# Insights into the toxicity of *Prymnesium parvum*

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Fotos: Stephanie Scholz/Vetmeduni, Michael Bernkopf/Vetmeduni, Thomas Suchanek/Vetmeduni, Niko Havranek/Vetmeduni

# Oder River incidence

- Massive fish killing event in the River Oder in summer 2022

Spektrum.de

15.08.2022 | UMWELTKATASTROPHE IN DER ODER

**»Die Dimensionen des Fischsterbens sind gewaltig«**

BBC NEWS

**Oder river: Mystery surrounds thousands of fish deaths**

14 August 2022

Watch: Tonnes of fish found dead in German-Polish river

By James FitzGerald

BBC News

The Guardian

**Poland pulls 100 tonnes of dead fish from Oder river after mystery mass die-off**

**More than 500 firefighters deployed to haul in dead fish, using dams, boats, quad bikes and even a drone**

Workers in Poland use an excavator and a dam to pull out dead fish from the Oder river after a mass die-off. Photograph: Marcin Bielecki/AFP/Getty Images

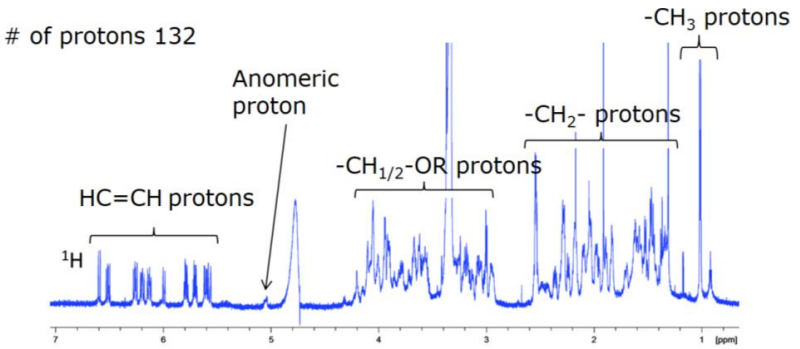
# *Prymnesium parvum* N. Carter – History

- 1961: first investigations [1]
- 1990s: causative agents identified [2, 3]
  - prymnesin 1 and 2 (A-types)
- Golden Algae Toxin (GAT) [4]
- 2016: novel toxins from *P. parvum* [5]
  - B-types (K-0081)
    + tentative identification of C-types


Roelke *et al.* (2011) J Plankton Research 33: 243-253.

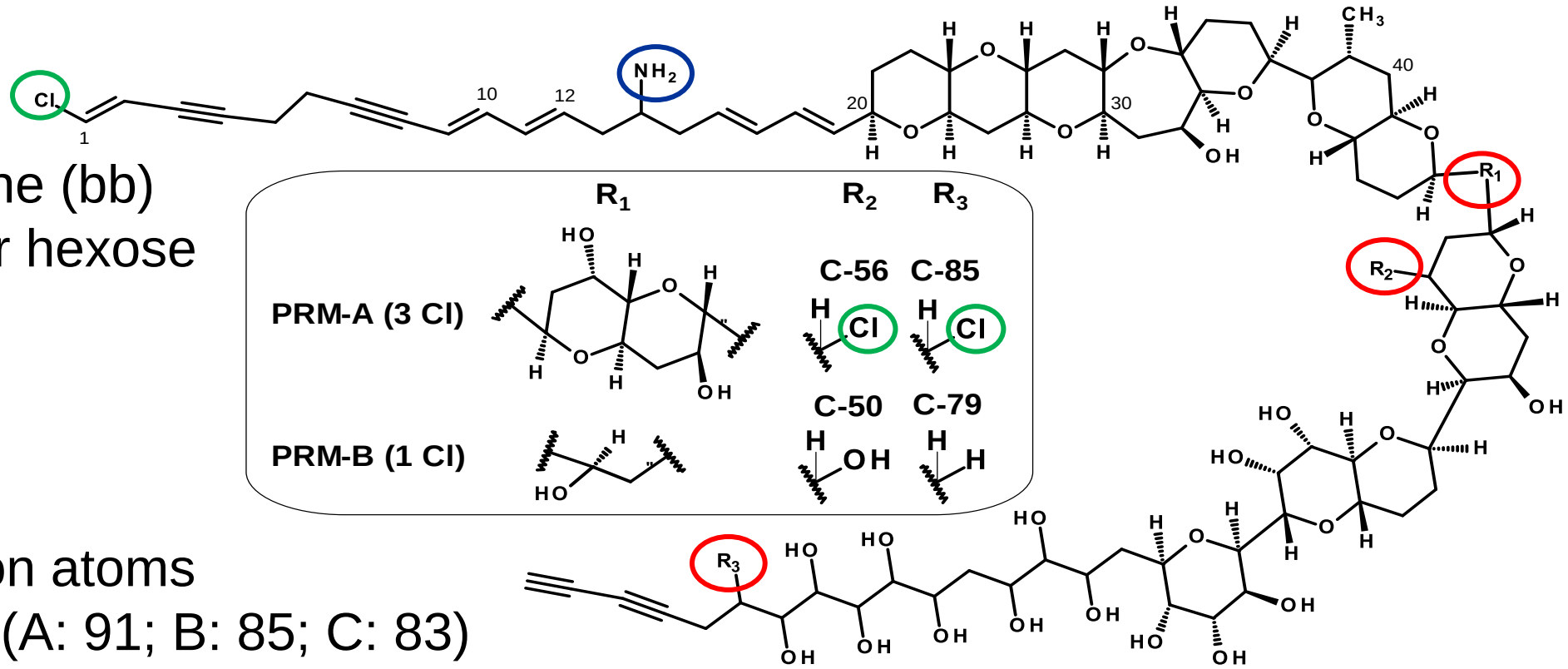
[1] Yariv & Hestrin J. Gen. Microbiol (1961) 24: 165-175.
[2] Igarashi *et al.* J. Am. Chem. Soc. (1996) 118: 479-480.
[3] Igarashi *et al.* J. Am. Chem. Soc. (1999) 121: 8499-8511.
[4] Henrikson *et al.* (2010). Toxicon 55, 1396-1404.
[5] Rasmussen *et al.* J. Nat. Prod. (2016) 79: 2250-2256.

