Open Meeting of the SETAC Plants Interest Group

CHAIRS:
DAVE OLSZYK (US EPA)
RENA ISEM ER (BAYER AG)
Not a member of the SETAC Plants Interest Group yet?

Make sure to join today! It is free and you will become part of a great scientific community.

https://www.setac.org/group/IGPlants
The SETAC Plants IG Steering Committee

How about you?

Verena Sesin
Canadian Nuclear Safety Commission

Judith Arnolds
South African National Biodiversity Institute

Joshua Arnie
Eurofins US

Valeska Contardo
Umweltbundesamt

Élisabeth M. Gross
Université de Lorraine

Aida M Farag
Columbia Environmental Research Center

Guido Gonsior
GG Biotech Design

Dave Olszyk
US EPA

Hank Krueger
Eurofins US

Rena Isemer
Bayer AG
The SETAC Plants Interest Group Steering Committee is looking for new members from **academia** sector.

Students are very welcome!!

Please send your application to Roel Evens *roel.evens@setac.org*.

For questions or additional information, please contact the current chairs of the Plants IG: David Olszyk (*Olszyk.David@epa.gov*) or Rena Isemer (*rena.isemer@bayer.com*).

*We are looking forward to your application.*
submit an abstract for the session

3.01 | Bridging ecotoxicology and risk assessment for aquatic and terrestrial plants
Track: Wildlife Toxicology, Ecology and Stress Response

scheduled at the SETAC North America 43rd Annual Meeting in Pittsburgh, PA, USA, from 13-17 November 2022.

The meeting is currently planned as a hybrid format with in-person and virtual components.

The deadline for abstract submissions is 17:00 EDT on 2 June 2022!

Please submit abstracts for poster and platform presentations via the meeting's website at:
https://pittsburgh.setac.org/scientific-program/abstract-submission/

Looking forward to seeing you in November!
VIRTUAL SETAC Plants IG Open Meeting

Minutes of the first VIRTUAL SETAC Plants IG Open Meeting held June 1st 2021 can be found at the group site >.

VIRTUAL SETAC Plants IG Open Meeting 2022

HOLD THE DATE

September 14th 2022

3-5 pm CEST, 9-11 am UTC-4, 6-8 am GMT-7

An invite, including a Teams Link as well as a detailed agenda will be send to you closer to the meeting.

Please feel free to contact the SETAC Plants IG chairs if you would like to propose any topics for the Virtual Open Meeting.
<table>
<thead>
<tr>
<th>time</th>
<th>topic</th>
<th>presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mins</td>
<td>Welcome and Opening</td>
<td>Rena Isemer (Bayer AG)</td>
</tr>
<tr>
<td></td>
<td>Update on SETAC Plants IG</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Glyceria Ring Test</td>
<td>Rita Wedeking (Bayer AG)</td>
</tr>
<tr>
<td>13</td>
<td>Reproduction</td>
<td>Andreas Duffner (Eurofins)</td>
</tr>
<tr>
<td>13</td>
<td>Visual Injury</td>
<td>Gwendolin Kraetzig (Adama)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sebastian Fellmann (Bayer AG)</td>
</tr>
<tr>
<td>13</td>
<td>Algae Ring Test</td>
<td>Eric Bruns (Bayer AG)</td>
</tr>
<tr>
<td>10</td>
<td>Call for New Topics e.g. possibility for a liaison with the Aquatic</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Toxicity Testing Interest Group</td>
<td>Watson-Leung (SETAC, on behalf of ATTIG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Closing</td>
<td>Rena Isemer (Bayer AG)</td>
</tr>
</tbody>
</table>
An OECD Test Guideline for *Glyceria maxima*: Results of the Final Ring-Test

Copenhagen, May 2022
Objective

Guideline development for the monocot *Glyceria maxima* based on TG 239 water-sediment test for *Myriophyllum spicatum*
Experimental

Validity criteria

• Mean total Shoot FW in control plants must at least double during the exposure phase, corresponding to a growth rate of at least 0.05 d\(^{-1}\) over 14 days.

