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Reflection paper on testing strategy and risk assessment for plants

Draft

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1. Introduction

The objective of this reflection paper is to address the recommended testing strategy and risk assessment for plants in the Phase II assessment.

2. Discussion

As the OECD 208 guideline for plant testing has been changed since the publication of the VICH Phase II guideline, guidance on how many plant species are needed for testing of veterinary pharmaceuticals is no longer available in the OECD 208 GL. As a consequence the CVMP recommends the following test strategy for veterinary medicinal products (VMPs) in relation to the risk for terrestrial plants.

Tier A. Six plant species from six different families are tested. The lowest EC_{50} value on the most sensitive endpoint is used in combination with an assessment factor of 100. If the resultant risk quotient (RQ) is below 1 the assessment can stop. If the RQ is ≥ 1 it is necessary to proceed to Tier B.

It is highly recommended, in order to enhance the representation of the plant kingdom, to use species belonging to six different families with four dicotyledonous and two monocotyledonous species.

Tier B. From the same plants species tested in Tier A, the lowest EC_{10} or NOEC values on the most sensitive endpoint is used in combination with an assessment factor of 10 in Tier B. If the resultant RQ is below 1 the assessment can stop. If the RQ is ≥ 1 it is necessary to proceed to a Higher Tier assessment.

It should be noted that the NOEC values very much depend on the experimental design, variation within the treatments, and the power of the statistical test. Experience has shown that NOEC values obtained from plant studies often are associated with effects significantly above 10%. For this reason preference is given to the EC10 values, which are interpolated within the test concentration range (including the controls). It is important to recognise extrapolation beyond the range of data adds significant uncertainty and needs to be justified. Where less than 10% effect is observed at the highest test concentration this can be used as a NOEC in Tier B.

Higher Tier assessment using statistical extrapolation techniques. If at Tier B a potential risk for plants is still identified, a statistical extrapolation technique (Species Sensitivity Distributions - SSD) can be used to derive a PNEC, provided the dataset is sufficient for its application. Using the SSD method, the concentration at which 95% of the species theoretically are protected (HC_5) can be estimated. More information about the SSD method can be found in Postuma et *al*. (2001)[1]

To obtain a good representation of the plant kingdom and to improve the statistical power of the SSD, two additional species – preferably from two new families - need to be tested in combination with the six species/families tested in Tier B.

The HC_5 of the SSD is used as the basis for deriving a PNEC. Within the REACH framework an additional assessment/uncertainty factor (AF) between 1 and 5 on the HC_5 is recommended to derive a PNEC. The AF is established on a case-by-case basis and depends on the quality and quantity of the available data. The most relevant points of uncertainty and hence the accuracy of the HC_5 prediction are in connection to plants related to: 1) The lack of information on the sensitivity of the endpoints outlined in OECD 208; 2) The extrapolation from short-term to long-term effects; 3) The extrapolation from laboratory tests to effects in the field, including the establishment of a safe level for the vast

[1] Species Sensitivity Distributions in Ecotoxicology. 2001. Edited by Leo Posthuma, Glenn W Suter II, Theo P Traas Edited by Leo Posthuma, Glenn W Suter II, Theo P Traas. CRC Press 2001, 616pp ISBN 1566705789

number of plant species found in for example European grasslands when based on results from a few tested species.

To move away from case-by-case decisions on the magnitude of assessment factors the CVMP recommends using the lower confidence level of the HC_5 as an estimate of the PNEC. This is believed to

- Increase transparency and objectivity of the assessment
- Encourage the inclusion of more data points
- Reward the generation of good quality and coherent data

An improved dataset in the SSD assessment, i.e. increased number of tested species covering the same endpoint, will lead to an enhanced confidence of the assessment and will automatically result in a narrower difference between the median (HC_5) and the lower confidence level (HC_5 LL) of the HC_5. Practical experience for a number of substances has shown that the difference between HC_5 and HC_5 LL is within a factor of 2 to 4, which correspond approximately with the recommended assessment factor used on the outcome of the SSD in the REACH framework for new and existing chemicals.

