European Precious Metals Federation

Derivation of a protective threshold value for silver towards freshwater organisms

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Introduction

- EU **REACH** registration silver
  - Chronic freshwater (fw) PNEC of 40 ng/L (2013)
  - Derived using statistical extrapolation (species sensitivity distribution, SSD)
  - Since 2013: several new studies published on chronic freshwater toxicity of silver ▶ re-evaluate PNEC derivation

Introduction

- EU **REACH** registration silver
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- EU **Water Framework Directive (WFD)**
  - Review Priority Substances (PS) list (2016) ▶ Ag under consideration as potential PS
  - Ultimate decision to depend on EU monitoring data + agreed PNEC/EQS (assessment of 'EU wide risk')
  - Discussions PNEC/EQS ongoing at European Commission level

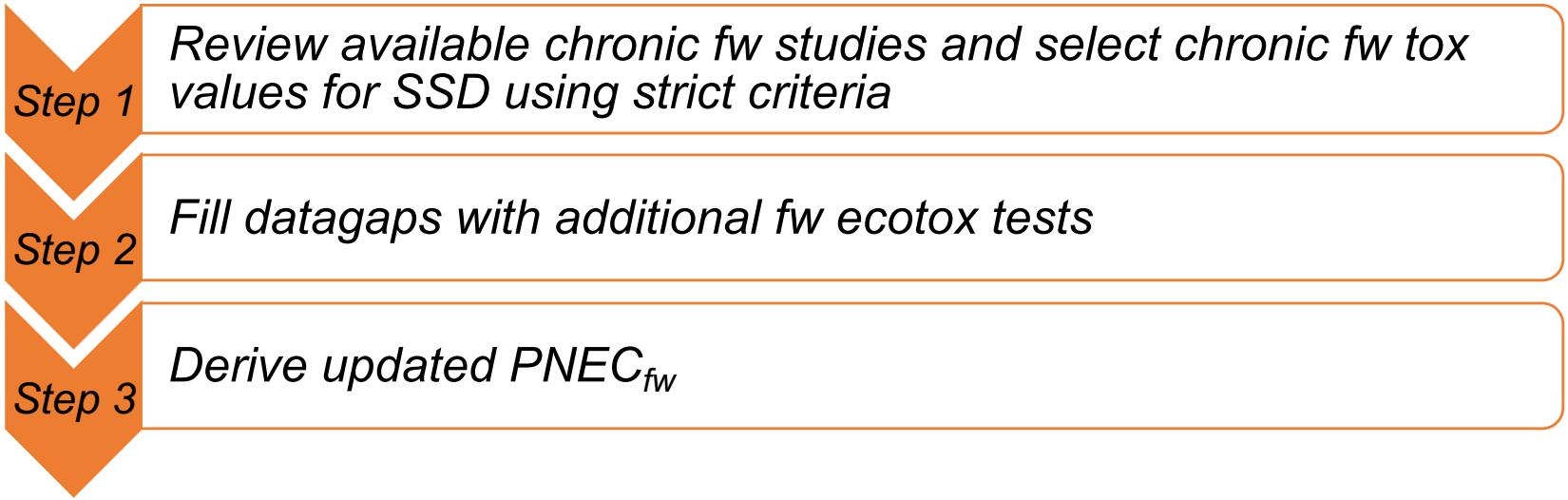
Approach

Step 1 *Review available chronic fw studies and select chronic fw tox values for SSD using strict criteria*

Step 2 *Fill datagaps with additional fw ecotox tests*

Step 3 *Derive updated $PNEC_{fw}$*

Approach



- Quality criteria for selection chronic fw studies in line with available guidances for REACH and WFD:
  - Measured dissolved Ag concentrations (<0.45 µm) only
  - Phys-chem properly described and within tolerance limits
  - EC_{10} values (most sensitive endpoint) preferred over NOEC
  - If tox values at ≠ hardness for same species ▶ value at lowest hardness retained

Results

- **Step 1**: **Review available chronic fw studies** ▶ dataset of 14 species covering 10 taxonomic groups

