



The Combined Algae Assay - A Promising Tool for Water Quality Assessment

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and Inge Werner

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WATER QUALITY

Too much pesticide on Swiss strawberries, says report

22/06/2016 BY LE NEWS

Laboratory analysis of conventionally grown strawberries picked from strawberry farms in **Thurgau** and Bern's **Seeland region**, along with samples from Switzerland's supermarkets, shows they contain high levels of pollution.

Problematic pesticide levels in Swiss streams

By *Susan Misicka*

SWI swissinfo.ch





Overview

- Why a combined algae assay?
- Overview on the test system
 - How is the assay performed?
 - What are the measurement parameters
 - Comparison to other standardised algae assays
- Performance of the combined algae assay
 - What is the variability of the assay?
 - Examples for dose-response curves
- Examples for projects
 - International studies
 - Ozonation test procedure
 - Module Ecotoxicology / EcolImpact
- Summary and Outlook



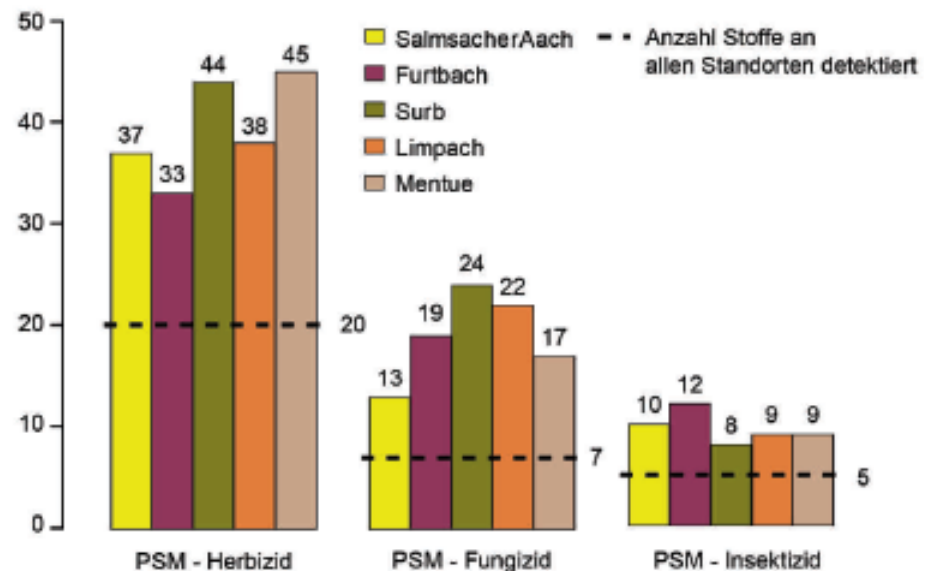
Why a combined algae
assay?



Pesticides in Swiss Streams

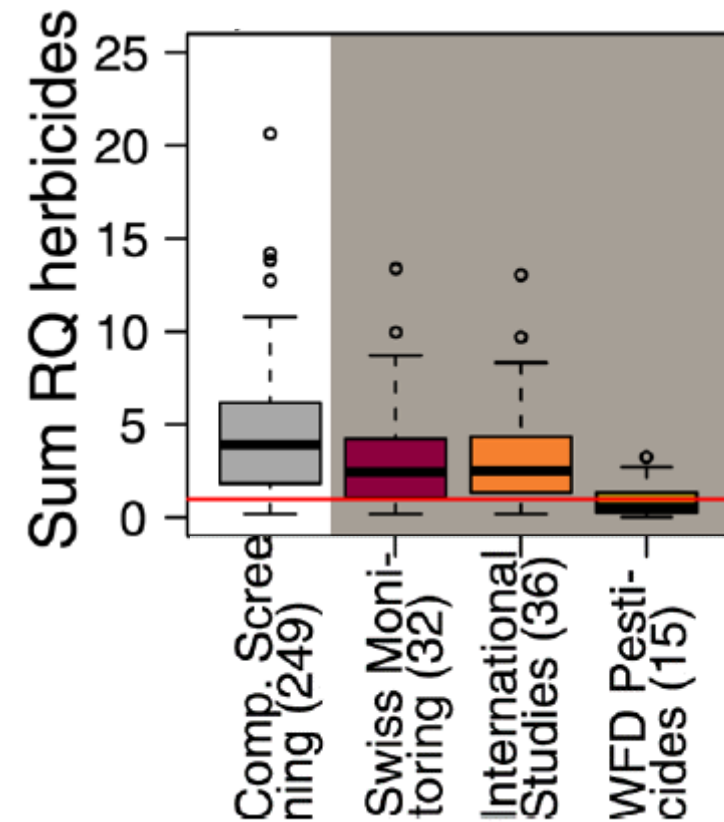
- Project NAWA SPEZ 2012
- 5 mid-sized streams in CH
- Diverse land use (e.g. wheat, fruits, vine)
- 5 regions (TG, ZH, AG, SO, VD)
- 2-week composite samples
- 300 active ingredients (plant protection products and biocides)

Number of detected herbicides, fungicides and insecticides per site



Herbicides in Swiss Streams

Name	Detektionshäufigkeit (Anteil Nachweise > 5ng/l)	Maximale Konzentration (ng/l)	Anzahl Über- schreitungen des CQK	Anzahl Standorte mit Nachweis der Substanz
S-Metolachlor	98%	960	9	5
Terbutylazin	62%	630	6	5
Isoproturon	67%	350	1	5
Diuron	60%	52	13	5
Flufenacet	44%	290	3	5
CMI	9%	510	2	2
Prosulfocarb	44%	690	1	4
Cyprodinil	38%	330	1	4
Linuron	38%	270	1	4
Dicamba	20%	1400	1	3





Why a Combined Algae Assay?

- **Six PSII inhibiting herbicides** included as priority substances under **EU WFD**
- **Environmental Quality standards:**
 - available and applied as regulatory tool in EU WFD
 - will soon be included in Swiss Water Protection Ordinance
- **PSII inhibitors** frequently applied and **present at environmentally relevant concentrations** in (Swiss) surface waters.
- **Specific action on primary producers:**
 - Direct effects on photosynthesis
 - Indirect effects on growth and organisms' capability of coping with multiple stressors.