# Prymnesins – Chemistry




## Common features

- 1600-2200 Da
- Aglycon backbone (bb) + pentose and/or hexose
- Primary amine
- Chlorine (1 to 4)



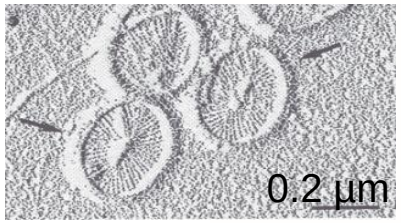

## Classification

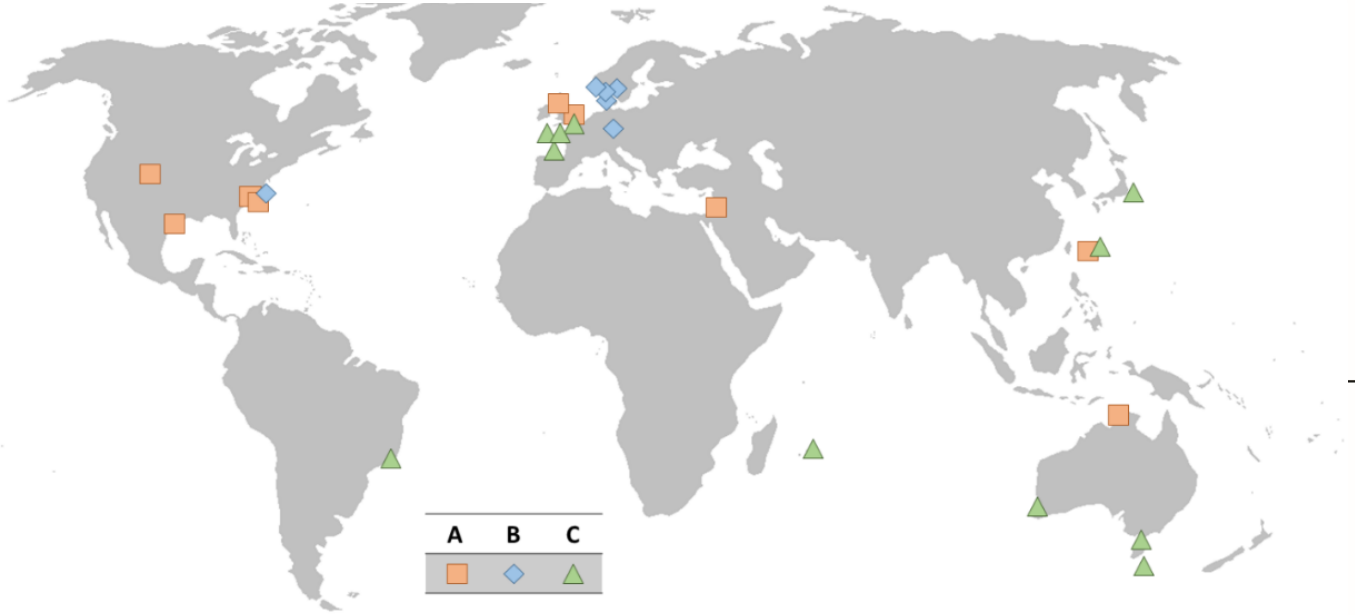
- Number of carbon atoms in the backbone (A: 91; B: 85; C: 83)

