

# Effects of short-chain chlorinated paraffins on the expression of key genes of *Gammarus pulex* at two exposure times

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## Take Home Message:

SCCPs affect *G. pulex* after 7 or 21 days

Multiple biological functions impacted

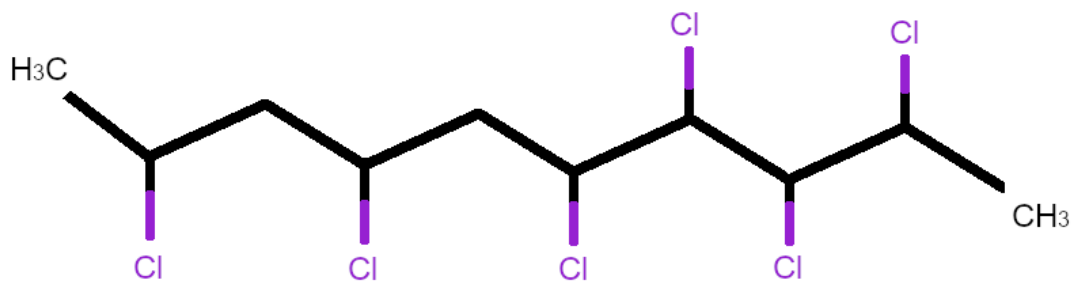
Evidence of adaptation after 21 days

Ambient t° plays a role (16°C vs 20°C)

**! SCCP effects on invertebrates under-studied!**

# What are SCCPs?

- Aliphatic alkanes, C<sub>10</sub>-C<sub>13</sub>, mass 40-70% chlorine
- Industrial additive:
  - Metalworking fluids
  - Plasticizer
  - Flame retardant
- >200 000 tons in the environment



*Fig. 1: 10 carbon SCCP, 61% chlorine mass*

**In this work: chloroparaffin C<sub>10-13</sub> 63% Cl formulation  
(CAS n°: 85535-84-8)**

# Properties & Occurrence

- Persistent Organic Pollutant (**POP**)
- **Bioaccumulable** + biomagnifiable
- Long range **transport** through air + water
- Developmental toxicity on fish and amphibians
- Thyroid hormone disruptor in fish
  
- Found in waters (ng and  $\mu\text{g/L}$  range) + biota (ng/g range)
- Present in Europe, North America, Asia, Australia, Arctic circle, Antarctica ...

# Problem & Scope

- Toxic at environmental concentrations
- Very few data on invertebrates

Can **realistic** SCCP exposure concentrations affect key **biological functions** of a common freshwater amphipod?

