify toxicity in a sample by measuring the results that exposure produces on standard test organisms.

Types of Toxicity Testing

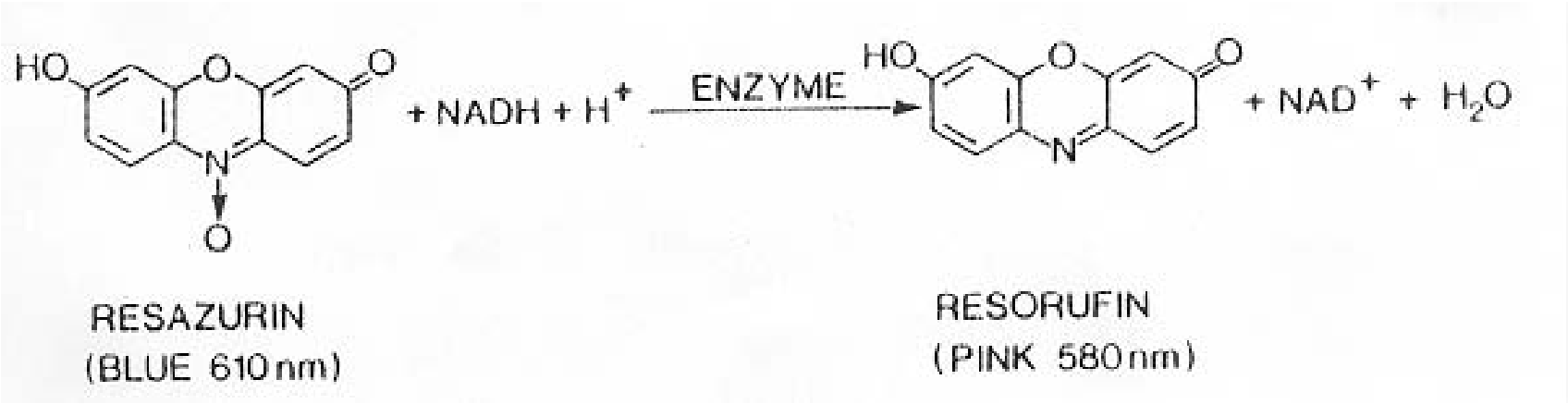
- Humans and other mammals. Best, but impractical.
- Fish. Complicated and expensive. Won't work for distribution systems.
- Daphnia. Easier than Fish, but still require care. May be too sensitive to interferences.
- Bacteria are a practical alternative for a test organism.

Current Methods of Bacteria Based Toxicity Testing

- Luminescent Methods
  - Functions by measuring effect of toxins on light output of luminescent bacteria
  - Shown to correlate with total toxicity.
  - Bioluminescence is not an essential metabolic pathway nor is it widespread in nature.
  - Toxins may be specific inhibitors of luciferase and may not be toxic to other organisms.
  - Requires an expensive instrument

Chemistry of the New Method

- Liu *et. al. (1981)* "A Rapid Test for Measuring Chemical Toxicity"
- Used Resazurin, a redox reactive dye.
- Blue to pink color change.

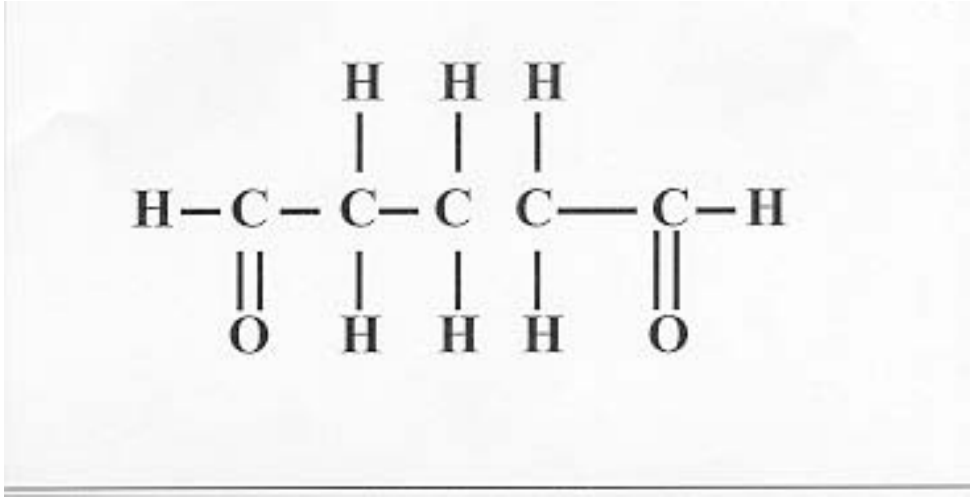


Problems with Liu's Method

- Requires large quantities of bacteria to complete a detectable color change in a reasonable test duration.
- Needs an organic extraction with n-amyl alcohol, which may be toxic itself.
- Requires centrifugation.

New Method Explained

- In routine testing using Liu's method it was discovered that Glutaric Dialdehyde accelerates the reaction with resazurin.



- The Gluteraldehyde most likely functions to speed up respiration by uncoupling oxidation form phosphorylation.
  - Due to increased speed, a smaller inoculum of bacteria is needed to get a change of color in a shorter time frame.
  - The smaller inoculum allows the color to be read without centrifugation or extraction. It can be read visually.
  - Gluteraldehyde works on a wide variety of Gram positive and Gram negative bacteria allowing indigenous cultures to be used.

