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Sublethal Toxicity of Diverse PFASs to Three Freshwater Invertebrates:

Comparisons Among Species, Structures, and Mixtures

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Overview

Background:

- Most eco-toxicity experimental data are for a small number of PFASs within the great structural diversity of compounds of interest
- PFAS risk assessment will be mainly concerned with sublethal effects

Project goals are to identify / understand:

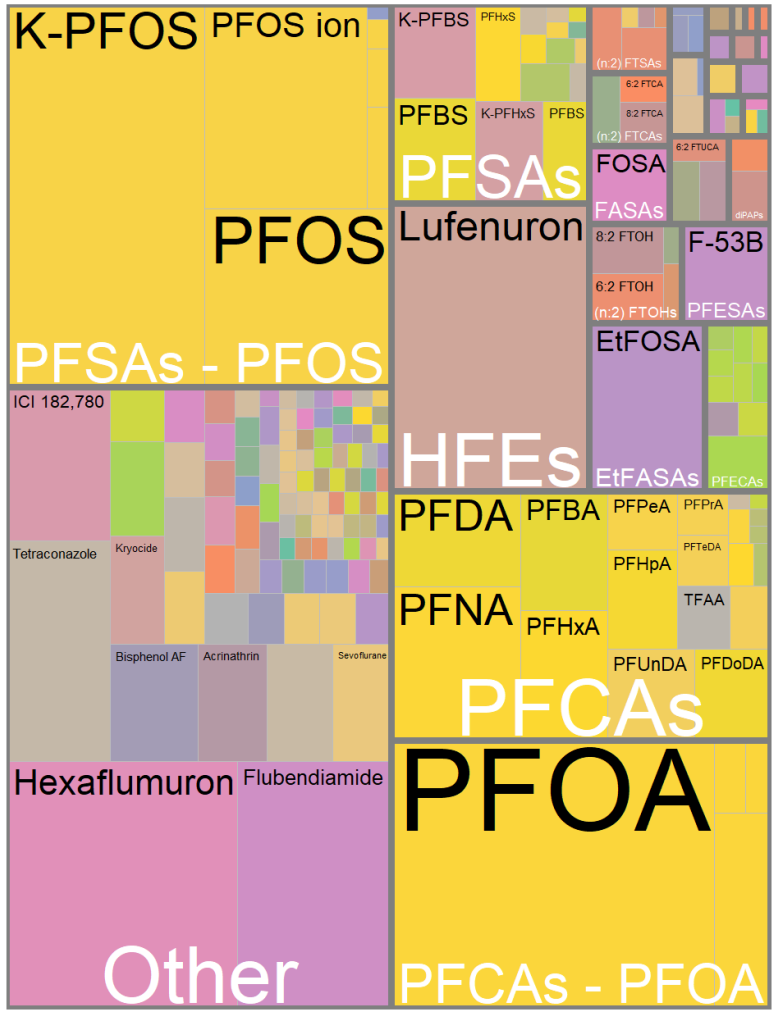
- Toxicity patterns related to PFAS structural characteristics (*e.g.* head group, chain length, partitioning)
- Groups of PFASs that appear to have the same mechanism of toxicity

Approach:

- Create a dataset with sublethal PFAS effect concentrations for a small number of freshwater species
- Describe patterns and develop hypotheses
- Test hypotheses with additional PFAS structures, mixture studies, tissue burden/ uptake studies, and partitioning measurements

1,348 Publications, 192 PFAS with Ecological Toxicity Data

Box size represents # references for each chemical



Data from ECOTOX Knowledgebase, Sept 2022 (J. Olker)

Short-term sublethal toxicity tests

- Balancing a need for chronic data *vs.* need for higher throughput
- 7-day chronic or sublethal toxicity tests with three invertebrate species
  - ***Ceriodaphnia dubia* (water flea)**
    Standard 7-day, 3-brood chronic test — Endpoints: survival and **reproduction**

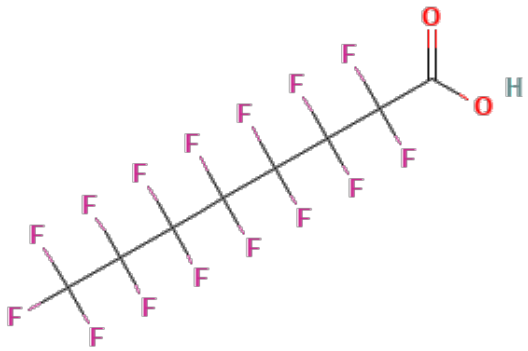
  - ***Hyalella azteca* (amphipod)**
    - 7-day static renewal test
    - 7-d old at start
    - Shortened from 10-day sublethal test — Endpoints: survival and **growth**

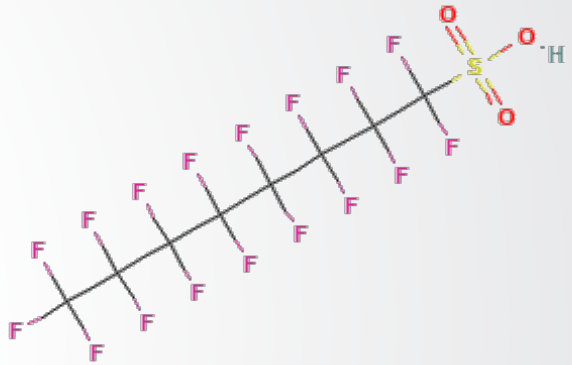
  - ***Chironomus dilutus* (midge)**
    - 7-day static renewal test
    - 3-d old at start
    - Shortened from 10-day sublethal test — Endpoints: survival and **growth**

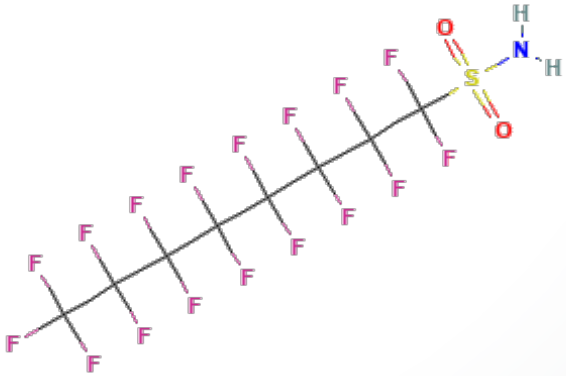
Short-term sublethal toxicity tests

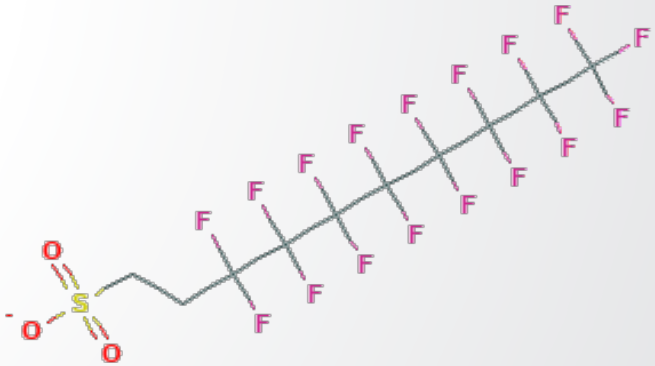
17 individual PFASs representing 4 classes

- Perfluorinated carboxylates (PFCAs)
- Perfluorinated sulfonates (PFSAs)
- Perfluorinated sulfonamides (FASAs)
- Fluorotelomer sulfonates (FTSs)









Number of fluorinated carbons:

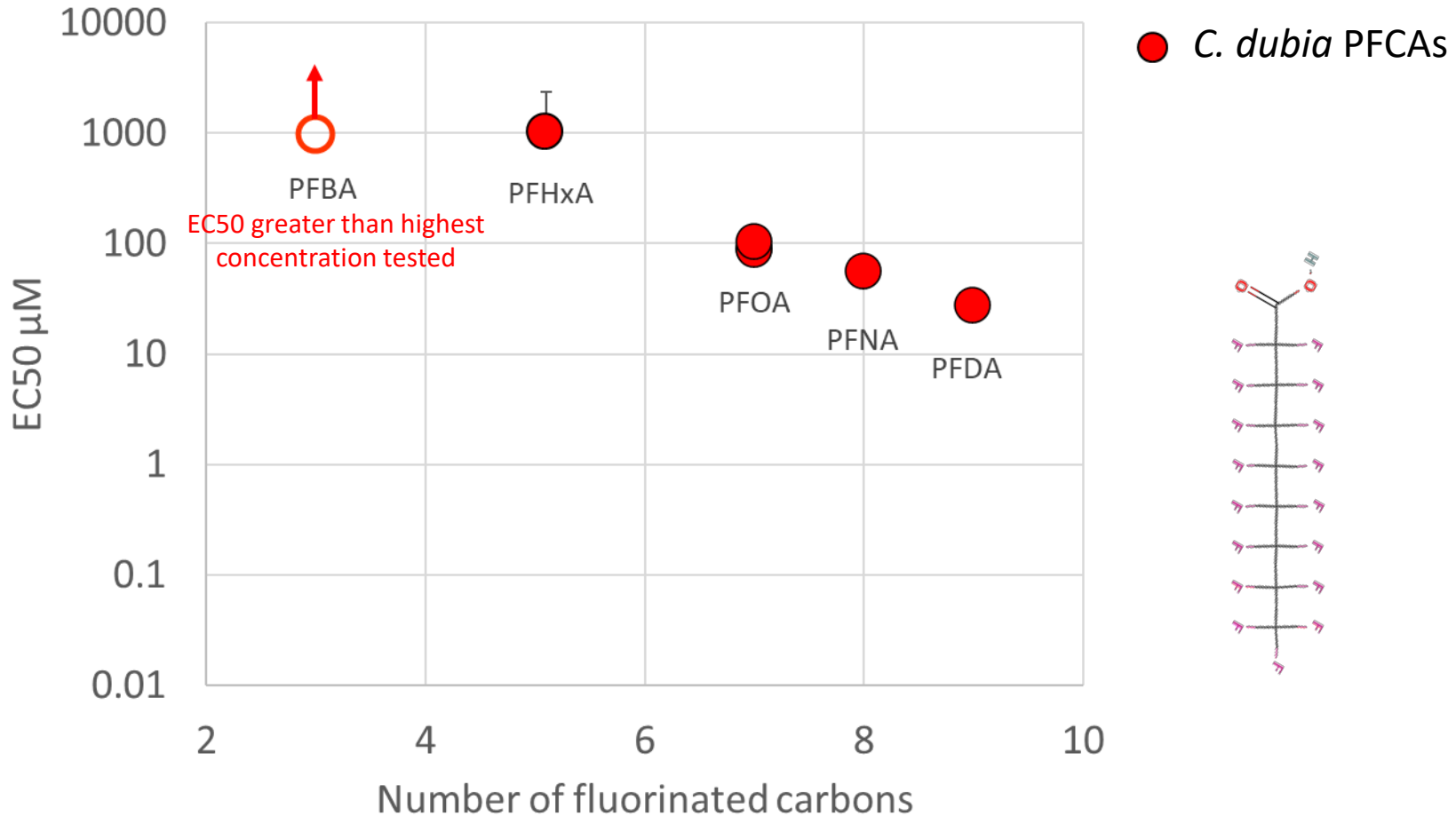
	3	4	5	6	7	8	9	10
PFCAs	X		X		X	X	X	
PFSAs		X		X	X	X	X	X
FASAs		X		X		X		
FTSs				X		X		X

