Effect of organophosphates malathion and temephos on cholinesterase activity in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae)

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Earthworms

- common test organisms and good indicator species for ecotoxicological research
- they have highly differentiated organs and tissues and an immune system that is comparable with that of vertebrates
- they can be exposed to pesticides either through dermal contact or by ingestion
many biomarkers have been identified in environmental pollution dealing with pesticides and organochlorine contaminants

acetylcholinesterase (AChE) activity is routinely used as a biomarker of the exposure to organophosphates and carbamates compounds

many unsolved questions in biomarker applicability in real time environmental pollution monitoring

effects of small so called sublethal doses
adult earthworms (*Eisenia fetida*) were exposed to the sub-lethal concentrations of organophosphates malathion and temephos

contact filter paper test procedure

ChE activity was determined according to the method of Ellman et al. (1961)
Malathion

- one of the most often used organophosphate insecticide in the world
- insecticide and acaricide with contact, gut and respiratory action
- in insects and mammals is metabolically converted to its structurally-similar metabolite, malaoxon
- malaoxon inhibits AChE resulting in respiratory, myocardial and neuromuscular transmission impairment

structure of malathion
Temephos

- Nonsystemic organophosphorus insecticide used to control mosquito, midge, and black fly larvae
- It is used in lakes, ponds, and wetlands
- Only organophosphate with larvicidal use
- Temephos affects the central nervous system through inhibition of cholinesterase
- In larvae, this results in death before reaching the adult stage

Structure of Temephos
## Experiment

<table>
<thead>
<tr>
<th>PESTICIDE</th>
<th>CLASS OF PESTICIDE</th>
<th>TYPE OF PESTICIDE</th>
<th>MODE OF ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALATHION</td>
<td>organophosphate</td>
<td>acaricide, insecticide</td>
<td>indirect inhibitor of cholinesterase</td>
</tr>
<tr>
<td>TEMEPHOS</td>
<td>organophosphate</td>
<td>insecticide (larvicidae)</td>
<td>cholinesterase inhibitor</td>
</tr>
</tbody>
</table>
Experiment

- **PRELIMINARY EXPERIMENT**
  - Determination of EC<sub>50</sub> and LC<sub>50</sub> values
  - Choosing the concentrations for final experiments

- **FINAL EXPERIMENT**
  - Determination of ChE activity

<table>
<thead>
<tr>
<th></th>
<th>MALATHION</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>TEMEPHOS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 ng/cm²</td>
<td>0.1 ng/cm²</td>
<td>0.2 ng/cm²</td>
<td>0.4 ng/cm²</td>
<td>0.6 ng/cm²</td>
<td>1 ng/cm²</td>
<td>4 ng/cm²</td>
<td>0.025 ng/cm²</td>
<td>0.01 ng/cm²</td>
<td>0.05 ng/cm²</td>
<td>0.12 ng/cm²</td>
</tr>
</tbody>
</table>
Concentration-response curves for 24, 48, and 72 h of exposure to different temephos concentrations with mortality as an endpoint, calculated from the first-preliminary experiment. Curves representing: (white) 24h, (grey) 48 h, and (black) 72 h of exposure.
Activity of ChE measured in *E. fetida* earthworms after exposure to different concentrations of temephos for 2 h.

*Significantly different vs. control activity (p<0.05).*
Results

Activity of ChE measured in *E. fetida* earthworms after exposure to different concentrations of malathion for 2 h.

*Significantly different vs. control activity (p<0.05).
Low dose stimulation, high dose inhibition.

Opposite effect in small doses (sub-lethal, sub-effect) compared to large doses.
Role of hormetic effect?

Importance of the hormetic effect?

INTERPRETATION OF RESULTS, ESPECIALLY IN INTERPRETATION OF ENVIRONMENTAL MONITORING

ENVIRONMENTAL RISK ASSESSMENT
Response to:

- Low-level concentrations
- Time!

Graph showing response vs. concentration and time, with notes: t = const. and conc. = const. (low-dose)
Objective of biomonitoring

- early detection of the biologically relevant concentrations of pollutants
- early detection of the effect of pollutants on biological systems

- Important to detect any variation from BENCHMARK VALUES for test organisms

- Mathematical models which describe response on presence of pollutant often show high sensitivity on so called starting conditions

↓

Mixtures!
Is hormesis doubtful?

Why is horpectic effect difficult to prove?

- **Small concentrations!**
  (researchers usually work with concentrations in range of expected response)

- **Biomarkers often show relatively large variance**
  (especially measured in situ organisms; in vivo)

  ↓

  using cell cultures could result in more accurate results and more precise determination of hormetic effect
Hormetic effect is NOT doubtful!


...and many others in which hormesis has been described, but not defined!


Conclusions

- Some dose-response curves can show an inverted U-shape characteristic for hormesis as it was seen by temephos and malathion in earthworms.

- This hormetic-like effect could be important for health status of an earthworm as well for correct interpretation of biomonitoring results.
Conclusions

The significance of hormetic effect detection:

1) in environmental biomonitoring, where very low concentrations are usually expected, it is obvious that hormetic effect can theoretically be anticipated as a marker of exposure to such concentrations

2) when earthworms are used for toxicological parameters research, models that include hormetic effect should be taken into account
THANK YOU FOR YOUR ATTENTION!

Questions... ?