COMMISSION OF THE
EUROPEAN COMMUNITIES

7032/VI/95 rev.5
22/7/97

Directorate General for Agriculture

VI B II-1

APPENDIX H

STORAGE STABILITY OF RESIDUE SAMPLES

1 Foreword

When compiling residue data it is essential to ensure that the residue situation of a sample remains accurately quantifiable from the time of sampling to analysis. Samples should be analysed as quickly as possible after collection, before physical and chemical changes take place. In cases where it is not possible to analyse samples immediately after they have been taken, the sample material must be stored until it is analysed.
In these cases the effect of storage conditions on the stability of residues must be investigated.

Initial indications of storage stability are obtained from hydrolysis behaviour, the behaviour of the technical active substance and its formulations during storage, preliminary adsorption/desorption tests and particularly from metabolic tests, if these tests are designed in such a way that such information can be obtained.

Generally speaking the active substances of plant protection products, which are also required to have a certain persistence in the field, are sufficiently stable to allow the residue situation to be retained by storing the samples at/below -18°C.

The various options for performing storage stability studies are described below with reference to the prevailing official regulations.

Storage stability tests should preferably be carried out prior to commencement of the residue trials programme in order to identify possible problems.

2 Objectives

The aim of these studies is to investigate the stability of residues during storage prior to analysis.

3 Extent of data required

Data are required to support residue trials data, livestock feeding studies, processing studies and studies in succeeding crops. Residue storage stability data may also be required in support of metabolism and distribution studies if these are to be used for quantitative purposes. Provided samples are frozen within generally 24 hours after sampling and unless a compound is otherwise known to be volatile or labile, data are not normally required for samples extracted and analyzed within 30 days from sampling (6 months in the case of radiolabelled material).
It may be necessary to perform studies on the stability of residues during storage.

4 Test procedure

4.0 Introduction

If accurate results are to be obtained from storage stability studies there must be a sufficiently large quantity of residue in the starting material to allow any degradation observed during storage to be described with sufficient precision.

4.1 Test substance(s)

If the starting materials are derived from outdoor or indoor field studies, they will generally have been treated with the formulation or types of formulation to be submitted for registration or maintenance of registration (see section 4.5.2).

If test substances are added to untreated sample materials in the laboratory (see section 4.5.3), it is usually the active substance and/or relevant metabolites that is added.

4.2 Residue level in test material

The residues should fall within the range of the expected residue values but should be at least ten times above the limit of determination of the analysis method so that any degradation can be accurately demonstrated.

4.3 Sampling dates

The sampling/analysis dates are dependent on previous knowledge of the stability of the residues.

If a relatively rapid reduction is likely, it is advisable to take samples soon after the "Day 0 value", e.g. after about 7, 14, 28, 56, 112 etc. days of storage.

If, on the basis of previous knowledge, stable residues are expected, longer intervals may be selected e.g. 0, 1, 3, 6 and 12 months.

The overall duration of the study is dependent on the period during which storage behaviour is/has to be investigated.

4.4 Selecting sample material

In the case of studies involving plant material, the number of sample materials is dependent on the use of the product. Normally a study should be performed on one plant or plant product from each of the following groups: predominantly water-, oil-, protein- or starch-containing materials.

In the case of studies involving animal material (i.e. animal transfer studies) e.g. tissues (muscle, liver), milk and eggs should be chosen.

4.5 Preparing samples for storage

4.5.1 Condition of samples

The sample material used to determine storage stability should be in the same prepared condition (e.g. chopped) as that in which the residue samples are stored.

Experiments should preferably be conducted on samples with incurred residues.

4.5.2 Using sample material from field studies

In a study of this type, sample material from outdoor or indoor studies, which is packed, despatched, prepared for analysis and examined using the analysis method which detects the relevant residue immediately after sampling, i.e. without intermediate storage, is used. It is particularly important that the starting material is well mixed, so that variations resulting from an unhomogeneous distribution which could in some circumstances necessitate repetition of the studies are minimised as far as possible. It is advisable to check the residue level by performing a minimum of three analyses on the starting material. At least two representative analytical samples (and corresponding control samples) from this material are then stored in each case, depending on the number of sampling dates planned. It appears sufficient to provide two analytical samples per sampling date for analysis, provided that repeat determinations are performed on at least three or four sampling dates.

The advantages of this method are that it is based on the situation in practice, i.e. incurred residues are considered. Its application is limited if no quantifiable residues or only residues at or about the limit of determination are available, since a reduction in the residue cannot be described.

4.5.3 Fortification experiments with the active substance or its relevant metabolites

In this variant the active substance or the metabolites forming the relevant residue are added to suitable sample material (e.g. from control plots) from a standard solution. Previous analysis of the sample material for residues is strongly advisable to prevent interference with other substances or from contamination with the test substances and consequently, in some circumstances, the need to repeat the study. When proceeding in accordance with 4.5.2 (aliquoting of a starting quantity), thorough homogeneous distribution is essential if a meaningful result is to be obtained.