- **Step 2**: **Additional fw ecotox tests** with ionic Ag ($AgNO_3$) using potentially sensitive species to strengthen SSD / resolve uncertainties:

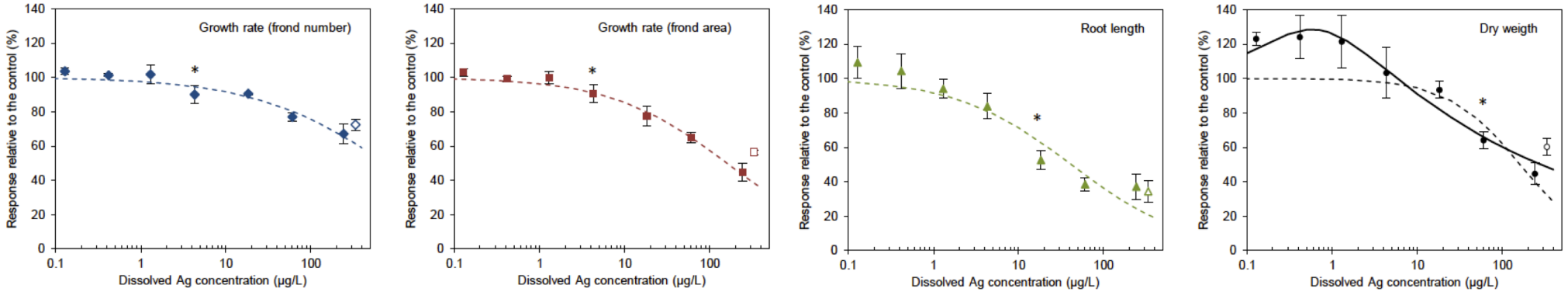
  1) *Lemna minor* (higher plant): 7-day growth rate test (OECD Test Guideline No. 221)
  2) *Anabaena flos-aquae* (cyanobacteria): 72-hour growth rate test (OECD Test Guideline No. 201)
  3) *Brachionus calyciflorus* (rotifer): 48-hour population growth rate test (APHA test procedure 8420)

  ➢ EDTA replaced by 1 mg/L natural DOC

  ➢ Dissolved Ag concentrations measured throughout tests

Results - Step 2: ecotox tests

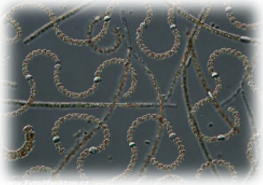
1) *Lemna minor* 7-d growth rate test (OECD 221)



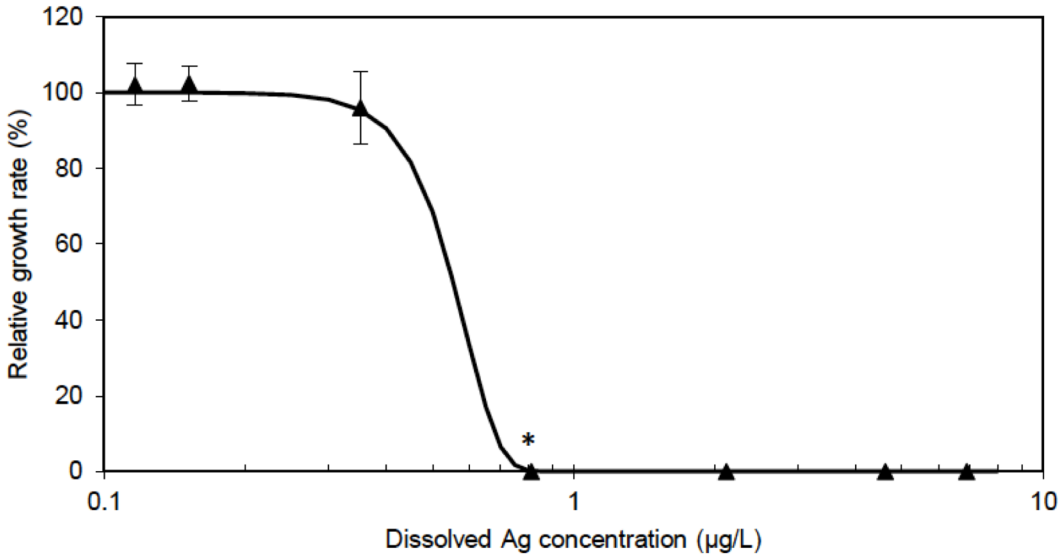
	EC10[b] (µg diss. Ag/L)	EC20[b] (µg diss. Ag/L)	EC50[b] (µg diss. Ag/L)
Growth rate (frond number)	14 (7-29)	62 (42-92)	*769[d] (381-1550)*
Growth rate (frond area)	5.2 (3.2-8.5)	18 (13-25)	159 (124-205)
Root length	1.4 (0.4-4.2)	4.8 (2.2-10.5)	42 (25.1-68.9)
Dry weigth	19.0 (3.9-91.9)	41.8 (14.5-120.4)	162 (78-336)
(hormesis)	10.7 (6.5-17.4)	20.6 (12.1-35.3)	283[e] (88-908)

