

Phytotoxicity

Andreas Baumgarten

Agency for Health and Food Safety, Vienna



Acknowledgement

This work has been carried out with financial support from the following EU Member States:
UK, Germany, France, Italy, Spain, Nordic countries, Netherlands, Denmark, Austria, EU DG
XI and JRC, Ispra.

CONTENTS

| | |
|--|----|
| LIST OF TABLES | 5 |
| LIST OF FIGURES | 5 |
| SUMMARY | 6 |
| 1. INTRODUCTION | 7 |
| 2. EXISTING METHODOLOGY | 8 |
| 2.1 Standards or draft standards | 8 |
| 2.2 Other than standard methods | 8 |
| 2.3 National law: | 8 |
| 3. EVALUATION OF DRAFTING A HORIZONTAL STANDARD | 9 |
| 3.1 General approach | 9 |
| 3.2 Test plants | 9 |
| 3.3 Experimental design | 9 |
| 3.4 Test parameters | 9 |
| 3.5 Reference material | 10 |
| 3.6 Growing conditions | 10 |
| 4. CRITICAL POINT AND RECOMMENDATIONS | 11 |
| 4.1 Reference material | 11 |
| 4.2 Fertilization | 11 |
| 4.3 Salt Content, dilution of the test material | 11 |
| 4.4 pH | 11 |
| 4.5 Test plants | 11 |
| 4.6 Water holding capacity | 12 |
| 5. DRAFT STANDARD (CEN TEMPLATE) | 13 |
| 5.1 Scope | 13 |
| 5.2 Normative references | 13 |
| 5.3 Terms and definitions | 13 |
| 5.4 Principle | 13 |
| 5.5 Reagents | 13 |
| 1.1.1 washed quartz sand, particle size ≤ 3 mm | 13 |
| 5.5.1 13 | |
| 5.5.2 Reference material (e.g. washed perlite, particle size 0.5-2 mm; mixture of equal masses of commercially available growing medium with low nutrient content (suitable for germination) and tennis court sand, particle size ≤ 2 mm, particles $< 0,063$ mm less than 25%) | 13 |
| 5.6 Apparatus | 14 |
| 5.6.1 glass trays, diameter = 120mm, height = 60mm ("Neubauer – tray") | 14 |
| 5.6.2 glass tube, height app. 60 mm, inner diameter 6 – 8 mm | 14 |
| 5.6.3 glass plate for covering the glass trays | 14 |
| 5.6.4 opaque plastic film for covering the glass trays | 14 |
| 5.6.5 balance, capable of weighing accurately to 0,01g | 14 |
| 5.6.6 testing facility: phytotron, plant growth room or greenhouse | 14 |
| 5.7 Sample preparation | 14 |
| 5.8 Procedure | 14 |
| 5.8.1 Experimental Design | 14 |
| 5.8.1.1 PREPARATION OF THE TRAYS | 14 |
| 5.8.1.2 TEST PLANTS | 14 |
| 5.8.1.3 PREPARATION OF THE SEEDS | 15 |
| HORIZONTAL - 7 | 3 |

| | |
|--|----|
| 5.8.1.4 GROWING CONDITIONS | 15 |
| 5.8.1.5 PERFORMING OF THE TEST | 16 |
| 5.9 Calculation and Expression of results | 16 |
| 5.10 Precision | 16 |
| 5.11 Test Report | 17 |
| <i>APPENDIX A FERTILIZATION OF THE TEST SAMPLE</i> | 18 |
| <i>A.1 Introduction</i> | 18 |
| <i>A.2 Test of the nutrient status</i> | 18 |
| APPENDIX B EXPERIMENTAL DESIGN | 19 |
| REFERENCES | 19 |
| REFERENCES | 20 |

LIST OF TABLES

| | | |
|---------|--|----|
| Table 1 | Test plants (in accordance with ISO 11269-2) | 15 |
| Table 2 | <i>Threshold values for fertilizer requirement</i> | 18 |

LIST OF FIGURES

| | | |
|----------|---------------------|----|
| Figure 1 | Experimental design | 19 |
|----------|---------------------|----|

SUMMARY

The assessment of phytotoxicity is one of the major criteria if using soil, sludge or composted biowaste as any kind of plant substrate. For testing, plants are either grown in the material directly or on inert substrates provided with leachates of the tested material. For this draft, the first approach has been chosen. A variety of test plants are available. The individual choice of the test plant can be made according to the scope of the test. In any case, at least one monocotyledonous and one dicotyledonous species have to be used.