- Purity analysis
- Exposure characterization

Results

***C. dubia* Reproduction**

Perfluorinated carboxylates

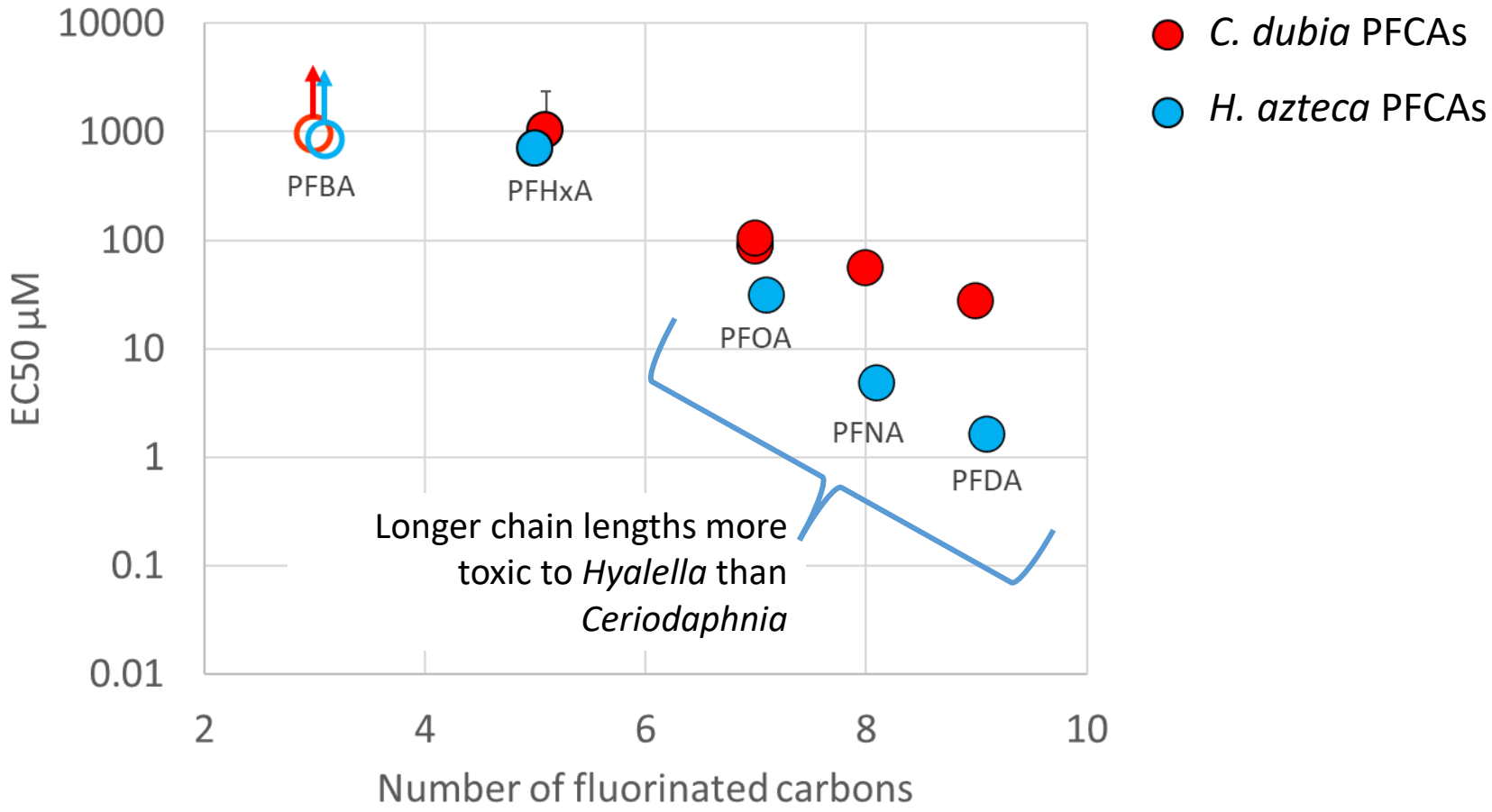


Results

Perfluorinated carboxylates

C. dubia **Reproduction**

H. azteca **Growth**



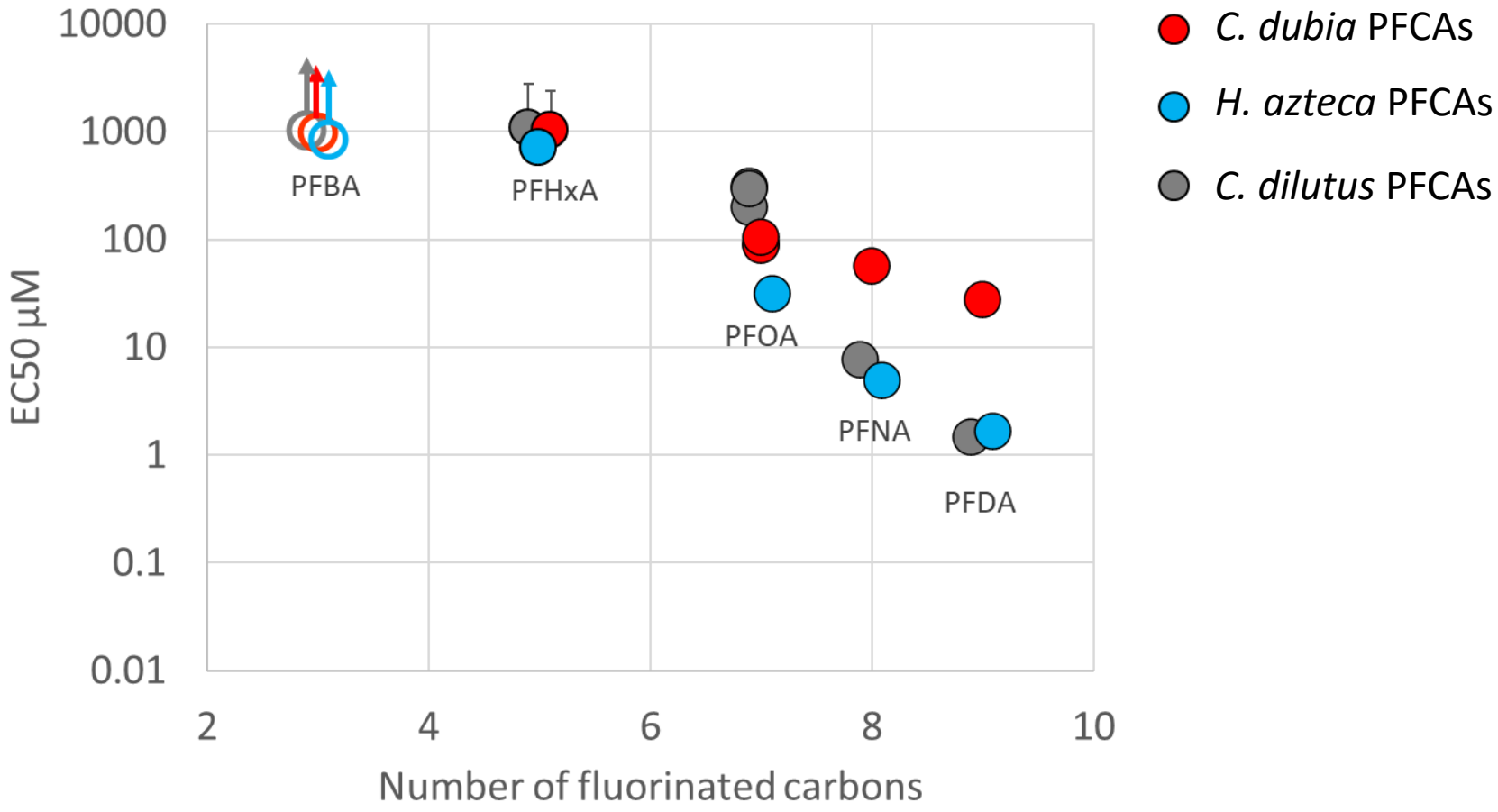
Results

Perfluorinated carboxylates

C. dubia **Reproduction**

H. azteca **Growth**

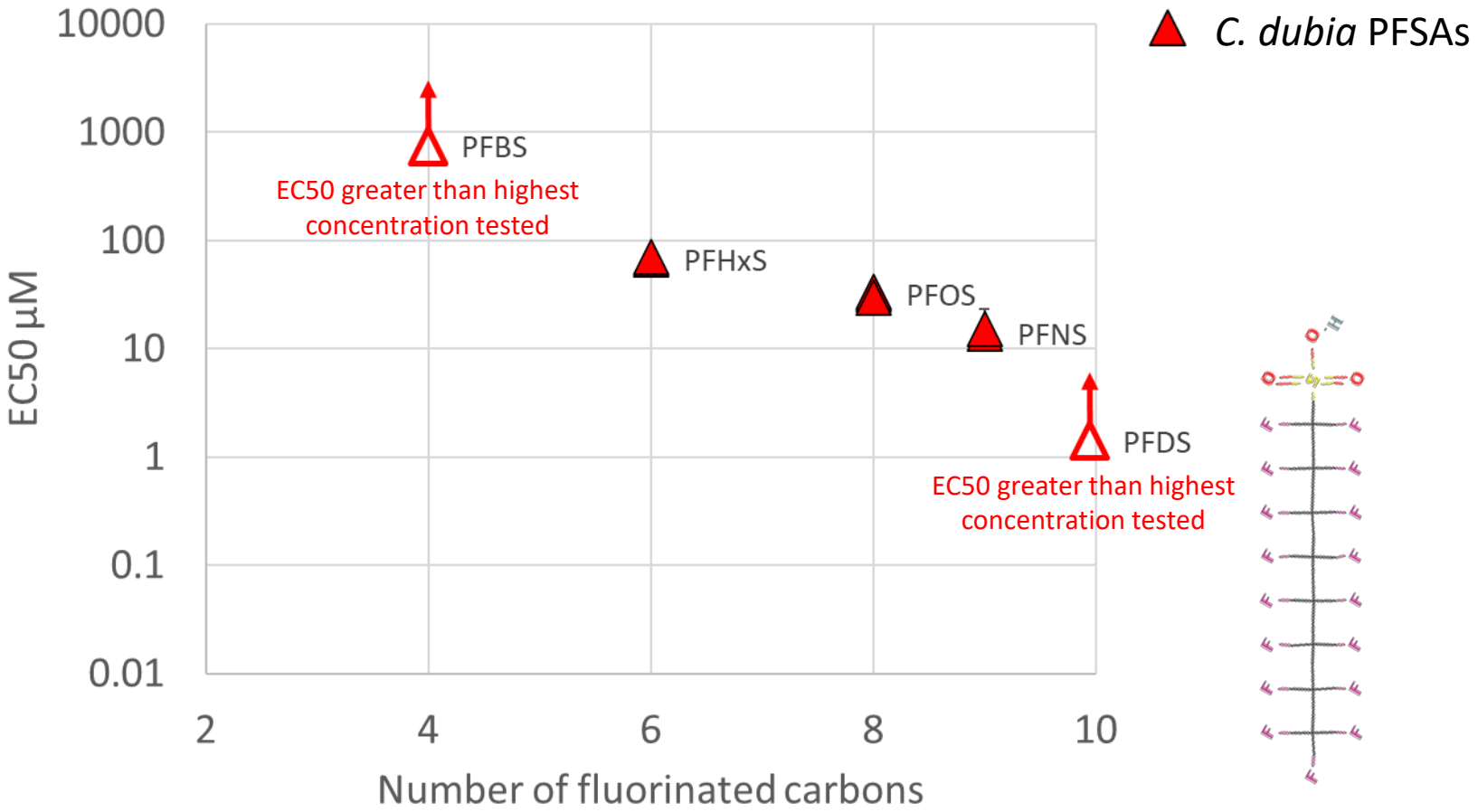
C. dilutus **Growth**



Results

***C. dubia* Reproduction**

Perfluorinated sulfonates



Results