• CoV for Yield Shoot FW of the control plants, based on measurements form test initiation to test termination, should be ≤ 35% between the replicates.

• Control plants must not show any visual symptoms of chlorosis.

Development and improvement of the key protocol

• Assessment parameters: Leaf Length (LL), Shoot FW, Shoot DW – no roots
• Importance of a sufficient establishment phase
• Re-specification of starting material to achieve the CoV validity criteria of 35%
• Defined water:sediment ratio 2.25:1

GG BioTech Design
Key results – Comparison between ring-tests

- 9 labs: 3 valid tests, 2 further valid tests after exclusion of outliers, 1 test excluded, 3 invalid tests.
- Failure to meet validity criteria can be attributed to high variability on Day 0 and deviations from the protocol.
- High variability in representative pants (used for FW & DW assessment, Day 0) combined with the lack of uniformity between representative and test plants was identified as key factor affecting test validity.

Mean CoVs for all assessment parameters on Day 0 were improved relative to the previous ring-tests, indicating improved standardization of test system and test plant material.

SFW: Shoot Fresh Weight / SDW: Shoot Dry Weight / LL: Leaf Length

SETAC Copenhagen, May 2022
Key results - Comparison between ring-tests

Control growth rates were similar in all-tests and control yields for SFW, SDW & LL were lower in Ring-tests 2 and 3 than in Ring-test 1 (larger starting material) and.

Repeatability CoVs (within the lab) for control plants and Day 14

SFW: Shoot Fresh Weight / SDW: Shoot Dry Weight / LL: Leaf Length
Key results – Sensitivity to imazapyr

EC$_{50}$ values were determined for all valid tests although mean values are only presented for parameters where $\geq$6 datapoints are available.

<table>
<thead>
<tr>
<th>Imazapry EC$_{50}$</th>
<th>(µg ai/L; mean &amp; 95% CI n $\geq$6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth rate</strong></td>
<td></td>
</tr>
<tr>
<td>SFW</td>
<td>SDW</td>
</tr>
<tr>
<td>512 (254 – 1031)</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
</tr>
<tr>
<td>SDW</td>
<td>LL</td>
</tr>
<tr>
<td>511 (259 – 1006)</td>
<td>n.d.</td>
</tr>
<tr>
<td>SFW</td>
<td>SDW</td>
</tr>
<tr>
<td>369 (187 – 725)</td>
<td>298 (193-458)</td>
</tr>
<tr>
<td></td>
<td>LL</td>
</tr>
</tbody>
</table>
Derivation of NOECs was possible for all test parameters in all tests. Minimum Detectable Differences (MDD) were improved, with the majority of datasets having an MDD of 40% or lower for most parameters.
Conclusions

• Reducing initial variability in plants is critical for the completion of the valid test.

• The protocol changes introduced for Ring-Test 3 have delivered reductions in variability and CoVs.

• The protocol is fit for the purpose of assessing the effects of chemicals on *Glyceria maxima* and generating endpoints for regulatory risk assessment.

• No further ring-tests required.
Next steps

Submission of the updated protocol to OECD and stakeholders for comment and consideration as OECD Test Guideline

Updates

• Additional recommendation for plant propagation
• Importance of minimizing variability in starting plant material, including the representative plants, will be reinforced
• Number of representative plants measured on Day 0 to be increased to 10 plants
The Glyceria working group operates under the Plant Interest Group of SETAC

Members represents different organizations and stakeholders

- Jo Davis Syngenta, UK (Chair)
- Gertie Arts WUR, NL
- Guido Gonsior GG BioTech Design GmbH, DE
- Johanna Kubitza BASF SE, DE
- Monika Ratte ToxRat, DE
- Rita Wedeking Bayer AG, DE

