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| INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS | | |
| Geneva | | |

enlarged editorial Committee

Geneva, January 7 and 8, 2015

Revision of document TGP/10: New section: Assessing uniformity by off-types   
on basis of more than one sample or sub-samples

Disclaimer: this document does not represent UPOV policies or guidance

The purpose of this document is to present a proposal for revision of document TGP/10 “Examining uniformity” to provide guidance on assessing uniformity by off-types on the basis of more than one sample or sub-sample.

The following abbreviations are used in this document:

TC: Technical Committee

TC-EDC: Enlarged Editorial Committee

TWA: Technical Working Party for Agricultural Crops

TWC: Technical Working Party on Automation and Computer Programs

TWF: Technical Working Party for Fruit Crops

TWO: Technical Working Party for Ornamental Plants and Forest Trees

TWPs: Technical Working Parties

TWV: Technical Working Party for Vegetables

The structure of this document is as follows:

[BACKGROUND 2](#_Toc405386456)

[DEVELOPMENTS IN 2014 2](#_Toc405386457)

[Technical Committee 2](#_Toc405386458)

[Technical Working Parties 2](#_Toc405386459)

[Technical Working Party for Ornamental Plants and Forest Trees 2](#_Toc405386460)

[Technical Working Party for Fruit Crops 2](#_Toc405386461)

[Technical Working Party on Automation and Computer Programs 2](#_Toc405386462)

[Technical Working Party for Vegetables 3](#_Toc405386463)

[Technical Working Party for Agricultural Crops 4](#_Toc405386464)

[Summary of approaches 4](#_Toc405386465)

[proposal 5](#_Toc405386466)

ANNEX I: Situation A: Two Growing Cycles in a single location

ANNEX II: Situation B: Two Growing Locations in the same year

ANNEX III: Situation C: More than one test/trial for a characteristics in the same growing cycle

ANNEX IV: Situation D: Assessing sub-samples within a single test/trial

ANNEX V: Use of a stepwise approach in the off-type procedure within the same growing cycle

# BACKGROUND

The background to this matter is provided in documents TC/48/14 “Assessing Uniformity by Off-Types on the Basis of More than One Sample or Sub-Sample” and TC/50/12 “Assessing Uniformity by Off-Types on the Basis of More than One Sample or Sub-Sample”.

# DEVELOPMENTS IN 2014

## Technical Committee

The TC considered document TC/50/12.

The TC noted that the TWC had proposed to provide more detailed information and further analysis on the consequences of the use of the approaches presented in situations A, B, C and D at its session in 2014, as set out in document TC/50/12, paragraph 33. The TC agreed that the document should be modified in order to explain that, in Situations A and B, a variety might be rejected after a single growing cycle under certain circumstances.

The TC noted that the TWC had proposed that the statistical basis for the acceptable number of off‑types in situation D be considered further at its session in 2014, as set out in document TC/50/12, paragraphs 34 and 35.

The TC agreed to develop guidance in document TGP/10 for situations A, B, C and D, as set out in document TC/50/12, Annexes I to IV, after consideration of the comments by the TWC.

The TC agreed that document TGP/10, paragraph 6 should be considered when considering the possible development of guidance on the approach presented in situation C, as set out in document TC/50/12, Annex III (see document TC/50/36 “Report on the Conclusions”, paragraphs 123 to 127).

## Technical Working Parties

### Technical Working Party for Ornamental Plants and Forest Trees

The TWO considered document TWO/47/9 and the situations described in the Annexes I to IV as a basis to develop guidance in document TGP/10.

The TWO agreed that clarification should be provided on the decision to be taken in Situation B, Alternative (a) “the trial is repeated at both locations for a second year”, in case after repeating a trial for the second year a variety is within the uniformity standard in one growing location but is not within the uniformity standard in the other growing location (see document TWO/47/28 “Report”, paragraphs 61 and 62).

### Technical Working Party for Fruit Crops

The TWF considered document TWF/45/9 and the situations described in the Annexes I to IV as a basis to develop guidance in document TGP/10.