Combined Algae assay as promising tool to measure these effects

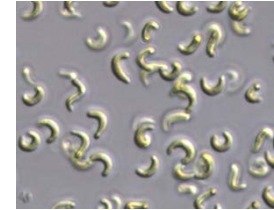


Overview on the test system



Combined Algae Assay (Escher et al., 2008, JEM 10)

- **Test organism:** single cell freshwater green algae (*Pseudokirchneriella subcapitata*)



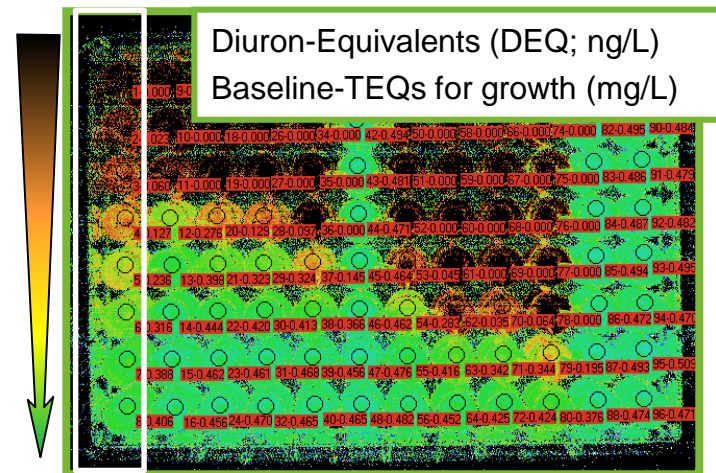
- **Test principle:**

Detection of effects on

- 1) photosynthesis (2h)
- 2) growth of algae (24h)

- **Endpoints:** Inhibition of photosynthesis-, growth (%)

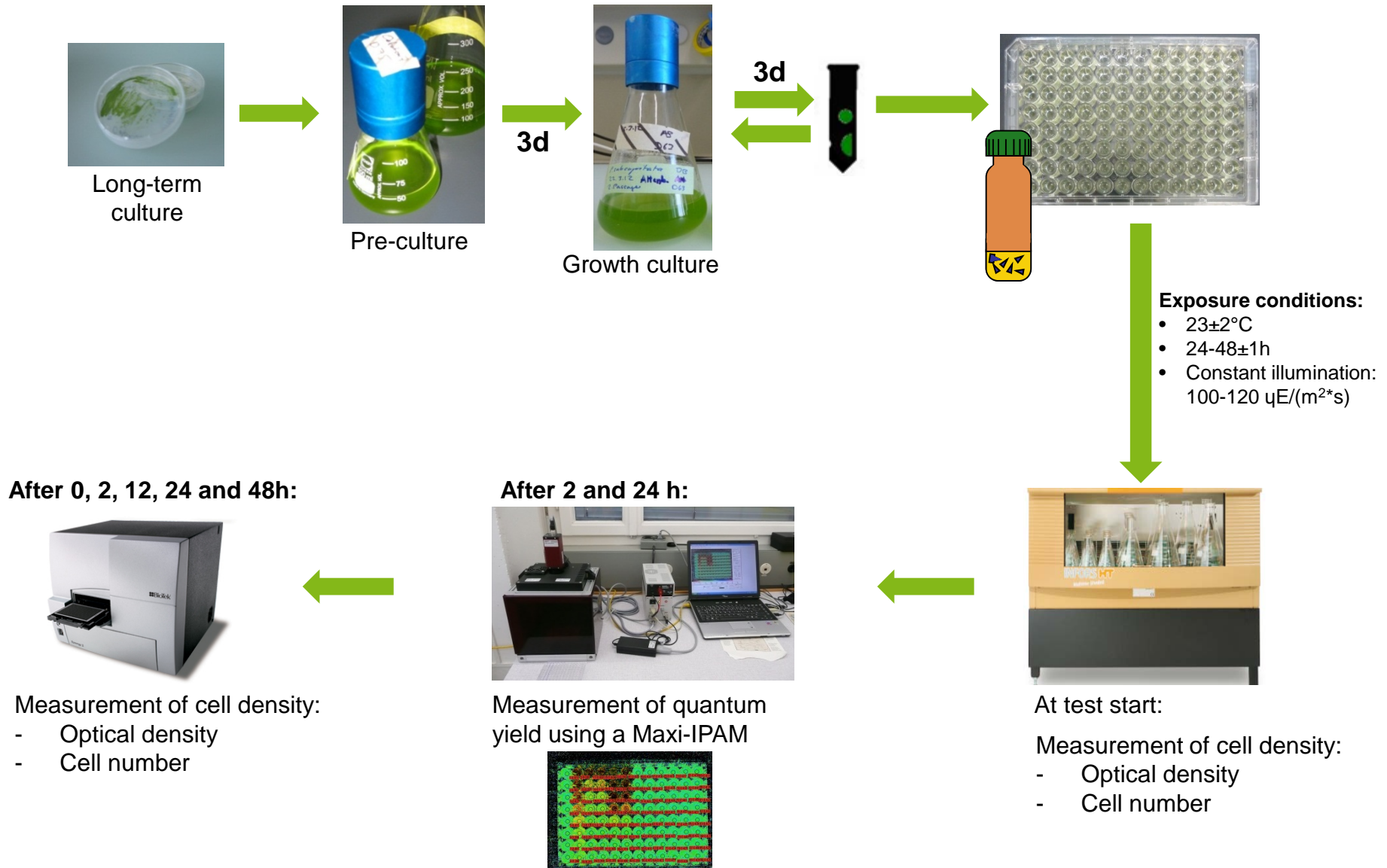
- **Toxicity parameters:** EC_x, Diuron-equivalent-concentration (DEQ), Baseline-toxicity-equivalent-concentration (baseline-TEQ)



Diuron



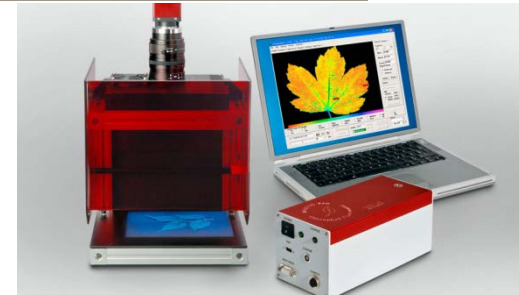
How is the Combined Algae Assay Performed?





Measurement of PS II Inhibition

Maxi-Imaging-Pulse-Amplitude-Modulation (Maxi-IPAM)



Walz, 2012

- Fluorescence excitation by
 - **Pulse-modulated measurement light** (no or very little photosynthetic activity)
 - **Actinic light** (photosynthetic activity)
 - **Saturation pulses** (complete closure of all PSII-reaction centers)
- **Photosystem II inhibiting herbicides** (e.g. diuron):
 - Lead to a **strong increase in fluorescent yield**
 - **Excitation energy** (normally converted to chemical energy) → **emitted as fluorescence**,
 - **Fluorescence** → can be measured with a **high sensitivity**,
 - Excitation of chlorophyll fluorescence reacts on a high number of compounds acting on the photosynthesis.