Perfluorinated sulfonates

C. dubia **Reproduction**

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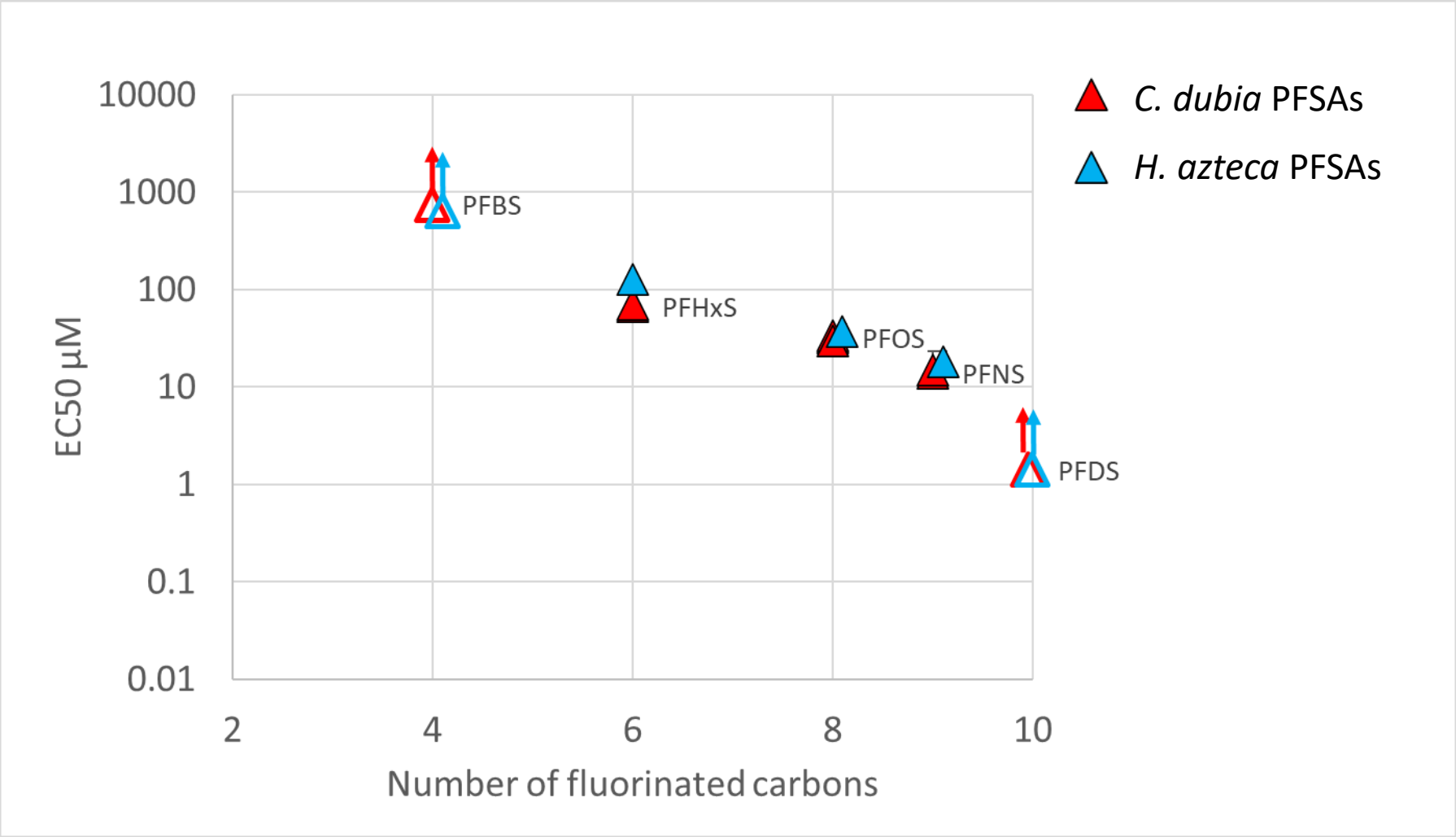


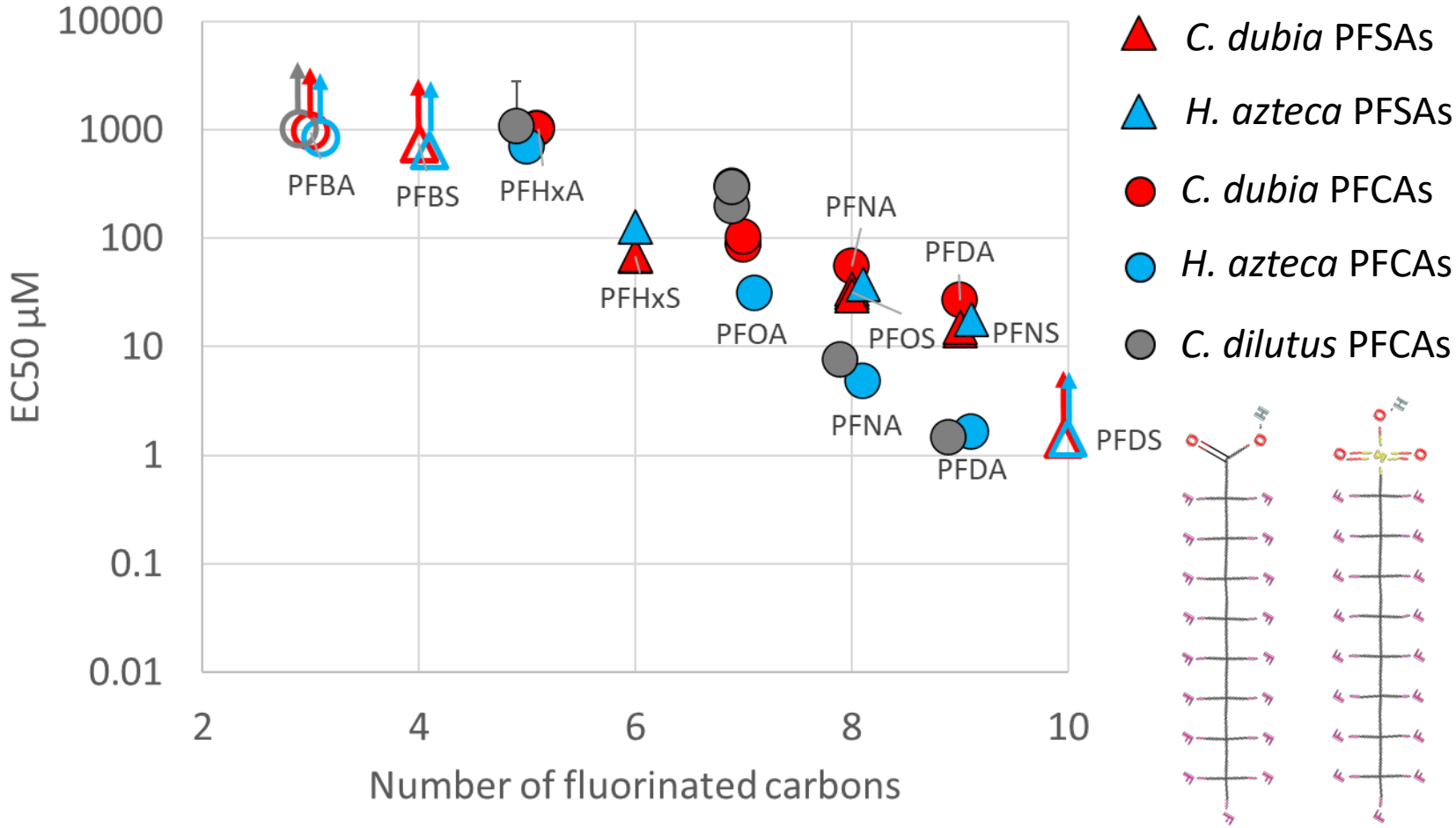
Results

Perfluorinated carboxylates and sulfonates

C. dubia **Reproduction**

H. azteca **Growth**

C. dilutus **(midge)**



Results

Perfluorinated carboxylates and sulfonates

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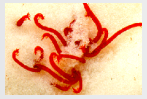
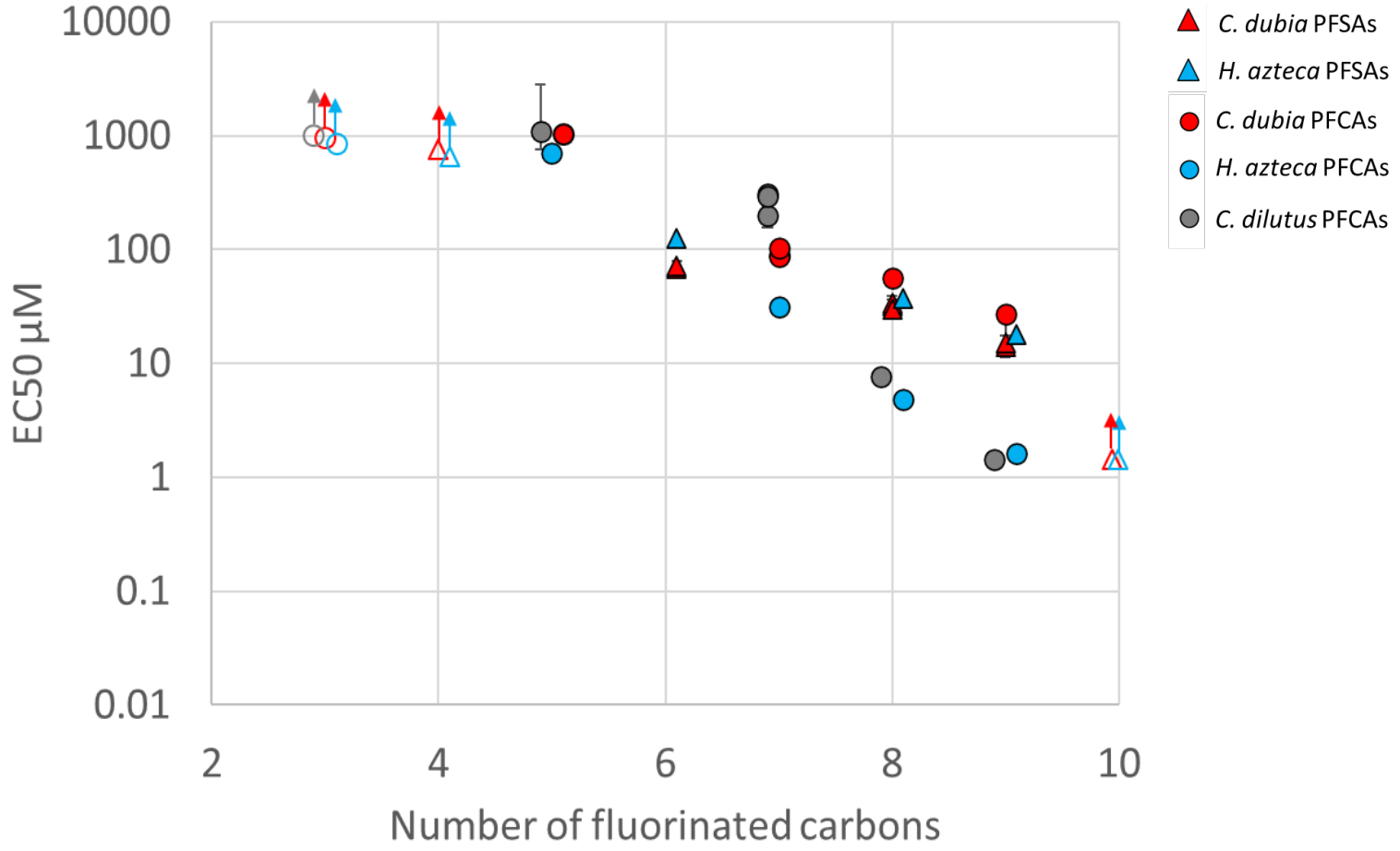
H. azteca **Growth**