The TWF agreed with the comment made by the TWO at its forty-seventh session that clarification should be provided on the decision to be taken in Situation B, Alternative (a) “the trial is repeated at both locations for a second year”, in case after repeating a trial for the second year a variety is within the uniformity standard in one growing location or year but is not within the uniformity standard in the other growing location or year.

The TWF agreed that the approaches were not relevant for the fruit sector, because vegetatively propagated varieties did not appear to be in the scope of the document (see document TWF/45/32 “Report”, paragraphs 66 to 68).

### Technical Working Party on Automation and Computer Programs

The TWC considered document TWC/32/9.

The TWC agreed that the values for type I and type II errors should be included in each of the examples described in situations A and B for the development of guidance in document TGP/10. The type I error is associated with a decision for non-uniformity (rejection of the true null hypothesis) and the type II error is associated with a decision for uniformity (acceptance of the alternative hypothesis).

The TWC agreed that the guidance provided in document TGP/10 “Examining Uniformity”, Section 6 “Combining all observations on a variety” was sufficient to address situation C “More than one sample or subsample for a characteristic in the same growing trial”, Annex III to document TWC/32/9. The TWC agreed that the example provided could be considered as a special test and that results of the uniformity assessment should be considered independently.

In relation to situation D, the TWC considered the use of a stepwise approach in the off-type procedure within the same growing cycle and the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants, as provided in Annex V to document TWC/32/9, which was introduced by an expert from Germany.

The TWC agreed that the type I and type II errors used in the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants were comparable to those of the entire sample for the example provided in wheat and barley.

The TWC noted that the stepwise approach in the off-type procedure was intended to reduce costs without increasing risks in the uniformity assessment. The TWC agreed to propose the guidance as follows (see document TWC/32/28 “Report”, paragraphs 19 to 24):

“SITUATION D: ASSESSING SUB-SAMPLES WITHIN A SINGLE TEST/TRIAL

**“Approach: Use of sub-sample as a first step of assessment**

“A variety is considered uniform if the number of off-types does not exceed a predefined lower limit in the sub-sample.

“A variety is considered non-uniform if the number of off-types exceeds a predefined upper limit in the sub‑sample.

“If the number of off-types is between the predefined lower and upper limits the whole sample is assessed. The lower and upper limits have to be chosen considering comparable type I and type II errors in the sub‑sample and the whole sample.

“Example:

“In a sample size of 100 plants, the acceptable number of off-types is 3 (based on a population standard of 1% and an acceptance probability of at least 95%).

“In a subsample of 20 plants used in the context of the sample size of 100 plants above:

“A variety is considered uniform if no off-types are observed in the sub-sample.

“A variety is considered non–uniform if the number of off-types in the sub-sample exceeds 3.

“If the number of off-types is 1 to 3, the whole sample of 100 plants is assessed.

“Annex V to document TWC/32/9 provides a full description of the statistical basis for this approach.”

### Technical Working Party for Vegetables

The TWV considered document TWV/48/9 and the situations described in the Annexes I to IV as a basis to develop guidance in document TGP/10.

The TWV agreed on the importance of assessing uniformity in each independent growing cycle and is not in favor of combining results from 2 cycles (see document TWV/48/43 “Report”, paragraphs 79 and 80).

### Technical Working Party for Agricultural Crops

The TWA considered document TWA/43/9, including Annexes I to IV, as a basis to develop guidance in document TGP/10.

The TWA noted that the TWC had been invited to provide an analysis of the consequences of the different approaches presented in the Annexes of document TWA/43/9 and, in particular, whether approach 2 in Situations A and B was appropriate.

The TWA agreed with the TWV on the importance of assessing uniformity in each independent growing cycle and was not in favor of combining results from 2 cycles.

The TWA agreed that an introduction paragraph should be added to Situation B to explain that years could be replaced by locations of DUS testing trials only when specific requirements are fulfilled such as no significant genotype x location interaction for any of the characteristics used in DUS examination.

