Acute and Chronic Effects of Short-chain Chlorinated Paraffins on Physiological and Biochemical Endpoints of Marine Zooplanktons

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Abstract

The LC50 values for SCCPs were determined to be 132 μg/L (24 h) for rotifers, 74.6 μg/L (24 h) for copepods, 44.5 μg/L (96 h) for juvenile mysids, and 108 μg/L (96 h) for adult mysids, respectively. When exposed to concentrations equivalent to 1/2 LC50 for SCCPs, a significant decrease in acetylcholinesterase (AChE) activity was observed, along with significant increases in oxidative stress parameters including malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST). The decreased AChE activity, observed consistently across all species, suggests that SCCPs affect nerve transmission and cholinergic function. The consistent increase in MDA, CAT, SOD, and GST levels indicates that SCCPs induce intracellular oxidative stress and trigger antioxidant responses.

Objective

We establish SCCP toxicity tests through LC50 on marine zooplanktons(mysid, rotifer, copepod), and perform chronic toxicity tests to determine whether SCCP affects oxidant and antioxidant response.

Methods

1 Experimental Animals and Water Condition

- The species used in the experiment are *Neomysis awatschensis*, *Brachionus koreanus*, *Tigropus japonicus* which live along the coast of South korea.
- Culture informations of each species are as same as table 1.

Table 1. Specific information for species's culture condition.

Species	Prey species	Salinity (psu)	Photo ratio	Temperature (℃)
Neomysis awatschensis	*Artemia salina* (nauplii)	30	14(L):10(D)	20
Brachionus pillicatillis	*Tetraselmis suecica*	17	12(L):12(D)	28
Tigropus japonicus	*Tetraselmis suecica*	32	12(L):12(D)	25

2 Chemical Information

- The commercial SCCP mixtures with different carbon chain length (C 10-13) and chlorine levels (55.5%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).
- In room temperature, DMSO was put in SCCPs solution , then cyclohexane was evaporated during a day. Finally, volume of stock solution was 0.5 mg (1000 ug/mL).

3 Chemical Exposure

3.1 Acute toxicity and chronic test on *N. awatschensis*

- 20 individuals of adult and juvenile mysids were assigned to 300 mL solution during 96 h and performed three replications for each condition (control, solvent control(0.005%), and 16 treatments (0~500 ug/L)).
- Based on the LC50 and NOEC values derived from the previous experiment, exposure was performed at ½ LC50 and NOEC concentrations for 96 h.
- AchE activity and feeding rate were measured as endpoints of activity, and MDA contents, GSH, CAT, and SOD activity were measured as endpoints of oxidation and antioxidant stress.
- At the concentration of NOEC and 1/10 NOEC, multi-generation test of three generations and chronic toxicity test for 4 weeks were performed.
- As endpoints, we measured total length, time to first molt, and number of newborn juveniles per female in multi-generation test of adult individuals, and survival rate in chronic toxicity test of adult and juvenile individuals.

3.2 Acute toxicity and chronic test on *B. manjavacas*

- 30 individuals of adult rotifers were assigned to 24-well plate during 24 h and performed three replications for each condition (control, solvent control(0.005%), and 16 treatments (0~500 ug/L)).
- Based on the LC50 and NOEC values derived from the previous experiment, exposure was performed at ½ LC50 and NOEC concentrations for 96 h.
- MDA contents, GPx, GR, GSH, CAT, and SOD activity were measured as endpoints of oxidation and antioxidant stress.
- At the concentration of NOEC and 1/10 NOEC, chronic toxicity test for 10 days were performed in 12-well plate. Then fecundity was measured.

3.3 Acute toxicity and chronic test on *T. japonicus*

- 30 individuals of adult rotifers were assigned to 24-well plate during 24 h and performed three replications for each condition (control, solvent control(0.005%), and 16 treatments (0~500 ug/L)).
- Based on the LC50 and NOEC values derived from the previous experiment, exposure was performed at ½ LC50 and NOEC concentrations for 24 h.
- AchE activity and feeding rate were measured as endpoints of activity, DCF fluorescence and MDA contents, GR, GPx, GSH, CAT, and SOD activity were measured as endpoints of oxidation and antioxidant stress.
- At the concentration of NOEC and 1/10 NOEC, three-generation and full life cycle test for 12 days were performed in 12-well plate.
- As endpoints, we measured survival rate, body length, time to molt, and reproductive rate.

Results

- The LC50 values for SCCPs were determined to be 132 μg/L (24 h) for rotifers, 74.6 μg/L (24 h) for copepods, 44.5 μg/L (96 h) for juvenile mysids, and 108 μg/L (96 h) for adult mysids, respectively (table 2).
- When exposed to concentrations equivalent to 1/2 LC50 for SCCPs, a significant decrease in acetylcholinesterase (AChE) activity was observed, along with significant increases in oxidative stress parameters including malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) (Fig 1,2,3).
- In chronic test, zooplanktons which treated with NOEC concentrations of SCCPs was reduced body length, survival rate and was disturbed reproduction (Fig 1,2,3).