C. dilutus **(midge)**



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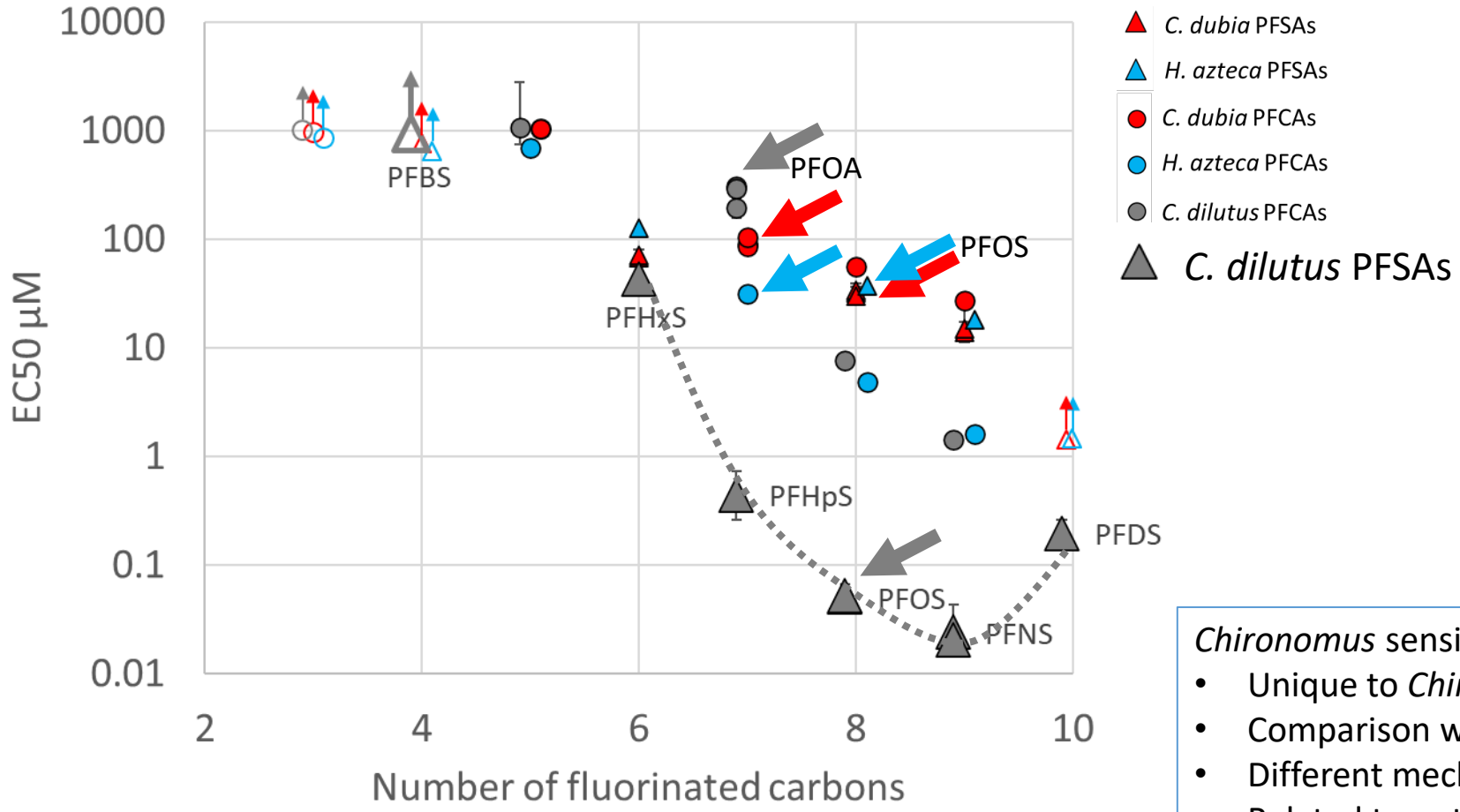


Chironomus sensitivity to long PFSAs

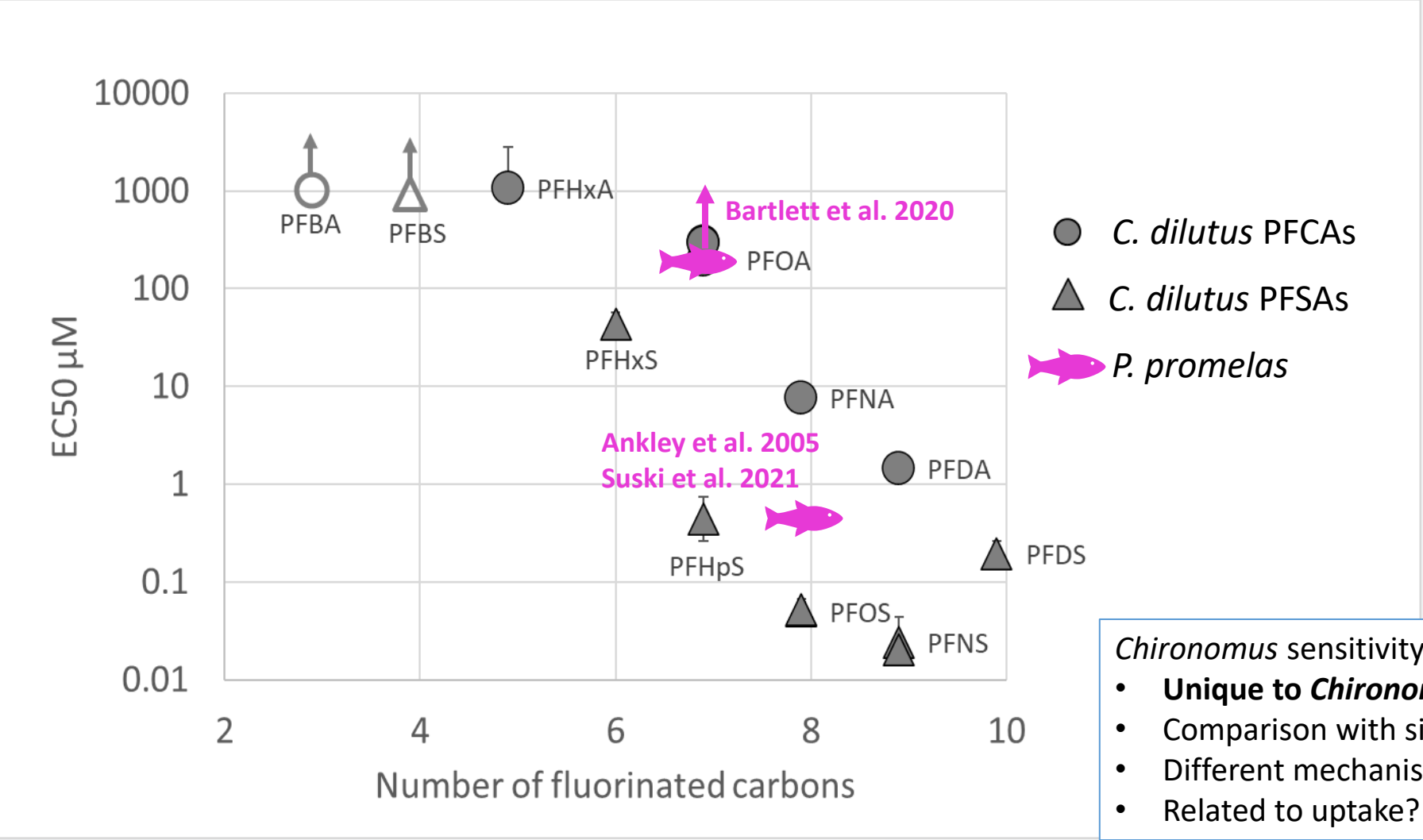
- Unique to *Chironomus*?
- Comparison with similar structures
- Different mechanisms of action?
- Related to uptake?

Results

Fathead minnow data from literature

P. promelas
Various endpoints, indicating toxicity of PFOS > PFOA

C. dilutus
Growth



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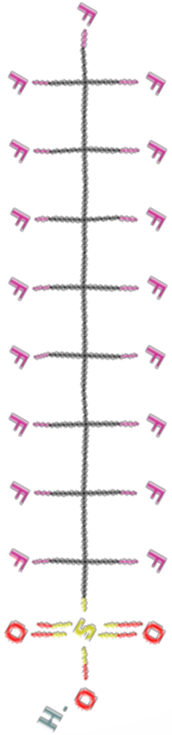
Results

