



PROJECT NUMBER 289706

COLLABORATIVE PROJECT

Assessing and Monitoring The Impacts of Genetically Modified Plants on Agro-ecosystems

Deliverable 9.3: Software implementing methods for analysis of single-environment field trial ERA data

Start date of the project: 01/12/2011 Duration: 54 months

Organisation name of lead contractor for this deliverable: **DLO: Wageningen University and Research centre, Plant Research International, Biometris, Wageningen, Netherlands**

Project funded by the European Commission within the Seventh Framework Programme (2007-2013)		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Deliverable 9.3 is a software which was produced by the authors and will be made freely available through the AMIGA web site. The present document is the user manual of the software.

AMIGA POWER ANALYSIS USER MANUAL

30 JUNE 2014 – AMIGA POWER ANALYSIS VERSION 1.0.0.0

1 INTRODUCTION

Welcome to Amiga Power Analysis for environmental risk assessment (ERA) using field trials. With this tool you can calculate the necessary replication for assessing differences and equivalences between a genetically modified organism (GMO) and a comparator plant variety.

This tool builds on EFSA recommendations (Perry et al. 2009, EFSA 2010) and work in the AMIGA project (Goedhart et al. 2013, 2014). It allows to specify the experimental design, additional factors in the experiment, and the method of statistical analysis that will be used. The power of difference tests and equivalence tests (Schuirmann et al. 1987, Perry et al. 2009) is calculated.

This program was developed in the AMIGA project (Assessing and monitoring the impacts of genetically modified plants on agro-ecosystems, <http://www.amigaproject.eu/>) on the amount of replication needed in field trials for GMO safety assessment.

The program was developed by the Biometris department of Wageningen University and Research centre (<http://www.biometris.nl/>).

Program developers: Johannes Kruisselbrink, Paul Goedhart, Hilko van der Voet

2 INSTALLATION INSTRUCTIONS

This section will guide you through the installation of Amiga Power Analysis.

2.1 PREREQUISITES

The current version of Amiga Power Analysis requires the software package GenStat (VSN 2012) for running the simulations. GenStat is available from <http://www.vsni.co.uk/software/genstat>. Future versions will use R instead of GenStat.

2.2 INSTALLATION STEPS

Step 1: Double click the installation file (AmigaPowerAnalysis.Installer.msi). This will run a standard installation. Follow the instructions on the screen – the suggested default settings should apply in most situations.

Step 2: Start Amiga Power Analysis using the desktop shortcut, from the start menu, or from the installation directory.



Step 3: Go to *Settings* → *Options* and specify the GenStat path by selecting **GenBatch.exe** (located in the bin directory of the GenStat installation directory).

3 GETTING STARTED

Start by opening an existing file or creating a new file. The user interface of Amiga Power Analysis is divided into tabs. In the sections below, the functionality of each tab will be explained separately.

3.1 ENDPOINTS

Enter a list of endpoints. For each endpoint indicate its group (retrieves default settings), and if needed adapt the measurement type and limits of concern (LoC). Endpoint groups can be edited under the Options menu. Note: currently only methods for Measurement type Count have been implemented.

Limits of Concern are ratios of the expected values for the GMO and the Comparator. Within these limits there is no concern about safety.

Provide a lower LoC, an upper LoC, or both. Unspecified (NaN) means no concern for changes in that direction.

3.2 ENDPOINTS DATA

For each endpoint, if needed adapt its distribution type, the binomial total (for fractions), and the power (for Taylor's Power law distribution).

If needed adapt expected values of mean and coefficient of variation (CV) for the comparator variety. Note: CV will be increased if incompatible with distribution type and mean.

Indicate if more there are observations in time series per plot (Repeated measures, not yet implemented).

Indicate if more zeroes are expected than corresponds to the chosen distribution (Excess zeroes, not yet implemented).