Table 2. Marine zooplanktons of medium lethal concentration (LC50)

Species	LC50 (ug/L)	Expousure period
Neomysis awatschensis (Adult)	108	96 h
Neomysis awatschensis (Juvenile)	44.5	96 h
Brachionus pillicatillis	74.6	24 h
Tigropus japonicus	132	24 h

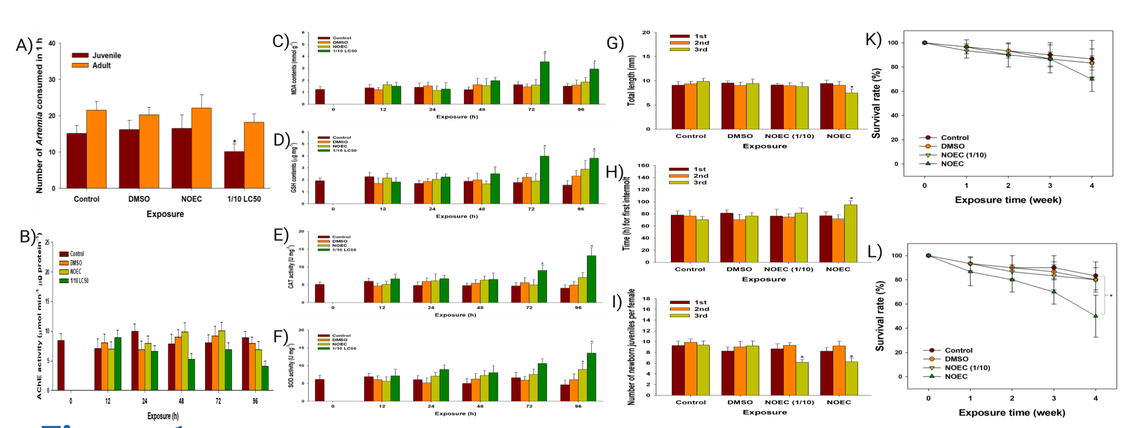

Figure 1. (A) Number of *Artemia sallina* nauplii consumed by individual exposed to SCCPs per hour; (B) activity acetylcoline of mysids exposed to SCCPs for 96 h; Activities of MDA (C), GSH (D), CAT (E) and SOD (F) in mysids exposed to SCCPs for 96 h; Total length (G), time for first intermolt (H) and number of newborn juveniles per female (I) of 1st-3th generation mysids exposed to SCCPs for 4 weeks; survival rate of adult mysids (K) and survival rate of juvenile mysids (L) exposed to SCCPs for 4 weeks.

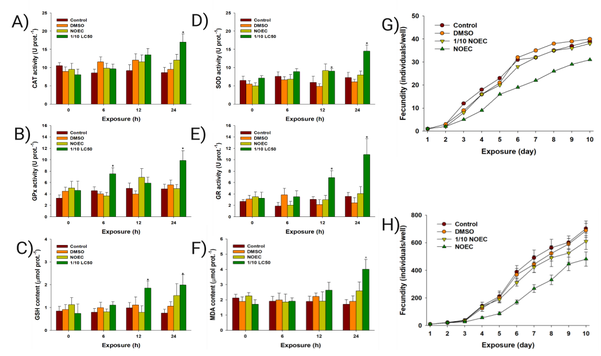

Figure 2. Activity of CAT (A), GPx (B), GSH (C), SOD (D) and GR (E) as well as content of MDA (F) in rotifer exposed to SCCPs for 24 h; Fecundity (G), (H) in rotifer exposed to SCCPs for 10 days.

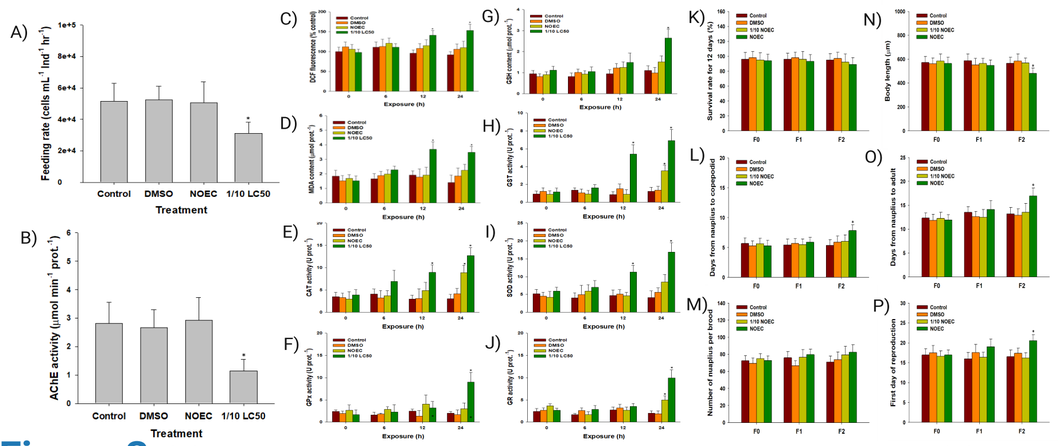

Figure 3. (A) Hourly feeding rate of rotifer exposed to SCCPs for 24 h; (B) acetylcoline activity of rotifer exposed to SCCPs for 24 h; DCF fluorescence (C), MDA content (D), activity of CAT (E), GPx (F), GSH(G), GST(H), SOD(I) and GR(J) of rotifer exposed to SCCPs for 24 h; Survival rate (K), Body length (N), development time to copepodid (L) and adult (O), offsprings number (M) and sexual maturity time (P) of 0-2 generation rotifers exposed to SCCPs for 12 days.