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Use the ToxTrak™ Toxicity Test Kit wherever you need to conduct toxicity assessment.

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Procedure

- Label tubes for a toxic free blank and n samples.
- Add 5 mL of toxic free water to the blank and 5 mL of sample to the appropriate tubes.
- Add contents of a foil pouch to each tube. Resazurin + Buffer.
- Add 2 drops of the accelerator solution.

- Add 0.5 mL of a 10-72 hour old stock or indigenous biomass sample that has been grown in Lauryl Tryptose or other broth
- Shake to mix.
- Read Abs. of the tubes vs DI water at 603 nm.
- Allow to sit for 45-75 minutes. Abs of blank should decrease 0.500-0.700 Abs.
- Read Abs. of the tubes vs DI water at 603 nm again.
- Calculate % Inhibition

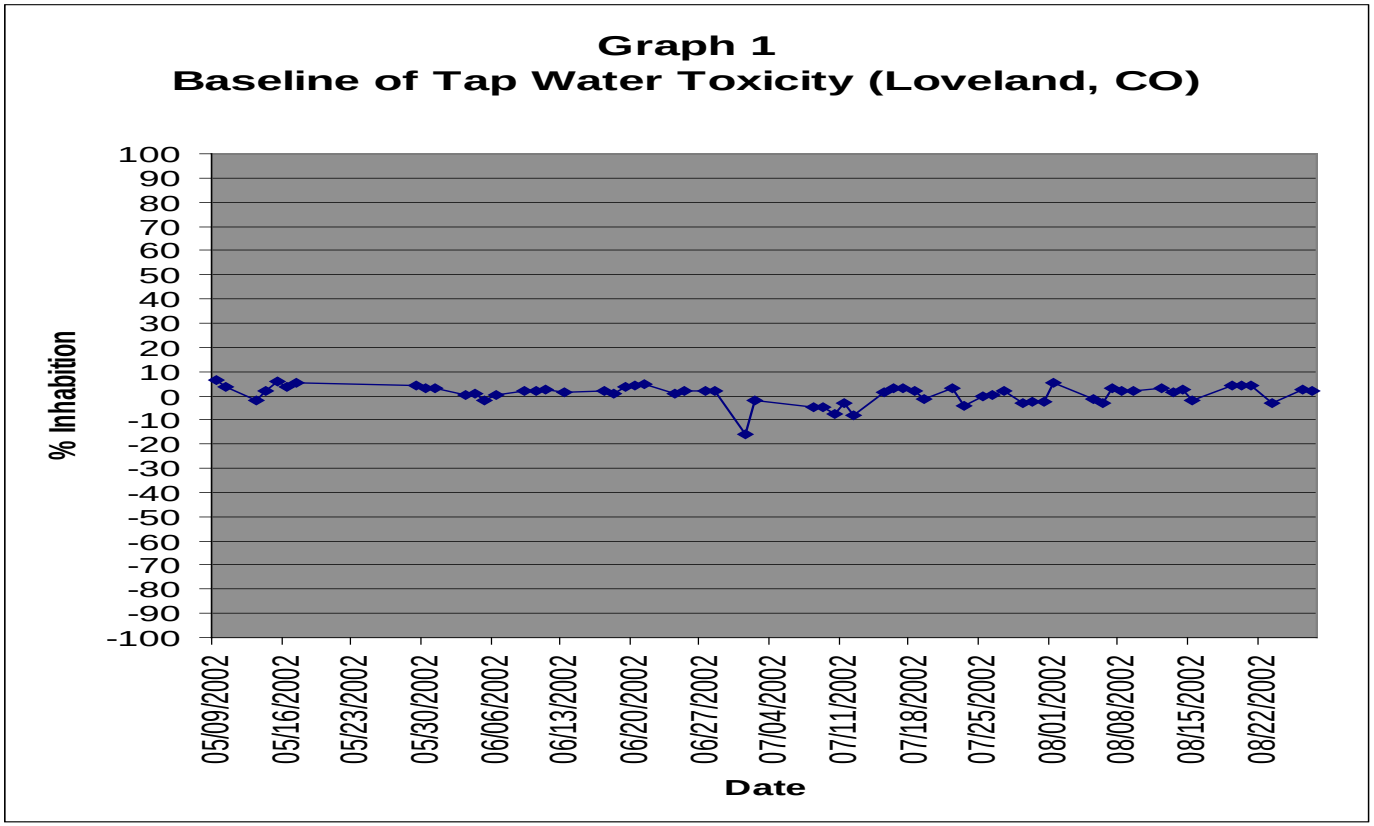
Calculate Inhibition as follows:

- $\%I = [1 - (\Delta A_{sample} \div \Delta A_{control})] \times 100$
  - Where
- ΔA = Initial abs. - Final abs.

Interpreting Results

- Toxicity can be positive or negative.
- Some noise in test.
- Limit of detection on the order of 10%
- Consistent negative or positive results < 10% can be interpreted as slightly toxic.

