Short-Chain Chlorinated Paraffin Effects on the Expression of Key Genes of *Gammarus pulex* Exposed at Two Temperatures

J. Jaegers*, C. Joaquim-Justo, E. Gismondi

University of Liège: Laboratory of Animal Ecology and Ecotoxicology (LEAE), Freshwater and Oceanic Sciences Unit of the University of Liège, B6c, 11 allée du 6 Août, 4000 Liège, Belgium

**Table 1**: list of studied genes, their biological functions, and their abbreviated names.

<table>
<thead>
<tr>
<th>Biological Functions</th>
<th>Genes</th>
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<tbody>
<tr>
<td>Regulation</td>
<td>Pharmacokinetics (PK)</td>
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<tr>
<td></td>
<td>Immune System</td>
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<tr>
<td></td>
<td>Prochemotaxis</td>
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<td></td>
<td>Methylmalonic acid (MMA)</td>
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<td>Endocrine System</td>
<td>Methylmalonic acid dehydrogenase (MMDH)</td>
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<td></td>
<td>Selenium-dependent glutathione 5-transferase (SeGPT)</td>
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<td></td>
<td>Catalase (CAT)</td>
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<td></td>
<td>Mitochondrial superoxide dismutase (MnSOD)</td>
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<td></td>
<td>Cu/Zn superoxide dismutase (CuZnSOD)</td>
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<tr>
<td>General Stress</td>
<td>Heat shock protein 70 (HSP70)</td>
</tr>
<tr>
<td></td>
<td>Thioredoxin (THX)</td>
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<tr>
<td></td>
<td>Thioredoxin reductase (THX red)</td>
</tr>
</tbody>
</table>

**Introductions:**
- Short-chain chlorinated paraffins (SCCPs) are ubiquitous pollutants found in air, sediments, water, biota, etc. included in the Stockholm Convention (2017).
- Persistent, bioaccumulative and toxic (PBT), readily bioaccumulates and biomagnifies.
- Present in surface waters at concentrations in the ng or µg/L range are what are the sublethal effects on exposed organisms?
- Freshwater amphipod crustacean *Gammarus pulex* studied to determine SCCP effects at relevant concentrations (10, 100 and 1000 ng/L).
- Measuring mRNA levels of genes for immune defenses, moulting-related endocrine cycle, osmoregulation, apoptosis, oxygen transport, antioxidant & anti-toxic defences (Table 1).
- Two temperature ranges simulate cooler and warmer climates – potential interactions of heat-related stress and toxicity of SCCPs?

**Materials & Methods:**
- G. pulex and collected and transferred to the lab
- Incubator at 16°C + 2
- 7 days of acclimatation + food
- Incubator 20°C + 2
- 7 day exposure at 16°C or 20°C to:
  - Solvent control (acetone 0.01%)
  - SCCP 100 ng/L
  - SCCP 10 ng/L SCCP 100 ng/L
  - SCCP 10 ng/L

**Fig. 1**: heatmaps of mRNA levels for the studied genes. Red = higher level of mRNA compared to controls, blue = lower level. Genes grouped by similarity of mRNA expression patterns.

**Results:**
- Genes with similar functions are similar responses to SCCPs in a given condition. Antioxidant, antitoxic stress = immunity loosely grouped together, same for endocrine genes (Fig. 1).
- 16°C: ↑ expression for MIH, HC, MnSOD Possible short term inhibition of moulting, issues with oxygen transportation and increase in oxidative stress.
- ↓ expression of ProPO and NaK = smaller immune response and less osmoregulatory capability. Most effects only at 1000ng/L SCCP.
- 16°C: ↑ expression of antioxidant + antistress genes, ProPO, NaK, and endocrine genes
  - Some function responses = , but others opposite. Ecr + MIH both lowest just before moult so do SCCPs encourage moult in females?
  - Most effects at 100ng/L, some at 100 ng/L. Females = more sensitive to SCCPs in the exposure conditions.
- 20°C: males: unpactected females: ↑↑↑ antioxidant + antistress genes at 100ng/L
  - Famet enzyme (expression which forms methyliosanolate = growth hormone).
  - Higher stress for females = endocrine disruption caused by heat stress and toxicity?

**Conclusions:**
- SCCPs = sublethal toxic effects in *G. pulex* males + females after 7 days at environmental concentrations: potentially endocrine disruption, oxidative stress, general stress, ...
- Cooler ℃ range: males over-express antioxidant defence + moulting cycle endocrine genes. Females = opposite. Immunity + osmoregulation impacted similarly in both sexes.
  - SEX DEPENDENT RESPONSE to SCCP EXPOSURE
- Warmer ℃ range: Female antioxidant genes highly overexpressed. Combined stress effect of ℃ and SCCPs?
  - Males seemingly unaffected. Effects hidden or compensated for by warm ℃ physiological response?
  - TEMPERATURE DEPENDENT RESPONSE to SCCP EXPOSURE
- Further research needed on SCCP effects on ecologically important but understudied groups. What are the mechanisms of endocrine disruption and sublethal toxicity?