1. INTRODUCTION

For the use of soil, sludge or biowaste compost any kind of toxic effect towards the environment has to be avoided. The organisms most likely to suffer immediately and visibly from adverse effects are higher plants. Therefore, without a detailed specification of the cause, the phytotoxic effect of a certain material can be used as an indicator.

Phytotoxicity is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions (PAS 100). If a phytotoxic effect of a certain material is stated, further investigations should be carried out to identify the specific cause.

There is a wide range of methods for examining phytotoxicity, although there are hardly any national standards. Therefore, both the test plants and the individual procedures differ to a large extent, mostly depending on the material under investigation. The proposed draft method is designed to create an optimum version by combining the most suitable parts of different test procedures.

2. EXISTING METHODOLOGY

2.1 Standards or draft standards

ISO 11269-2:1995 Soil Quality – Determination of the effects of pollutants on soil flora – Effects of chemicals on the emergence and growth of higher plants

ISO/CD 17126 Soil Quality – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (*Lactuca sativa* (L.))

ÖNORM S 2021 (draft): Requirements for growing media

2.2 Other than standard methods

The following methods have been supplied by organisations other than standardisation bodies:

WRAP, The Composting Association (2002): Public Available Specification 100 – Specification for composted material, Annex D: Method to assess contamination by weed propagules and phytotoxins in composted material

Federal Compost Quality Assurance Association, Germany (1998): Determination and evaluation of phytotoxicity of compost by means of a germinating plant test with summer barley

Fuchs, J.G. (2000): New biotests to measure the biological qualities of compost. *AgrarForschung* 7(7): 314 – 319

Petersen, L.: Water Extract – a new method for a bioassay; DEG Green Team, Denmark

Rijkslaboratorium Gent: Phytotoxicity

KIWA: Test of Phytotoxicity

RHP-foundation: Phytotoxicity

2.3 National law:

Austrian compost Ordinance

German Ordinance on the utilization of Bio-Wastes on land used for agricultural, silvicultural and horticultural purposes

3. EVALUATION OF DRAFTING A HORIZONTAL STANDARD

3.1 General approach

Generally, there are two possible approaches to assess phytotoxicity:

- To grow certain plants directly in the test material or in diluted samples
- To grow certain plants in hydroponic systems (e.g. rockwool granules) supplied with leachate or mixtures of leachate and nutrient solution

For the purpose of this draft, it seems more convenient to use the first approach. Furthermore, in some cases the use of closed systems is suggested to assess possible effects of volatile phytotoxins as well.

3.2 Test plants

Depending on the scope of the method, several plants can be used for the test, ranging from monocotyledons like barley or ryegrass to dicotyledons like cress, lettuce or tomato. It seems important to leave a certain choice to the operator, however, at least one monocotyledonous and one dicotyledonous species has to be selected.

3.3 Experimental design

There are a great variety of experimental designs available. The most important demands are

- To offer sufficient rooting space
- To ensure the sufficient supply of water and
- To ensure the sufficient supply of nutrients.

The design described in this draft is believed to meet all these requirements.

3.4 Test parameters

For all methods, the key parameter is the fresh weight of the shoot. Additionally, depending on the test plants several other criteria are suggested to allow a more detailed interpretation of possible phytotoxic effects:

- Root weight
- Root length
- Development of the root system
- Germination rate
- Shoot/root ration
- Plant abnormalities

In this draft, it is suggested to use some of these criteria in addition to the fresh weight.

3.5 Reference material

All the parameters described above have to be related to the results obtained from a reference substrate. Again, a variety of materials are suggested by the different authors:

- Sand
- Rock wool granules
- Perlite
- Commercially available standardised substrates
- Peat based growing media (PBGGM) according to a specific composition
- Commercially available substrates, low in nutrient content (e.g. for germination)
- Mixtures of commercially available substrates with inert materials (e.g. tennis court sand)

However, the standard substrate has to be available any time all over Europe in constant quality.