Performance of the assay



Test Parameters of the Combined Algae Assay

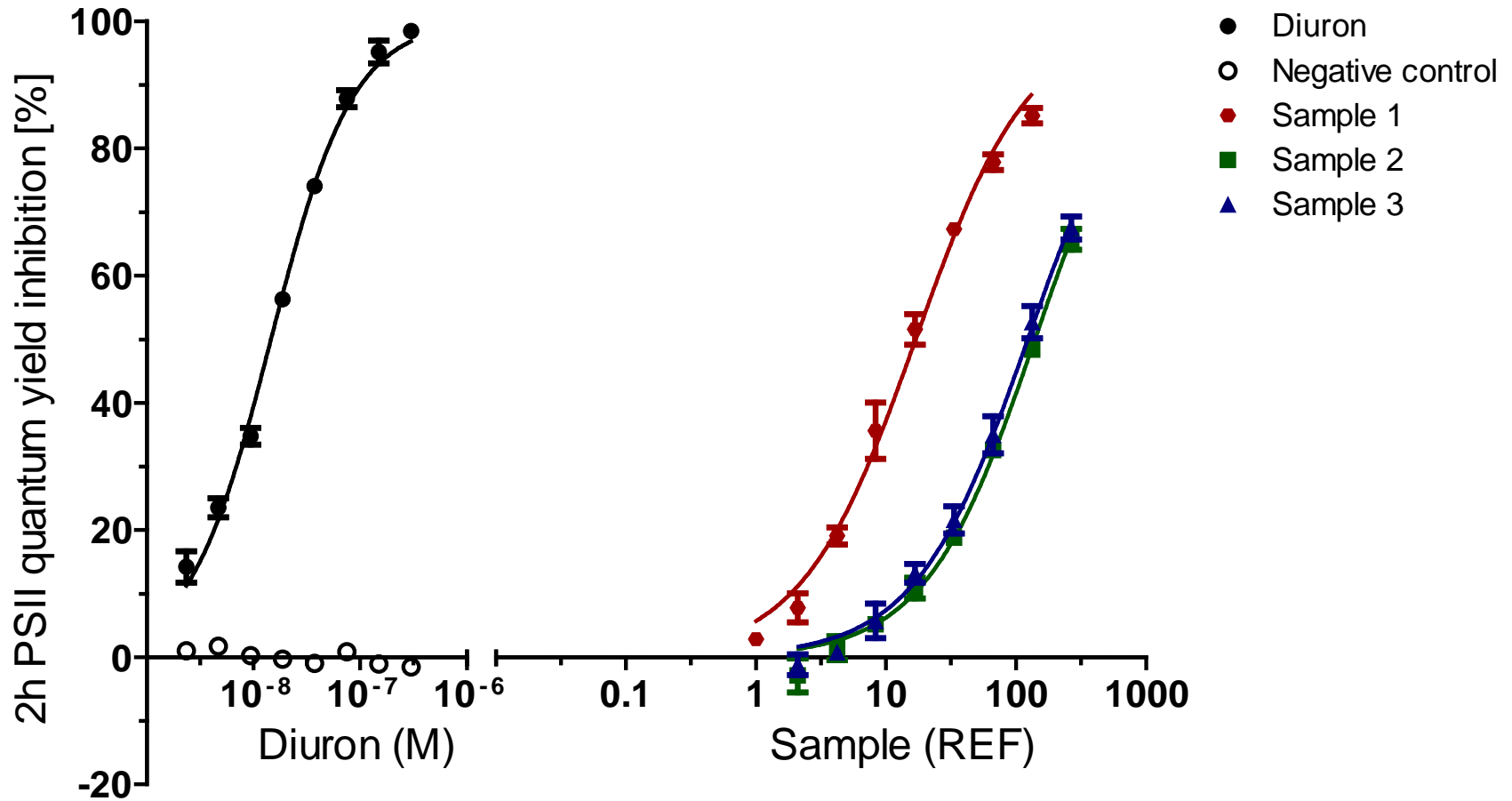
In comparison to standardized algae assays

Assay	Starting cell density / ml	Reference compound(s)
ISO	$5 \cdot 10^3 - 10^4$	3,5-DCP, $K_2Cr_2O_7$
OECD	$5 \cdot 10^3 - 10^4$	3,5-DCP, $K_2Cr_2O_7$
Environment Canada	ca. 10^4	Phenol, $ZnSO_4$, $CuSO_4$
Combined algae assay (48h, Altenburger medium)	ca. $1 \cdot 10^5$	Diuron
Combined algae assay (24h, Talaquil medium)	ca. $2 \cdot 10^5$	Diuron



Dose Response Curves

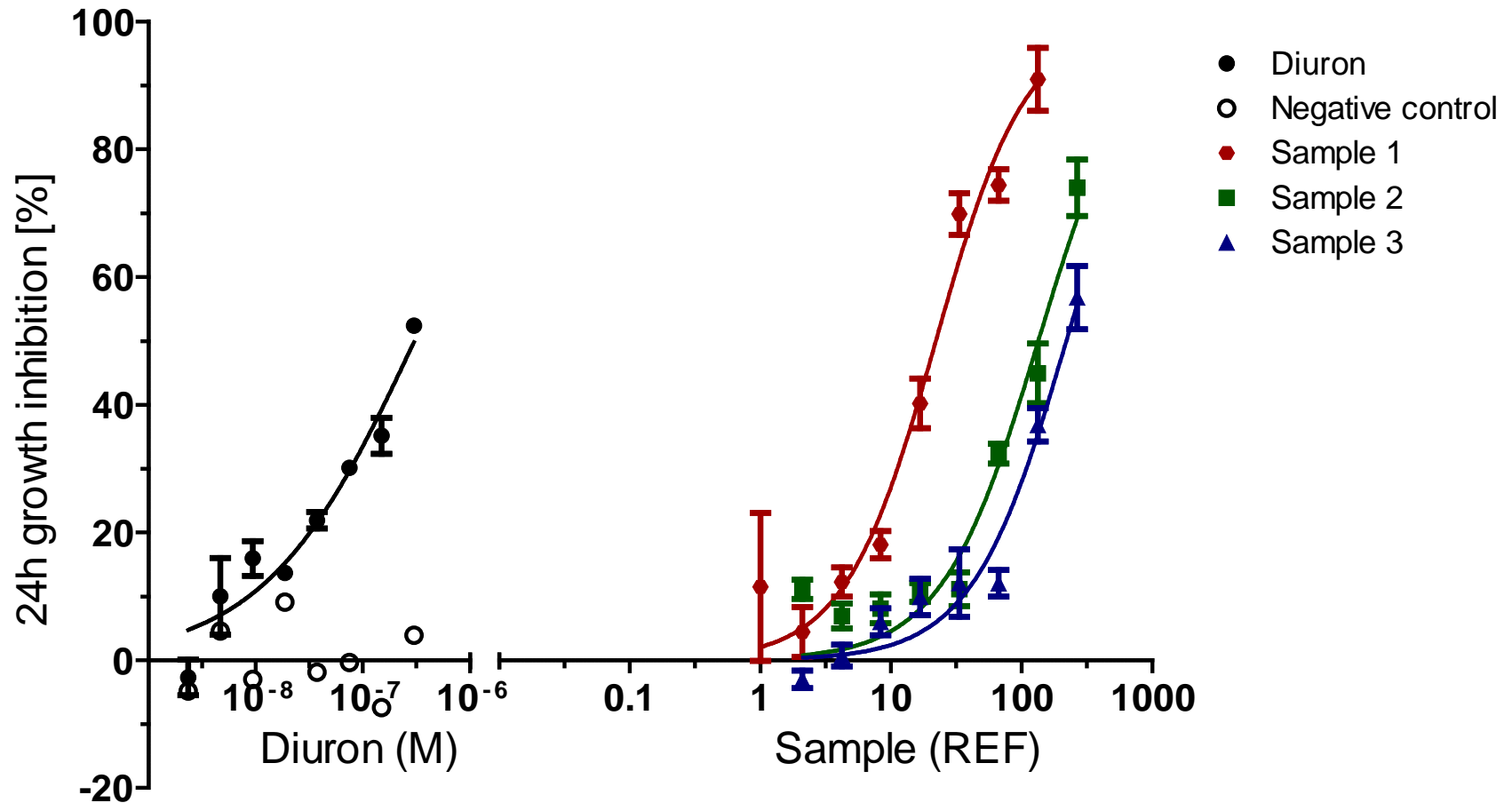
PSII-Inhibition (2h)