Fluorotelomer sulfonates

PFOS

8 fluorinated



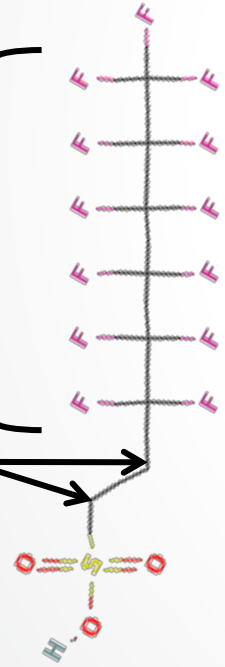
Results

Fluorotelomer sulfonates

6:2 FTS

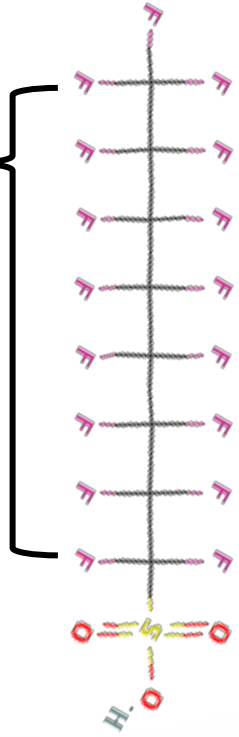
6 fluorinated

2 unfluorinated



PFOS

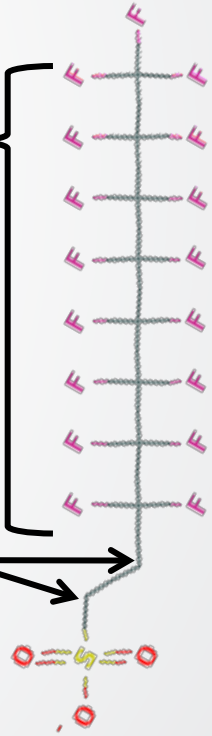
8 fluorinated



8:2 FTS

8 fluorinated

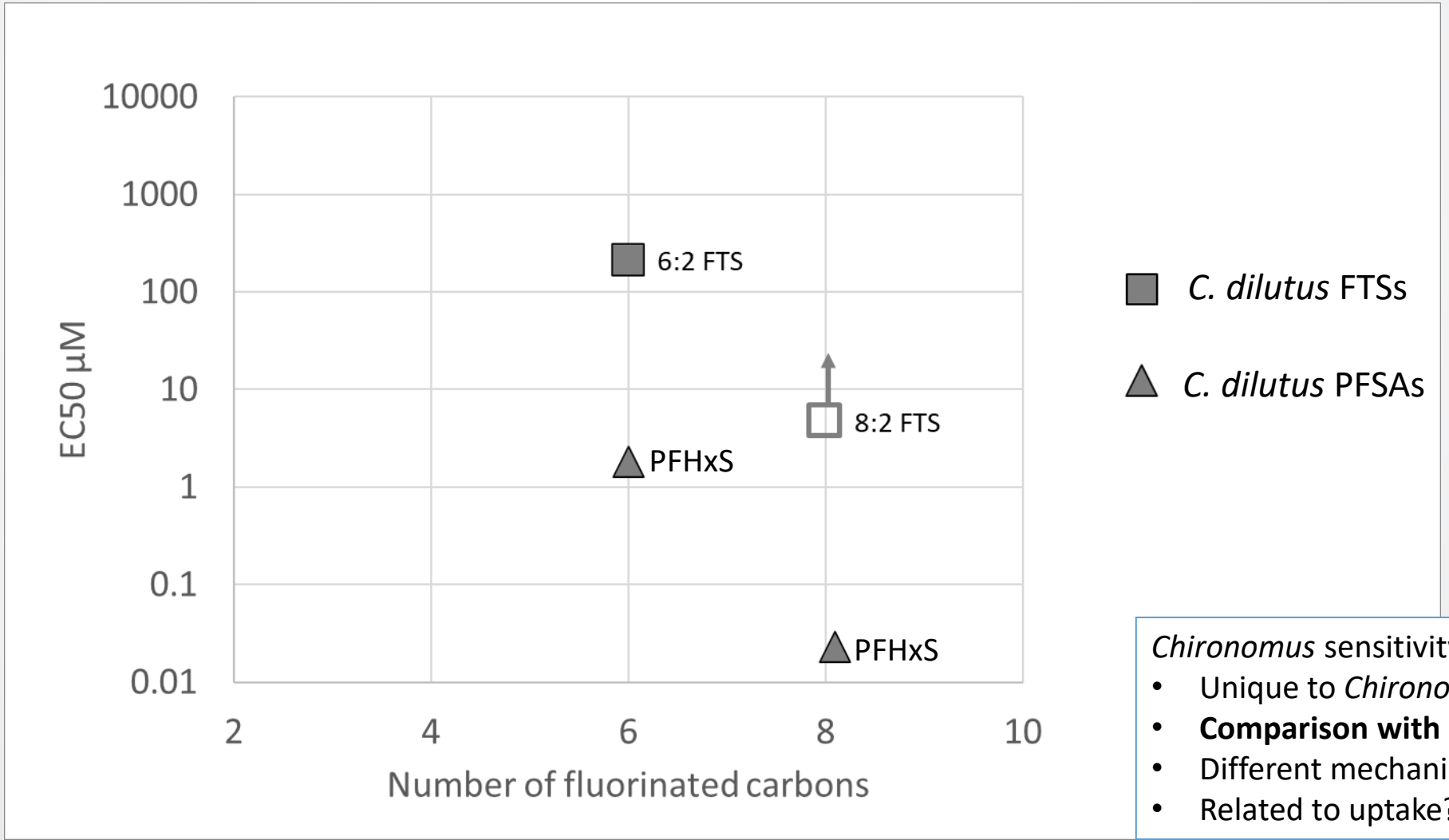
2 unfluorinated



Results

Fluorotelomer sulfonates

C. dilutus **(midge)**



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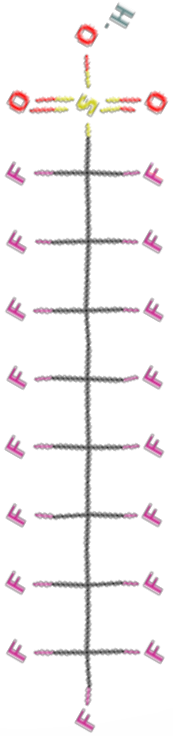
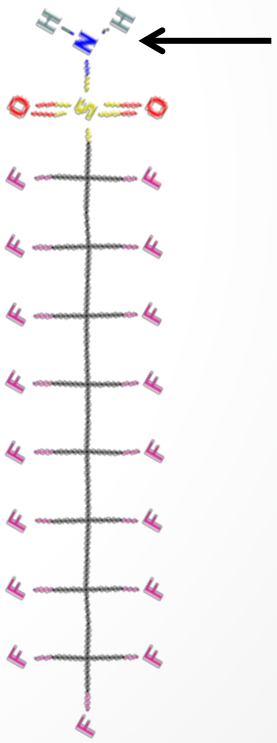
Results

Perfluorinated sulfonamides

PFOS



PFOSA



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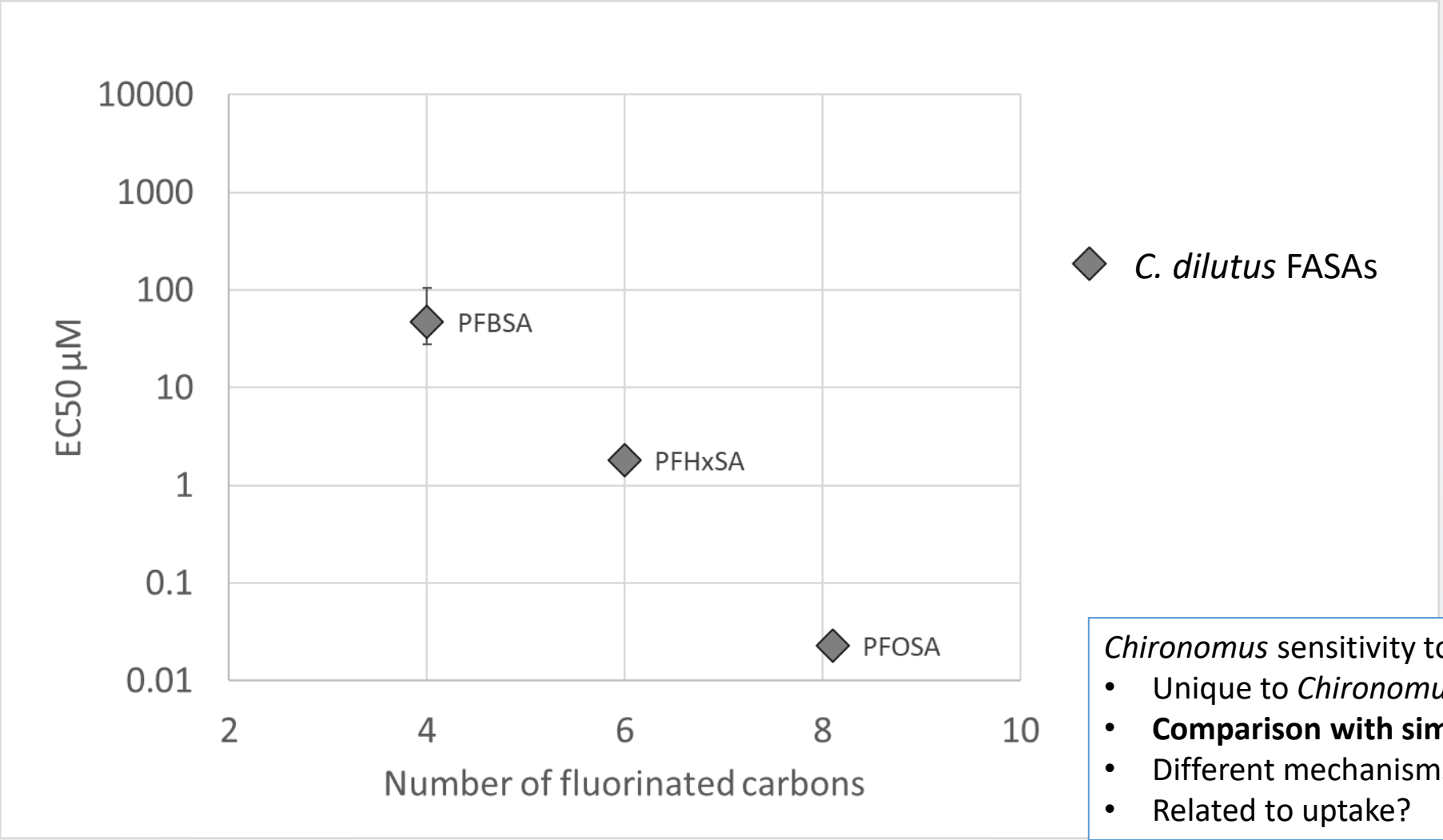

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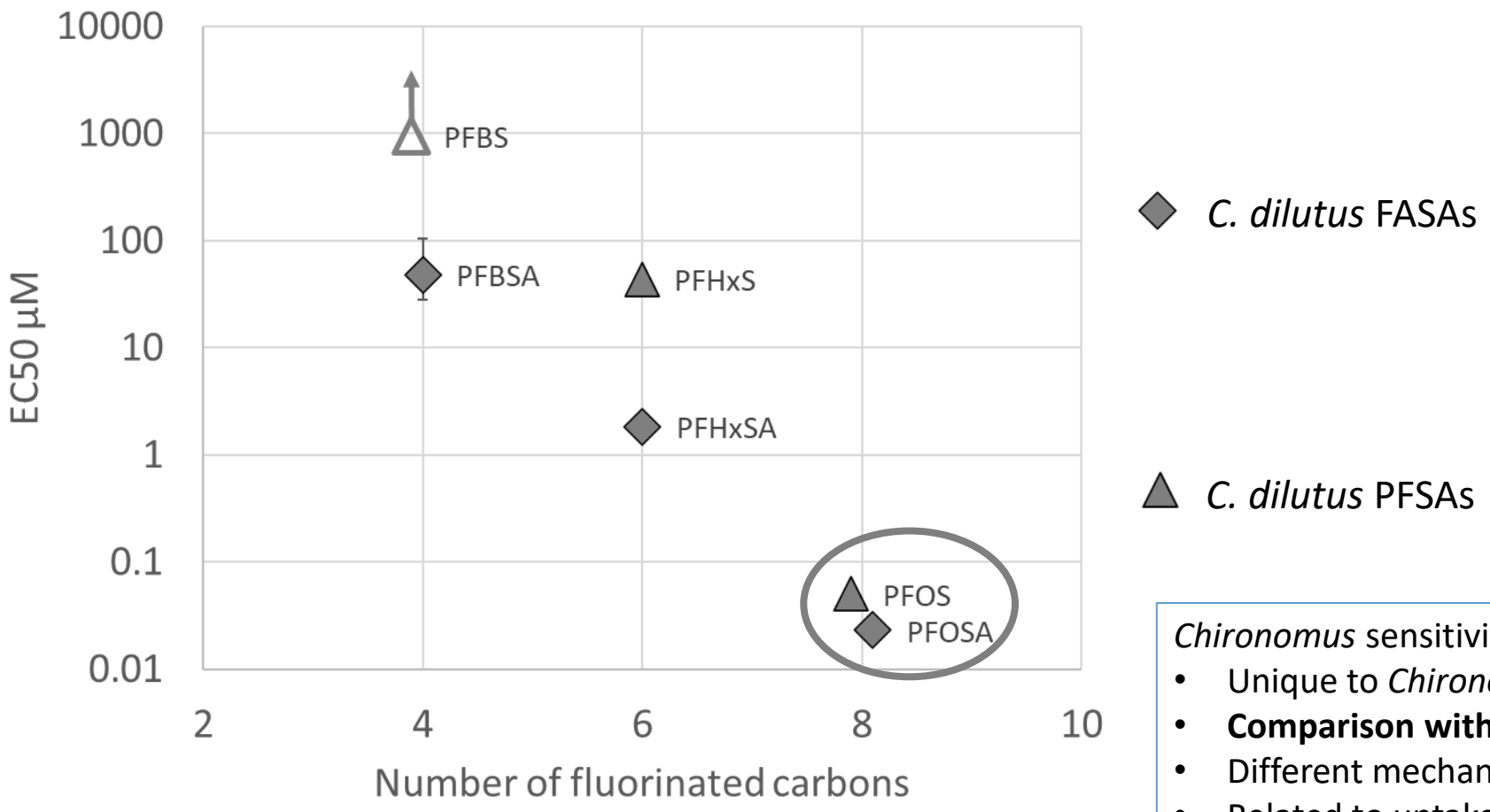