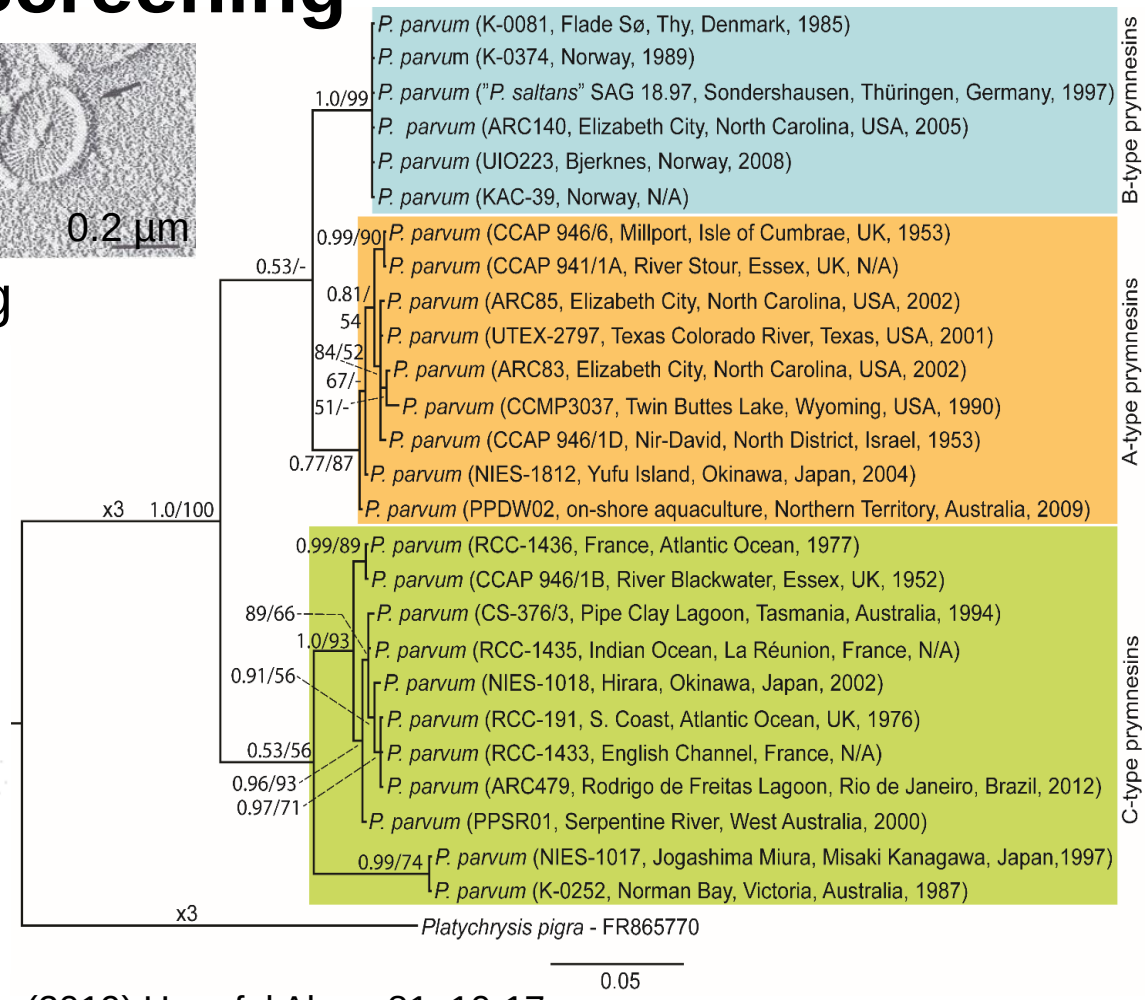
Rasmussen *et al.* J. Nat. Prod. (2016) 79: 2250-2256.
Binzer & Svenssen, Daugbjerg, Alves-de-Souza, Pinto, Hansen, Larsen, Varga (2019) Harmful Algae 81: 10-17.

# *Prymnesium parvum* N. Carter – Screening

- 27 strains
  - Chemical profile
  - Internal Transcribed Spacer (ITS) sequencing



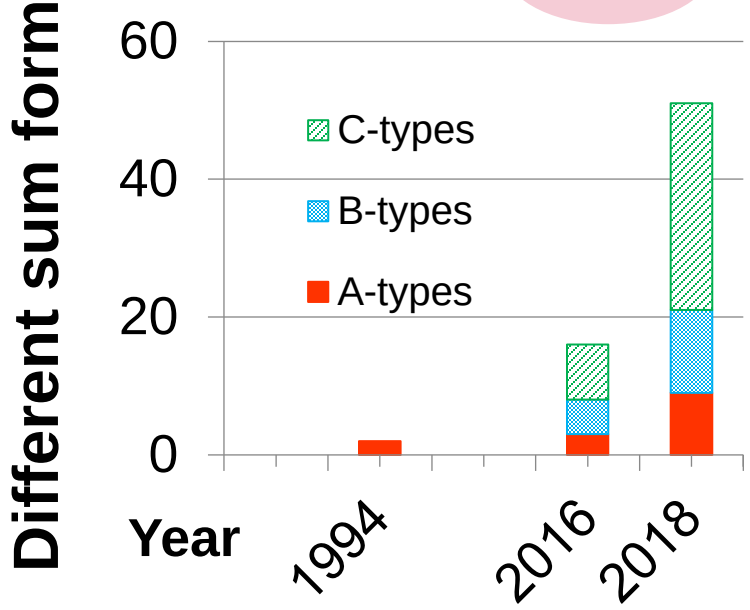




Binzer & Svenssen, Daugbjerg, Alves-de-Souza, Pinto, Hansen, Larsen, Varga (2019) Harmful Algae 81: 10-17.

# Prymnesins – Diversity

- Screening study
  – 27 strains

HRMS!