Dose Response Curves

Growth (24h)

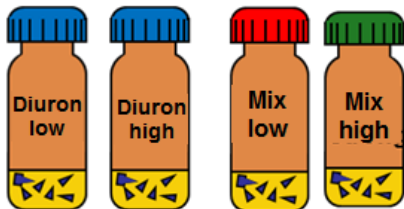




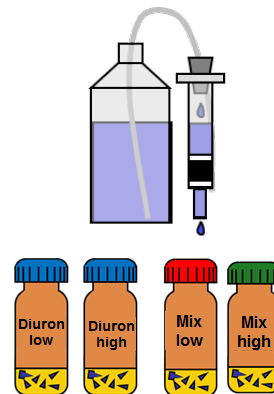
Validation of the Combined Algae Assay at the Ecotox Centre

- Preparation of **reconstituted environmental samples** with environmentally relevant concentrations of PSII inhibitors (diuron or mixture of diuron, isoproturone, terbutryne and terbutylazine):
 - Representing WWTP effluent → **Diuron_{high} / Mix_{high}: 500 ng DEQ/L**
 - Representing surface water → **Diuron_{low} / Mix_{low}: 50 ng DEQ/L**

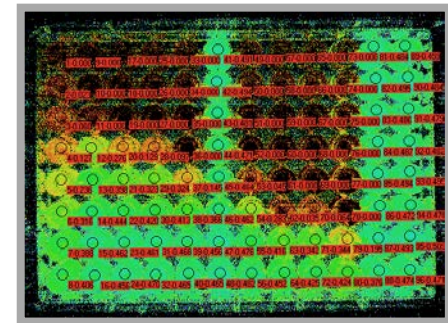
Standards



Standards + Extraction



Bioassay



✓ Intraday / interday variability

✓ Variability including extraction



Validation of the Combined Algae Assay: Summary

- **Detection limits** enable measurement of samples below the chronic environmental quality standard for Diuron (70 ng/L in CH, 200 ng/L in EU) (with sample enrichment by solid phase extraction (SPE)).
 - **Recovery:** in mixtures with low and high PSII inhibitor concentrations between 81 and 102%
 - **Bioassay variability:** between 5 and 11%
 - **Variability of bioassay and SPE:** between 7 and 17%
 - **Recovery in spiked environmental sample:** between 99 and 125%
- ➡ **Bioassay results correspond to requirements for chemical analysis**
- ➡ **Combined algae assay can be applied for water quality evaluation of wastewater and polluted surface waters.**



Project Examples



Examples for application of the assay



Available online at www.sciencedirect.com



Biosensors and Bioelectronics 22 (2007) 2554–2563

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www.elsevier.com/locate/bios

Methodology and evaluation of a highly sensitive algae toxicity test based on multiwell chlorophyll fluorescence imaging

Ulrich Schreiber^a, Pamela Quayle^b, Sven Schmidt^c, Beate I. Escher^d, Jochen F. Mueller^{b,*}

Toxic pressure in the Dutch delta measured with bioassays

Trends over the years 2000–2009

Report 607013013/2010

J. Struijs | E. van der Grinten | T. Aldenberg

National Institute for Public Health and the Environment

Ministry of Health, Welfare and Sport

Science of the Total Environment 576 (2017) 785–795

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journal homepage: www.elsevier.com/locate/scitotenv



Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams

Peta A. Neale^{a,b,1}, Nicole A. Munz^{c,d,1}, Selim Ait-Aïssa^e, Rolf Altenburger^f, François Brion^e, Wibke Busch^f, Beate I. Escher^{a,b,f,g,*}, Klára Hilscherová^h, Cornelia Kienleⁱ, Jiří Novák^h, Thomas-Benjamin Seiler^j, Ying Shao^j, Christian Stamm^e, Juliane Hollender^{c,d}

PAPER

www.rsc.org/jem | Journal of Environmental Monitoring

Monitoring of the ecotoxicological hazard potential by polar organic micropollutants in sewage treatment plants and surface waters using a mode-of-action based test battery^{†,‡}

Beate I. Escher,^{a,*} Nadine Bramaz,^a Pamela Quayle,^{ab} Sibylle Rutishauser^{ab} and Étienne L. M. Vermeirssen^a

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Article

pubs.acs.org/est

Identification of Photosynthesis Inhibitors of Pelagic Marine Algae Using 96-Well Plate Microfractionation for Enhanced Throughput in Effect-Directed Analysis

Petra Booij,^{*,†} A. Dick Vethaak,^{†,§} Pim E. G. Leonards,[†] Sascha B. Sjollem,[‡] Jeroen Kool,^{||} Pim de Voogt,^{‡,⊥} and Marja H. Lamoree[†]

Available online at www.sciencedirect.com



Biosensors and Bioelectronics 21 (2006) 2086–2093

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Phytotoxicity of surface waters of the Thames and Brisbane River Estuaries: A combined chemical analysis and bioassay approach for the comparison of two systems