Utilizing the System as a Water Security Tool (Baseline)



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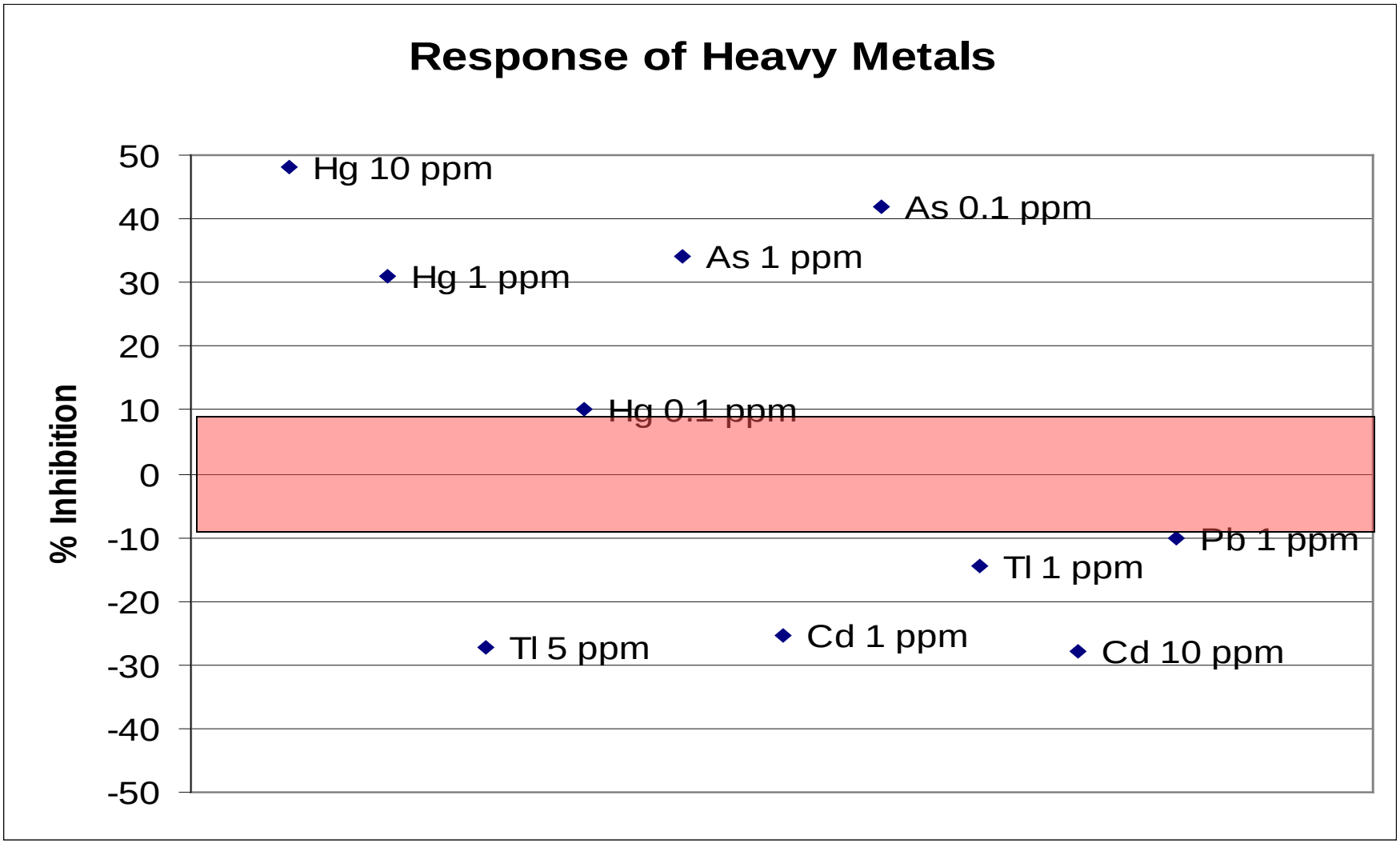
Baseline

- Baseline run against reservoir.
- All fell between 6.4 and -8.1% Inhibition except for 1 data point.
- Possible Forrest fire explanation.

Heavy Metals

- Highly toxic to humans
- Fairly easy to obtain in quantity
- Stable in water.
- Salts are readily soluble.
- Tested Arsenic, Cadmium, Lead, Mercury, and Thallium.