3.6 Growing conditions

There is also a variety in growing conditions reported by the different authors. The main task is to ensure optimal germination and growing conditions for the plants. In this draft, the description is in accordance with ISO 11269-2.

4. CRITICAL POINT AND RECOMMENDATIONS

4.1 Reference material

The reference material should both serve as a reference regarding plant growth and a possible mixing constituent for the test material as well. Therefore, it has to be low but sufficient in nutrient content and to provide an optimum range of water holding capacity. Already, mixtures of commercially available substrates with certain inert constituents like tennis court sand have proved to be suitable. However, as the availability of these materials might be limited, research work has to be done to identify proper materials as mixing components or pure substrates available on a large scale (e.g. perlite).

Furthermore, possible ranges of plant fresh weight obtained by growing on the reference material have to be identified to enable the assessment of the performance of the test procedure. Below certain amounts of harvest, the results of the test must not be accepted. Also in this respect further research work is necessary.

4.2 Fertilization

If initially a material is low in nutrients, deficiencies in plant growth might occur with no regard to phytotoxicity. In this case, additional fertilization will be necessary. To identify nutrient deficiencies, the determination of plant available N, P and K according to EN 13652 (water extraction) or EN 13651 (CAT-extraction) is suggested. For setting the thresholds, additional research work is required. Preliminary, this part is included in the draft but may be removed until the stating of final results of this research work.

4.3 Salt Content, dilution of the test material

Some authors suggest a dilution of the test material in case of high salt contents/electric conductivity. As this parameter can be regarded as one of the most important factors of phytotoxicity (especially regarding sludges and composted biowaste), it has to be clarified whether there is phytotoxicity due to high salt content. Therefore, the material has to be tested undiluted. Besides, there might be additional phytotoxic parameters with no regard to the salt content. To enable a clarification in this respect, certain dilution ratios are proposed.

4.4 pH

The pH contributes to the phytotoxicity of a material as well. However, sometimes a low pH might be required due to the purpose of the use. In these cases, if the initial pH of the material meets this demand, pH can be adjusted to a value ranging between 5 and 7 using lime.

4.5 Test plants

As pointed out before, there are a variety of test plants available to be chosen regarding certain methods or purposes. At least, one monocotyledonous and one dicotyledonous species have to be used for the test. The choice may be up to the client or the performing laboratory. A suggestion is included in the draft method.

4.6 Water holding capacity

Probably, the application of this method is limited to materials with sufficient water holding capacity. Materials with a large amount of coarse particles seem unlikely to provide optimum moisture for growing seedlings. Again, there is an urgent need for further research work to define the scope of the method.

Furthermore, an exact definition of the optimum water content for plant growth is hardly possible. To ensure suitable moisture conditions, it is recommended that only staff experienced in plant growing should carry out the test.

5. DRAFT STANDARD (CEN TEMPLATE)

NOTE: Where italics appear in the draft method it indicates an area that requires additional work and confirmation

5.1 Scope

This European Standard specifies a method to assess the phytotoxicity of soil, sludge and treated biowaste. The method is not applicable for materials with a water volume (EN 13041:1999) *less than 50%*.

5.2 Normative references

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this method only when incorporated in it by amendment or revision. For undated references the latest edition of the publications referred to apply.

EN 00000 *HORIZONTAL Sampling procedures*

EN 13040 Soil improvers and growing media - Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density

EN 13041 Soil improvers and growing media - Determination of physical properties

ISO 11269-2 Soil quality – Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the emergency and growth of higher plants

5.3 Terms and definitions

For the purpose of this standard, the definitions given in PD CR 13456, EN 12579, EN 13040 and PAS 100 apply.

5.4 Principle

The germination and development of indicator plants in the test sample is monitored in relation to a standard substrate under controlled growing conditions.