- **Root length** most sensitive endpoint
- **EC_{10}** of **1.4 µg/L** used for SSD

Results - Step 2: ecotox tests

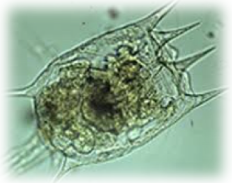


2) *Anabaena flos-aquae* 72-h growth rate test (OECD 201)

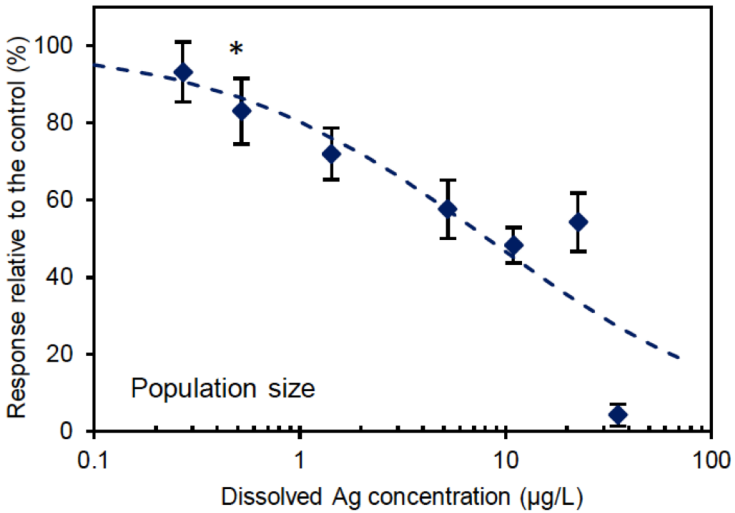


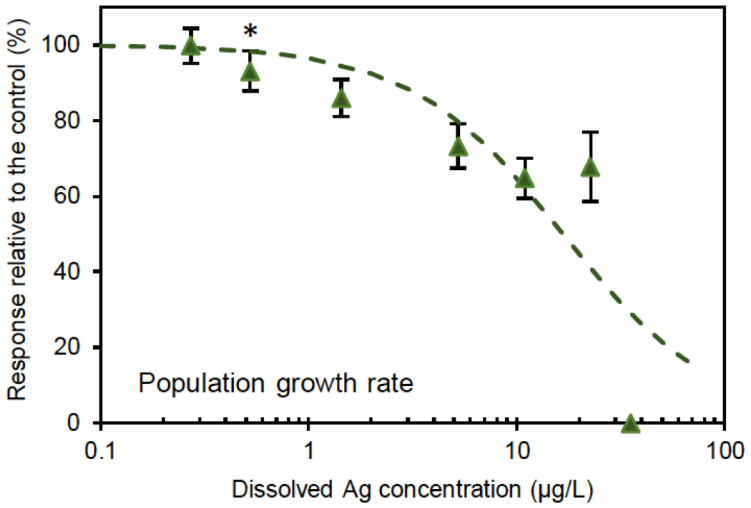
	Effect concentrations (µg diss Ag/L)[a]
EC10[b]	0.41 (0.29-0.52)
EC20[b]	0.46 (0.25-0.67)
EC50[b]	0.56 (0.16-0.96)
NOEC[c]	0.35 (4±10)
LOEC[c]	0.84 (100±0)