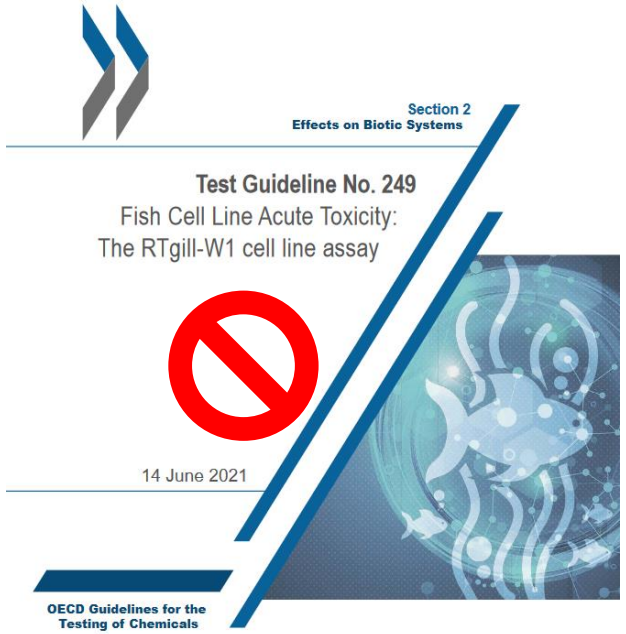

| | A-type | B-type | C-type |
|---|---|---|---|
| **# C in backbone** | 91 | 85 | 83 |
| **# compounds (2016 / 2018)** | 9 (3 / 6) | 12 (5 / 7) | 30 (8 / 22) |
| **# Cl** | 2 or 3 | 1 or 2 | 2, 3 or 4 |
| **# pentose[1)]** | 0, 1 or 2 | 0 or 1 | 0, 1 or 2 |
| **# hexose[1)]** | 0 or 1 | 0, 1 or 2 | 0 or 1 |
| **add. doublebonds** | 1 | 1 | 1 or 2 |
| **add. modifications** | + O | - | + 3O, +3O +O |

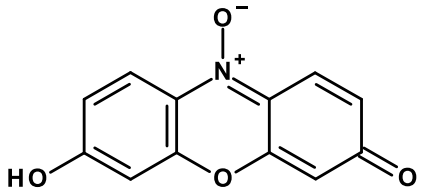
**[1)] up to three in total**

Rasmussen *et al.* J. Nat. Prod. (2016) 79: 2250-2256.
Binzer & Svenssen, Daugbjerg, Alves-de-Souza, Pinto, Hansen, Larsen, Varga (2019) Harmful Algae 81: 10-17.

# Cytotoxicity

- Fish gill cell line (RTgill-W1)
- CellTiter-Blue®
  ~ Presto Blue®
  ~ Alamar Blue®

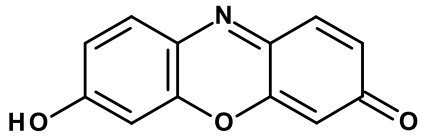


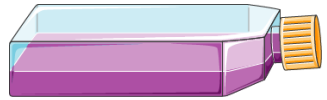
**Resazurin**
(no fluorescence)

NADH / H⁺

NAD⁺, $H_2O$



**Resorufin**
fluorescence
(540 nm / 590 nm)



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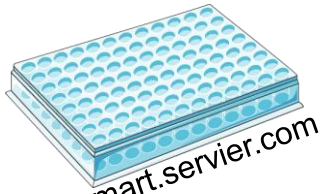

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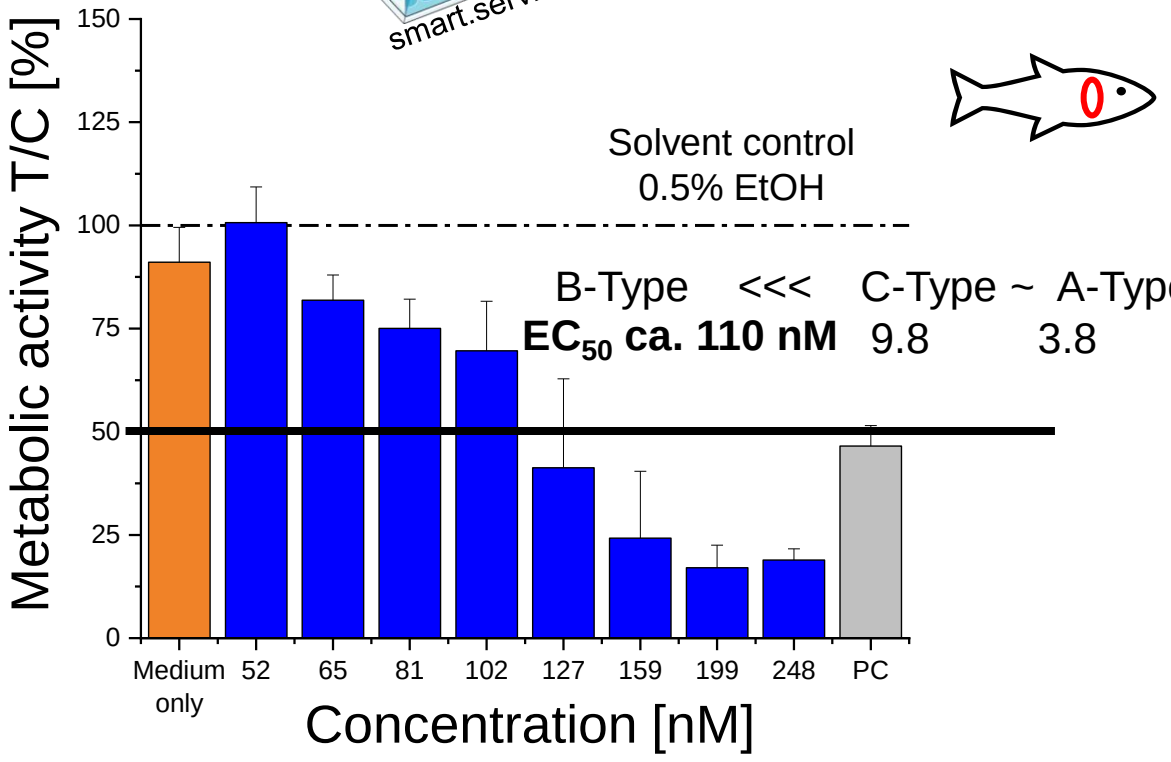
Kristin Schirmer




Incubation time 3 h (n ≥ 3), PC 0.1 % Triton™ X-100

Varga & Prause *et al.* (2024) Archives of Toxicology 98: 999-1014.

