LemnaTec High-Content Screening

PLANT SCREENING FOR OECD TESTING

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Regulatory Framework

The OECD Guideline 227 (adopted 2006) “Terrestrial Plant Test: Vegetative Vigour Test” and the OECD Guideline 208 (adopted 2006) “Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test” are both used to test the effects of chemicals on plants. Depending on the chemicals and their path into the environment, the substances may be applied via spraying or form a part of the initial soil mixture.
In both cases growth and development of the tested plants should be quantified to finally allow for a quantitative measurement of inhibition or toxicity in relation to an untreated control plant group. As both test designs include dose response measurements, there is a strong need for continuous quantitative data. Only such data enables the application of different kinds of statistics, including calculation of EC values.

Measurement Parameters

The measurement parameters of OECD 208 are described in § 29 as follows:

29. During the observation period, i. e. 14 to 21 days after 50 % of the control plants (also solvent controls if applicable) have emerged, the plants are observed frequently (at least weekly and if possible daily) for emergence and visual phytotoxicity and mortality. At the end of the test, measurement of percent emergence and biomass of surviving plants should be recorded, as well as visible detrimental effects on different parts of the plant. The latter include abnormalities in appearance of the emerged seedlings, stunted growth, chlorosis, discoloration, mortality, and effects on plant development. The final biomass can be measured using final average dry shoot weight of surviving plants, by harvesting the shoot at the soil surface and drying them to constant weight at 60 °C. Alternatively, the final biomass can be measured using fresh shoot weight. The height of the shoot may be another endpoint, if required by regulatory authorities. A uniform scoring system for visual injury should be used to evaluate the observable toxic responses. Examples for performing qualitative and quantitative visual ratings are provided in references (23) (24).

OECD 227 uses an almost identical wording in § 27:
27. During the observation period, the plants are observed frequently (at least weekly and if possible daily) for visual phytotoxicity and mortality. At the end of the test, measurement of biomass of surviving plants should be recorded as well as visible detrimental effects on different parts of the plant. The latter include abnormalities in appearance of the young plants, stunted growth, chlorosis, discoloration, mortality, and effects on plant development. The final biomass can be measured using final average dry shoot weight of surviving plants, by harvesting the shoot at the soil surface and drying them to constant weight at 60 °C. Alternatively, the final biomass can be measured using fresh shoot weight. The height of the shoot may be another endpoint, if required by regulatory authorities. A uniform scoring for visual injury should be used to evaluate the observable toxic responses. Examples for performing qualitative and quantitative visual ratings are provided in references (12) (25).

As a general conclusion to be drawn from these guidelines on quantification of growth and development, a reproducible and, if possible, quantitative assessment is essential. This assessment needs to be highly effective; otherwise the “frequent” assessment will cost too much time and consequently money if performed on an appropriate quality level. Due to the length of the test (up to 3 weeks), changes in the staff performing the classification/quantification must be taken into account. Thus the need for reproducibility of the classifications and measurements becomes even more urgent. As tests are repeated at different times of the year or under different conditions in places remote from those persons finally making the evaluation, reproducible quantitative information helps to increase the test quality and to avoid false conclusions.

Intermediate measurement of phytotoxicity finally means that colour changes shift between dark and light green, and chlorosis as well as significant changes in growth or size should also be measured. The guidelines grant a great deal of flexibility, taking into account that different plants and the different technologies available may result in quite different kinds of random observations. Obviously any kind of visual assessment will contain a great amount of subjective factors, thus preventing high reproducibility of results. Due to the comparatively low amount of replicates and the need include more than just final fresh or dry weight in the evaluation and bias in reproducibility of results can easily provoke unintentional higher or lower EC-value estimates.

By contrast, image processing with the LemnaTec Plant Scanalyzer system can provide non-destructive, reproducible and efficient measurements by including measurements of plant colours, projected leaf areas (including correlative biomass parameters), leaf lengths, shoot heights or factors such as changes in phenotype (e. g. rolling or hanging leaves).

Growth Dynamics and Consequences

As these measurements are made accurately at various measurement points, data of the whole growth dynamic of a plant is acquired. Such a huge amount of information makes test results much more stringent, in consequence dramatically enhancing quality control by contractors and helping to avoid artefacts.

Although this is discussed in detail in other LemnaTec papers ("Final biomass and area under the growth curve calculation strongly depend on absolute growth rate of controls", "Arabidopsis Assessment in Time and Space", "Corn Morphology – Leaf Rolling") some aspects should be highlighted here shortly:

1. Most plants grow exponential rather than linear during the first weeks. This has severe consequences as any direct comparison of size at the end of the test (by measuring either fresh or dry weight) explicitly uses linear growth as a basic (and thus often incorrect) growth model. As a result test conditions with almost unlimited height growth (i. e. good plant growth conditions) may lead to higher inhibition values than tests limited, for example, by poor light (darker greenhouse in winter etc.) or fewer nutrients. This may significantly influence the EC values calculated afterwards.
2. A high level of heterogeneity is produced by different seed germination times which in most cases are natural and only rarely influenced by external factors. Not by fault, but by long-term experience, seed quality controls always accept a wide distribution of germination times. In some cases (e. g. for cress) germination time and related root length are closely connected to initial seed size (as shown by Dirk Vandenhirtz, diploma thesis, RWTH Aachen). As a result, small differences in size at

the beginning of a test can lead to quite significant differences at the end, suggesting a high biological variability. High standard deviations can lead to high NOEC values if controls show such values as a mere result of different germination times. Especially for non-crop plants a higher variability can be expected, which could then result in high NOEC values, being in fact merely the result of inappropriate measurement.

3. Due to the large number of different species that could be used in testing and a limited knowledge of their control growth dynamics, deviating growth patterns can hide toxicity or suggest greater effects than really exist, because the test just comprises a restricted (limited) part of the life cycle which is only determined by time. If plants already approach their respective maximum growth during the test, it may happen that treatments catch up with controls, reducing the measured degree of damage and thus suggesting lower toxicity. If treated plants, for example, merely slow down in growth during the last week, the effect may not look very significant. But the example shows in fact a real and profound problem so far underestimated. If a substance provokes just a retardation/lag phase at the beginning but is rapidly degradable (normally a highly profitable trait), this may result in a huge difference of size at the end of the test due to retarded exponential growth and consequently massive overestimation of toxicity.

Using growth estimates based on image processing can easily include quantitative assessment of growth dynamics and thus allow a risk assessment based on reliable measurement facts. Artefacts of mathematically false models (linear growth) can be avoided and changes in growth dynamics properly assessed. At least, such information can be a very important supplement to properly interpret the fresh or dry weight measured at the end of a test, particularly as this figure alone cannot provide sufficient information on the vegetative vigour or seedling growth.

Conclusion

To back up the OECD Guidelines 208 and 227 with reliable data, quantitative image processing under reproducible conditions can definitely help to measure parameters and values appropriate to the respective plants, reflecting (providing) substantial values for both growth and vigour.

For further information please do not hesitate to contact

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