A **biomarker-based** approach

# Materials and methods

Adult male *G. pulex*  
collected in the wild

Incubator: 16 °C



Incubator: 20 °C

7-day acclimation  
to lab conditions



RT-qPCR



RNA extraction

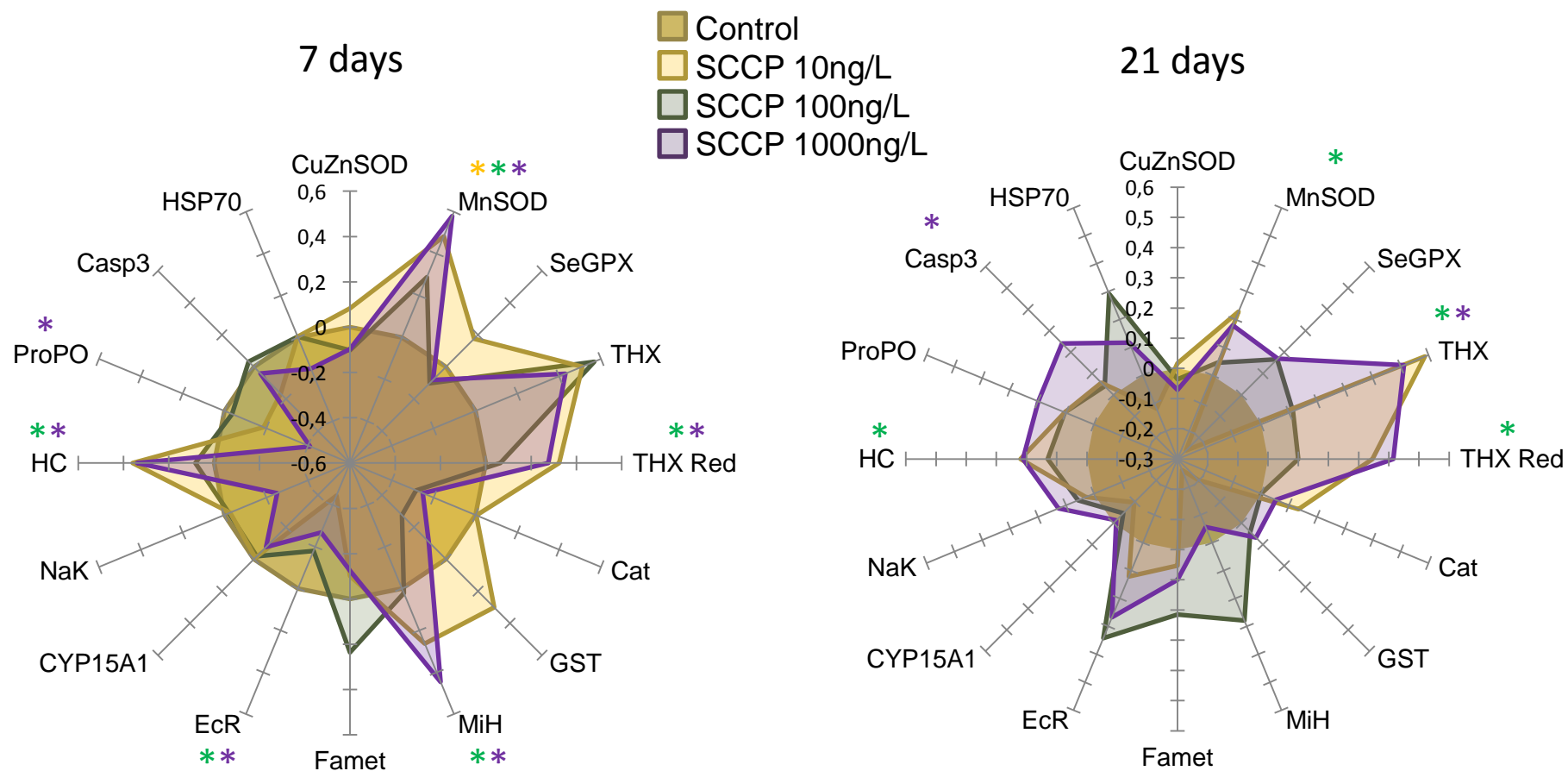


Reverse-transcription  
to cDNA

7- or 21-day exposure:  
solvent control (acetone 0.01%)  
SCCP 1000 ng/L  
SCCP 100 ng/L  
SCCP 10 ng/L