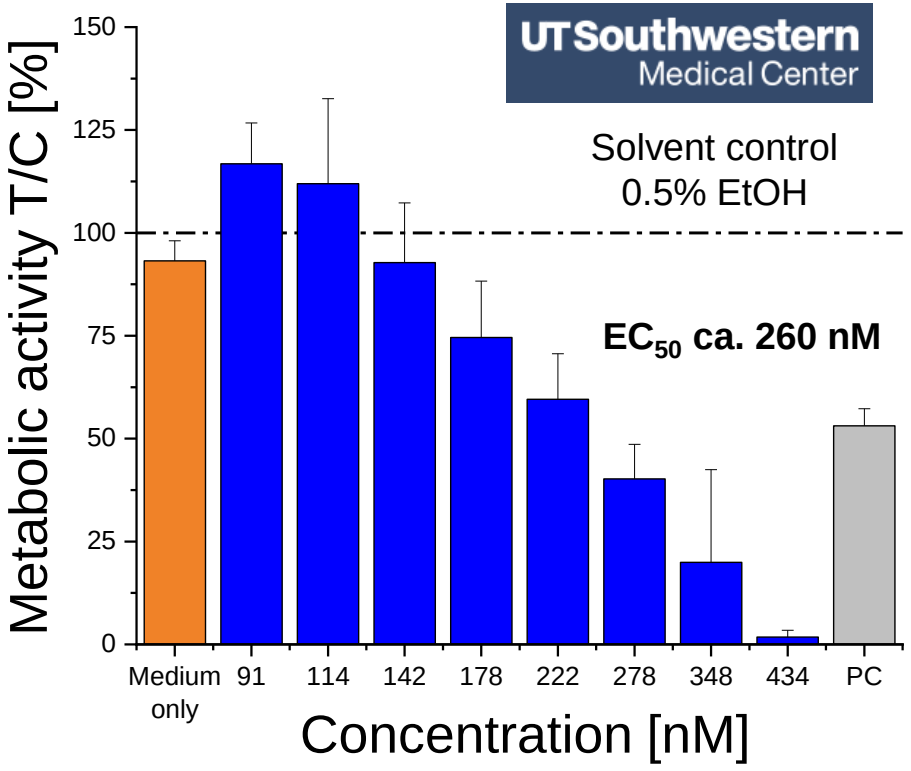
# Cytotoxicity


©B. Gross

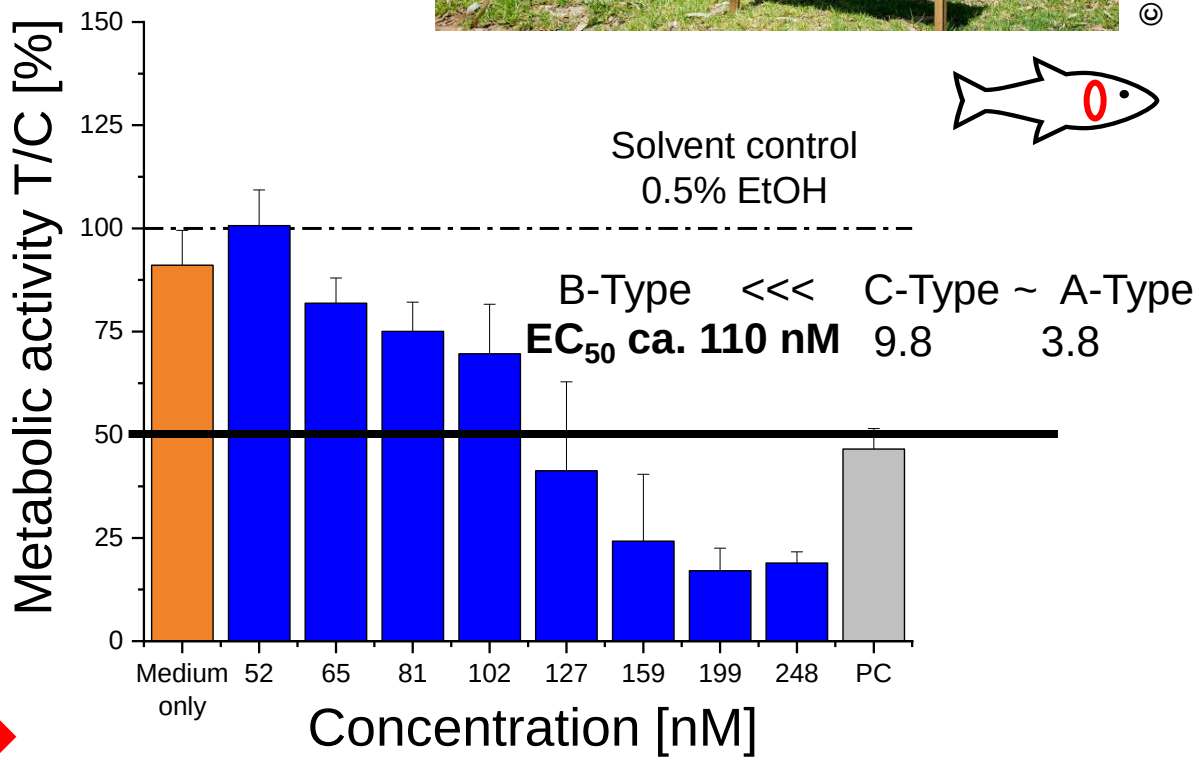
Jerry W. Shay

UT Southwestern Medical Center

HCEC-1CT