S.M. Bengtson Nash^{a,*}, J. Goddard^b, J.F. Müller^a

^a The National Research Centre for Environmental Toxicology (EnTOX), The University of Queensland (UQ), Coopers Plains, Qld, Australia

^b The UK Environment Agency, South East Area, Thames Region, Surrey GU16 7SQ, UK

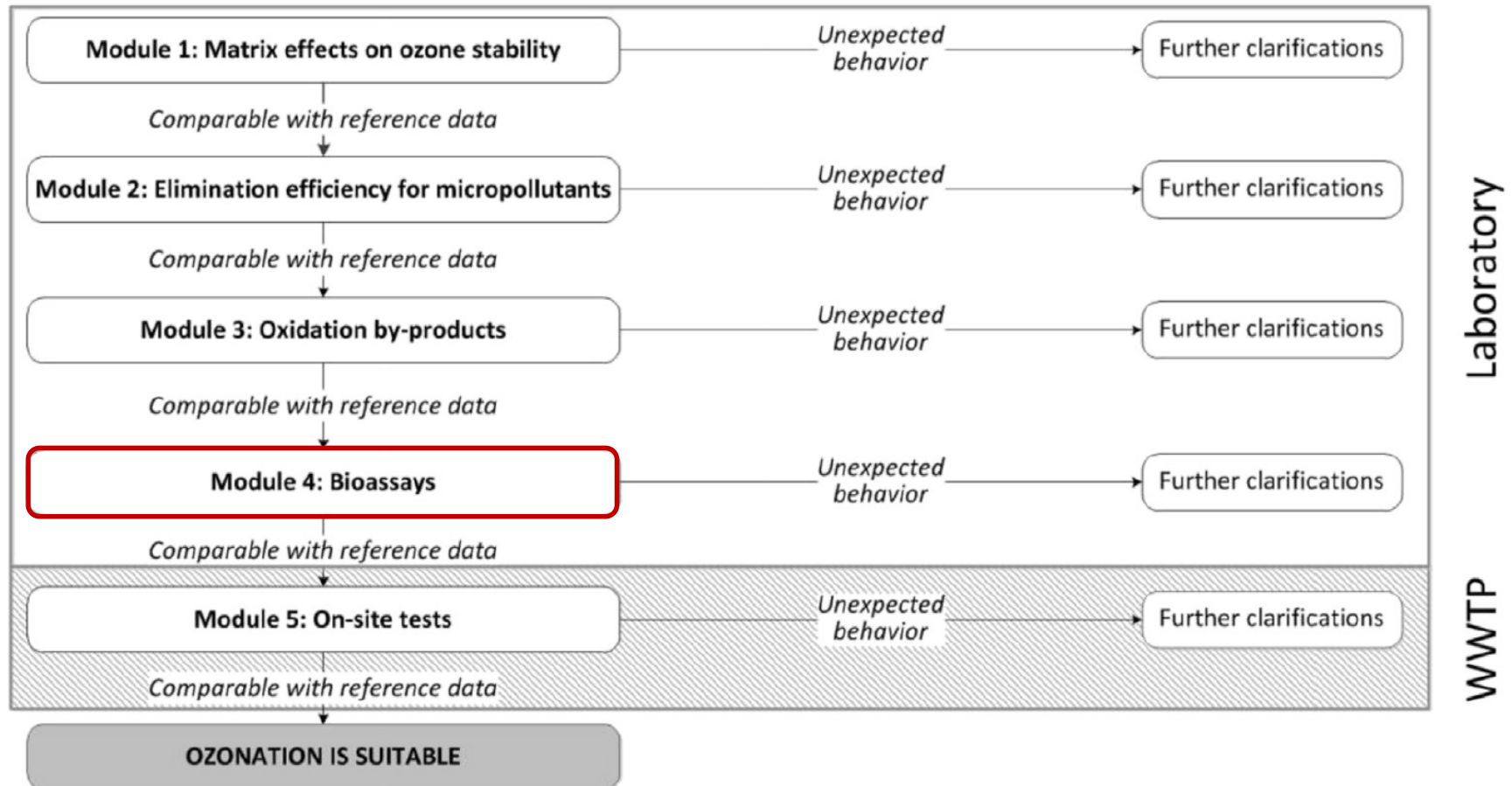
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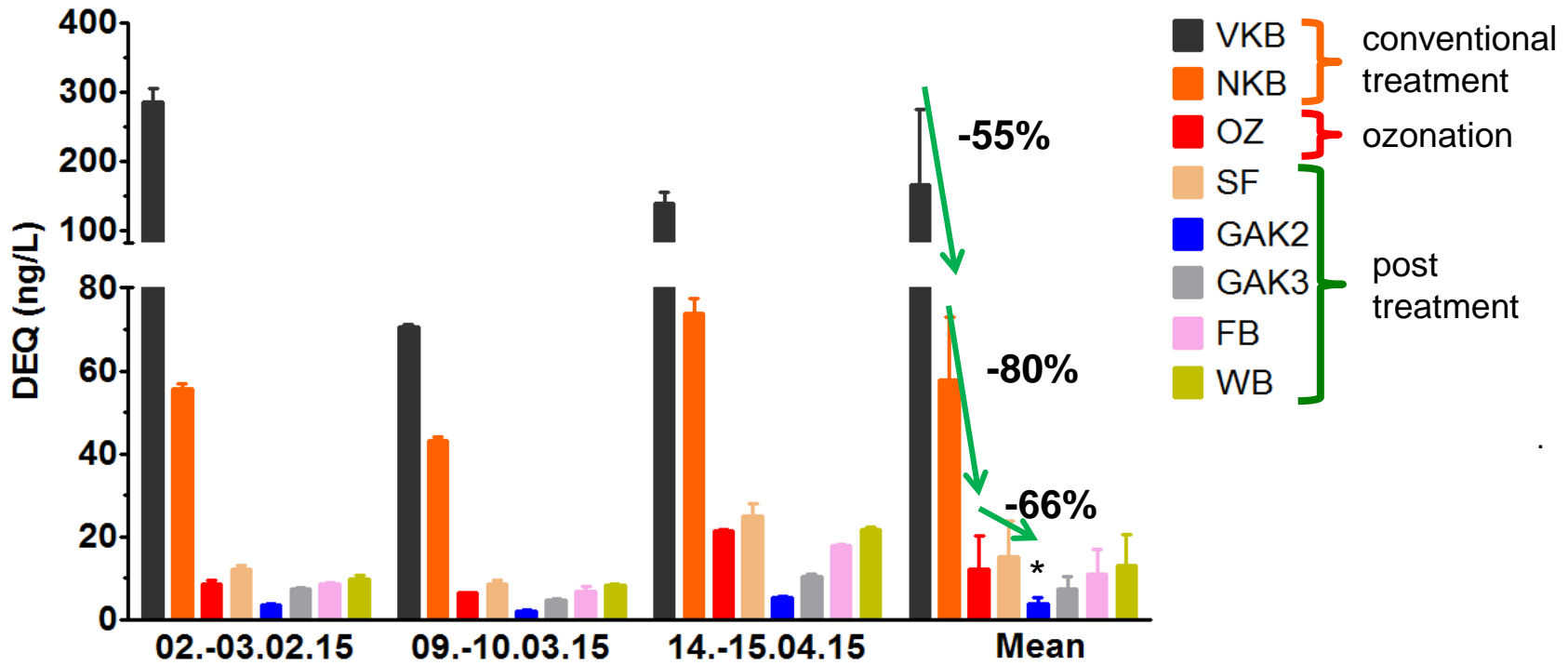
Evaluation of Advanced Wastewater Treatment