Acknowledgements to all participation Laboratories

- BASF SE (DE)
- Bayer AG (DE)
- BioChem Agrar GmbH (DE)
- ECT Oekotoxikologie GmbH (DE)
- Eurofins (DE)
- FERA (UK)
- Fraunhofer (DE)
- GG BioTech Design GmbH (DE)
- IME (DE)
- Ibacon GmbH (DE)
- Noak GmbH (DE)
- Mesocosm GmbH (DE)
- Rheinland Pfalz AgroScience GmbH (DE)
- Smithers Viscient (US)
- Toxi-Coop (HU)
- Wageningen Environmental Research
Introduction

Regulation in the EU (1107): A test with a second macrophyte is required when:

- terrestrial plant data indicate high selectivity for monocot or dicot species
- standard *Lemna* and algae test species are not sensitive enough (e.g. EC50 > 1 mg ai/L)

Suitable additional tests species: *Myriophyllum spicatum* (dicot) & *Glyceria maxima* (monocot)

OECD TG 239 for *Myriophyllum spicatum* in a water-sediment test system has been adapted for *Glyceria maxima* (reed sweet grass) and the modified protocol has been trialed in 3 ring tests between 2016 and 2021.
Objectives
Definition of test duration
Identification of robust assessment parameters

Objectives
Assessment of suitability of roots for deriving endpoints
Increased standardization of the experimental set-up to reduce variability

Objectives
Significant improvement of CoV by increased standardization of test system & uniformity of test plants

Outcome
Roots are less reliable to shoots for generating endpoints
Variability must be reduced to <35% control CoV criterion

Outcome
14 days test duration is adequate to achieve doubling of shoot biomass
Leaf length is more robust than shoot height

Outcome
A protocol suitable for adoption as an OECD TG

2016
Ring Test 1 – Isoproturon¹
13 labs

2018
Ring Test 2 – Imazapyr²
11 labs

2021
Ring Test 3 – Imazapyr
9 labs
## Experimental – Key protocol development

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>Ring Test 1 Isoproturon</th>
<th>Ring Test 2 Imazapyr</th>
<th>Ring test 3 Imazapyr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Establishment phase</td>
<td>3 days</td>
<td>1</td>
<td>3-7 days</td>
</tr>
<tr>
<td>Exposure phase</td>
<td>14 and 21 days</td>
<td>14 days</td>
<td>14 days</td>
</tr>
<tr>
<td>Water:sediment ratio</td>
<td>Not defined</td>
<td>Not defined</td>
<td>2.25 : 1</td>
</tr>
<tr>
<td>Starting material</td>
<td>1-3 shoots / pot</td>
<td>1 shoot / pot</td>
<td>1 shoot / pot with 1-3 leaves Height x= 15-25 cm ± 10%</td>
</tr>
<tr>
<td>Water depth over sediment</td>
<td>3 cm</td>
<td>5 cm</td>
<td>3 cm</td>
</tr>
<tr>
<td>Experimental design</td>
<td>Control: 6 replicates</td>
<td>Control: 6 replicates</td>
<td>Control: 6 replicates</td>
</tr>
<tr>
<td></td>
<td>5 Test Concentrations: 4 replicates</td>
<td>6 Test Concentrations: 4 replicates</td>
<td>7 Test Concentrations: 4 replicates</td>
</tr>
<tr>
<td>Assessment parameters</td>
<td>Shoot height, Leaf Length (LL), Shoot FW, Shoot DW</td>
<td>LL, Shoot FW, Shoot DW, Root FW, Root DW</td>
<td>LL, Shoot FW, Shoot DW</td>
</tr>
<tr>
<td>Temperature</td>
<td>22 ± 2 °C</td>
<td>23 ± 2 °C</td>
<td>22 ± 2 °C</td>
</tr>
<tr>
<td>Participated Labs</td>
<td>13</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>
Background
Background
Goals of this working group