Factor 2.3

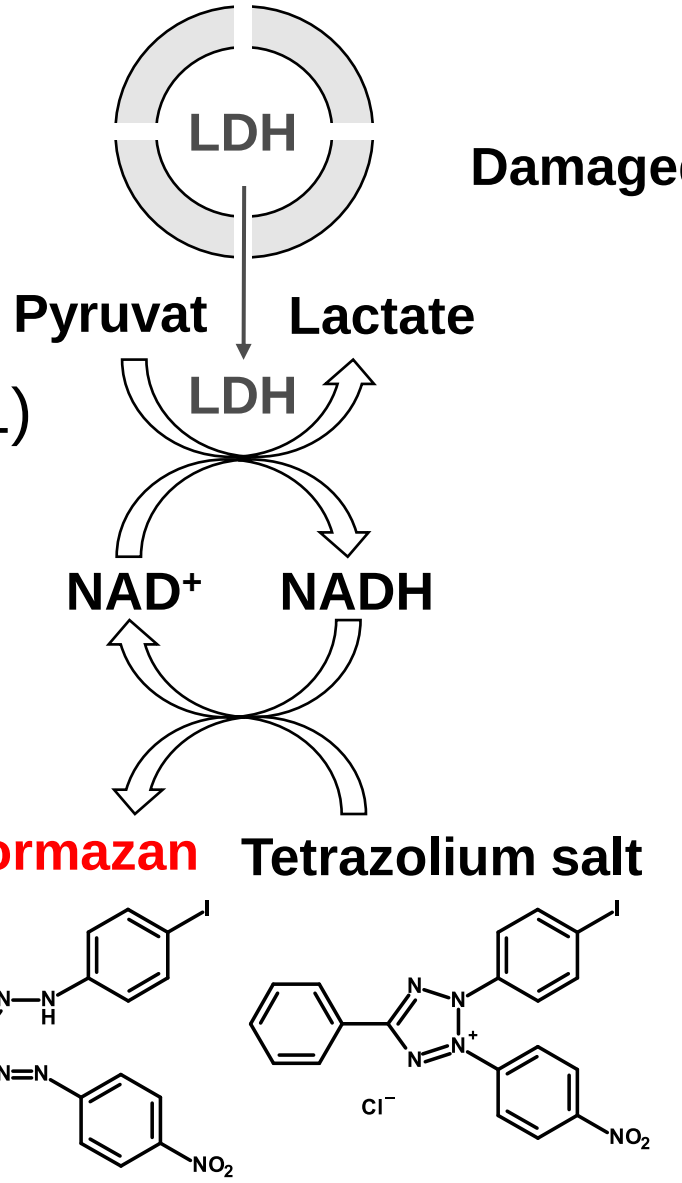



Incubation time 3 h (n ≥ 3), PC 0.1 % Triton™ X-100

Varga & Prause *et al.* (2024) Archives of Toxicology 98: 999-1014.