Results - Step 2: ecotox tests



3) *Brachionus calyciflorus* 48-h population growth rate test (APHA 8420)





Endpoint	EC10[b] (µg diss. Ag/L)	EC20[b] (µg diss. Ag/L)	EC50[b] (µg diss. Ag/L)
Population size	0.31 (0.13-0.73)	1.0 (0.6-1.9)	8.2 (5.9-11.3)
Population growth rate	2.6 (1.5-4.7)	5.2 (3.5-7.7)	16.7 (13.5-20.7)

- **Population size** most sensitive endpoint
- **EC_{10}** of **0.31 µg/L** used for SSD

Results - Step 3: derivation updated $PNEC_{fw}$

- **17 species in 12 tax groups**

Taxonomic group		Species	NOEC/EC_{10} (µg Ag/L)	DOC (mg/L)	Hardness (mg $CaCO_3$/L)
Fish	Cyprinidae	*Pimephales promelas*	0.38	2.4	30.5
	Salmonidae	*Oncorhynchus mykiss*	0.46	1.4	28.5
		Salmo trutta	0.23	0.8	27.9
Crustaceans	Cladocera	*Ceriodaphnia dubia*	4.36	3.4	85.2
		Daphnia magna	0.80	1.0	69.0
	Amphipoda	*Hyalella azteca*	1.54	1.5	23.4
Insects	Ephemeroptera	*Isonychia bicolor*	0.16	2.0	34.8
		Stenonema modestum	1.00	<2.0	48.5
	Diptera	*Chironomus tentans*	12.54	<2.0	52.1
Rotifera		***Brachionus calyciflorus***	**0.31**	**1.6**	**48.0**
Molluscs	Bivalvia	*Corbicula fluminea*	0.84	2.0	34.8
	Gastropoda	*Lymnaea stagnalis*	1.48	0.76	116
Cyanobacteria		***Anabaena flos-aquae***	**0.41**	**1.8**	**25.0**
		Synechococcus leopoliensis	1.87	<2.0	40.0
Algae	Chlorophyceae	*Chlamydomonas reinhardtii*	7.50	<2.0	40.0
		Pseudokirchneriella subcapitata	0.10	0.63	10.0
Higher plants	Tracheophyta	***Lemna minor***	**1.40**	**1.6**	**10.4**