• Developing and publishing a standard protocol for testing reproductive endpoints of (non-)crop species under greenhouse conditions
  • Collecting & analyzing available data and experiences
  • Providing guidance for studies, where reproductive endpoints shall be assessed in a regulatory context

• Potentially establishing a ring-test, based on the agreed protocol
Working Group Members

Andreas Duffner (Eurofins, Group lead)
Chris Mihan (Bayer, Group lead)
Bianca Flickinger (BASF)
Dave Olszyk (EPA)
Eugenia Soler (Eurofins)
Fernando Huerta (Eurofins)
Gwendolin Kraetzig (Adama)
Holger Teresiak (agro-check)
Jo Davies (Syngenta)
Sandrine Charles (University of Lyon)

-> New persons are welcome, especially from academia / authorities!
Status update

- Provision of a Sharepoint accessible to all WG members for the collection of working documents, publications, data etc.

- Roadmaps incl. timeline, tasks and responsibilities

- Currently running subgroups:
  - Plant species selection
  - Challenges, Restrictions, Open Questions
  - Protocol elements

  ➢ All subgroups are in close exchange with each other
Subgroup Plant species selection

- Criteria for selection of species
- Collection of species already tested by group members or in literature
- Discussion of species selection
- Species-specific vs. general repro endpoints
- Recommendations for test species
Subgroup Challenges, Restrictions, Open Questions

- Continuous collection over duration of the project
- Research & Discussion
- Alignment & Conclusion / Transparent presentation of options
Subgroup Protocol elements

• Utilization of already existing guidelines
• Adaptation or creation of protocol elements specific to repro needs, incl. test conditions, endpoint selection, assessment dates etc.
• Integration of output from other dedicated subgroups
Next steps

• **Initiating (potential) follow-up subgroups:**

  • Ecological relevance of assessment endpoints, ER$_x$ values, Modelling

  • Drafting Protocol

-> New persons are welcome, especially from academia / authorities!
Thank you!

For any questions: 
AndreasDuffner@eurofins.com OR christine.mihan@bayer.com
Introduction of the SETAC Plant Visual Injury Working Group

Gwendolin Kraetzig (Adama) and Sebastian Fellmann (Bayer AG)
Background

In the virtual open meeting (2021) of the SETAC Plants Interest Group the topic of plant visual injury was identified as relevant and discussed based on the results of a survey within the Plant Interest Group community.

<table>
<thead>
<tr>
<th>ANSWER CHOICES</th>
<th>RESPONSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical Evaluation of Visual Injury Scores in Non-Target Terrestrial Plant Studies</td>
<td>42.31% 11</td>
</tr>
<tr>
<td>Higher Tier NTTP Testing – Reproduction testing</td>
<td>61.64% 16</td>
</tr>
<tr>
<td>Reclamation of plant communities at contaminated sites</td>
<td>30.77% 8</td>
</tr>
<tr>
<td>Other topics of interest (please specify)</td>
<td>30.77% 8</td>
</tr>
<tr>
<td>Total Respondents: 26</td>
<td></td>
</tr>
</tbody>
</table>


Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology

European Food Safety Authority
Current activities – Working group

• A **working group** was established and a **Kick-off workshop** was held in February 2022

• The aim was to discuss the **status quo**, identify the **challenges** of plant visual injury assessment (PVI) and how to follow-up

→ More Details: [https://www.setac.org/group/IGPlants](https://www.setac.org/group/IGPlants)
## Discussion points – Pros and Cons

<table>
<thead>
<tr>
<th>Visual scores</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td><strong>Disadvantages</strong></td>
</tr>
</tbody>
</table>
| • Data available on the same day as harvest  
• Cheap  
• Non-destructive | • Subjective assessment open to human bias  
• Discontinuous data  
• Less precise, especially around ER$_{50}$  
• Estimation of control variability may need harmonization |
| • Unequivocal data, not open to human bias  
• Continuous data  
• More precise estimate of ER$_{50}$  
• Estimation of control variability is possible | • Data not available on the same day as harvest  
• More expensive  
• Destructive |