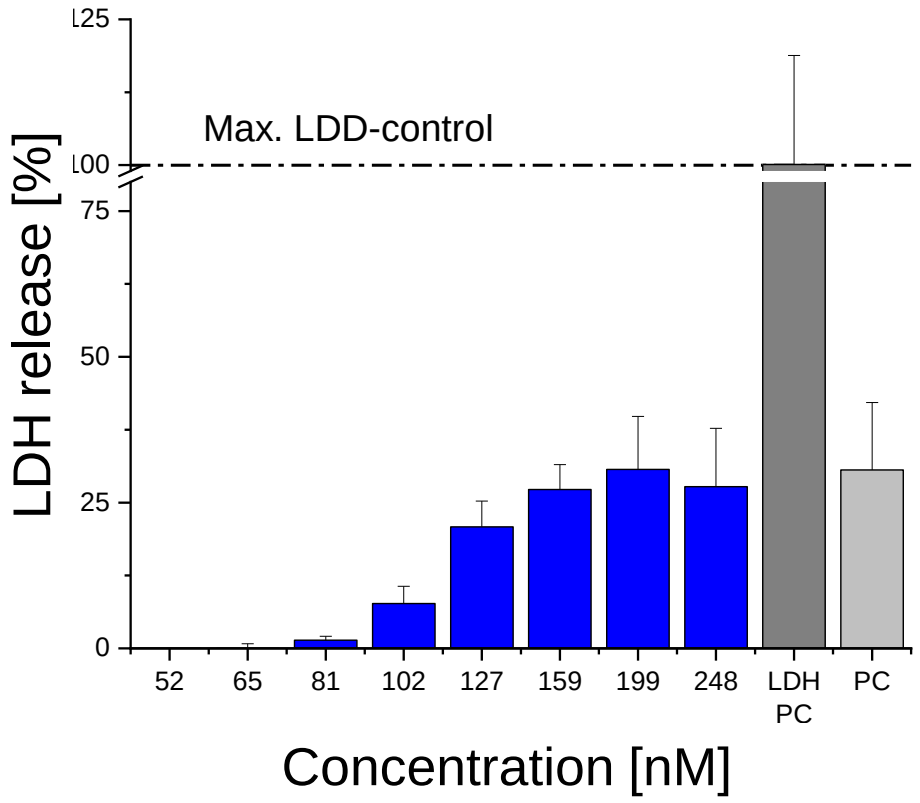
# Membrane damage

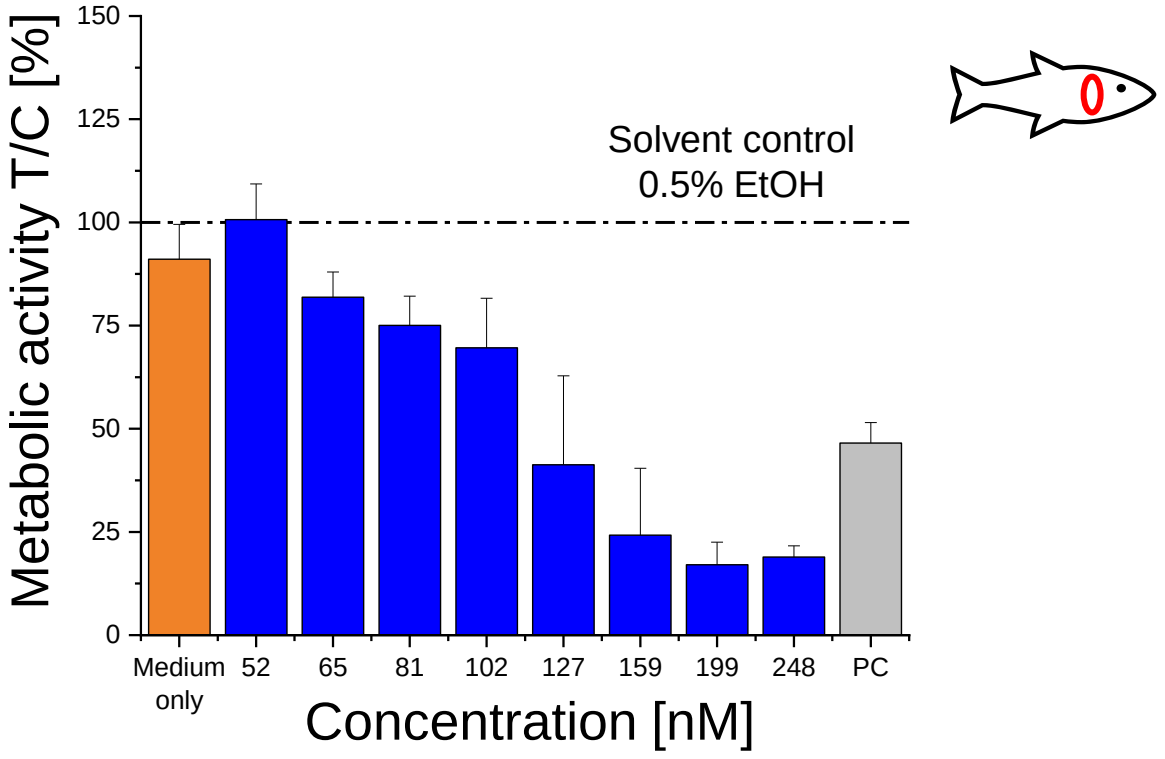
- Fish gill cell line (RTgill-W1)
- LDH
  - Lactate-dehydrogenase
  - Cytosolic enzyme