All data used in the SSD assessment need to meet the general requirement on quality applicable also in the lower Tier of the risk assessment of VMPs, e.g. documentation of meeting the validity criteria of the OECD 208 or coming from a source in the open literature, which enable a similar evaluation. However, in order to use the SSD method the CVMP have set out a set of minimum criteria which need to be fulfilled in addition to the general quality criteria described. These are:

1. The minimum set of plant species tested must be eight from at least six different families.
2. The minimum number of monocotyledonous and dicotyledonous plant species must be three and five, respectively.
3. Only definitive EC10 or NOEC values can be used in the SSD calculation. It is highly recommended to base the SSD on EC10 values, but it can be acceptable to use a combination of NOEC and EC10 values in cases where for example new EC10 data generated by the applicant is combined with (older) NOEC data from the open literature.
   To ensure that the correct SSD is fitted with the appropriate confidence intervals, all less than (<) and greater than (>) values as such should not be used in the SSD calculation, e.g. in cases where no significant effects where observed at the highest test concentration, this concentration cannot be used as a NOEC value in the SSD.
4. The endpoint needs to be of similar nature, e.g. biomass, and should not include seedling emergence if biomass is the most sensitive endpoint.
5. If a plant species has been tested more than once a geometric mean of the same endpoint is used in the SSD assessment
6. Preferably the HC_5 is calculated based on a log-normal distribution. The likelihood of the data coming from a log-normal distribution must be tested by "Goodness of Fit" methods. The Anderson-Darling test normal distribution is recommended to datasets below twenty. If the Anderson-Darling statistic is above the 5% critical value (i.e. 0.752), normality must be rejected and data cannot be used for SSD.

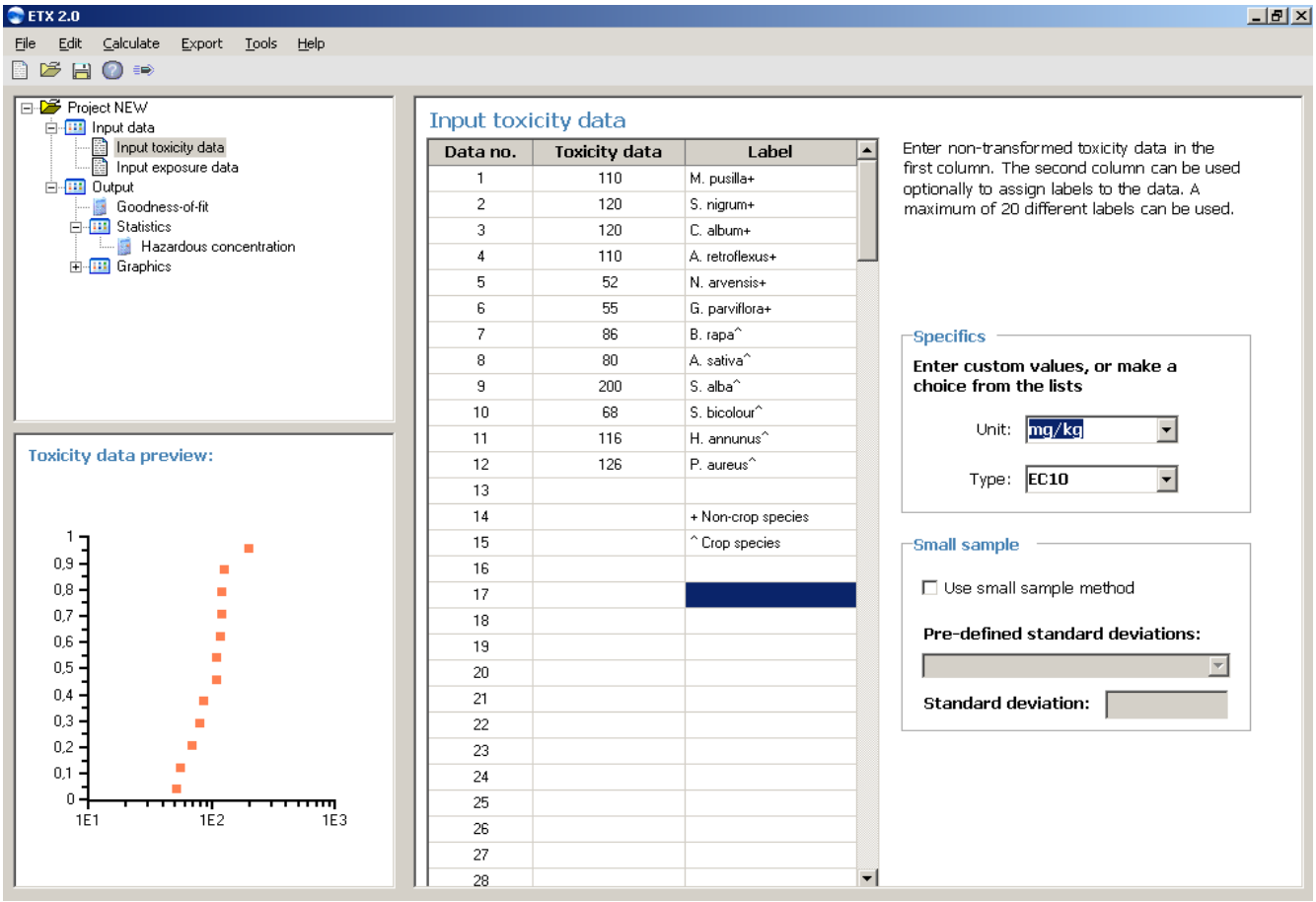
If there is evidence that plants are sensitive for the substance under evaluation, then the stepwise approach can be avoided and the SSD method can be used at the start of the risk assessment. This can be done by testing eight or more plants species, in the first instance and provided that the criteria mentioned above are met these data can be used directly in the SSD method. In such cases it is recommended to choose the plant species at random, in order to get the best fit of the sensitivity to a normal distribution. A better fit to a normal distribution will lead to higher confidence in the data and also in a narrower confidence interval around the HC_5 value.