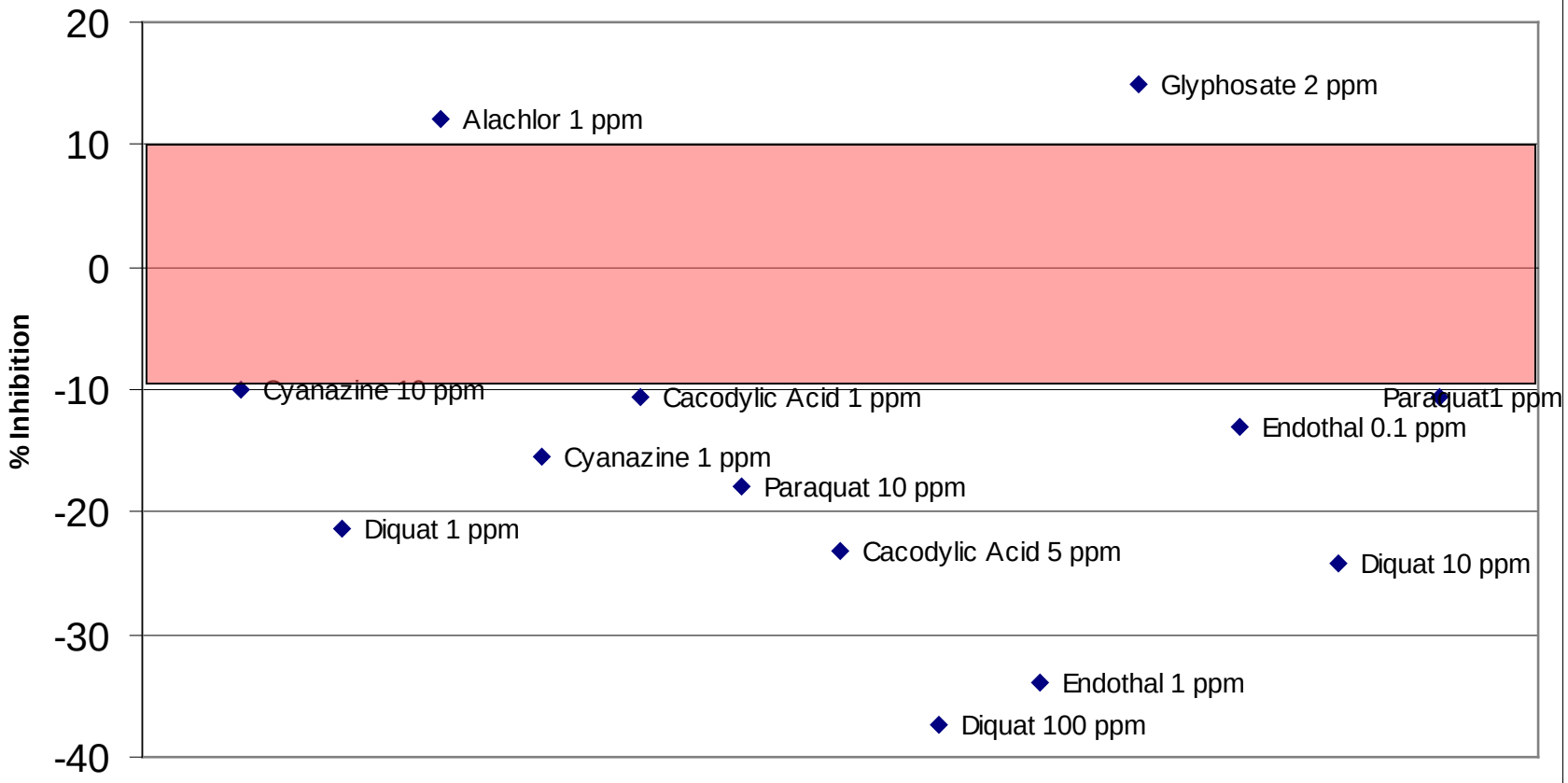
Heavy Metals

- Response from all metals tested.
- Mercury down to 0.1 mg/L
- Arsenic over 40% at 0.1 mg/L
- Other (Lead, Cadmium, Thallium) down to 1 mg/L.
- Test seems to be very good at detecting metal contamination.

Herbicides

- Some can be extremely toxic.
- Easy to obtain in large quantities from agricultural usage.
- Inexpensive.
- Many are quite soluble.
- Alachlor, Cacodylic Acid, Cyanazine, Diquat, Endothal, Glyphosate, and Paraquat were tested.

