Complex Mixture-associated Hormesis and Toxicity: The Case of Leather Tanning Industry

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Testing Complex Mixtures

Toxicological and genotoxicological investigation of complex mixtures is one of the main focus of the recent research in toxicology. Testing complex mixtures presents a formidable scientific problem since most recently available toxicological data have been obtained from single substance studies and are not simply transferable to mixtures of chemicals.

Groten et al. 2001
Main laboratory procedures and endpoints in sea urchins embryo and sperm bioassays

<table>
<thead>
<tr>
<th>Testing object</th>
<th>Duration</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos</td>
<td>up to 72 hrs</td>
<td>1) larval malformations&lt;br&gt;2) developmental arrest&lt;br&gt;3) embryonic mortality&lt;br&gt;4) cytogenetic aberrations</td>
</tr>
<tr>
<td>Sperm</td>
<td>1 hr</td>
<td>1) fertilization rate&lt;br&gt;2) offspring quality</td>
</tr>
</tbody>
</table>
Mitotoxic Agents

Zygote
Blastula

Embryo-selective Agents

Gastrula
Prism
Pluteus

Cleavage
Larval Differentiation

Fertilization
Hatching
Hours post-fertilization

0
10
20
50

N
P1
P2
Published literature from sea urchin embryo tests
(from MedLine and Toxline, April 2008, and author’s archives)

<table>
<thead>
<tr>
<th>Classes of agents</th>
<th>No. agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganics</td>
<td>12</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>28</td>
</tr>
<tr>
<td>Environmental/occupational agents</td>
<td>21</td>
</tr>
<tr>
<td>Natural products</td>
<td>7</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>9</td>
</tr>
</tbody>
</table>
Cd(II) + Zn(II)

Fertilization Rate (% of Control)

\[ [M] \]

Pagano et al. 1986
STUDY DESIGN IN EVALUATING HORMETIC EFFECTS

The evaluation of concentration-related shifts from hormesis to toxicity requires adequate design in bioassays, including:

a) broadly ranging agent concentrations, not confined to NOAEL;

b) adequate definition of controls.
Defining control quality criteria in toxicity bioassays

- control quality is commonly assumed to be “optimal” (zero frequency of adverse events);
- by definition, “100% normal” controls do not permit any observation of hormetic effects;
- controls are required to be characterized by low (sub-optimal) culture quality;
- control quality was re-defined by accepting controls with >30% developmental defects.

*De Nicola et al. 2006*
Hormetic effects in sea urchin bioassays have been reported as changes in fertilization success, by maintaining fertilization rate (FR) in controls at suboptimal levels, i.e. 50 to 70% (Pagano et al., 1986; De Nicola et al., 2004).

In order to evaluate any hormetic effects also in the embryotoxicity bioassays, the acceptance criteria for control cultures were re-defined, by assuming that “low-quality” control cultures (assigned as having <70% viable pluteus larvae) provide information allowing us to discern both toxic and hormetic responses in terms of either developmental toxicity or amelioration of larval quality or viability.
SOME REPORTS ON HORMESIS FROM OUR TEAM


## Selected information on tannin-related toxic and hormetic effects

<table>
<thead>
<tr>
<th>Agents</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camellin B</td>
<td>induced apoptosis in HeLa cell line</td>
<td>Wang et al. (2001)</td>
</tr>
<tr>
<td><em>Hypericum perforatum</em></td>
<td>↑ immunostimulating activity   ↓ immunosuppressing activity</td>
<td>Anonymous (2001)</td>
</tr>
<tr>
<td>extract &amp; oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>non- toxic &lt;5 g/ kg body weight in mice</td>
<td>Rajalakshmik et al. (2001)</td>
</tr>
<tr>
<td>Areca nut</td>
<td>oral cancer promotion</td>
<td>Ieng et al. (2001)</td>
</tr>
<tr>
<td>polyphenols and tannin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Terminalia arjuna</em></td>
<td>↓ 2AF - induced mutagenicity</td>
<td>Kaur et al. (2000)</td>
</tr>
<tr>
<td>tannin extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannic Acid</td>
<td>↑ metabolic activation of a few mutagens</td>
<td>Chen, Chung (2000)</td>
</tr>
<tr>
<td>Tannins</td>
<td>↑ inhibitory activity on lipid peroxidation</td>
<td>Sasaki et al. (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hong et al. (1995)</td>
</tr>
</tbody>
</table>
**Vegetable tannin (Acacia sp.)**

**Embryo Exposure**

- **N(ctr) < 70%**
- **N(ctr) ≥ 70%**

**Graph:**
- Larval malformations (P1)
- Developmental arrest (P2)
- Embryonic mortality (D2)

**Exposure Levels:**
- Control
- VTWE (mg/l): 0.1, 0.3, 1, 3, 10
Vegetable or Synthetic Tannin (Acacia sp.)

**Sperm Exposure:**
Fertilization Success

**Graph:**
- **P. lividus**
- **Fertilization Rate (FR)** measured across different concentrations of Tannin water extract (mg/l) in control and treated groups.
- **FR*(ctr)* < 70%** for the control group
- **FR*(ctr)* ≥ 70%** for treated groups

- **Graph a:** Control versus treated groups with significant differences indicated by asterisks.
- **Graph b:** Detailed concentration-response relationship showing the impact of Tannin water extract on fertilization success.
**P. Lividus**

Vegetable vs. synthetic tannins

<table>
<thead>
<tr>
<th>Tannin water extract (mg/l)</th>
<th>Control</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Viable Larvae (N+R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VTWE</td>
<td>50</td>
<td>75</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>STWE</td>
<td>50</td>
<td>75</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
</tr>
</tbody>
</table>
Dunaliella tertiolecta

Selenastrum capricornutum

(Tannin water extract (mg/l))

(Cells / mL) x 10^3

Control 0.1 0.3 1 3 10 30

D. tertiolecta

S. capricornutum

a

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*
Tannery wastewater sewage in Marrakesh, Morocco

De Nicola et al. 2007
CONCLUSION

♦ Vegetable tannery effluent (TTE) results in lesser toxicity vs. chromium tannery effluent (CTE)

♦ Concentration-response trends are found:
  a) non-linear for TTE (hormesis/toxicity shift)
  b) monotonic for CTE (toxicity also at low levels)

♦ Prospect to renewing the interest to extending applications of vegetable tanning process
ACKNOWLEDGEMENTS

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