There are different software programs available to calculate the HC_5 and HC_5 LL and to assess whether the data follow a normal distribution, e.g. the E_TX 2.0 program developed by RIVM and the SSD Generator developed by EPA CADDIS[2]. The choice of software program is optional.

The E_TX 2.0 program is available at http://www.rivm.nl/rvs/risbeoor/Modellen/ETX.jsp. An example of the outcome of the E_TX 2.0 program is presented below. No publicly available data set for (veterinary) pharmaceuticals regarding phytotoxicity was available for instructive purposes. Instead a dataset for the phytotoxicity of the narcotic acting detergent linear alkylbenzene sulphonate (LAS) was used[3]. This theoretical example covering twelve EC10 values with an industrial narcotic-acting chemical demonstrates a change from a PNEC of 5.2 mg/kg in Tier B to a PNEC (the HC_5 LL) of 33.7 mg/kg in the Higher Tier Assessment.

The input data are shown in Fig. 1.

Fig.1. The input-screen from the E_TX 2.0 program developed by RIVM.



[2] the SSD Generator developed by EPA CADDIS is available at http://www.epa.gov/caddis/da_software_ssdmacro.html
[3] Jensen, J, Smith, SR, Krogh, PH, Versteeg, DJ & Temara, A 2007, 'European risk assessment of LAS in agricultural soil revisited: Species sensitivity distribution and risk estimates', Chemosphere, vol. 69, nr. 6, s. 880-892