5.5 Reagents

5.5.1 washed quartz sand, particle size ≤ 3 mm

5.5.2 Reference material (e.g. washed perlite, particle size 0.5-2 mm; mixture of equal masses of commercially available growing medium with low nutrient content (suitable for germination) and tennis court sand, particle size ≤ 2 mm, particles $< 0,063$ mm less than 25%)

5.6 Apparatus

5.6.1 glass trays, diameter = 120mm, height = 60mm ("Neubauer – tray")

5.6.2 glass tube, height app. 60 mm, inner diameter 6 – 8 mm

5.6.3 glass plate for covering the glass trays

5.6.4 opaque plastic film for covering the glass trays

5.6.5 balance, capable of weighing accurately to 0,01g

5.6.6 testing facility: phytotron, plant growth room or greenhouse

5.7 Sample preparation

The sampling is performed in accordance with EN 00000 (HORIZONTAL sampling procedure); the sample is prepared in accordance with EN 13040:1999, clause 8. Initially, the sample has to be tested without dilution. Depending on the scope of the test, further dilution with the standard material is possible.

NOTE: For dilution, the following ratios (test material + reference material) are proposed:
1 + 1; 1 + 3; 1 + 7

5.8 Procedure

5.8.1 Experimental Design

5.8.1.1 Preparation of the trays

Fill the tray (5.6.1) with app. 200g (app. 100ml) washed quartz sand (5.5.1) and spread it evenly on the bottom of the tray. Place a small glass tube (5.6.2) vertically in the middle of the tray and add as much test sample as necessary to leave a distance of appr. 10 mm to the upper edge of the tray after gentle compression. Dry test samples have to be moistened before filling. The same procedure is carried out with the standard substrate.

NOTE 1: If the purpose of the material requires a pH less than 5 (e.g. growing medium for Azalea-culture) and the initial material meets this condition, the pH has to be increased to pH 5 to 7 with lime before starting.

NOTE 2: *If the nutrient status of the test sample is low, it has to be adjusted using suitable fertilizers (see Appendix A)*

5.8.1.2 Test plants

At least, one monocotyledonous (Category 1) and one dictyledonous plant (Category 2) has to be used as test plants (see Table 1). The use of Barley (*Hordeum vulgare* L.) and Chinese Cabbage (*Brassica campestris* L. var. *chinensis*) is suggested.

Table 1 Test plants (in accordance with ISO 11269-2)

| Category | Test plant | Scientific name |
|----------|--|--|
| 1 | Barley (spring or winter) | <i>Hordeum vulgare</i> L. |
| 1 | Rye | <i>Secale cereale</i> L. |
| 1 | Ryegrass, perennial | <i>Lolium perenne</i> L. |
| 1 | Rice | <i>Oryza sativa</i> L. |
| 1 | Oat (common or winter) | <i>Avena sativa</i> L. |
| 1 | Wheat, soft | <i>Triticum aestivum</i> L. |
| 1 | Sorghum, common (or shattercane or durra, white or millet, great) | <i>Sorghum bicolor</i> (L.) Moench |
| 1 | Sweetcorn | <i>Zea mays</i> L. |
| 2 | Chinese cabbage | <i>Brassica campestris</i> L. var. <i>chinensis</i> |
| 2 | Cress, garden | <i>Lepidium sativum</i> L. |
| 2 | Mustard, white | <i>Sinapis alba</i> |
| 2 | Rape (or rape (summer) or rape (winter)) | <i>Brassica napus</i> (L.) ssp. <i>napus</i> |
| 2 | radish, wild | <i>Raphanus sativus</i> L. |
| 2 | Turnip, wild | <i>Brassica rapa</i> ssp. (DC.) Metzg. |
| 2 | Bird's foot clover, Fenugreek | <i>Trifolium ornithopodioides</i> L. |
| 2 | Lettuce | <i>Lactuca sativa</i> L. |
| 2 | Tomato | <i>Lycopersicon</i> <i>esculentum</i> Miller |
| 2 | Bean | <i>Phaseolus aureus</i> Roxb. |

5.8.1.3 Preparation of the seeds

Spread 20 seeds evenly on the surface and cover with appr. 100 g (appr. 50 ml) quartz sand (5.5.1). If the seeds are too small to handle properly (e.g. garden cress), use 0,4 g \pm 0,01 g of seeds instead. Afterwards, the substrate is moistened using the glass tubule or gentle spraying. Back water has to be avoided. For each test plant, 4 replicates have to be prepared both for the test sample and the standard substrate.

