Steven J. Brooks[1], Tânia Gomes[1], Ana Catarina Almeida[1], Maria Christou[1], Congying Zheng[2], Sergey Shaposhnikov[2], Daria G. Popa[3], Florin Oancea[3]

Norwegian Institute for Water Research

1 Norwegian Institute for Water Research (NIVA), Økernveien 94, N-0579 Oslo, Norway; 2 NorgenoTech, Ullernchassern,64/66 Oslo, Norway;
3 ICECHIM, National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM, Splaiul Independentei No. 202, Sector 6, 060021 Bucharest, Romania.

An ecotoxicity assessment of the plant biostimulant strigolactone (SL-6)

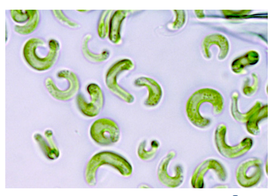
Background

Strigolactone (SL-6) is a phytohormone and plant biostimulant developed to enhance crop yields and improve agricultural outputs on a commercial scale. Application of SL-6 in the field will inevitably lead to its run-off into streams, rivers and coastal marine waters. However, little is known of the toxic effects of SL-6 in the environment, including aquatic organisms, and this information is needed before its widespread application.

The study involves a risk assessment of the toxicity of SL-6 to aquatic organisms using a range of standardised bioassays representing different trophic groups and acute and chronic endpoints from both freshwater and marine environments. In addition, the genotoxicity of SL-6 was determined using the comet assay, using algal, oyster and zebra fish embryo cells.

Methods

OECD 201 Microalgal growth *Raphidocelis subcapitatata*



OECD 202 Immobilisation *Daphnia sp.*

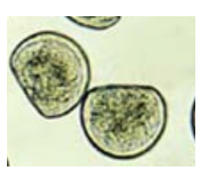
OECD 236 Zebra fish embryo *Danio rerio*

Freshwater *in vivo* bioassays

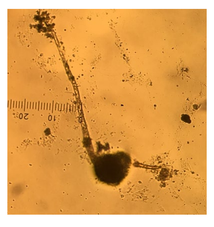
ISO10253 Microalgal growth *Skeletonema pseudocostatum*

ISO 14699 *Tisbe battagliai* acute toxicity

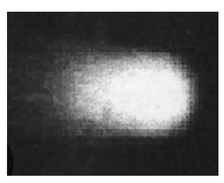
ASTM E724-89 Oyster embryo *Crassostrea gigas*

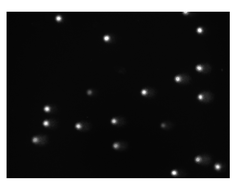


Fucus sp. germling growth

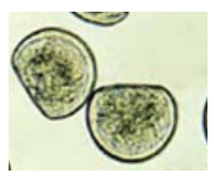


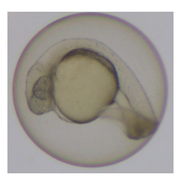
Marine *in vivo* bioassays

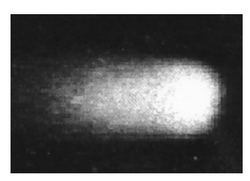




Genotoxicity assessment (comet assay)