Response of Herbacides



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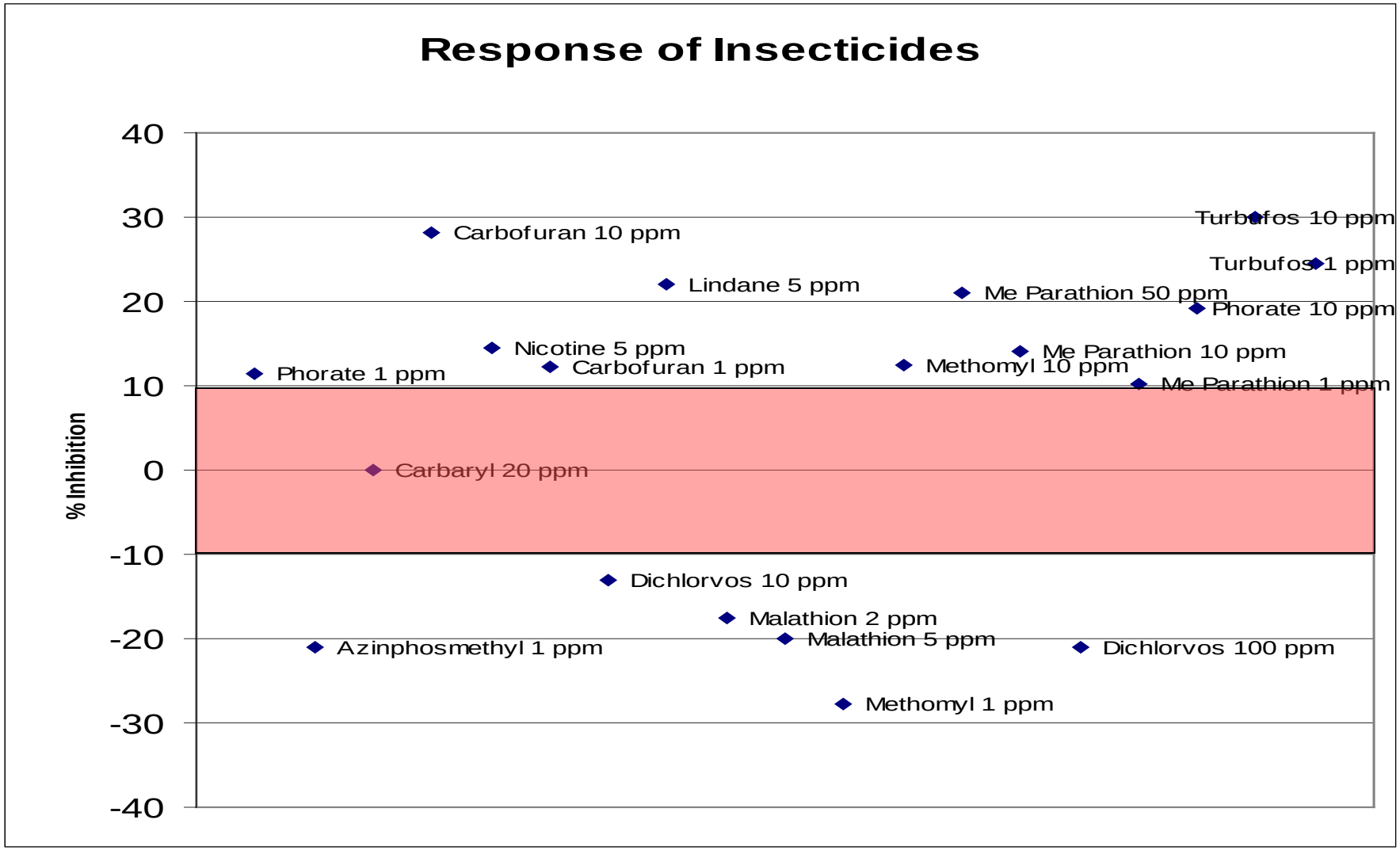
Herbicides

Summary

- Wide range of responses, both positive and negative inhibition was exhibited.
- Cyanazine more toxic at lower levels. Within noise of test, but may indicate competing modes of toxicity.
- Endothal -34% at only 1 ppm.
- Good indicator for herbicide contamination.

Insecticides

- Tend to be more harmful than herbicides.
- Some structures similar to chemical warfare agents. Cholinesterase inhibitors.
- Readily available in large quantities.
- Some are limited by solubility. Others are not.
- Azinphosmethyl, Carbaryl, Carbofuran, Dichlorvos, Lindane, Malathion, Methomyl, Methyl Parathion, Nicotine, Phorate, Turbufos tested.