→ More Details: [https://www.setac.org/group/IGPlants](https://www.setac.org/group/IGPlants)
Discussion points – scoring system

• Different rating systems are currently used

Scoring by categorization:
  e.g.:
  1 = no symptoms,
  2 = slight symptoms,
  3 = moderate symptoms,
  4 = severe symptoms

<table>
<thead>
<tr>
<th>Score</th>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Effect</td>
<td>No noticeable effect</td>
</tr>
<tr>
<td>10</td>
<td>Slight Effect</td>
<td>Effect barely noticeable</td>
</tr>
<tr>
<td>20</td>
<td>Slight Effect</td>
<td>Some effect, not apparently detrimental</td>
</tr>
<tr>
<td>30</td>
<td>Slight Effect</td>
<td>Effect more pronounced, not obviously detrimental</td>
</tr>
<tr>
<td>40</td>
<td>Moderate Effect</td>
<td>Effect moderate, plants appear able to recover</td>
</tr>
<tr>
<td>50</td>
<td>Moderate Effect</td>
<td>Probable lasting effect, recovery doubtful</td>
</tr>
<tr>
<td>60</td>
<td>Moderate Effect</td>
<td>Lasting effect, recovery doubtful</td>
</tr>
<tr>
<td>70</td>
<td>Severe Effect</td>
<td>Heavy injury, loss of individual leaves</td>
</tr>
<tr>
<td>80</td>
<td>Severe Effect</td>
<td>Plant nearly destroyed, a few surviving leaves</td>
</tr>
<tr>
<td>90</td>
<td>Severe Effect</td>
<td>Occasional surviving leaves</td>
</tr>
<tr>
<td>100</td>
<td>Complete Effect</td>
<td>Death of entire plant</td>
</tr>
</tbody>
</table>

More Details: [https://www.setac.org/group/IGPlants](https://www.setac.org/group/IGPlants)
Discussion points – Evaluation and statistics

• How to use the data for endpoint determination? Example: Can procedures in Toxicological Histology inform visual injury scoring for plants?

Severity Scoring of Microscopic Findings in Toxicological Pathology

Jeff Wolf, DVM, DACVP
Experimental Pathology Laboratories, Inc., Sterling, Virginia

→ More Details: https://www.setac.org/group/IGPlants
Current activities – Working group

→ Mission statement:
“to evaluate and improve the Reproducibility, Reliability and regulatory Relevance of plant visual injury (PVI) end points in an aligned approach within the SETAC scientific community”

• Three pillars were identified in order to address the challenges of PVI assessments

https://www.setac.org/members/group_content_view.asp?group=90693&id=150260
Next steps

• Survey was initiated to collect feedback of the SETAC plant interest community on the topic of PVI assessments

→ Please respond to the survey (still open until 27th of May)

• Second workshop (to be announced) in order to:
  • discuss the results of the survey
  • define follow-up work to address the questions identified in the three pillars

• If you are interested to participate in the working group please get in contact with us or register for the Plant Interest Group through SETAC:
  • https://www.setac.org/group/IGPlants.
  • You do not need to be a current member of SETAC to join the group
Thanks for your attention!

Steering committee members:
Patricia Lopez-Mancisidor (Corteva), Joshua Arnie (Eurofins), Gunther du Hoffmann (Eurofins), Henry Krueger (Eurofins), Eileen Paterson (Corteva), Gwendolin Kraetzig (Adama), Jeff Wolf (EPL) Tim Springer (Springer Ecotox), John Green (Green-Ecostats), Ashlee Kirkwood (Smithers), Andreas Duffner (Eurofins), Rena Isemer (Bayer AG), Sebastian Fellmann (Bayer AG)