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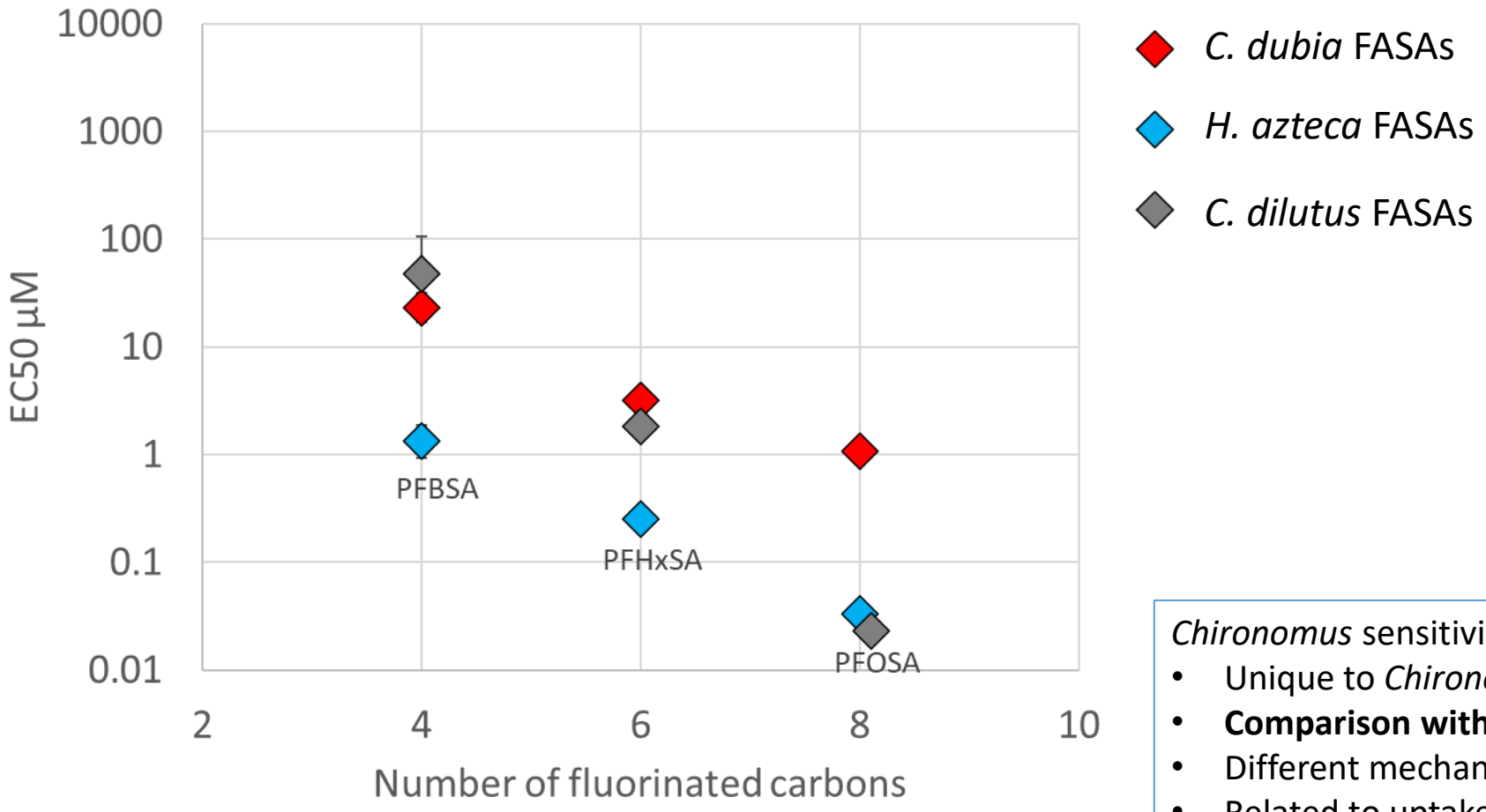
H. azteca **(amphipod)**

C. dilutus **(midge)**

Perfluorinated sulfonamides



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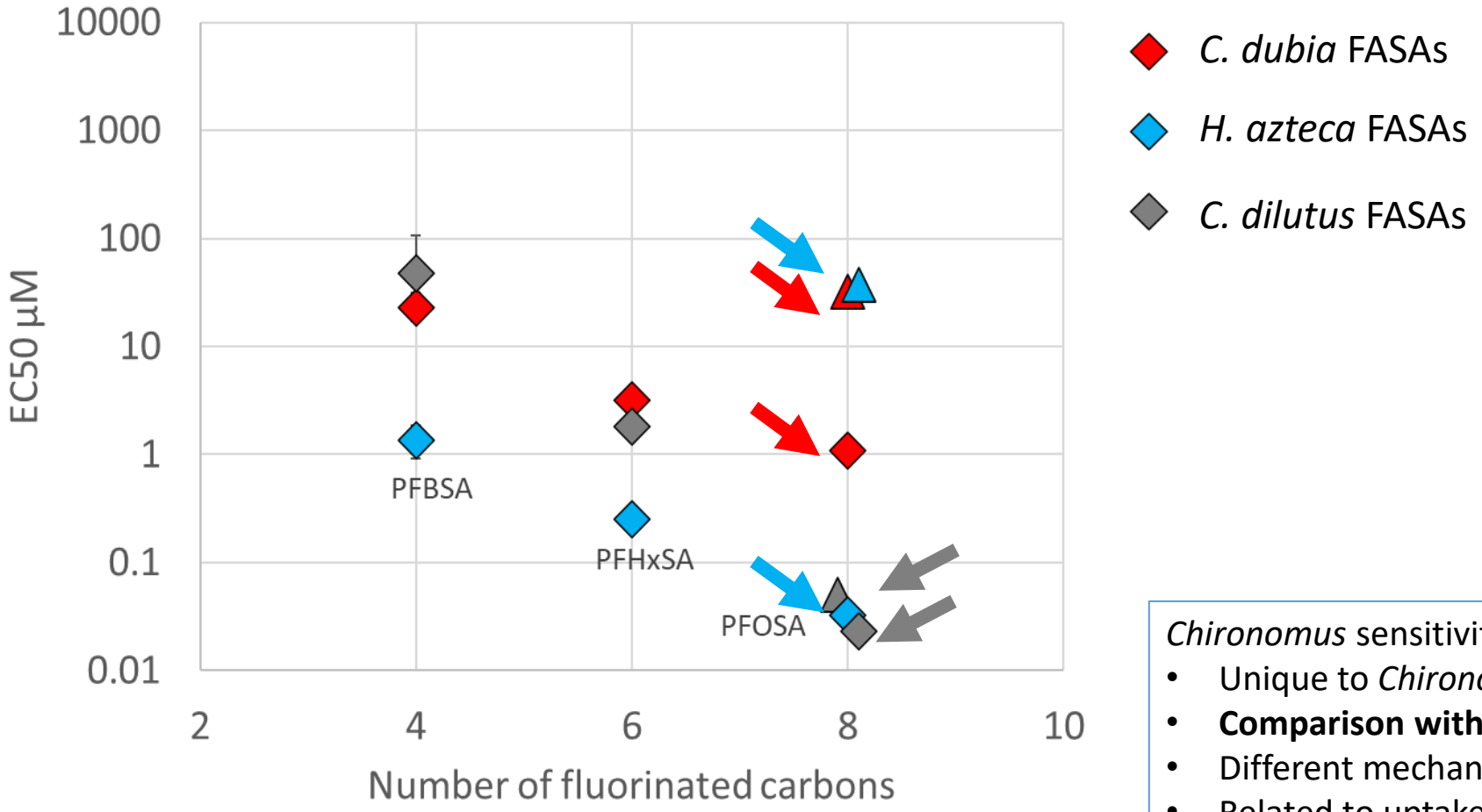

Chironomus sensitivity to long PFSAs

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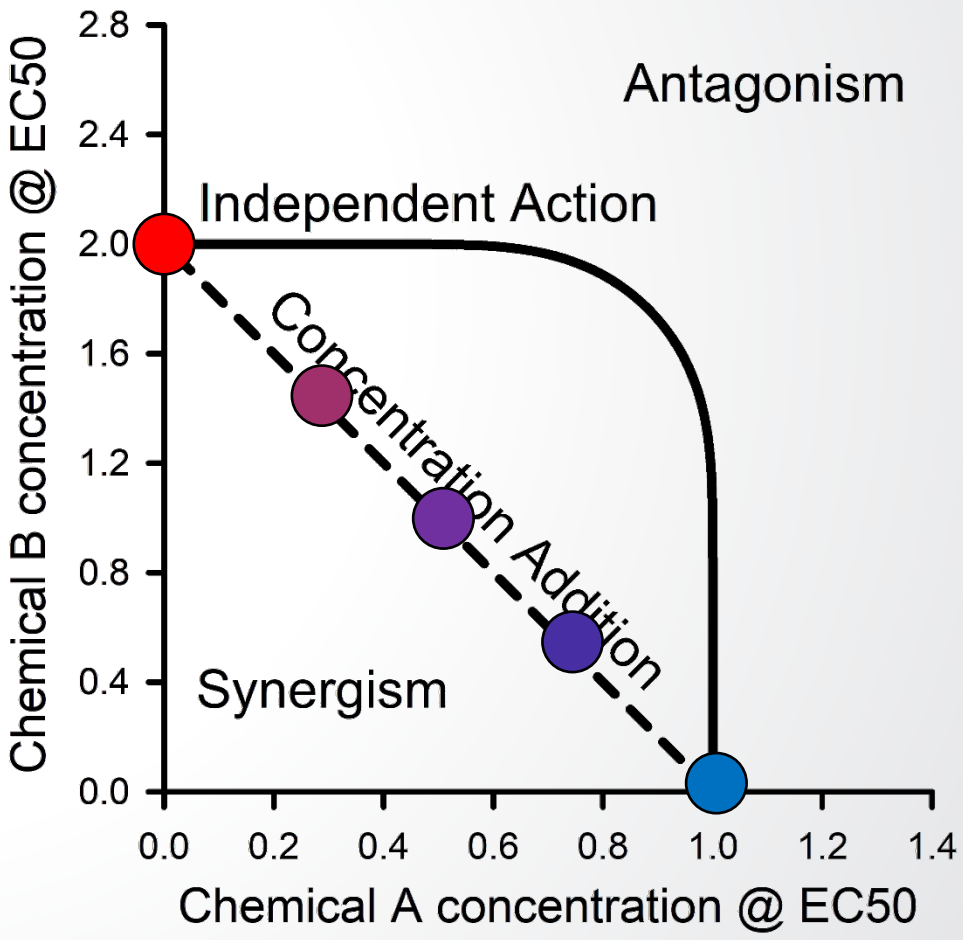
Binary mixture studies