The TWA agreed with the TWC that the guidance provided in document TGP/10 “Examining Uniformity”, Section 6 “Combining all observations on a variety” was sufficient to address situation C “More than one sample or subsample for a characteristic in the same growing trial”, Annex III to document TWA/43/9.

The TWA agreed with the TWC that guidance in Situation D should read as follows (see document TWA/43/27 “Report”, paragraphs 66 to 71):

“SITUATION D: ASSESSING SUB-SAMPLES WITHIN A SINGLE TEST/TRIAL

**“Approach: Use of sub-sample as a first step of assessment**

“A variety is considered uniform if the number of off-types does not exceed a predefined lower limit in the sub-sample.

“A variety is considered non-uniform if the number of off-types exceeds a predefined upper limit in the sub‑sample.

“If the number of off-types is between the predefined lower and upper limits the whole sample is assessed. The lower and upper limits have to be chosen considering comparable type I and type II errors in the sub‑sample and the whole sample.

“Example:

“In a sample size of 100 plants, the acceptable number of off-types is 3 (based on a population standard of 1% and an acceptance probability of at least 95%).

“In a subsample of 20 plants used in the context of the sample size of 100 plants above:

“A variety is considered uniform if no off-types are observed in the sub-sample.

“A variety is considered non–uniform if the number of off-types in the sub-sample exceeds 3.

“If the number of off-types is 1 to 3, the whole sample of 100 plants is assessed.

“Annex V to document TWC/32/9 provides a full description of the statistical basis for this approach.”

# Summary of approaches

Annexes I to IV to this document summarize different situations when different samples are combined for the overall assessment of uniformity of a variety in accordance with the conclusions of the TC at its fiftieth session on the basis of the proposals made by the TWPs at their sessions in 2014, as follows:

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| Note for revisions of Annexes I to IV  **~~Strikethrough~~ (highlighted)** indicates proposed deletion of text according to amendments proposed by the TWPs, at their sessions in 2014.  **Underlining (highlighted)** indicates proposed insertion of text according to amendments proposed by the TWPs, at their sessions in 2014. |

*Annex I: Situation A: Two growing cycles in a single location*

Approach 1: Third growing cycle in the case of inconsistent results

Approach 2: Combining the results of two growing cycles

*Annex II: Situation B: Two growing locations in the same year*

Approach 1: Third growing cycle in the case of inconsistent results

Approach 2: Combining the results of two locations

*~~Annex III: Situation C: More than one sample/sub-sample for a characteristic in the same growing cycle~~*

~~Approach: Additional growing cycle in the case of inconsistent results~~ (to be deleted)

*Annex IV: Situation D: Assessing sub-samples within a single test/trial*

Approach*:*  Sub-sample as a first step of assessment

The summary in Annexes I to IV only relates to situations where more than one sample, or sub‑sample, concern the examination of the same characteristic. In the case of different samples, or sub‑samples (e.g. special test), to examine a different characteristic there is no requirement to combine the results because a variety is required to be uniform for all relevant characteristics.

Annex V to this document presents a memorandum provided by the experts from Germany on the use of a stepwise approach in the off-type procedure within the same growing cycle in Situation D.

# proposal

It is proposed that Annexes I, II and IV to this document are considered as guidance on “assessing uniformity by off-types on the basis of more than one sample or sub-sample” for inclusion in a future revision of document TGP/10 “Examining distinctness”.

The TC-EDC is invited to note the information in this document to be presented to the TC and propose any improvements to the document in that regard.