116 *Fig.2.The HC_5 estimations from the E_TX 2.0 program developed by RIVM.*

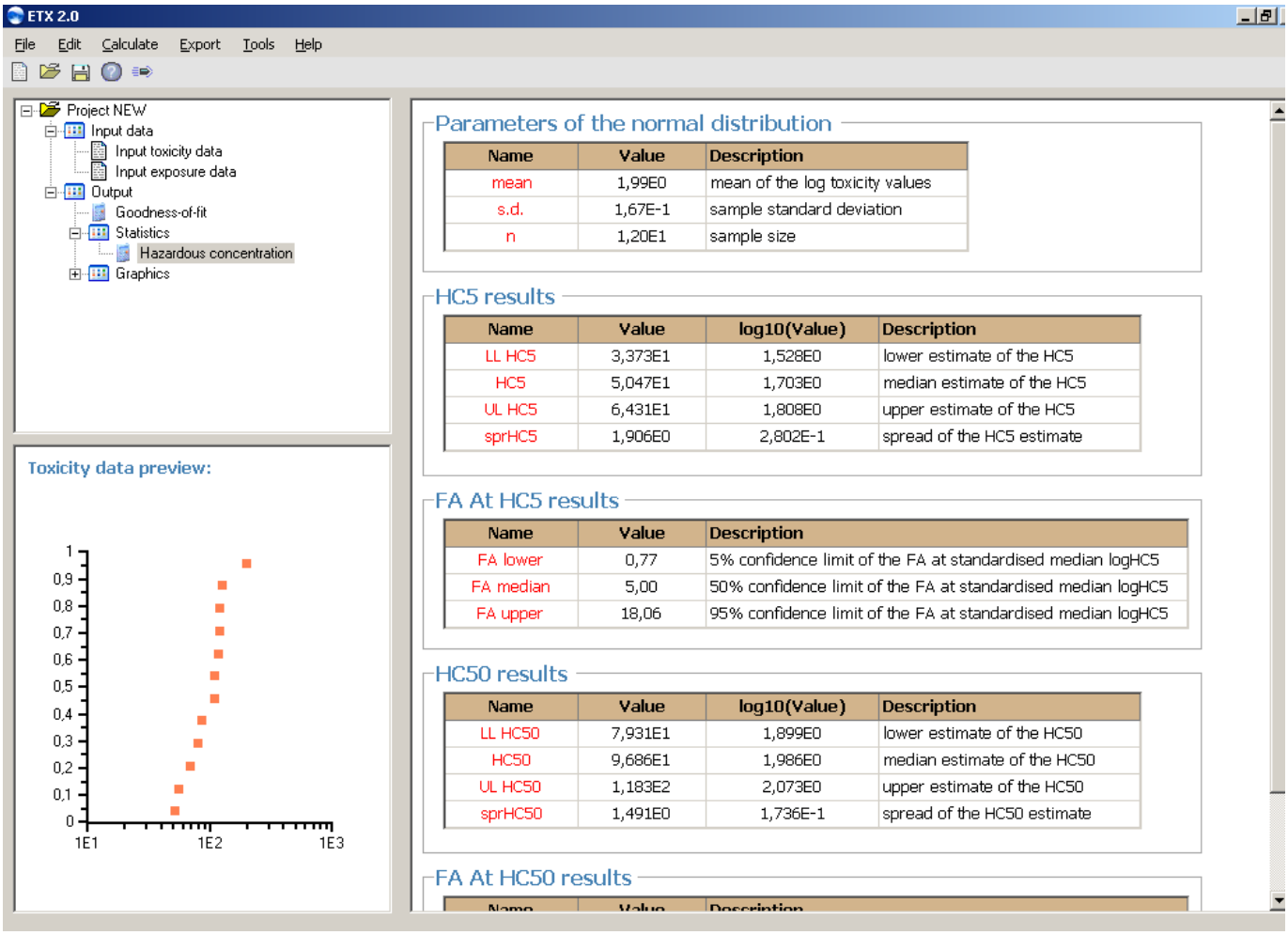


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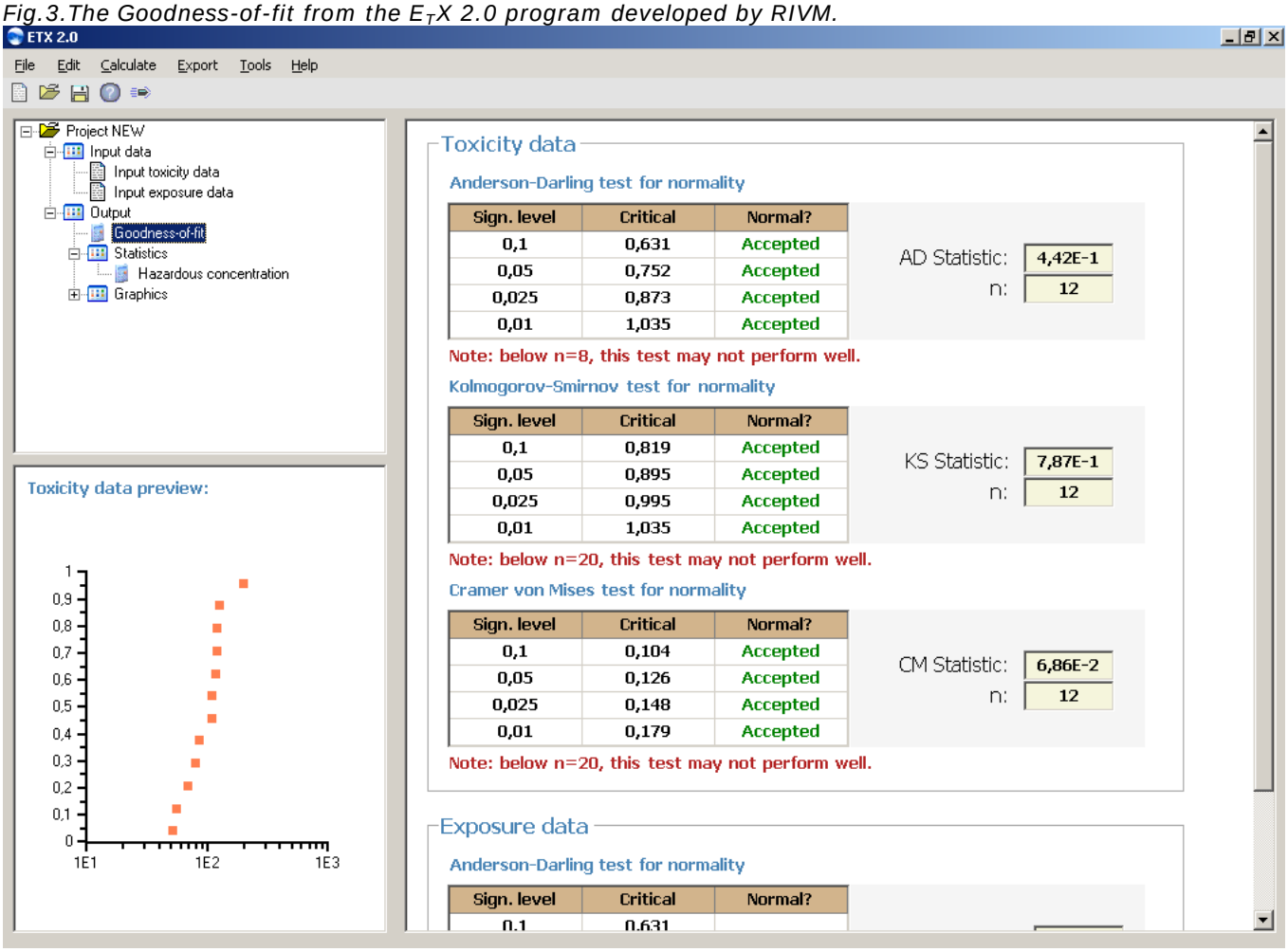
Fig.3.The Goodness-of-fit from the E_TX 2.0 program developed by RIVM.



3. Conclusion

Following the revision of OECD 208 this reflection paper updates the testing strategy for plants according to the VICH guideline that enables applicants to assess the risk for plants in a Tiered approach including a higher Tier based on the SSD method. If desired the stepwise approach can be avoided and the SSD method can also be used at the start of the risk assessment in Phase II.