3.3 FACTORS

The main factor in a GMO comparative evaluation experiment is always Variety, with levels 1 (labelled GMO) and 2 (labelled Comparator).

If the design contains more varieties enter additional rows in the Levels table.

If numbers of plots per variety are not equal, change the (relative) frequencies.

If the design contains more factors (e.g. spraying treatments), add additional rows in the Factor table, and specify the levels and relative frequencies in the Levels table.

3.4 DESIGN

Specify the type of experimental design. When other factors have been specified, the GMO-CMP Variety comparisons can be expected to be the same for all levels of such a factor (no interaction) or different (interaction). Indicate if such interactions are expected for one or more endpoints. Uncheck the box 'Use interactions for all endpoints' will allow you to specify specific endpoints in the next screen. Note: Interactions with Variety will lower the effective replication, because comparisons are now needed at the separate levels of the other factor. For specified interactions in a split-plot design, indicate the level where the factor is randomised. For specified interactions, indicate both for the GMO and the CMP the levels of the additional factor that have to be compared.

3.5 COMPARISONS

The comparison GMO to CMP is made separately for levels of other factors. Per endpoint, indicate which levels of these other factors should be included for the GMO and for the CMP.

3.6 MODIFIERS

The comparison GMO to CMP is made separately for levels of other factors. Per endpoint, indicate which levels of these other factors should be included for the GMO and for the CMP.

3.7 SIMULATION

Specify how to perform the power analysis and which methods of analysis are to be compared. It is advised first to use the Approximate method (Lyles) because it is much faster. Currently, only the Approximate method is implemented (Lyles et al. 2007).

3.8 OVERALL RESULTS

The power analysis is based on the minimum power across the primary comparisons, in terms of Concern Standardized Differences (CSD, equals 1 at the Limit of Concern).

Select primary comparisons. Choose method of analysis if more have been investigated.

Power is shown for difference tests (upper graphs) and equivalence tests (lower graphs), both as a function of the number of replicates (left) and the Concern Standardized Difference (right).

Note: Number of plots in design is Number of replicates times Number of plots per block

3.9 RESULTS PER COMPARISON

Choose endpoint in table. Choose method of analysis if more have been investigated. Power is shown for difference tests (upper graphs) and equivalence tests (lower graphs), both as a function of the number of replicates (left) and the Ratio GMO/CMP (right).

Note: Number of plots in design is Number of replicates times Number of plots per block.

REFERENCES

- EFSA (2010). EFSA Panel on Genetically Modified Organisms (GMO). Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal*, 8(11): 1879. [111 pp.], doi:10.2903/j.efsa.2010.1879.
- Goedhart PW, Van der Voet H, Baldacchino F & Arpaia S (2013). Environmental Risk Assessment of Genetically Modified Organisms: Overview of field studies, examples of datasets, statistical models and a simulation tool. Deliverable 9.1, AMIGA project, project number 289706.
- Goedhart PW, van der Voet H, Baldacchino F & Arpaia S (2014). A statistical simulation model for field testing of non-target organisms in environmental risk assessment of genetically modified plants. *Ecology and Evolution*. 4: 1267–1283. <http://dx.doi.org/10.1002/ece3.1019>.
- Lyles RH, Lin H-M & Williamson JM (2007). A practical approach to computing power for generalized linear models with nominal, count, or ordinal responses. *Statistics In Medicine*, 26(7): 1632-1648.

- Perry JN, ter Braak CJF, Dixon PM, Duan JJ, Hails RS, Huesken A, Lavielle M, Marvier M, Scardi M, Schmidt K, Tothmeresz B, Schaarschmidt F & van der Voet, H (2009). Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. *Environmental Biosafety Research*, 8: 65-78.
- Schuirman DJ (1987). A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics*, 15(6): 657-680.
- VSN International (2012). *GenStat for Windows 15th Edition*. VSN International, Hemel Hempstead, United Kingdom. Web page: www.GenStat.co.uk.