5.8.1.4 Growing conditions

The temperature, humidity and light conditions shall be such that they are suitable for maintaining "normal" growth of all selected species.

NOTE: The following conditions and procedures are recommended:

- Temperature: to meet the normal growing conditions of the species selected (normally between 15°C and 25°C)
- Lighting: 16 hours per day; 7000lx minimum light intensity in the wavelength suitable for photosynthesis. Therefore, in a greenhouse, additional lighting may be necessary during times of low natural light
- Moisture: The moisture of the substrate has to be kept constant by daily gentle spraying. To monitor the variation of water content, randomly weigh several trays during culture. The variation of the moisture content must not exceed 10%. Furthermore, monitor the moisture distribution within the test sample by visual control. If necessary, add water through the glass tube.

5.8.1.5 Performing of the test

The trays are covered with a glass plate (5.6.3) and an opaque plastic film (5.6.4) until germination starts.

After germination of the first plants in the standard substrate, the covers are removed from all the trays. The trays are kept 8 to 11 days in the testing facility, then the plants are cut near the substrate surface and the mass of the fresh plant (accuracy 0,1g) is determined.

5.9 Calculation and Expression of results

The mean fresh mass of the test plants germinated on the test sample is given by Equation (1)

$$\bar{M}_{sample} = (M1_{sample} + M2_{sample} + M3_{sample} + M4_{sample}) \div 4 \quad (1)$$

where

\bar{M}_{sample} is the mean fresh mass of the test plants germinated on the test sample and

$M1 - 4_{sample}$ are the masses of the test plants germinated on the test sample repetitions.

The mean fresh mass of the test plants germinated on the standard substrate is given by Equation (2)

$$\bar{M}_{std} = (M1_{std} + M2_{std} + M3_{std} + M4_{std}) \div 4 \quad (2)$$

where

\bar{M}_{std} is the mean fresh mass of the test plants germinated on the standard substrate and

$M1 - 4_{std}$ are the masses of the test plants germinated on the standard substrate repetitions.

If the deviation between the mean and the individual repetitions is more than 15%, the results are not valid.

The percentage of plant fresh mass germinated on the test sample in relation to the plant fresh mass germinated on the standard substrate (PFM), is given by equation (3)

$$PFM(\%) = \frac{100 \cdot \bar{M}_{sample}}{\bar{M}_{std}} \quad (3)$$

Furthermore, anomalies of plants or plant growth have to be recorded.

NOTE:

Additionally, it is recommended to monitor the following criteria as well:

- a) germination rate in % compared to the standard substrate
- b) delay of germination in days compared to the standard substrate

5.10 Precision

No data available at the moment

5.11 Test Report

The test report shall include the following information:

A reference to this method

A complete identification of the sample

The kind of diluent

The dilution ratios

The percentage of plant fresh mass germinated on the test sample in relation to the plant fresh mass germinated on the standard substrate.

Anomalies of plants or plant growth

Any deviations to the prescribed method.

Any factors that may have affected the test

NOTE: Further parameters as suggested in the NOTE in clause 5.9 may be included.

APPENDIX A FERTILIZATION OF THE TEST SAMPLE

A.1 Introduction

Problems in germination and growth are not only a function of possible phytotoxic substances, but of nutrient supply as well. Whereas a nutrient surplus might be seen as a possible phytotoxic attribute, a lack of nutrients can be overcome easily by fertilizing. Therefore, prior to performing the test, the nutrient status of the test sample has to be investigated.

A.2 Test of the nutrient status

To assess the nutrient status of the test sample, the following determinations have to be carried out:

Determination of calcium chloride/DTPA (CAT) soluble nutrients in accordance with EN 13651 or