Varga & Prause *et al.* (2024) Archives of Toxicology 98: 999-1014

# Membrane damage







Incubation time 3 h (n ≥ 3), PC 0.1 % Triton™ X-100

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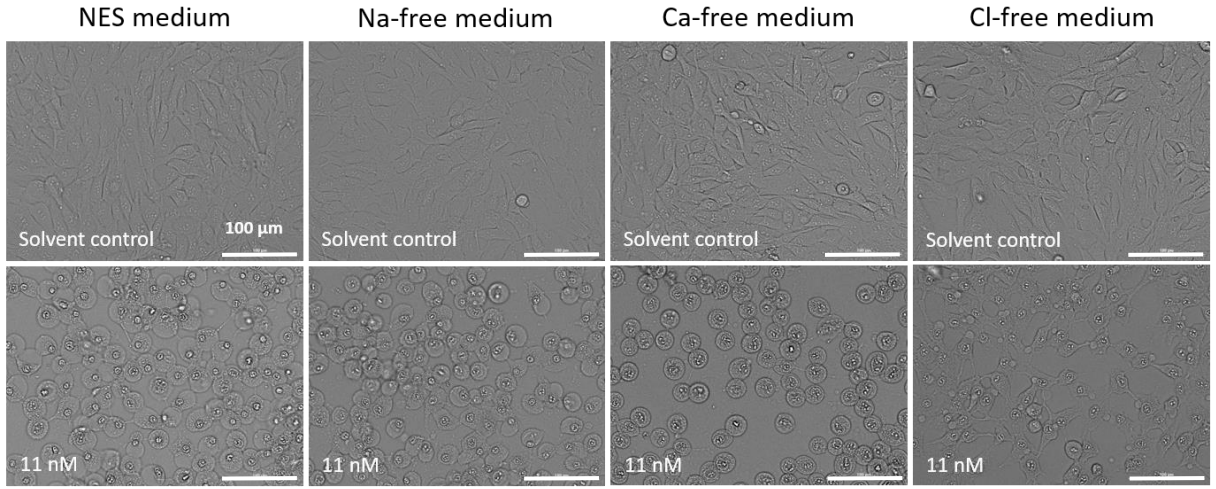
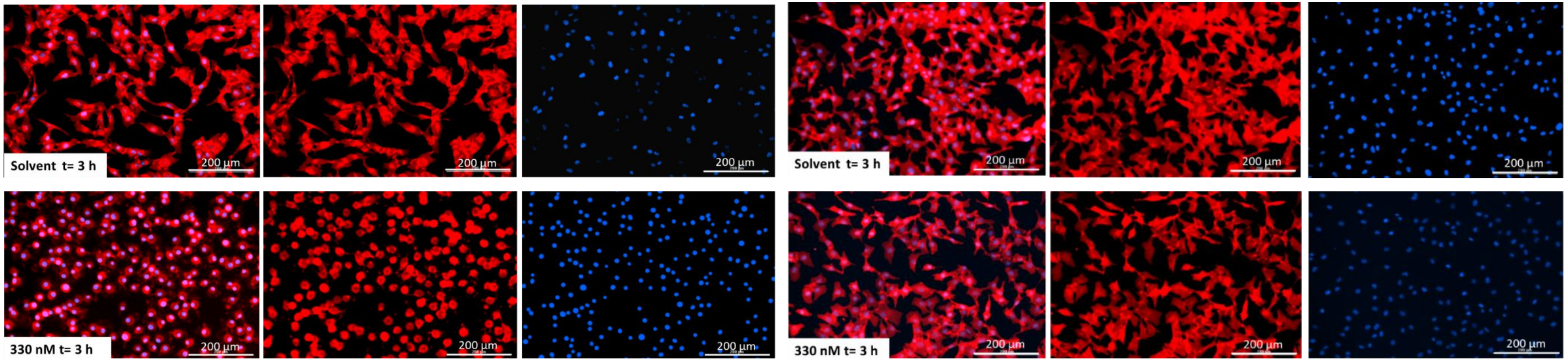
# Ion free media




HCEC-1CT

NES – normal external solution (control)

Cl- free medium

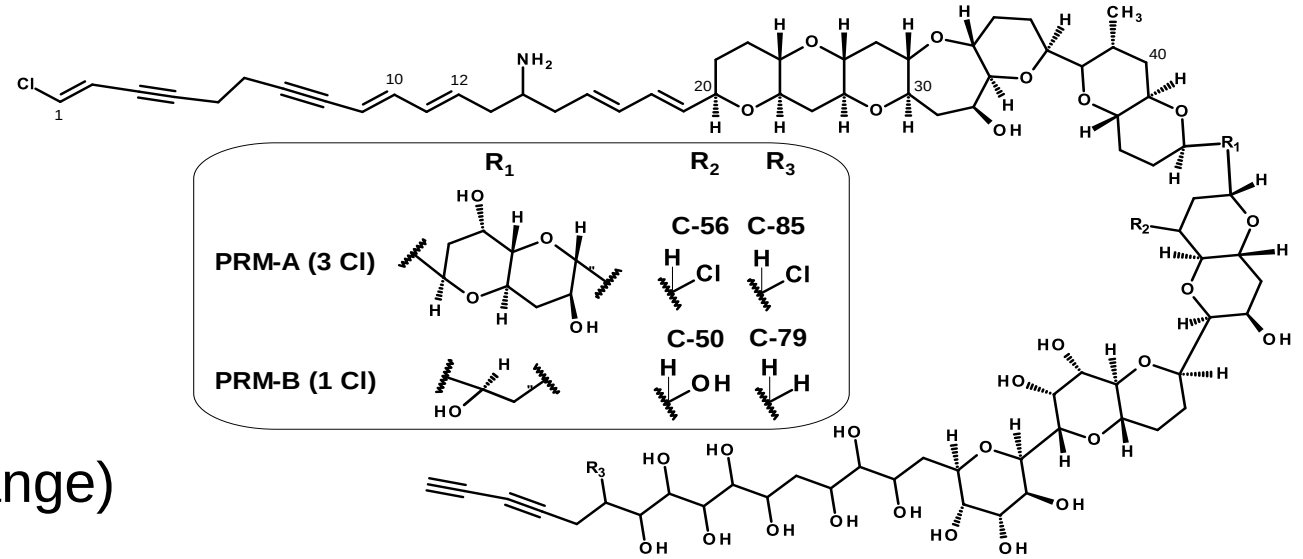



Varga & Prause *et al.* (2024) Archives of Toxicology 98: 999-1014

# Take-home-messages

- *Prymnesium parvum*
  - 3 toxin types with different toxicities
  - Complex prymnesin profile
  - Cytotoxic & lytic (low to medium nM range)
  - Hemolytic
  - Sterol interaction?

- Oder catastrophe 2022
  - algal bloom of *Prymnesium parvum*
  - “Blessing in disguise”

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# Thanks

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**Stephanie Spahr**

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Urban Tillmann
Bernd Krock
Jan Tebben

# Global Impacts of Biotoxins on the Safety and Sustainability of Food and Water

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STONEHILL COLLEGE Stonehill College, Easton Massachusetts (45 mins from Boston)

# Thank you very much for your attention

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