Novel test procedure to evaluate the treatability of wastewater with ozone





ReTREAT: Algae Test – Photosynthesis Inhibition



- Considerable decrease of toxicity after biological treatment (NKB) and ozonation (Oz)
- GAK2 shows a significant difference between post treatments (-66%)
- Overall elimination 89 – 97%

VKB:	mechanical treatment
NKB:	secondary clarifier/biological treatment
OZ:	ozonation
SF:	sand filtration
GAK2:	granulated activated carbon (fresh)
GAK3:	granulated activated carbon (preloaded)
FB:	fixed bed
TFB/WB:	turbulent fluidized bed

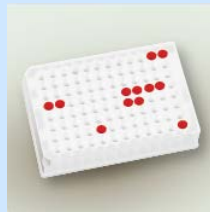


A Bioassay-Based Evaluation Concept

High % wastewater in the waterbody



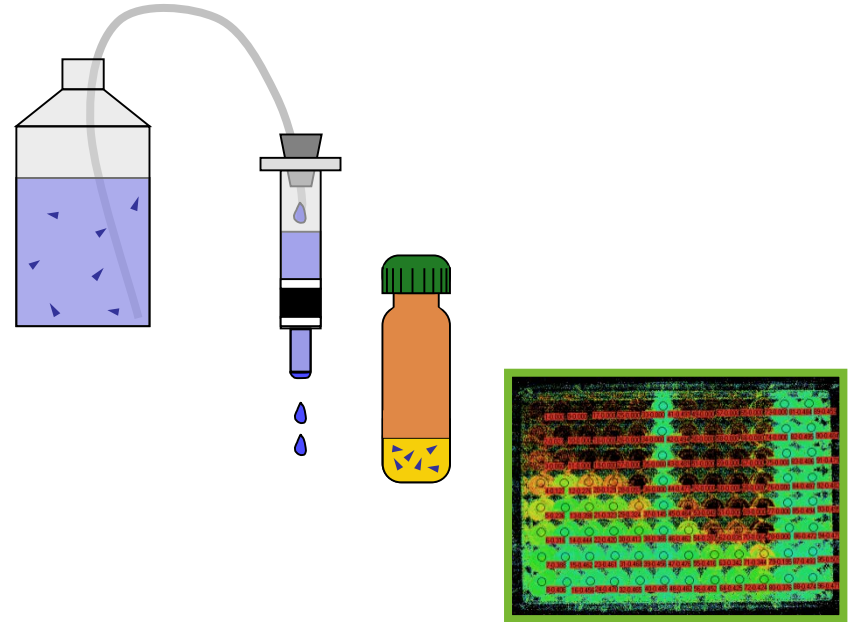
Effect-based screening of wastewater



Effect concentrations in the water body



Ecotoxicological assessment of contamination



Evaluation		Compliance with quality criterion / trigger value
	very good	passed
	good	
	moderate	exceeded
	insufficient	
	poor	



Water quality assessment

based on risk assessment procedures

$$\text{Risk quotient (RQ)} = \frac{\text{BEQ}}{\text{EQS}} =$$

<1 Trigger value passed

>1 Trigger value exceeded

With:

BEQ = Bioanalytical equivalent concentration

EQS = Environmental quality standard

Applied chronic EQS proposal for the evaluation:

- Photosystem II-inhibiting effects: EQS for Diuron = 70 ng/L



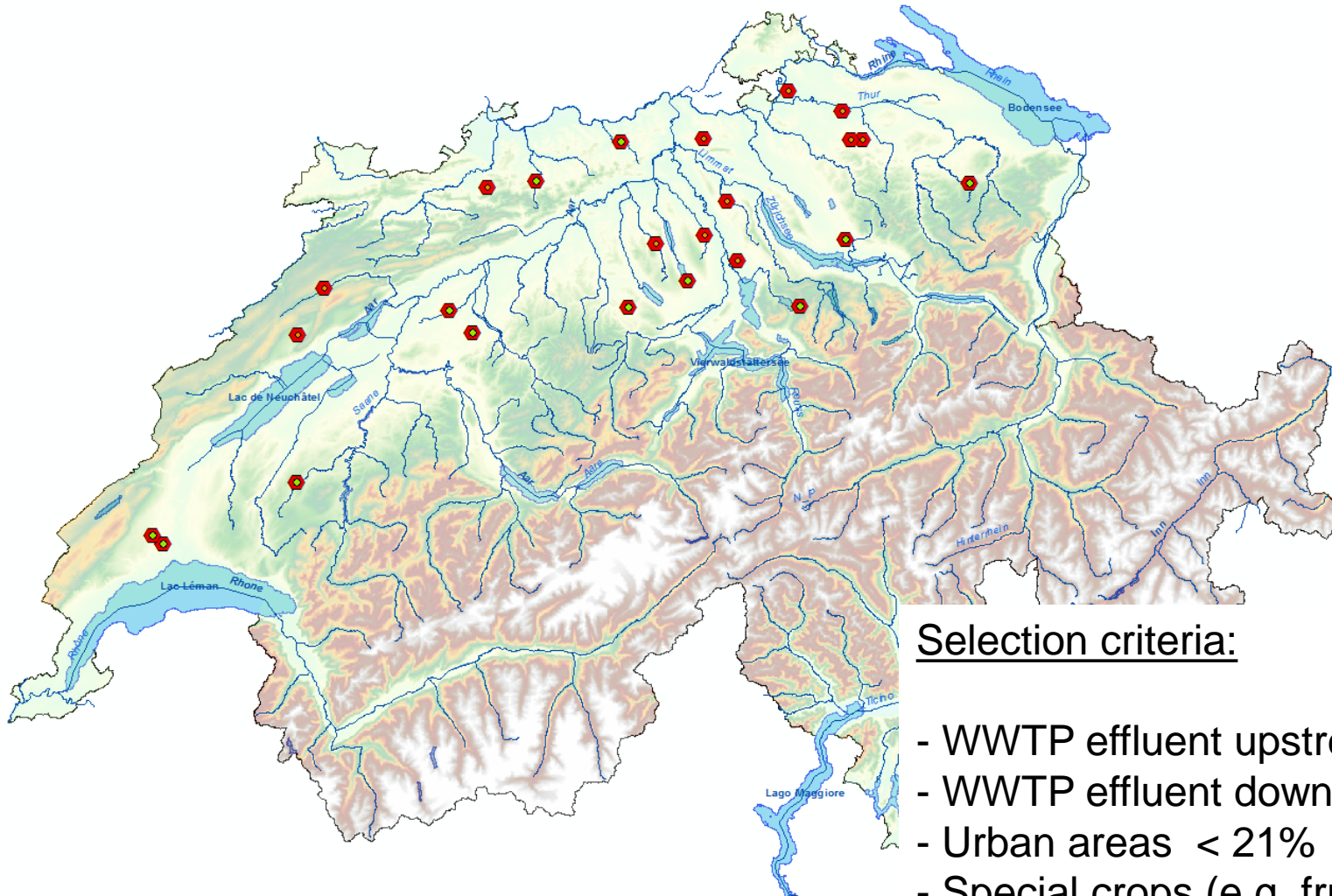
Ecotoxicological Assessment of the Results