[Annexes follow]

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| SITUATION A: TWO GROWING CYCLES IN A SINGLE LOCATION |
| **Approach 1: Third growing cycle in the case of inconsistent results**  A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.  A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.  If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but is not within the uniformity standard in the other growing cycle, then uniformity is assessed in a third growing cycle after consultation with the applicant. If in the third growing cycle the variety is within the uniformity standard, the variety is considered uniform. If in the third growing cycle the variety fails to meet the uniformity standard, the variety is considered non-uniform.  Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  A variety may be rejected after a single growing cycle, under certain circumstances.  **Approach 2: Combining the results of two growing cycles**  A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.  A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.  If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but is not within the uniformity standard in the other growing cycle, a variety is considered uniform if the total number of off-types at the end of the two growing cycles does not exceed the number of allowed off-types for the combined sample.  Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  A variety may be rejected after a single growing cycle, under certain circumstances.  Example:   |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | |  | | Population Standard = 1% | | | | | | |  | | Acceptance Probability ≥ 95% | | | | | | | Sample Size in each of growing cycles 1 and 2 = 50 | | | | | | | Maximum number of Off-Types = 2 | | | | | | | Sample Size in growing cycles 1 and 2 combined = 100 | | | | | | | Maximum number of Off-Types = 3 | | | | | | |  | |  |  |  | | |  | Growing cycle | | | Decision | | | |  | First | Second | | Approach 1 | | Approach 2 | | Number of  Off-Types | 2 | 2 | | uniform | | uniform | | 0 | 3 | | third growing cycle | | uniform | | 1 | 3 | | third growing cycle | | non-uniform | | 0 | 10\* | | third growing cycle\* | | non-uniform\* | | 10\*\* | 0 | | third growing cycle\*\* | | non-uniform\*\* |   \* Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  \*\* A variety may be rejected after a single growing cycle, under certain circumstances. |

[Annex II follows]

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| SITUATION B: TWO GROWING LOCATIONS IN THE SAME YEAR |
| **Introduction:** Differentyears can be replaced by different locations of DUS testing trials only when specific requirements are fulfilled, such as no significant genotype x location interaction for any of the characteristics used in DUS examination.  **Approach 1: Third growing cycle for inconsistent results**  A variety is considered uniform if it is within the uniformity standard in both of the growing locations.  A variety is considered non-uniform if it fails to meet the uniformity standard in both of the growing locations.  If the variety is within the uniformity standard in one growing location but is not within the uniformity standard in the other growing location, then the trial is repeated at the leading station (location)  ~~Alternative (a) the trial is repeated at both locations for a second year;~~  ~~Alternative (b) the trial is repeated at the Leading station (location)~~  Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  A variety may be rejected after a single trial in one growing location, under certain circumstances.  **Approach 2: Combining the results of two locations**  A variety is considered uniform if it is within the uniformity standard in both locations.  A variety is considered non-uniform if it fails to meet the uniformity standard in both locations.  If the variety is within the uniformity standard in one growing location but is not within the uniformity standard in the other growing location, a variety is considered within the uniformity standard if the number of off-type plants or parts of plants does not exceed the allowed number of off-types for the combined sample (two locations).  Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  A variety may be rejected after a single trial in one growing location, under certain circumstances.  Example:   |  | | --- | | Population Standard = 1% | | Acceptance Probability ≥ 95% | | Sample Size in each of growing locations 1 and 2 = 50 | | Maximum number of Off-Types = 2 | | Sample Size in growing locations 1 and 2 combined = 100 | | Maximum number of Off-Types = 3 |  |  |  |  |  |  | | --- | --- | --- | --- | --- | |  | Growing location | | Decision | | |  | First | Second | Approach 1 | Approach 2 | | Number of  Off-Types | 2 | 2 | uniform | uniform | | 0 | 3 | repeat trial | uniform | | 1 | 3 | repeat trial | non-uniform | | 0 | 10\* | repeat trial\* | non-uniform\* | | 10\*\* | 0 | repeat trial\*\* | non-uniform\*\* |   \* Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle.  \*\* A variety may be rejected after a single growing cycle, under certain circumstances. |

[Annex III follows]