- Same 7-day sublethal toxicity test designs as for individual chemicals
- Binary mixtures, varying ratios on basis of toxic units

	Chemical A	Chemical B	EC50
Test 1	100%	0%	
Test 2	75%	25%	
Test 3	50%	50%	
Test 4	25%	75%	
Test 5	0%	100%	

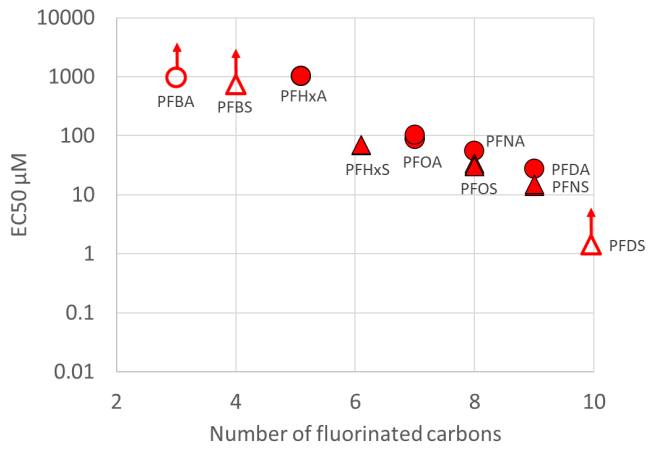
- Isobole analysis: plot effect concentrations for mixtures of chemicals A and B



Binary mixture studies

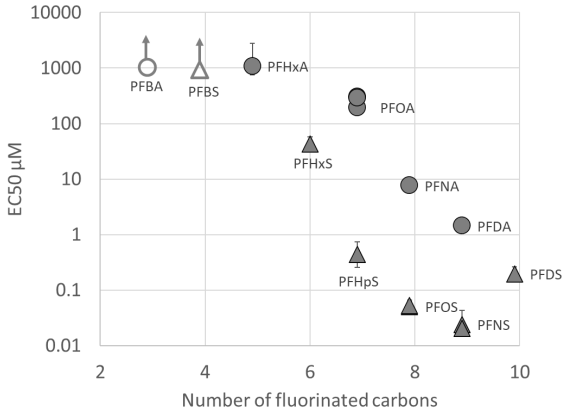
HYPOTHESIS: RESULTS:

Ceriodaphnia



	Same	Different
PFCA+PFSA	X	
PFCA+PFCA	X	
PFSA+PFSA	X	

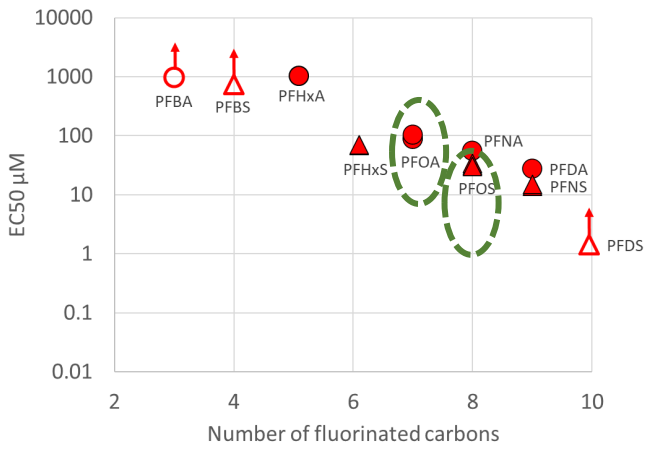
Chironomus



	Same	Different
PFCA+PFSA		X
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

Binary mixture studies

Ceriodaphnia



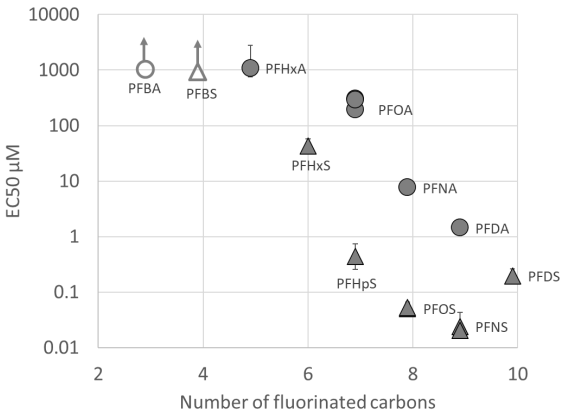
HYPOTHESIS:

	Same	Different
PFCA+PFSA	**X**	
PFCA+PFCA	X	
PFSA+PFSA	X	

RESULTS:

C. dubia PFCA+PFCA:

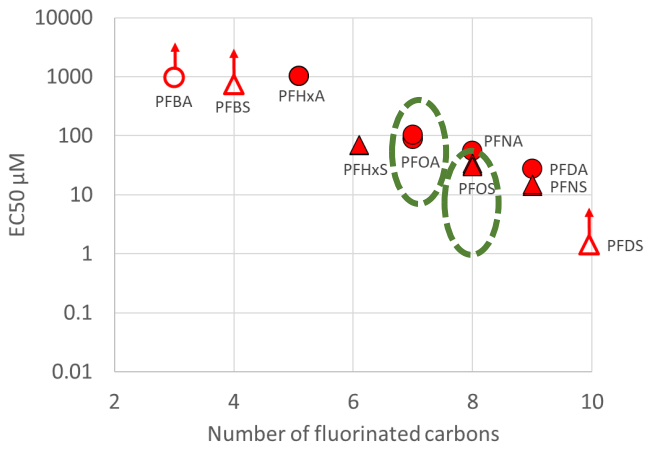
Chironomus



	Same	Different
PFCA+PFSA		X
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

Binary mixture studies

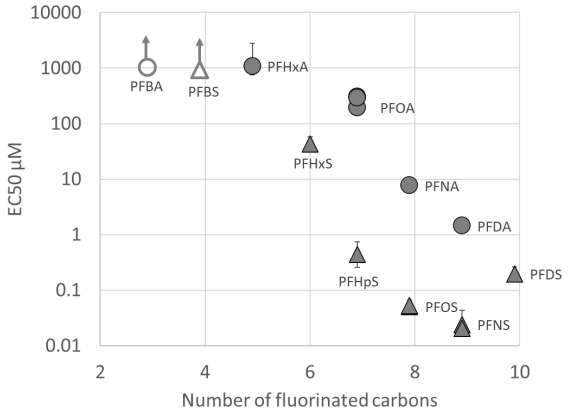
Ceriodaphnia



HYPOTHESIS:

	Same	Different
PFCA+PFSA	**X**	
PFCA+PFCA	X	
PFSA+PFSA	X	

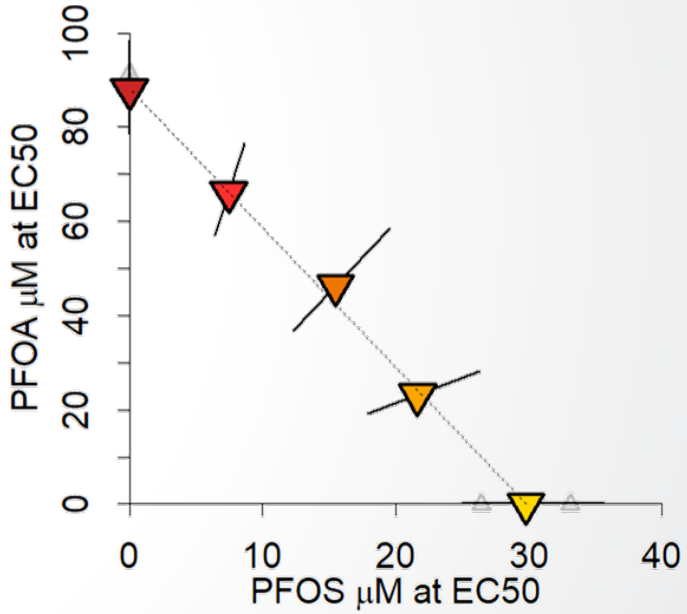
Chironomus



	Same	Different
PFCA+PFSA		X
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

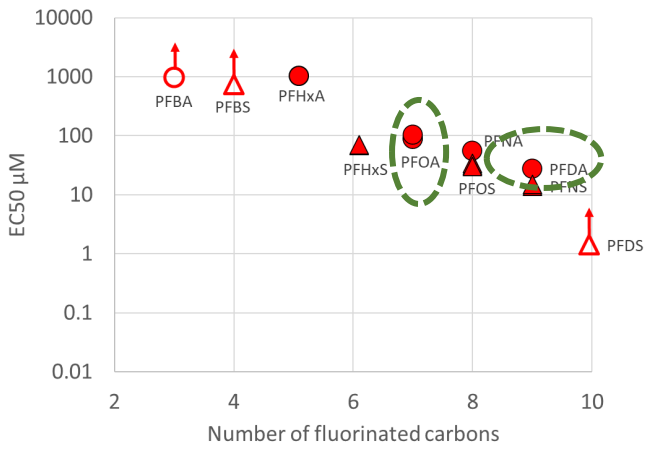
RESULTS:

C. dubia PFCA+PFSA: Additive



Binary mixture studies

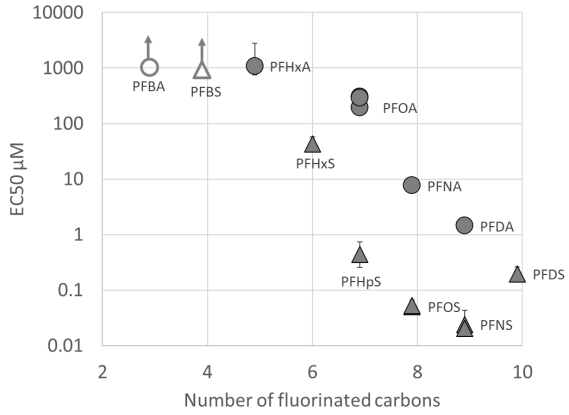
Ceriodaphnia



HYPOTHESIS:

	Same	Different
PFCA+PFSA	X	
PFCA+PFCA	**X**	
PFSA+PFSA	X	

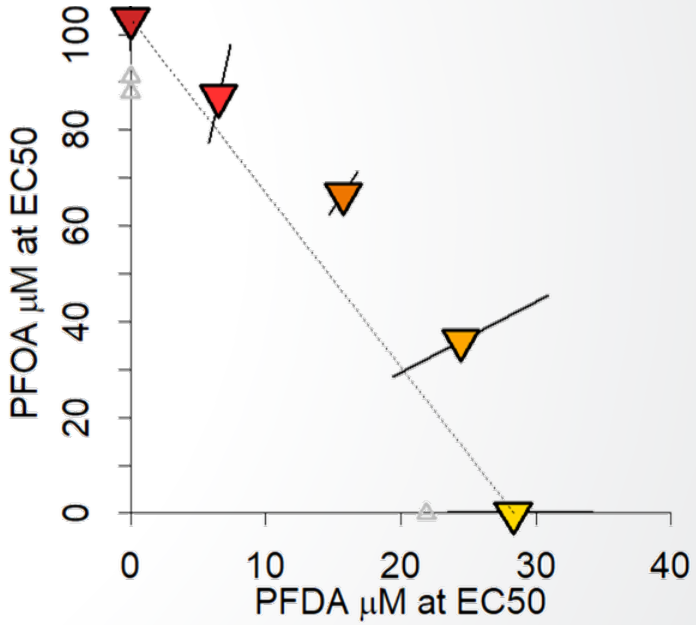
Chironomus



	Same	Different
PFCA+PFSA		X
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