Suggestion for an evaluation scheme in 5 classes

Evaluation		Criterion/description		Compliance with quality criterion / trigger value
	very good	DEQ <	7 ng/L	passed
	good	7 ng/L ≤ DEQ <	70 ng/L	
	moderate	70 ng/L ≤ DEQ <	175 ng/L	exceeded
	insufficient	175 ng/L ≤ DEQ <	700 ng/L	
	poor	DEQ ≥	700 ng/L	



Case study: Photosystem II-inhibiting effects in Swiss WWTP effluents and streams (Project EcolImpact)

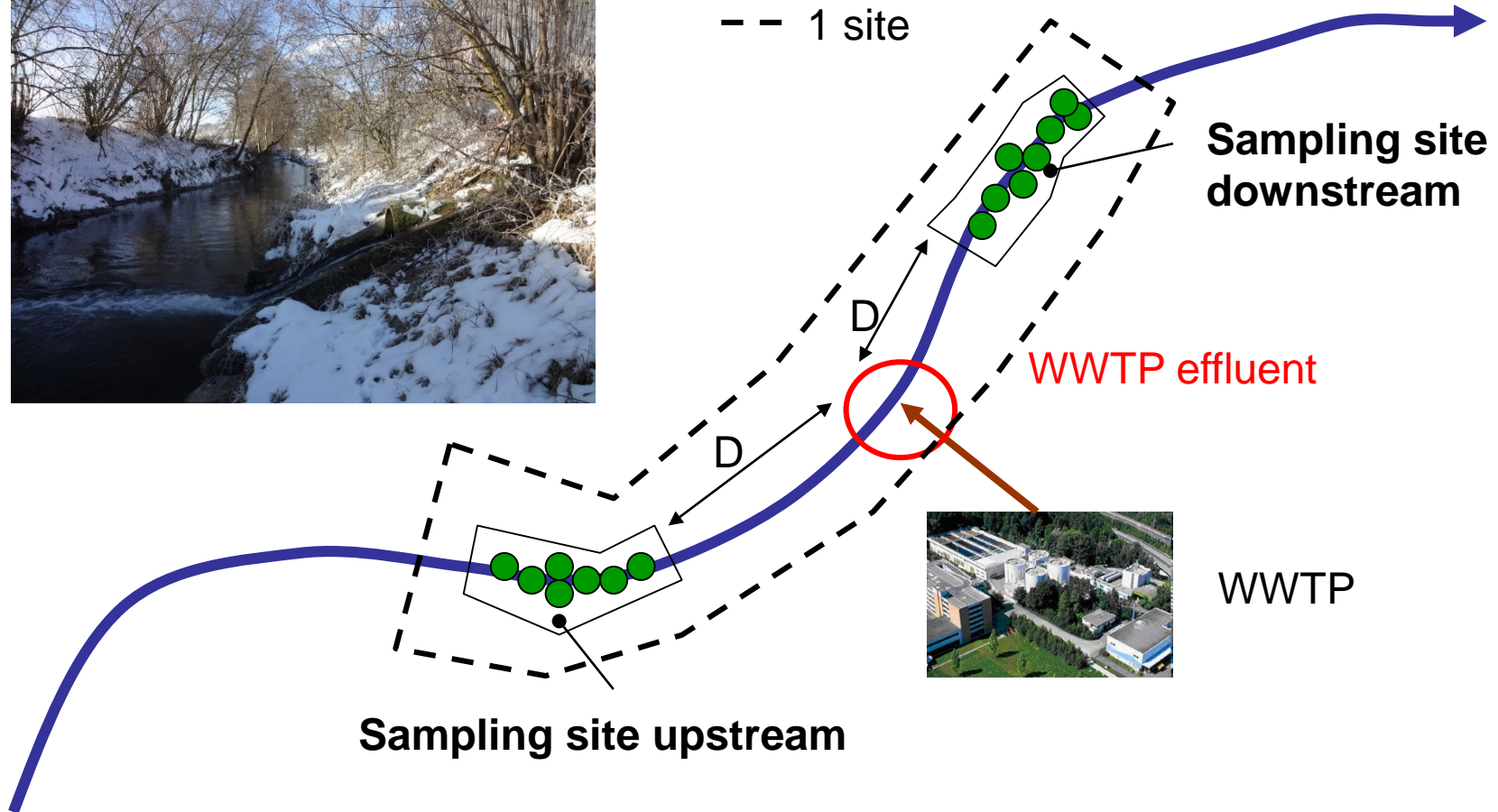


Selection criteria:

- WWTP effluent upstream < 5%
- WWTP effluent downstream > 20%
- Urban areas < 21%
- Special crops (e.g. fruits) < 10%