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| ~~SITUATION C: MORE THAN ONE SAMPLE OR SUBSAMPLE FOR A CHARACTERISTIC IN THE SAME GROWING CYCLE~~ |
| **~~Approach: Additional growing cycle in the case of inconsistent results~~**  ~~A variety is considered to be uniform for a characteristic if it is within the uniformity standard for the characteristic in all samples or subsamples.~~  ~~A variety is considered non-uniform if it fails to meet the uniformity standard for the characteristic in all samples or subsamples.~~  ~~In the case where a variety is within the uniformity standard for the characteristic in one sample or subsample (e.g. main trial) and not in another sample or subsample (e.g. ear-row plot), both samples or subsamples are examined in a further growing cycle.~~  ~~Note: to consider whether Situation C is already covered by document TGP/10 “Examining Uniformity”, Section 6 “Combining all observations on a variety”.~~  ~~Extract from document TGP/10 “Examining Uniformity”~~  ~~“SECTION 6: Combining all observations on a variety~~  ~~[…]~~  ~~“6.3 Off-types only: characteristics observed on different samples~~  ~~“In many cases, uniformity is assessed by observations on different samples of plants or parts of plants. For example, for uniformity in wheat (see UPOV Test Guidelines for Wheat:  TG/3), some characteristics are observed on a sample of 2,000 plants, whilst some other characteristics are observed on a sample of 100 parts of plants taken from 100 plants. Off‑type plants observed in the plot of 2,000 plants can be excluded from further observations. For the plant parts taken from 100 plants, it is not normally possible to trace back the plant part to the original plant in the plot. Therefore, the sample of 100 plant parts needs to be considered to be independent from the 2,000 plants. Another independent sample of the variety is observed for seed characteristics. In such cases, a uniformity assessment should be carried out on all the independent samples, using the appropriate population standard. A variety should be considered to be uniform if the uniformity requirements are fulfilled in all samples.”~~ |

[Annex IV follows]

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| SITUATION D: ASSESSING SUB-SAMPLES WITHIN A SINGLE TEST/TRIAL |
| **Approach: Use of sub-sample as a first step of assessment**  A variety is considered uniform if ~~no~~ the number of off-types ~~are observed in a~~ does not exceed a predefined lower limit in the sub-sample.  A variety is considered non–uniform if the number of off-types ~~in the~~ exceeds a predefined upper limit in the sub-sample ~~exceeds the accepted number of off-types for the whole sample~~.  If the number of off-types is ~~1 or more, but below the accepted number of off-types for the whole sample~~ between the predefined lower and upper limits, the whole sample is assessed. The lower and upper limits have to be chosen considering comparable type I and type II errors in the sub‑sample and the whole sample.  Example:  In a sample size of 100 plants, the acceptable number of off-types is 3 (based on a population standard of 1% and an acceptance probability of at least 95%).  In a subsample of 20 plants used in the context of the sample size of 100 plants above:  A variety is considered uniform if no off-types are observed in the sub-sample.  A variety is considered non–uniform if the number of off-types in the sub-sample exceeds 3.  If the number of off-types is 1 to 3, the whole sample of 100 plants is assessed.  ~~(The background to this example is provided in Annex V to this document)~~  Annex V to document TWC/32/9 provides a full description of the statistical basis for this approach. |

[Annex V follows]

MEMORANDUM FROM THE EXPERT FROM GERMANY

Use of a stepwise approach in the off-type procedure within the same growing cycle

*Background*

The TC noted that the TWC had proposed that the statistical basis for the acceptable number of off‑types in situation D be considered further at its session in 2014, as set out in document TC/50/12, paragraphs 34 and 35.

The following text is based on document TWC/31/22 Annex V.

The method of uniformity assessment on the basis of off-types (off-types procedure) was described in document TGP/8. Paragraph 8.1.7 provides guidance on the use of the off-types procedure on more than one single test, including a combined test, a two-stage test and sequential tests.

A combined test is described as follows:

Make a decision after two (or three) years based on the total number of plants examined and the total number of off-types recorded.

A two-stage test is described as follows:

Use the result of the first year to see if the data suggests a clear decision (reject or accept). If the decision is not clear then proceed with the second year and decide after the second year.

A sequential test is a multi-stage test where decision rules can be defined dependently or independently on results of the test.