Results

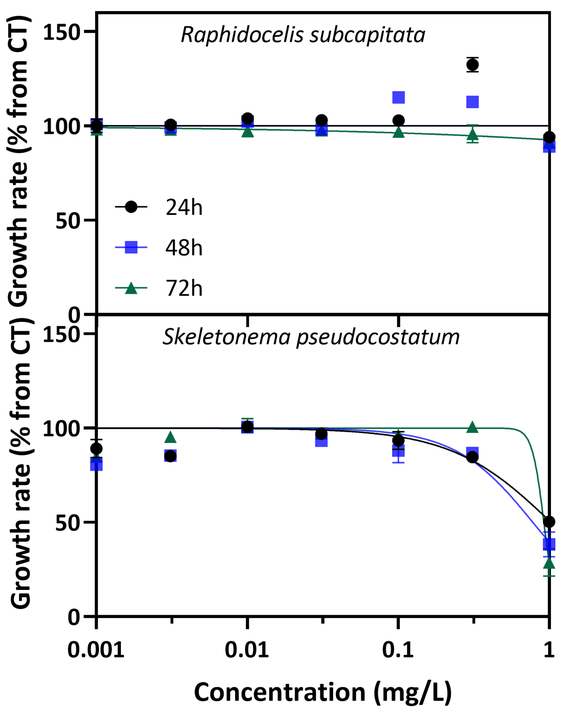


Fig 1. Growth of *Raphidocelis subcapitata* (freshwater) and *Skeletonema pseudocostatum* (marine) microalgae following 72 h exposure to increasing concentrations of SL-6.

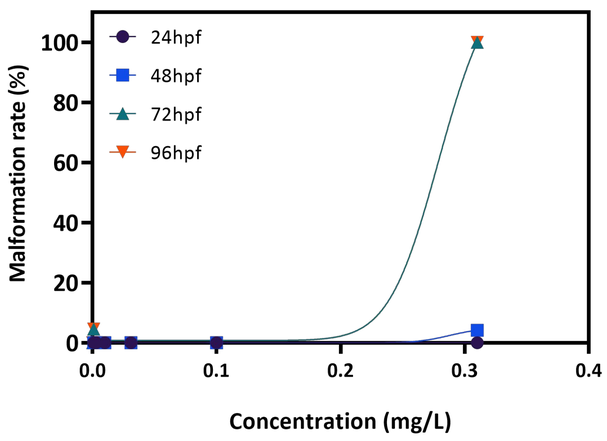


Fig 2. Malformation rate in zebrafish larvae exposed to SL-6 for 96h, recorded at 24-, 48-, 72- and 96-hours post fertilization (hpf).

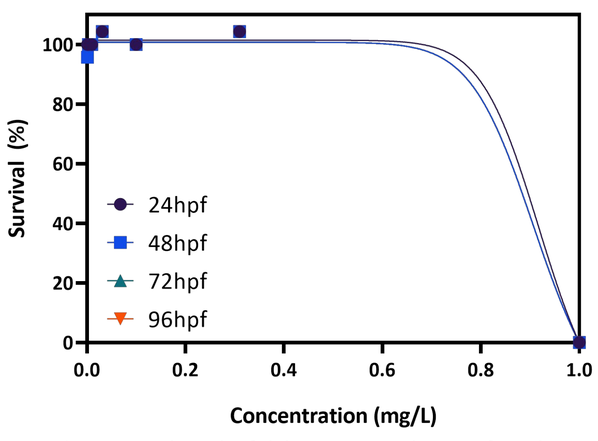


Fig 3. Survival in zebrafish larvae exposed to SL-6 for 96h, recorded at 24, 48, 72 and 96 hpf.

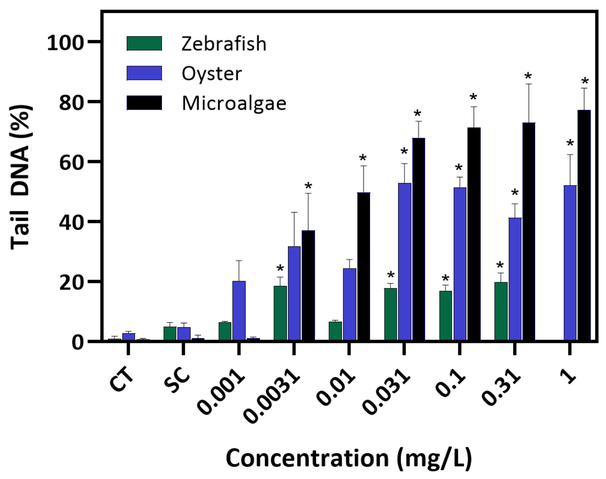


Fig 5. Percentage DNA strand breaks in the marine microalgae *S. pseudocostatum*, and embryo larvae of the oyster (*C. gigas*) and zebrafish (*D. rerio*), after 72h, 24 h and 96h exposure to SL-6, respectively. CT- control, SC – solvent control, * statistically significant differences to CT ($p < 0.05$).