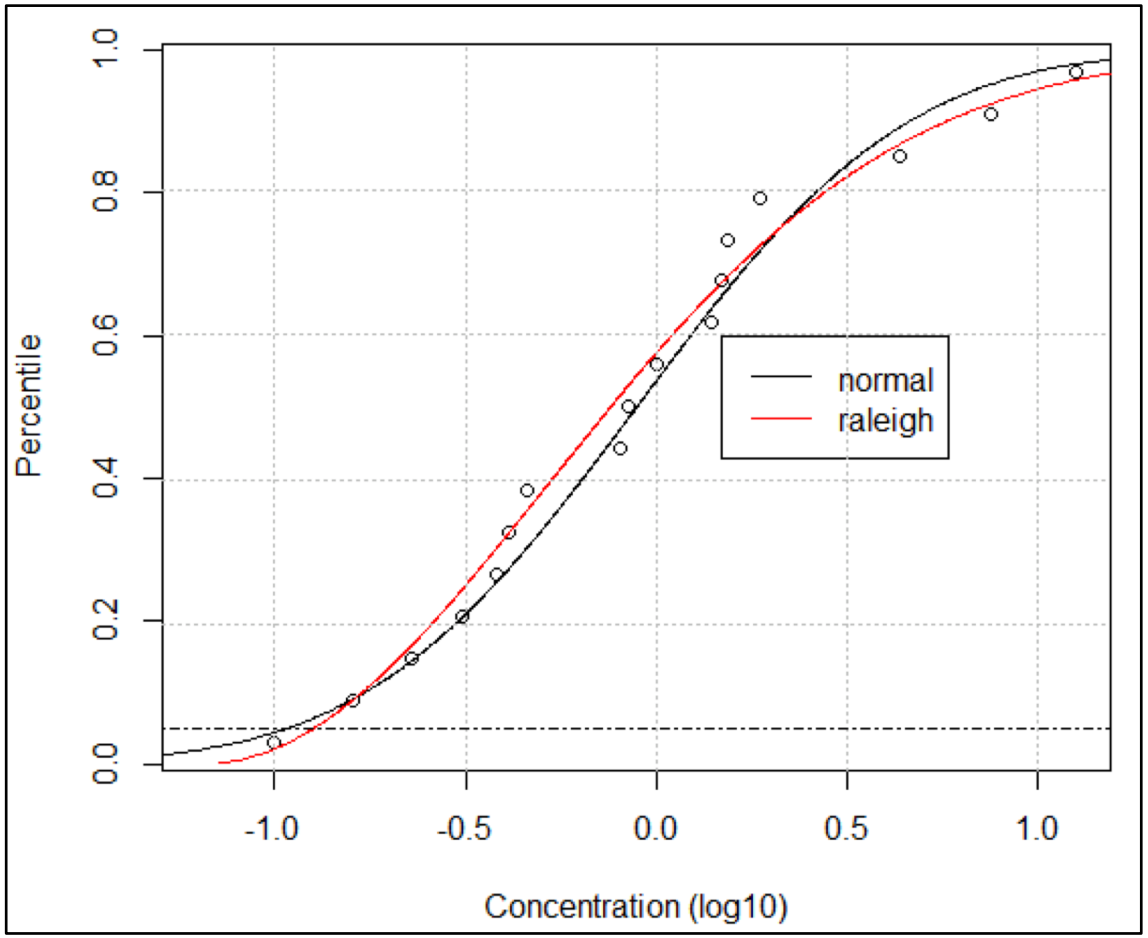
➢ Conservative dataset in terms of **bioavailability**

DOC: 0.63 - 3.4 mg/L (average 1.7 mg/L)

Hardness: 10 - 116 mg/L $CaCO_3$ (average: 42.7 mg/L CaCO3)

Results - Step 3: derivation updated $PNEC_{fw}$

- **Species Sensitivity Distribution**



	EC_{10}
min (µg/L)	0.100
max (µg/L)	12.54
HC_5 (µg/L) - BestFit	**0.125 (0.071-0.23)**

Results - Step 3: derivation updated $PNEC_{fw}$

- **Assessment factor:**
  - Should reflect residual **uncertainty**; to be decided on **case-by-case** basis
  - **maximum AF of 3 is proposed**:
    - main remaining uncertainty: limited availability of field/mesocosm data (but available microcosm data suggest small-scale laboratory experiments may overestimate environmental responses ▶ unlikely that field/mesocosm data will suggest a lower threshold)
    - conservatism, related to data selection and bioavailability, used in the PNEC derivation

- **$PNEC_{freshwater}$ = 0.125 / AF (1-3) = 0.042 µg/L - 0.125 µg/L (<0.45 µm fraction)**

- PNEC ≤ lowest EC_{10} in the ecotox database (*Pseudokirchneriella subcapitata*) ▶ **adequately protective for sensitive freshwater organisms**

Conclusions

- Critical review of chronic freshwater toxicity data for silver + additional testing has **strengthened the SSD for silver** (covering 17 species in 12 taxonomic groups).
- Dataset includes data for **cyanobacteria**, which were considered to be particularly sensitive to silver (cfr. anti-microbial properties of silver ions).
- PNEC is **0.042 µg/L - 0.125 µg/L** (< 0.45 µm fraction), and ≤ lowest EC_{10} in the SSD (*P. subcapitata)* ▶ **adequately protective** for sensitive freshwater organisms.
- Silver PNEC is also conservative in terms of **bioavailability**: selected chronic toxicity data typically reflect conditions of high bioavailability (hardness and DOC well below median values in EU natural waters).
- Industry is currently considering the development of a **bioavailability model** for silver.

THANK YOU

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