A specific example for the use of a two-step test is provided in document TWC/29/09 “Assessing uniformity by off-types on the basis of more than one sample or sub-sample”, Annex I, page 13. The following specific approach is applied by several European examination offices in wheat and barley for the assessment of uniformity in case of characteristics observed on a sample size of 100 plants or parts of plants. The population standard is fixed at 1% with an acceptance probability of 95% for each decision.

In the first step 20 plants or parts of plants are observed.

* If there are no off-type plants in 20 plants then the variety does not exceed the number of allowed off-types for this characteristic for this growing cycle
* If there are more than 3 off-type plants then the variety exceeds the number of allowed off-types for this characteristic for this growing cycle.
* If there are 1, 2 or 3 off-type plants then the second step follows.

In the second step further 80 plants or part of plants are observed.

* If there are 3 or less off-type plants in 100 (20 of step 1 + 80 of step 2) plants then the variety does not exceed the number of allowed off-types for this characteristic for this growing cycle
* If there are more than 3 off-type plants in 100 (20 of step 1 + 80 of step 2) plants then the variety exceeds the number of allowed off-types for this characteristic for this growing cycle

The decision rule is defined as follows:

A variety is considered to be within the uniformity standard in a given growing cycle if the number of off-types in all samples does not exceed the number of allowed off-types in either of the samples.

A variety is considered to be uniform if it is within the uniformity standard in both of the two growing cycles.

If at the end of two growing cycles the variety is within the uniformity standard in one cycle but not in the other cycle, the test will be continued for a third growing cycle. If at the end of the third growing cycle the variety is within the uniformity standard, the variety is considered to be uniform. If at the end of the third growing cycle the variety fails to meet the uniformity standard, the variety is considered to be non-uniform.

This so-called stepwise approach can be used for a number of characteristics in each growing cycle in which the steps are represented by the samples (20 plants or 20 + 80 plants).

In order to compare different tests and decision rules it is useful to compare appropriate type-I and type-II errors. Basic ideas were described by experts from France in document TWC/13/17 “Sequential analysis”.

It should be reminded that a decision for uniformity of a variety has always a so-called type-II error (acceptance of null hypothesis) whereas a decision for non-uniformity of a variety has a so-called type-I error (rejection of null hypothesis). The following applies for the described cereal example:

It is assumed that the population standard is 1% and the acceptance probability is 95%. The number of allowed off-types within 100 plants is 3. All risks are evaluated on the basis of the binomial distribution. To compute the type-II error the population standard for the non-uniform varieties is assumed to be 2% (two times 1%).

For 20 plants the number of allowed off-types is normally 1. However, for 20 plants in the first step of the two-step procedure (see above) it is defined that no off-type is allowed. If we have 20 plants in the first step and no off-type the type-II error (beta risk) is 66.8%. This is high but comparable with 2 off-types in 100 plants. In this case the type-II error is 67.7%.

Looking on the type-I error, the actual error is 1.7 % in case of 20 plants and 1 off- type, and 1.8 % in case of 3 off-types in 100 plants. A decision on the basis of a sample of 20 plants is only taken if there are no off-types which represents a smaller error than the decision taken on 100 plants.

In case of 20 plants in the first step and more than 3 off-types the type-I error (alpha risk) is almost zero. It is very small and smaller than in case of more than 3 off-types in 100 plants. In this case the type-I error (alpha risk) is 0.3 %.

If in the first step there are 1, 2 or 3 off-types in 20 plants the next 80 plants are assessed and the decision is taken on the basis of 100 plants. In that case the type-I and type-II errors have to be evaluated using special formulas or using the software from France (<http://www.seedtest.org/en/stats_tool_box_content---1--1143.html> ).

So it is possible to compare all the other situations.

The open question is: Do we need a statement regarding to the different types of errors for each decision or do we need a statement over all decisions. This should be discussed with statistical experts.

Because of underlying nonlinear formulas there are no general rules for all practical cases usable in DUS tests.

[End of Annex V and of document]