The NORMAN interlaboratory study on biotesting of spiked water extracts

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NORMAN WG2
Bioassays and biomarkers in water quality monitoring

Introduction: The **NORMAN network** working group on **Bioassays and Biomarkers (Bio WG)** focuses on the application of biotools for environmental quality monitoring. A main objective is to provide recommendations for the implementation of **effect-based tools into regulatory frameworks**. In 2013/2014, a blind **interlaboratory study** (ILS) applying **bioassays** to evaluate **spiked surface water extracts** was performed.

The **Aim** was to verify whether a **battery of bioassays** conducted in **different laboratories** following their **own methods and protocols** would produce **comparable results** when applied to evaluate spiked water extracts. The ILS is expected to **promote the use of biotests for water quality monitoring** at the level of EU policy-makers.

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Planning & organization: ESA-Bio5, **RWTH Aachen University** (DE)
Bioassay battery selection: by participants of the **WG Bio meeting**
ILS participants selection: **query** on interest & bioassay availability

Clean water sample extract: solid phase extraction by UFZ → 10,000x concentrated
Chemicals for spiking: **selection** considering bioassay effects and environmental relevance
Composition: **single chemicals & mixtures, concentrations** for **complete curves** in bioassays

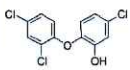
Table 1: Battery of bioassays performed by participant institutes, and composition of spiked samples per bioassay.

Bioassay battery	Participant institutes and respective countries: RWTH (DE)	BfG (DE)	Ifremer (FR)	IVM (NL)	Recetox (CZ)	INERIS (FR)	Entox (AU)	ITM (SE)	Ecotox Centre (CH)	ISSeP (BE)	Waternet (NL)	Sample code	Chemical spiking of water extract
Algae - Freshwater algal growth inhibition test with unicellular green algae					X		X		X		X	A	TCS 0.1 µg/µL
												B	ACR 10 µg/µL
												C	TCS 0.1 µg/µL, ACR 10 µg/µL, EE2 100 ng/µL
FET - Fish embryo acute toxicity test with Danio rerio	X		X		X							A	TCS 3 µg/µL
												B	ACR 2 µg/µL
												C	TCS 3 µg/µL, ACR 2 µg/µL, EE2 100 ng/µL, 3-NBA 2 pg/µL
Daphnia - Daphnia magna acute immobilisation test						X		X		X	X	A	TCS 1 µg/µL
												B	ACR 15 µg/µL
												C	TCS 1 µg/µL,ACR 15µg/µL, EE2 100 ng/µL, 3-NBA 2 pg/µL
YES - Yeast Estrogen Screening Assay		X			X					X		A	EE2 100 ng/µL
												B	TCS 1 µg/µL, ACR 2 µg/µL, EE2 100 ng/µL,
ER-Luc - Cell-based estrogen receptor reporter gene assay	X			X		X	X					A	EE2 1 ng/µL
												B	TCS 1 µg/µL, ACR 2 µg/µL, EE2 1 ng/µL
Ames - Ames fluctuation assay		X		X			X					A	3-NBA 2 pg/µL
												B	TCS 0.11 µg/µL,ACR 2 µg/µL, EE2 100 ng/µL, 3-NBA 2 pg/µL

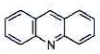
Spiking in water extract 10.000 x

DE: Germany, FR: France, NL: the Netherlands, CZ: Czech Republic, AU: Australia, SE: Sweden, CH: Switzerland, BE: Belgium

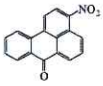
Chemicals for spiking:



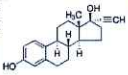
Triclosan (TCS)
CAS 3380-34-5



Acridine (ACR)
CAS 260-94-6



3-nitrobenzanthrone (3-NBA)
CAS 17117-34-9



17-α-ethinylestradiol (EE2)
CAS 57-63-6

Inter-laboratory comparison

Dose-response curves → quite comparable results across different laboratories:

Daphnia:
2-parameter Weibull function

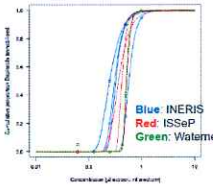


Fig. 1: Dose-response curves for the 48h Daphnia test for sample A (TCS).

Inter-sample comparison

Dose-response curves for the single compounds or the mixture spiking:

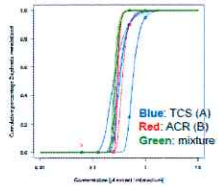


Fig. 2: Dose-response curves for the 48h Daphnia test from INERIS.

ER-Luc:
3-parameter logit function

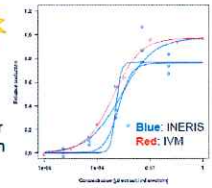


Fig. 3: Dose-response curves for the ER-Luc assay for sample A (EE2).

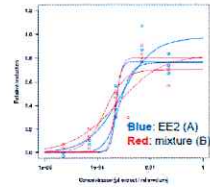


Fig. 4: Dose-response curves for the ER-Luc assay from INERIS.

EC_{50} values from the different laboratories in the same range for the different samples:

Table 2: EC_{50} values in µl extract / ml medium from the 48h acute Daphnia immobilisation test (95% confidence limits).

Sample	INERIS	ISSeP	Waternet
A	0.351 (0.320-0.382)	0.478 (0.372-0.584)	0.516 (0.471-0.560)
B	0.340 (0.196-0.484)	0.254 (0.239-0.269)	0.269 (0.206-0.332)
C	0.265 (0.212-0.319)	0.221 (0.207-0.234)	0.255 (0.193-0.317)

Table 3: EC_{50} values in µl extract / ml medium from the ER-luc assay (95% confidence limits).

Sample	INERIS	IVM
A	$5.90*10^{-4}$ ($2.66*10^{-4}$-$9*10^{-4}$)	$4.59*10^{-4}$ ($3.75*10^{-4}$-$5*10^{-4}$)
B	$3.34*10^{-4}$ ($1.48*10^{-4}$-$5*10^{-4}$)	$8.62*10^{-4}$ ($2.55*10^{-4}$-$1.5*10^{-3}$)

Outlook & Expected outcomes:

- Integrated statistical analysis of the data was performed
- Bioassays produced mostly highly comparable results, even when protocols differed strongly
- This exercise is considered to be a very important step towards the implementation of bioanalytical monitoring tools, where harmonised methods for data analysis and results evaluation are crucial
- The experience and outcomes of this study will shortly be published in a peer review journal, discussing the capabilities, advantages and also the limitations of bioanalytical water quality monitoring and management

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