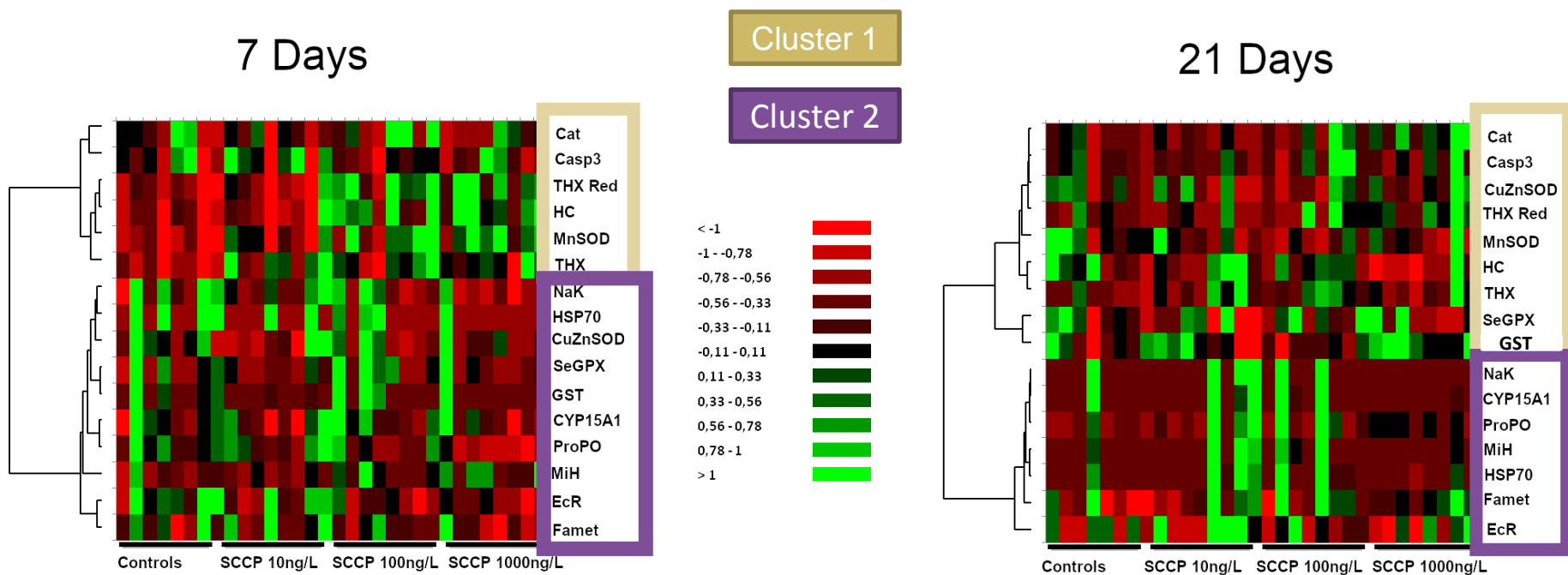
Studied genes	Acronym	Biological function
Cu/Zn-Superoxide dismutase	CuZnSOD	<b>Antioxidant defences</b>
Mn-Superoxide dismutase	MnSOD	
Se-dependant glutathione peroxidase	SeGPX	
Thioredoxin	THX	
Thioredoxin reductase	THX Red	
Catalase	Cat	
Glutathione-S-transferase	GST	
Moult-inhibiting hormone	MIH	<b>Endocrine system</b>
Farnesoic acid O-methyltransferase	Famet	
Ecdysteroid receptor	EcR	
Methylfarnesoate epoxidase	CYP15A1	
Na/K ATPase	NaK	<b>Osmoregulation</b>
Hemocyanin	HC	<b>Respiration</b>
Prophenoloxidase	ProPO	<b>Immunity</b>
Caspase 3	Casp3	<b>Apoptosis control</b>
Heat-shock protein 70	HSP70	<b>Heat stress</b>
Actin + Elongation factor 1-alpha	--	<b>Reference genes</b>

# Results & Discussion



*Fig. 2: log<sub>2</sub> fold-change values for gene expressions after SCCP exposure. Genes marked with (\*) displayed a significant ( $p < 0.05$ ) difference with controls, colour coded to the corresponding SCCP exposure condition.*

# Results & Discussion



*Fig. 3: heatmaps with hierarchical clustering of genes by similarity of expression levels between exposure conditions. The 2 main clusters have been highlighted.*

## Results & Discussion

- Up-regulation of antioxidant genes
- Short-term inhibition of moulting
- Variations in HC and ProPO linked to moult inhibition?
- Increase in caspase3 mRNA after 21 days