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Insecticides

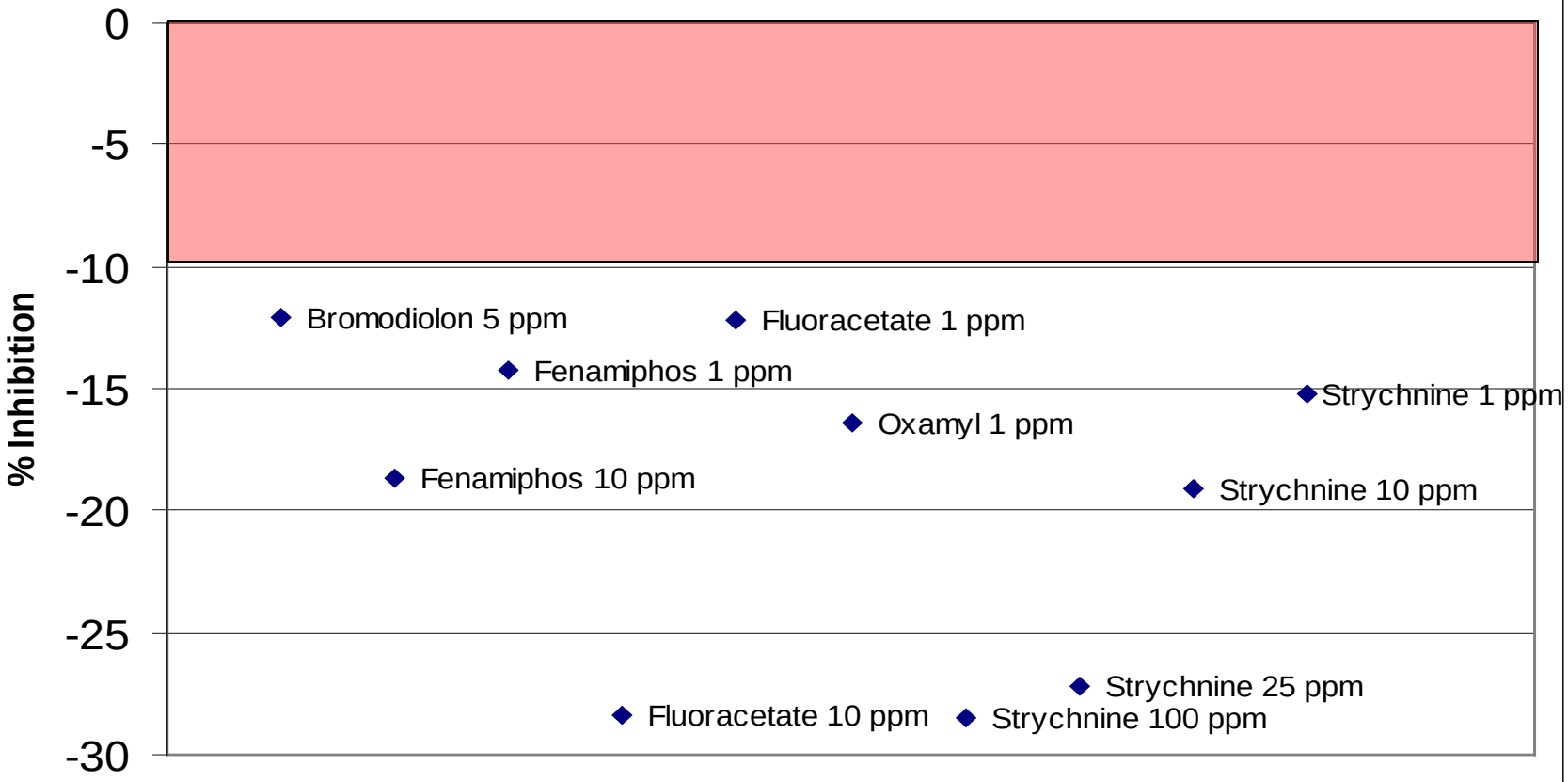
Summary

- Variety of responses. Both positive and negative inhibition.
- Response to all insecticides tested except Carbaryl.
- Carbaryl was difficult to dissolve. May have degraded during ultrasonication.
- Fairly good detection of insecticides.

Nematocides and Rodenticicdes

- Nematocides similar to insecticides.
- Some similar to warfare agents.
- Some cholinesterase inhibitors.
- Rodenticides designed to kill mammals.
- Bromodiolon, Fenamiphos, Fluoracetate, Oxamyl, and Strychnine were tested.

Response of Nematicides & Rodenticides



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Nematocides and Rodenticides

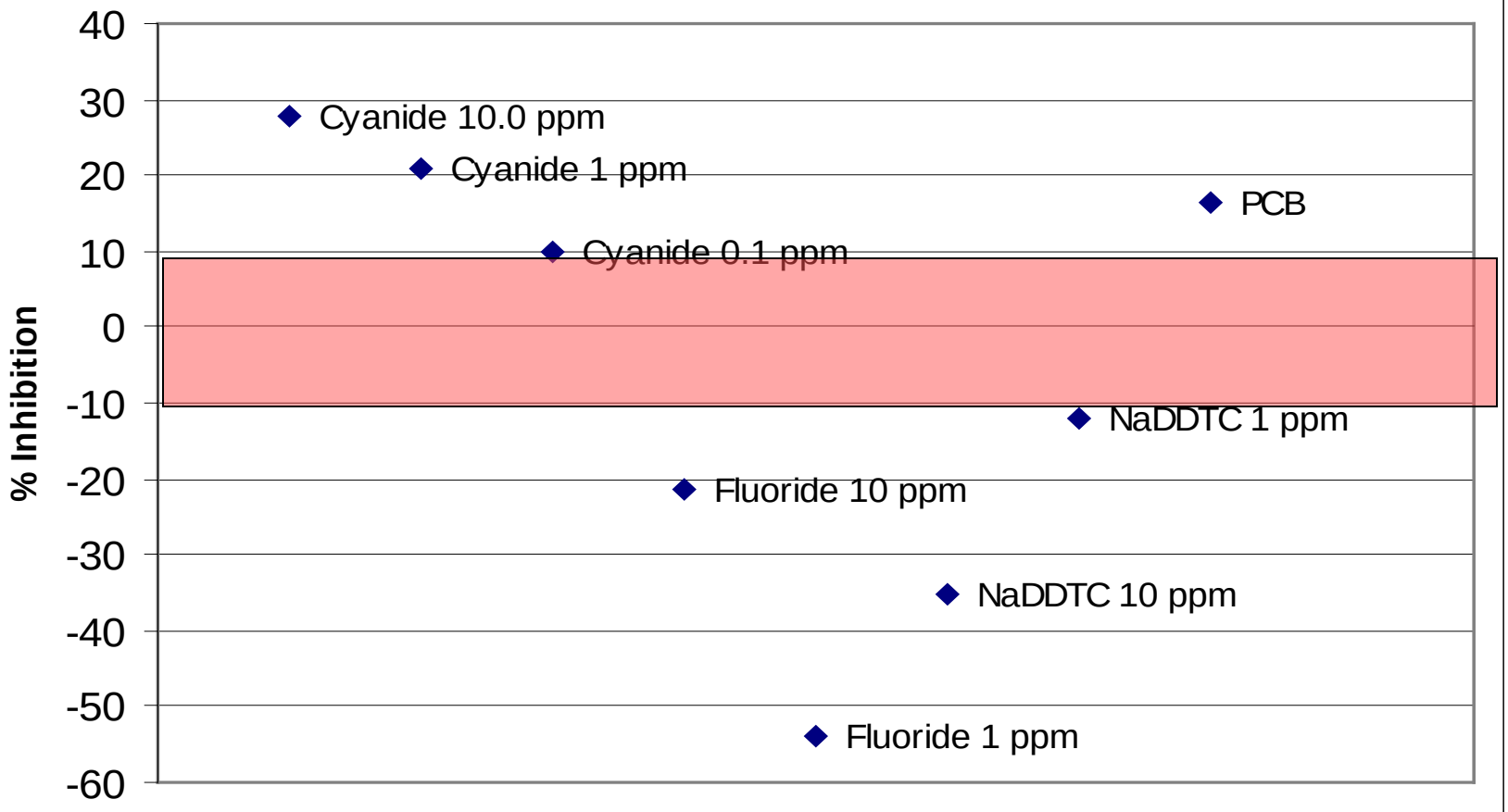
Summary

- All the compounds tested gave a response.
- All showed negative inhibition.
- Common poisons like Strychnine and Fluoracetate were capable of being detected at levels as low as 1 mg/L.