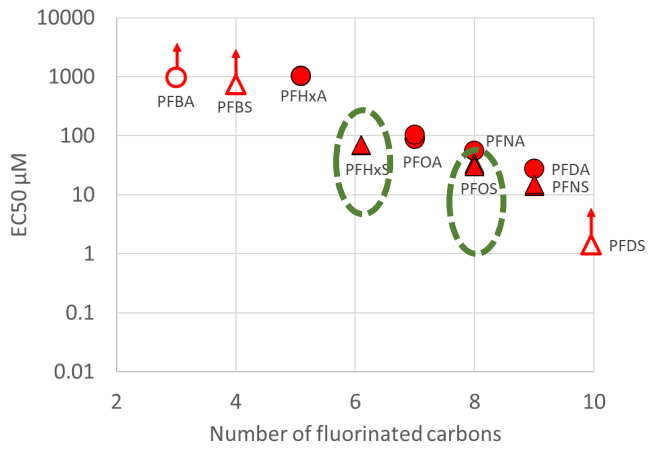
RESULTS:

C. dubia PFCA+PFCA: Additive



Binary mixture studies

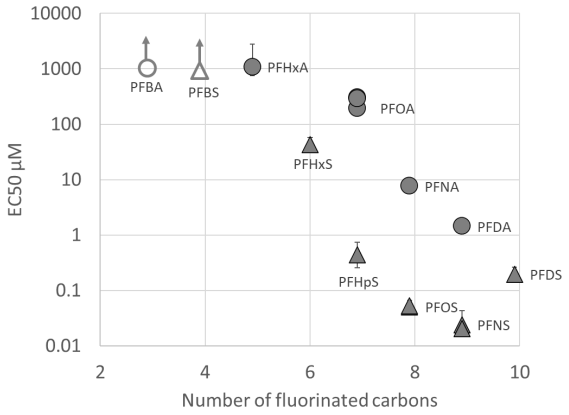
Ceriodaphnia



HYPOTHESIS:

	Same	Different
PFCA+PFSA	X	
PFCA+PFCA	X	
PFSA+PFSA	**X**	

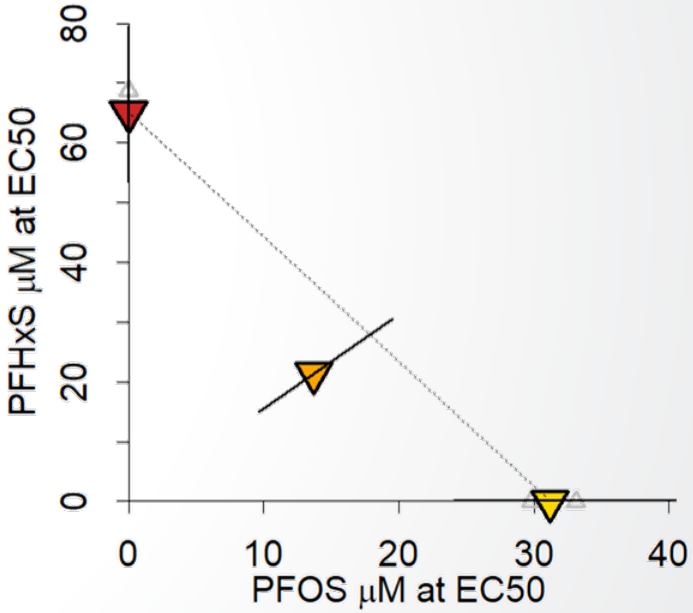
Chironomus



	Same	Different
PFCA+PFSA		X
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

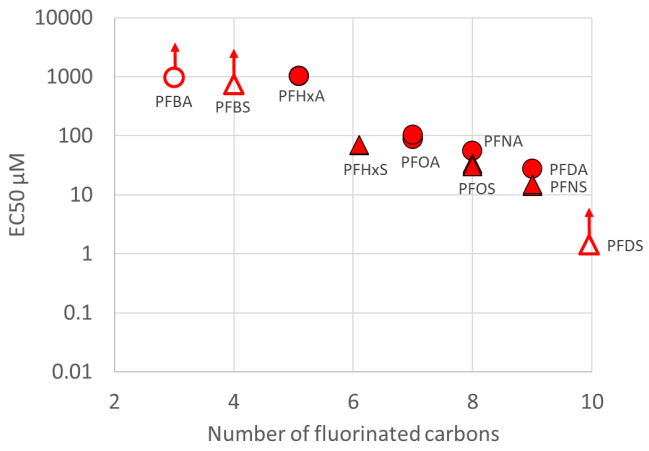
RESULTS:

C. dubia PFSA+PFSA: Additive



Binary mixture studies

Ceriodaphnia



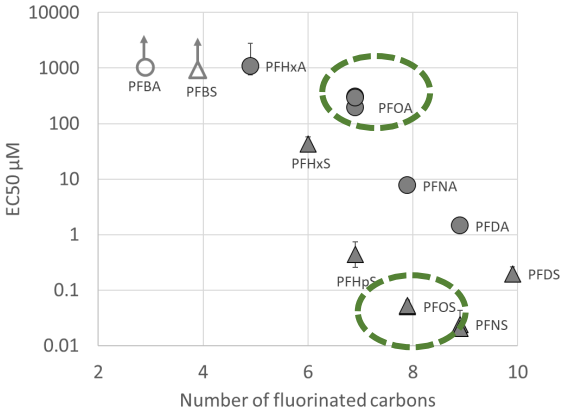
HYPOTHESIS:

	Same	Different
PFCA+PFCA	X	
PFSA+PFSA	X	
PFCA+PFSA	X	

RESULTS:

C. dilutus PFCA+PFSA:

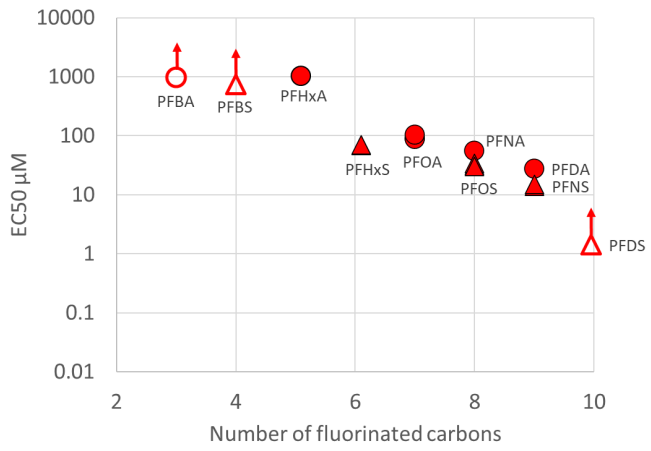
Chironomus



	Same	Different
PFCA+PFSA		**X**
PFCA+PFCA	X	
PFSA+PFSA	X	
PFSA+FASA		X

Binary mixture studies

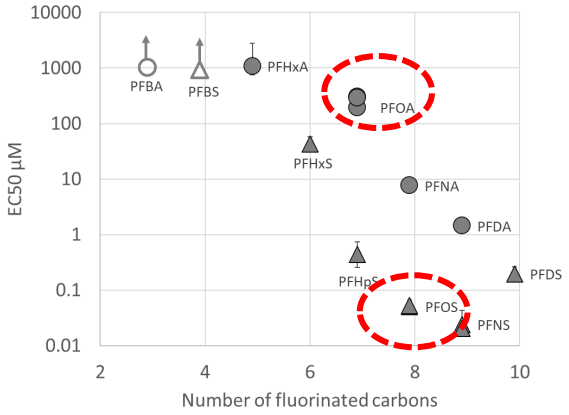
Ceriodaphnia



HYPOTHESIS:

	Same	Different
PFCA+PFCA	X	
PFSA+PFSA	X	
PFCA+PFSA	X	

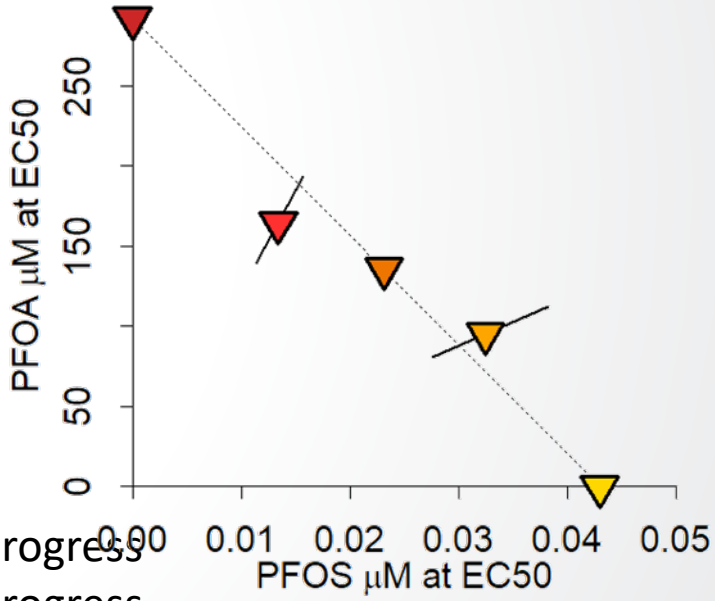
Chironomus



	Same	Different	
PFCA+PFSA	**X**		
PFCA+PFCA	X		in progress
PFSA+PFSA	X		in progress
PFSA+FASA		X	in progress

RESULTS:

C. dilutus PFCA+PFSA: Additive!



Next steps

- Tissue uptake / body burden studies
- Partitioning measurements
- SERDP ER22-3175 Collaboration with Univ. Delaware to model partitioning coefficients and potency of anionic PFAS
- Fathead minnow studies
- Further mixture work
- Effects of varying water chemistry on PFAS toxicity

Our posters:

3.04.P-Th055 - Determination of Bioconcentration Factors for Per- and Polyfluorinated Alkyl Substances in Aquatic Invertebrates.

Ian Mundy[1], Will Backe, Ph.D.[1], Leah Glimsdal[1], Sarah Howe[2], Sarah Kadlec[1], David Mount[1], Edward Piasecki[2], Henry Sluka[1] and Lauren Votava[1], (1)U.S. Environmental Protection Agency, (2)Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency

5.04.P-Th090 Measurement of Octanol-Water and Butanol-Water Partitioning Coefficients for Relevant Per- and Poly-fluorinated Alkyl Substances.

Henry Sluka[1], Tyler Shonrock[2], Lawrence Burkhard[3], Ian Mundy[3], Will Backe, Ph.D.[3] and David Mount[3], (1)Oak Ridge Institute for Science and Education (ORISE) participant at U.S. Environmental Protection Agency, (2)Oak Ridge Associated Universities, (3)U.S. Environmental Protection Agency

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Disclaimer: the statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of the U.S. Environmental Protection Agency.