# Comparison with 20°C exposure

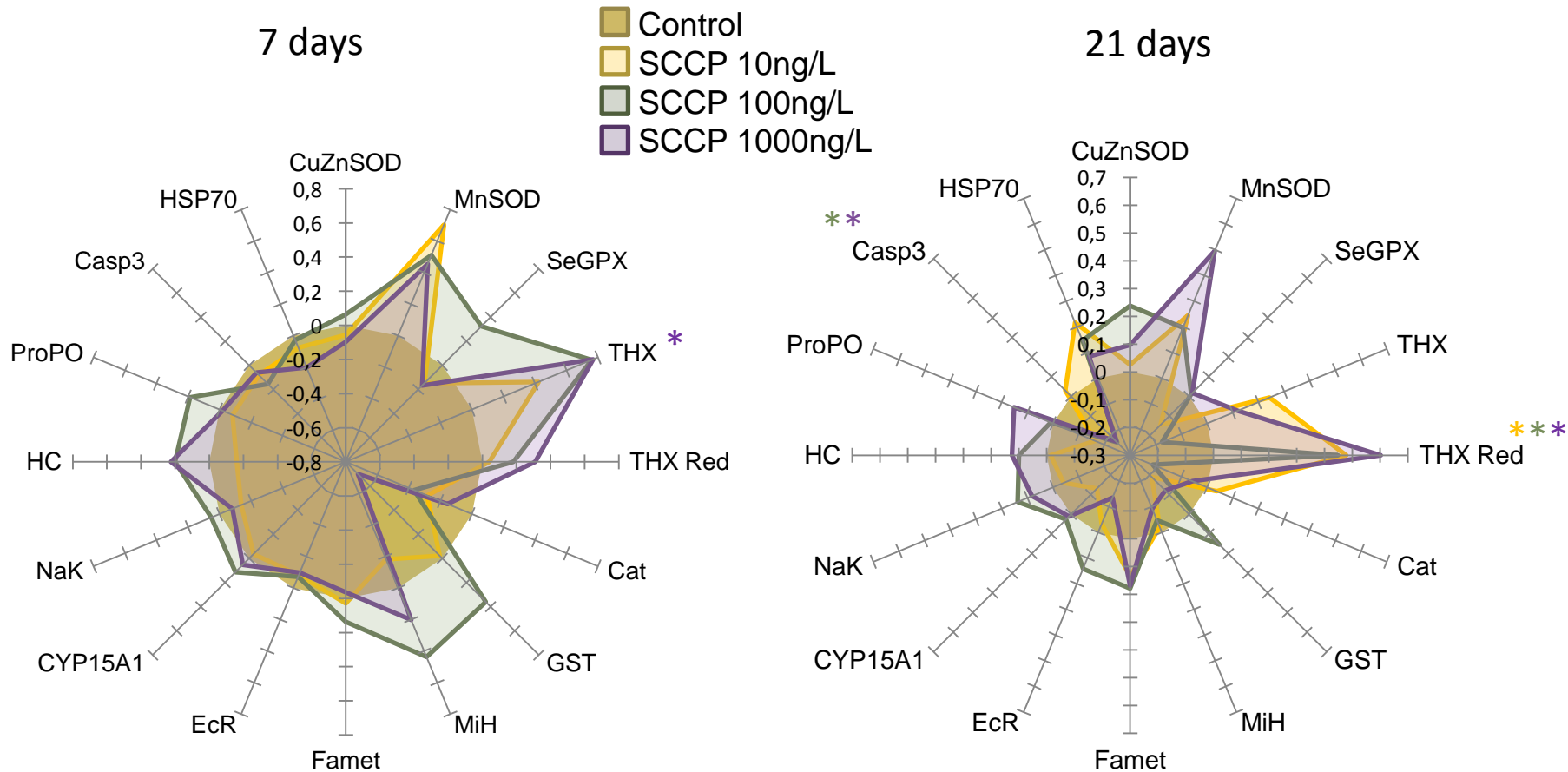
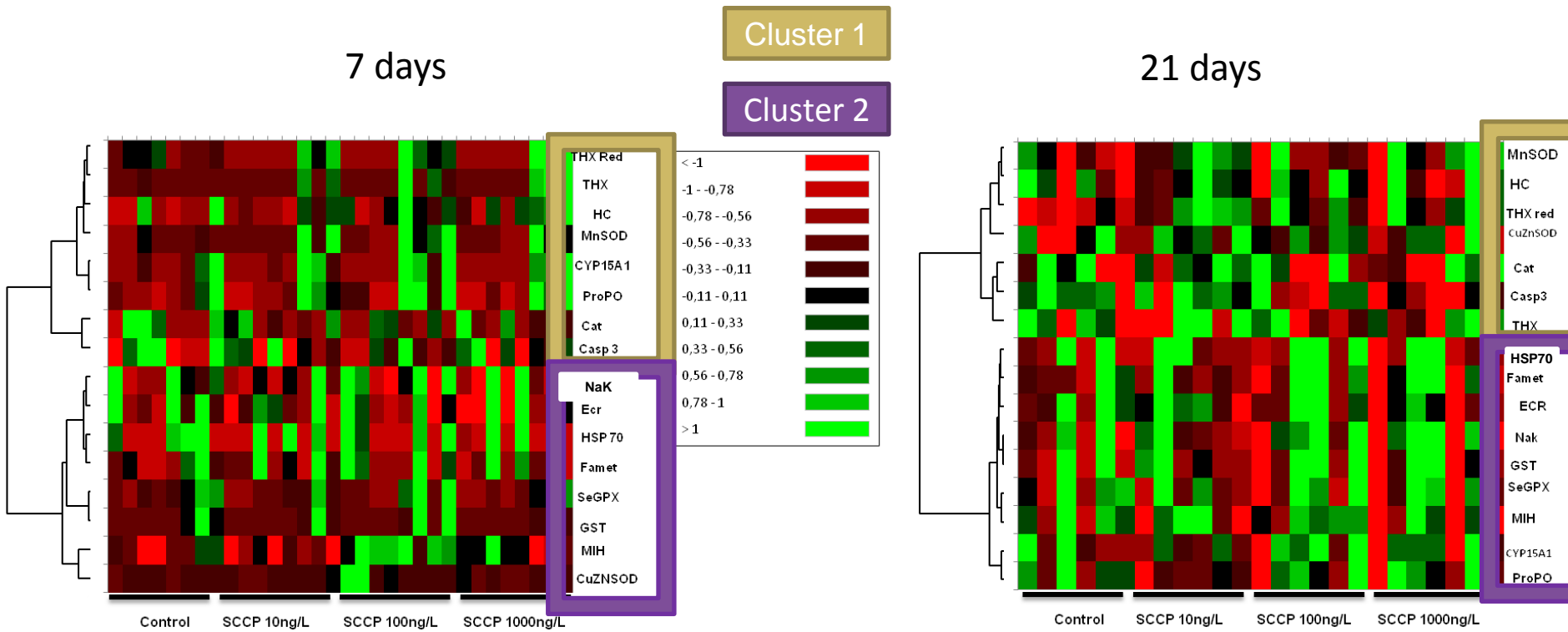


Fig. 4: log<sub>2</sub> fold-change values for gene expressions after SCCP exposure at 20°C. Genes marked with (\*) displayed a significant ( $p < 0.05$ ) difference with controls, colour coded to the corresponding SCCP exposure condition.

# Comparison with 20°C exposure



*Fig. 3: heatmaps with hierarchical clustering of genes by similarity of expression levels between 20°C exposure conditions. The 2 main clusters have been highlighted.*

# Conclusions

- 16°C range: **many biological functions** impacted (endocrine, antioxidant, respiration)
- 20°C range: **antioxidant system** highly up-regulated

**=> At environmental concentrations! ( $\leq 1000$  ng/L)**

- Evidence of **adaptation** between 7 and 21 days
- SCCP effects need to be further **studied in invertebrates**

# Acknowledgements

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**Thank you for attending this talk!**