Toxic Industrial Chemicals (TICs)

- Many industrial compounds are very toxic and easy to obtain in large quantity.
- Cyanide is a prime example. Used in mining, metal finishing and other industriesd.

Response of Industrial Chemicals



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Toxic Industrial Chemicals

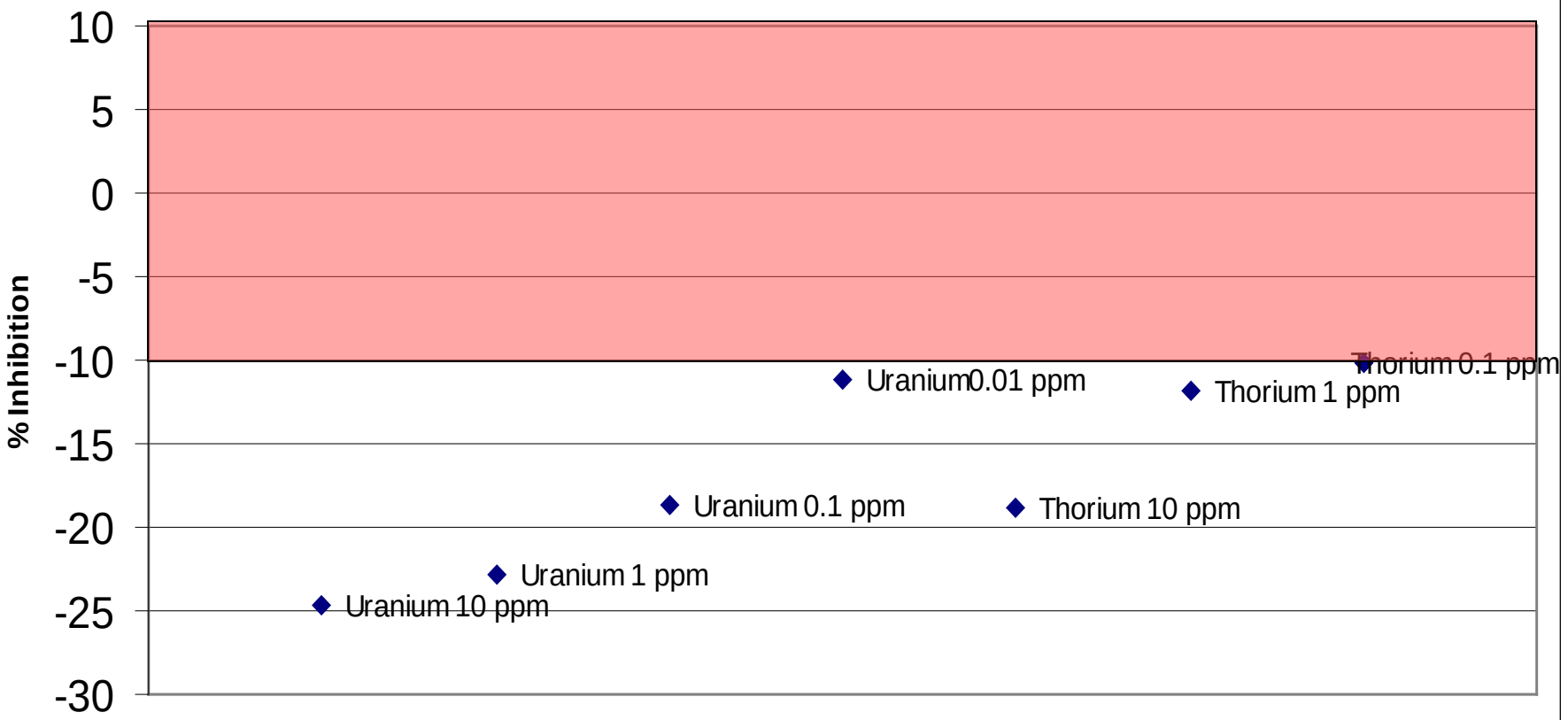
Summary

- All the compounds tested gave a response.
- Some showed negative inhibition. Some showed positive inhibition.
- Very sensitive to cyanide down to 0.1 ppm

Radionuclides

- Obtaining high purity material is difficult and would not likely be targeted at a water supply.
- Possible secondary contamination with high level material.
- Possible use of low level material against a water system.
- Uranium and Thorium salts were studied.