Determination of water-soluble nutrients in accordance with EN 13652

Evaluation of the results

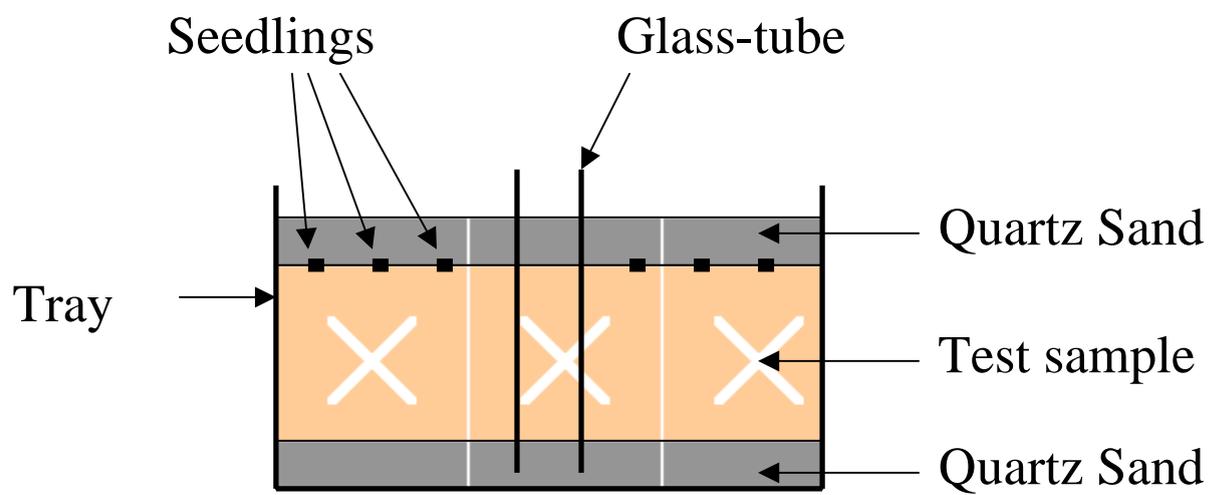
If the nutrient contents are below critical values, the sample has to be fertilized accordingly (Table 2).

Table 2 Threshold values for fertilizer requirement

| Nutrient | lower limit CAT-extraction | lower limit Water-extraction | amount to be added (g/l) | possible fertilizers |
|----------|-------------------------------|---------------------------------|-----------------------------|-------------------------|
| N | | | | NH_4NO_3 |
| P | | | | KH_2PO_4, H_3PO_4 |
| K | | | | K_2SO_4 |

APPENDIX B EXPERIMENTAL DESIGN

Figure 1 Experimental design



REFERENCES

ISO 11269-2:1995 Soil Quality – Determination of the effects of pollutants on soil flora – Effects of chemicals on the emergence and growth of higher plants

ISO/CD 17126 Soil Quality – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (*Lactuca sativa* (L.))

ÖNORM S 2021 (draft): Requirements for growing media

EN 13651 Soil improvers and growing media – extraction of elements using CaCl_2 /DTPA (CAT)

EN 13652 Soil improvers and growing media – water extraction of elements

WRAP, The Composting Association (2002): Public Available Specification 100 – Specification for composted material, Annex D: Method to assess contamination by weed propagules and phytotoxins in composted material

Federal Compost Quality Assurance Association, Germany (1998): Determination and evaluation of phytotoxicity of compost by means of a germinating plant test with summer barley

Fuchs, J.G. (2000): New biotests to measure the biological qualities of compost. *AgrarForschung* 7(7): 314 – 319

Kipp, J.A., G. Wever and C. de Krej (ed.) (2000): International Substrate Manual: Analysis Characteristics Recommendations. Elsevier Doetinchem The Netherlands

Petersen, L.: Water Extract – a new method for a bioassay; DEG Green Team, Hvidkærvej 29 5250 Odense SV, Denmark

VITO, (2001): Standard Operating Procedure: Phytotoxicity (Fytotoxiciteit); <http://www.vito.be/milieu/milieumetingen8a1.htm>

Wever, G. (2003): Set of appendices on analytical methods. BRL-K10001 Substrate materials for the product certificate for Substrate materials. KIWA BRL-K10001, The Netherlands.

Austrian compost Ordinance. Federal Ministry of Agriculture, Forestry, Environment and Water Management, Federal Law Leaflet (BGBl.) II Nr. 292/2001. Vienna, 2001

German Ordinance on the utilization of Bio-Wastes on Land used for Agricultural, Silvicultural and Horticultural Purposes. (Biowaste ordinance – BioAbfV) Federal Ministry for Environment, Nature Protection and Reactor Safety. Status 21st of September 1998: Federal Law Leaflet I 1998, page 2955. Bonn, 1998