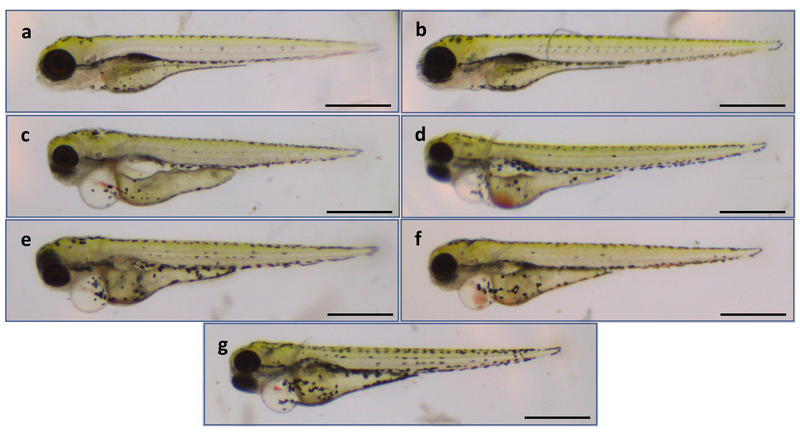


Fig 4 . Zebrafish larvae exposed for 96h to a,b) Solvent control (0.1% DMSO), or c-g) 0.31 mg/L SL-6. Larvae exposed to SL-6 **developed pericardial and yolk sac oedemas** of different severity and haemorrhage (d,f). Scale bars represent 0.5 mm.

Table 1. Summary of SL-6 ecotoxicity tests for freshwater and marine species as well as genotoxicity (comet)

FW/SW	Species	Endpoint	NOEC (mg/L)	LOEC (mg/L)	LC/EC_{50} (mg/L)
Freshwater	Microalgae (72h)	Growth	0.1	0.31	n.d.
	Daphnia acute (24h)	Mortality	1.0	>1.0	n.d.
	Fish embryo (96h)	Embryo malformation	0.1	0.31	0.35
		Survival	0.33	1.0	0.78
		Hatching rate	0.1	0.33	n.d.
Marine	Microalgae (72h)	Growth	0.31	1.0	0.95
	Fucus germling (14 d)	Growth	1.0	>1.0	n.d.
	Tisbe acute (48h)	Mortality	0.1	>0.1	n.d.
	Oyster embryo (24 h)	Embryo development	1.0	> 1.0	n.d.
Genotoxicity	Microalgae (72h)	DNA damage (% strand breaks)	0.001	0.0031	
	Oyster embryo (48h)		0.01	0.031	
	Zebrafish (96 h)		0.001	0.0031	

Risk Assessment

$$\frac{\text{Predicted Environmental concentration (PEC)}}{\text{Predicted No Effect Concentration (PNEC)}} = \text{Risk Quotient (RQ)}$$

Table 2. Summary of the risk assessment of SL-6 based on the lowest NOEC from both the ecotoxicity and the genotoxicity tests. Assessment factor (AF) taken from the European Union Technical guidance document on risk assessment (ECB, 2003).

	NOEC (mg/L)	Assessment factor (AF)	Calculated PNEC (µg/L)
Ecotoxicity	0.1	100	1.0
Genotoxicity	0.001	100	0.01

Conclusions

- Exposure to 0.31 mg/L SL-6 caused pericardial and yolk sac oedemas in zebrafish larvae.
- The lowest NOEC of 0.1 mg/L SL-6 was observed in FW microalgae and zebrafish (embryo malformations and hatching success).
- Genotoxicity was observed at 100 fold lower SL-6 concentrations, with a NOEC of 0.001 mg/L for both the microalgae (*S. pseudocostatum*) and zebrafish (*D. rerio*) larvae.
- A PNEC of 1.0 µg/L SL-6, was calculated with the lowest NOEC from the ecotoxicity tests.
- A PNEC of 0.01 µg/L SL-6 was calculated for genotoxicity with the lowest NOEC from the comet assay.

Contact info:
Norwegian Institute for Water Research (NIVA), Section for Ecotoxicology and Risk Assessment, Økernveien 94, N-0579 OSLO, Norway. Email: sbr@niva.no

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