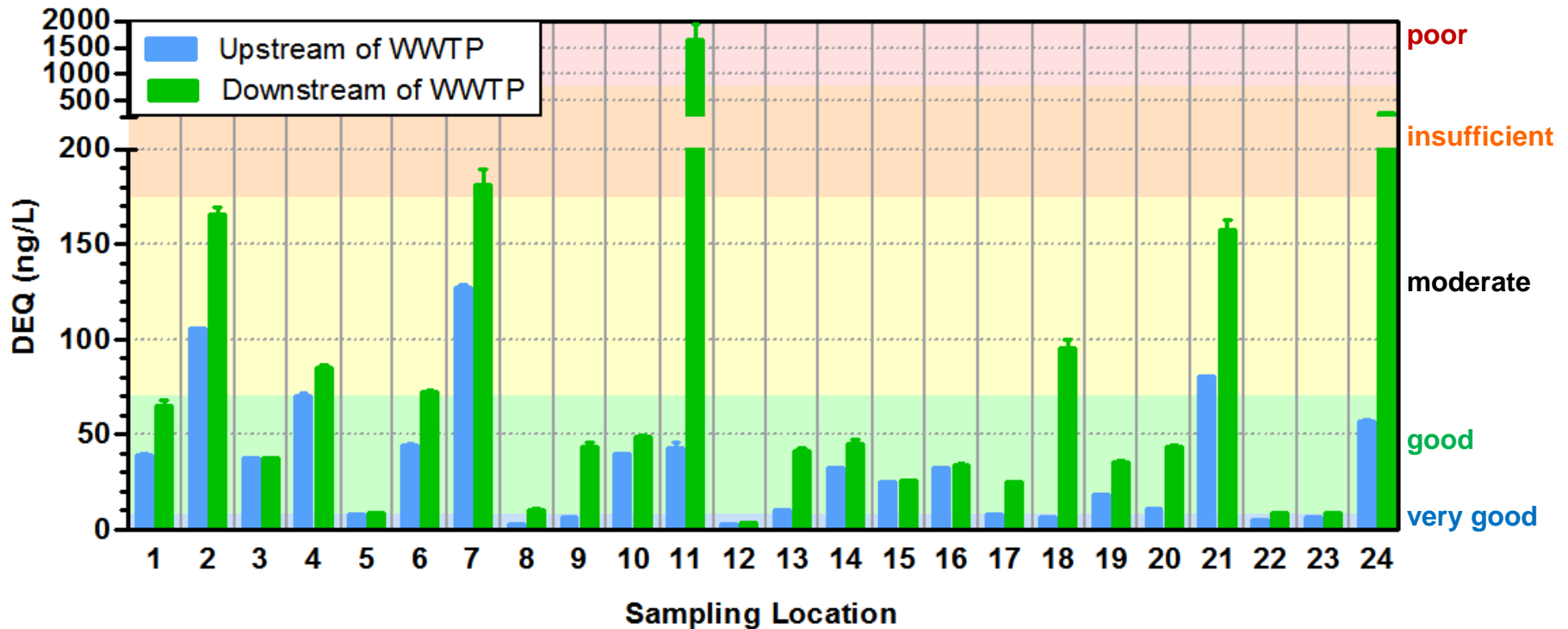
EcolImpact: Sampling sites in the rivers



Distance D: a dozen up to a few 100 m (dependent on mixture conditions)



Combined Algae Assay – Results Photosynthesis Inhibition



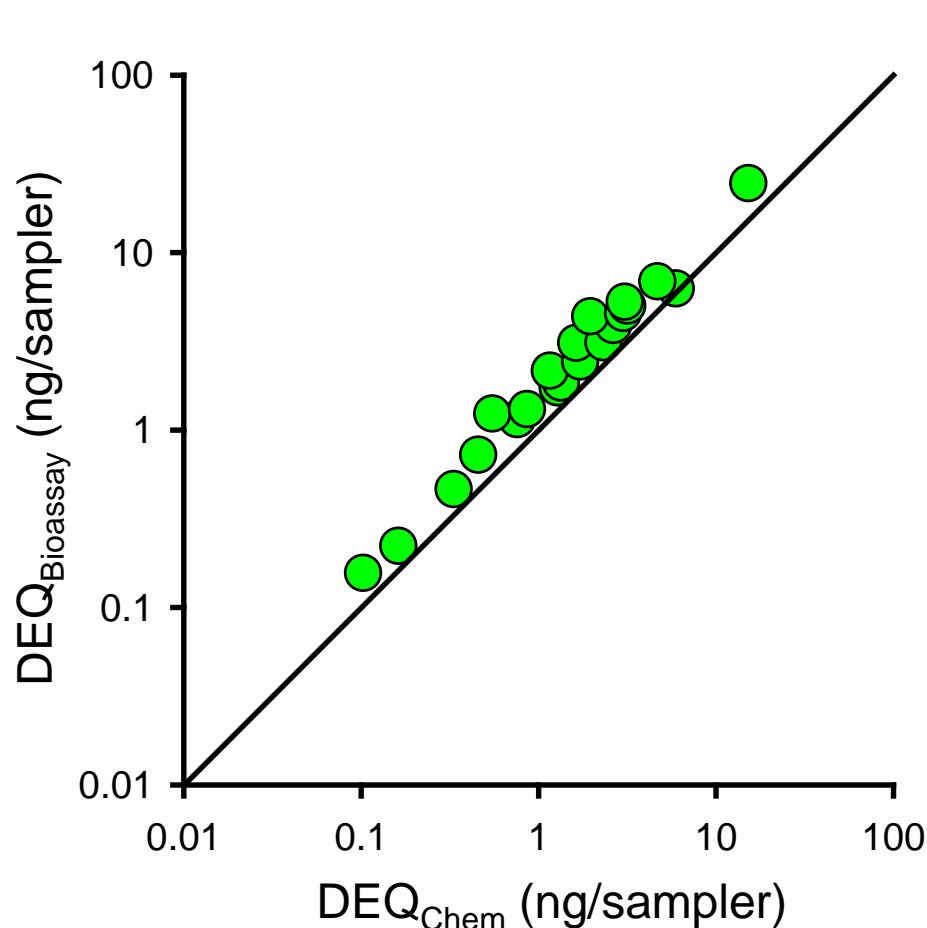
→ Diuron EQS exceedance upstream in 3 of 24 sampling locations and downstream in 8 of 24 sites

DEQ = Diuron-Äquivalenz-Konzentration



Algae Assay: Correlation DEQs from Bioassay and Chemical Analysis

21 WWTP effluents sampled with passive samplers



DEQ_{chem} based on concentrations of measured PSII inhibitors (using LC-MS/MS):

- Atrazine
- Diuron
- Irgarol
- Isoproturon
- Terbutryn
- Terbutylazin

Conc. summed up taking into account their respective relative potencies



Summary and Conclusions

Possibilities

- Combined algae assay enables measurement of several relevant endpoints,
- Specific effects of PSII inhibitors and growth inhibition can be detected with a single assay,
- Test duration of 24-48 h shorter as for already standardised assays (e.g. ISO, OECD),
- Use of 96-well-plate enables measurement of higher numbers of samples and/or dilution steps.



Summary and Conclusions

Points to address

- Optimisation of growth endpoint needed
- Adjustment of cell density to enable exponential growth over 48 h



Aim: ISO standardisation of the assay

Would you be interested?



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