Response of Radionuclides



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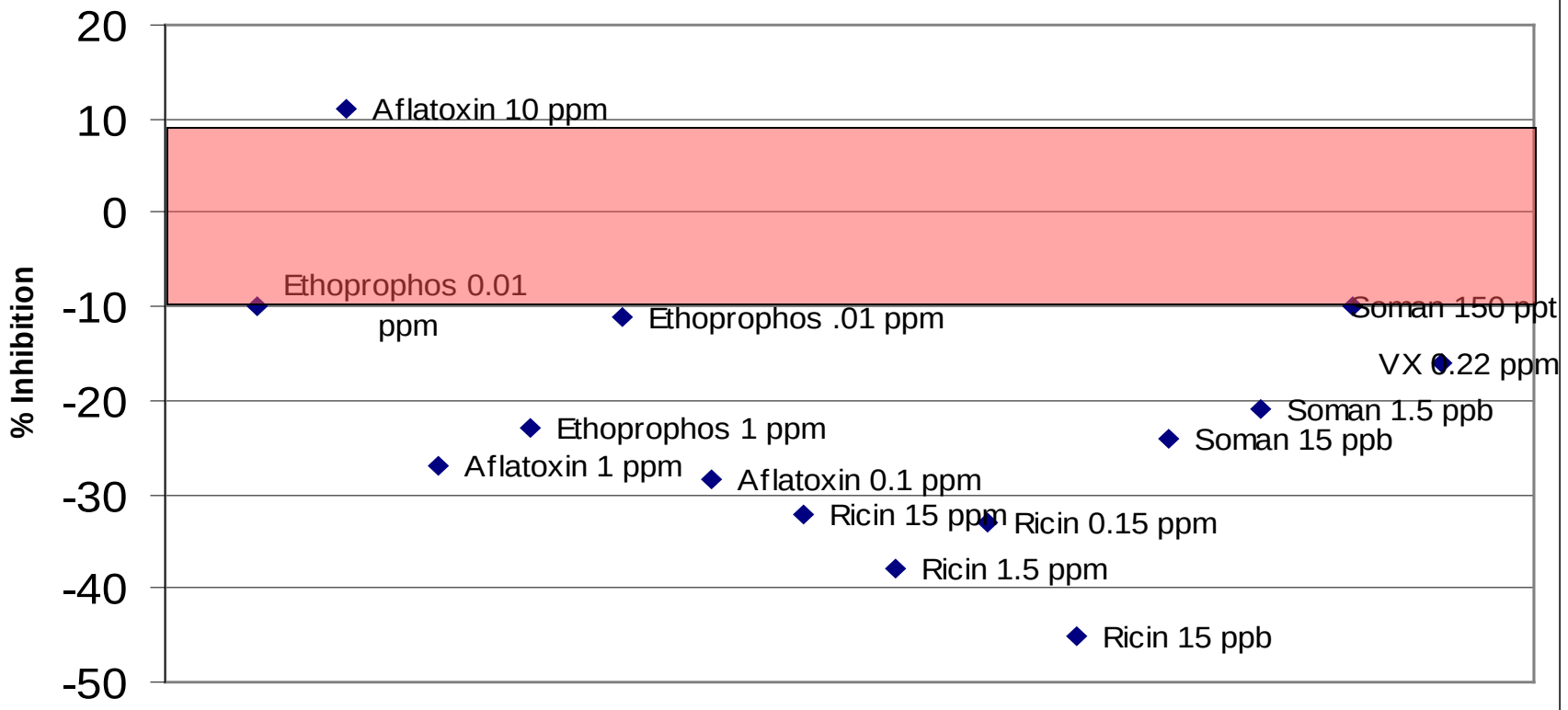
Radionuclides
Summary

- All tested compounds exhibited negative inhibition.
- Toxicity may or may not be due to radioactivity.
- Good sensitivity to both Uranium and Thorium. Uranium down to 0.01 mg/L and Thorium down to 0.1 mg/L.

Chemical Warfare Agents

- Wide variety of agents.
- Most likely mode of attack is aerosol. May result in secondary contamination of water.
- Most agents not available for testing.
- Aflatoxin and Ethoprophos a VX surrogate were tested at Hach.
- Ricin, Soman, BoTox and VX tested at Battelle

Graph 10
Warfare Agents



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Chemical Warfare Agents

Summary

- Aflatoxin showed competing modes of action.
- Surrogate for VX Ethoprophos could be detected at levels as low as 0.01 mg.L
- VX gave varying results but was detected.
- Very good detection of Ricin
- BoTox not detected.

Discussion

- New method was shown to be effective in detecting a wide variety of chemical threat agents
- Response seen form each category of agent.
- Only 2 agents Carbaryl and BoTox showed no response.
- Extremely effective at detecting low levels of some agents such as heavy metals, Radionuclides, Ricin, and cyanide.

Discussion

- Tests can be run on any spectrophotometer or colorimeter capable of measuring abs at around 600 nm.
- Most facilities already have such an instrument.
- Portability or use of color disc system make it mobile for first responders.

Discussion

- Can cut down on false positives by using acclimatized bacteria.
- Versatile: Can be used for drinking water waste water, as a prescreen for WET tests or as an investigative tool to help track pollution plumes.
- Easy Procedure.
- Low cost. Around $2 per test

Conclusion

The proposed tests simplicity, versatility and its low cost for instrumentation and reagents should make it a valuable tool in the arsenal of solutions we deploy to help safeguard our Nation’s drinking water supplies.