An alternative is not to add until after sub-sampling, i.e. to add to the individual analysis samples. The sub-samples should be measured in such a way that they correspond to the net weight specified for the analysis method, i.e. are analysed as a whole. Addition, e.g. by means of a microlitre spray, and brief mixing should suffice. The advantage of this method is the elimination of errors resulting from non-homogeneous distribution, since the entire sample is analysed in each case. The disadvantage of this method can be that the free unconjugated residue in the test material may be suject to greater degradation than the incurred residue in the field samples where some of the residue may have been conjugated (see 4.5.2).

4.6 Storage conditions

It is recommended that the samples be stored in a cool dark place in inert non-contaminating containers. The study must be carried out under controlled temperature conditions.

The storage conditions are based on the practical conditions for storage of residue samples.

Previous experience has shown that temperatures of - 18°C and below are usually sufficient to retain accurately quantifiable pesticide residues.

Extended storage in freezers can cause moisture to migrate to the surface of the sample then to the freezer coils, slowly dessiccating the sample. This effect may be of importance if water content affects the subsequent analysis and can affect the calculated residue concentration.

4.7 Analysis and number of analyses per sampling date

A minimum of two parallel analysis (= two samples) should be carried out for each sampling date. It is recommended that a number of additional samples are also taken on each date; these may be used if required for repeating analyses.

The analytical method must be able to determine the parent compound and/or its relevant metabolites. Recovery validation data should be generated for each analytical series using the control material.

5 **Report**

5.0 Introduction

A report on storage stability studies should include all relevant data in a suitable format. This can, for example, be achieved by using the method outlined below.

5.1 Summary report

A report on storage stability studies should include all relevant data in a suitable format.

The summary presentation of a storage stability study is, for example, sub-divided into the following sections:

- Objective
- Sample background
- Preparing samples
- Analysis, measuring, assessment
- Evaluation, discussion of results.

5.1.1 Objective

The Objecitve section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

5.1.2 Sample background

This section of the report describes the origin of the sample materials used in the storage stability study.

5.1.3 Preparing and storing samples

This section should be used to describe the methods used to prepare (such as chopping, adding of the test substance(s) ...) and to store samples.

5.1.4 Method of analysis and results

This essentially describes the analytical method used to analyse the samples and to measure the residues.

This section of the report contains the results and the methods used to analyse the samples. Reports should state: residue definition, recoveries from fortified samples and limits of determination. Representative chromatograms should be included (minimum of standard: untreated sample, fortified untreated sample and typical sample).

5.1.5 Discussion of the results

This section of the report discusses and evaluates the reported analysis results in the light of the questions outlined in the Objective section.

The likely effect of prolonged storage on residues should be considered.

The results are presented in tabular form including average values, where appropriate with comments e.g. indicating outliers, special circumstances etc. The individual results should not be corrected to 100 % yield; the recovery rates determined for each sampling date should, however, be indicated.

In the case of a perceptible reduction in particular, a reduction graph showing the storage time on the X-axis and the content in percentage concentration ("Day O value" = 100 %) on the Y-axis, is plotted from the average values or the individual values determined. This graph can be used to determine the percentage reduction at any point in time by means of interpolation.

Where study samples are stored under conditions different from those in the residues storage stability study this must be stated.

Where the degradation during storage is significant (more than 30%) it may be necessary to use a shorter sample storage period prior to analysis.

6 References

"P. Beutel, H. Frehse, B. Krebs, H. Morgenthaler, H.-J. Scheuermann und G. Timme (1992):
Rückstandsversuche- Teil II: Lagerstabiliät von Rückstandsproben, Industrieverband Agrar e.V. (IVA), 2. Auflage 1992.

P. Beutel, H. Frehse, B. Krebs, H. Morgenthaler, H.-J. Scheuermann and G. Timme (1992):
Residue Studies - Part II/ Starage stability or residue samples, Industrieverband Agrar e.V. (IVA) 1st impression 1992."

Egli, H. (1982):
Storage Stability of Pesticide Residues.
J. Agric. Food Chem. 30, 861-866.

K. Hohgardt, J.-R. Lundehn, H. Parnemann und A. Wilkening,
1990:
Prüfung des Rückstandsverhaltens - Allgemeine Hinweise zu Planung, Anlage und Durchführung von Rückstandsversuchen, Richtlinie der Biologischen Bundesanstalt für Land- und Forstwirtschaft (Studying residue behaviour - General information on planning, setting up and performing residue trials, Federal Biological Research Centre for Agriculture and Forestry Guidelines), Part IV, 3-3, January 1990, Saphir Verlag (Ribbesbüttel/Germany)

US Department of Commerce (1988):
Pesticide Assessment Guidelines, Subdivision O, Effects of Storage
Storage Stability - on Validation of Pesticide Residue Data.
National Technical Information Service, October, EPA.

Deutsche Forschungsgemeinschaft (German Research Association):
Rückstandsanalytik von Pflanzenschutzmitteln. Methodensammlung der Arbeitsgruppe "Analytik". 1. bis 10. (Residue analysis of pesticides. Method compilation of the "Analysis" working group, 1 to 10) 1989 issue, Chapter XI, in conjunction with Chapter V, 4-7.
VCH-Verlagsgesellschaft mbH, Weinheim, Germany.

UK-MAFF (1992):
Data Requirements for Approval under the Control of Pesticides Regulations 1986.