codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 7 a) **CRD 5**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON PESTICIDE RESIDUES
Thirty-fourth Session
The Hague, The Netherlands, 13 - 18 May 2002

German Comments on CL 2001/29 PR; Methods of Analysis for the Determination of Pesticide Residues

Background

The Codex Committee on Pesticide Residues has discussed the criteria for the selection of recommended methods of analysis on several occasions. In its 33th session the Committee agreed to circulate both the Proposed Draft Amendment to the Introduction of the Recommended Methods of Analysis for Pesticide Residues (ALINORM 24A, Appendix VIII, CL 2001/29-PR) and the Proposed Draft Amendments to the Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (ALINORM 24A, Appendix VII) at step 3 of the Codex Procedure.
In its 31st Session the Codex Committee on Pesticide Residues agreed to maintain the present list until appropriate validation criteria were developed (ALINORM/24A, para 128). As those criteria are presently being elaborated under the Codex Procedure Germany proposes the inclusion of a number of recently developed European Standards in the List of Recommended Methods of Analysis for Pesticide Residues
The attached methods of analysis for the determination of food additives and contaminants have been validated nationally or internationally according to ISO 5725, ISO/IUPAC/AOAC-protocol or equivalent requirements, and were subsequently standardized by the Technical Committee 275 "Food analysis - Horizontal methods" of the European Committee for Standardization (CEN). As the scope of the methods also includes a number of contaminants it has also been presented at the 23rd session of the CCMAS and, in agreement with the chairman of CCMAS and the Codex procedures, it has been decided to address these methods at the responsible Codex Committee on food additives and contaminants.
The attached extracts of the European standardized methods contain information on their scope, principle, validation data and some further aspects, if appropriate.

Proposal

Germany proposes to endorse the attached methods of analysis for the determination of pesticide residues in foods as CODEX-methods.

European Standards elaborated by CEN/TC 275 “Food analysis – Horizontal methods” which are proposed by the German delegation to be endorsed as CODEX-methods by the CCFAC

Determination of pesticides and PCBs

EN 1528-1: 1996-10	Fatty food - Determination of pesticides and polychlorinated	Type III

(confirmed 2001)	biphenyls (PCBs) - Part 1: General considerations	
EN 1528-2: 1996-10 (confirmed 2001)	Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 2: Extraction of fat, pesticides and PCBs and determination of fat content	Type III
EN 1528-3: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 3: Clean-up methods	Type III
EN 1528-4: 1996-10 (confirmed 2001)	Fatty food – Determination of pesticides and polychlorinated biphenyls (PCBs) - Part 4: Determination, confirmatory tests, Miscellaneous	Type III
EN 12393-1:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 1: General considerations	
EN 12393-2:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 2: Methods for extraction and clean-up	
EN 12393-3:1998-10	Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues – Part 3: Determination and confirmatory tests	
EN 12396-1:1998-10	Non fatty food - Determination of dithiocarbamate and thiuram disulfide residues - Part 1: Spectrometric method	
EN 12396-2:1998-10	Non fatty food - Determination of dithiocarbamate and thiuram disulfide residues - Part 2: Gaschromatographic method	
EN 12396-3:2000-05	Non fatty food – Determination of dithiocarbamate and thiuram disulfide residues - Part 3: UV-spectrometric xanthogenate method	
EN 13191-1:2000-04	Non fatty food - Determination of bromide residues Part 1: Determination of total bromide as inorganic bromide	
EN 13191-2:2000-04	Non fatty food - Determination of bromide residues Part 2: Determination of bromide	

EN 1528 Fatty food - Determination of pesticides and polychlorinated biphenyls (PCBs) – General Introduction

EN 1528 consists of the following Parts:

Part 1 "General" presents the scope of the standard and describes general considerations with regard to reagents, apparatus, gas chromatography etc., applying to each of the analytical methods selected.

Part 2 "Extraction of fat, pesticides and PCBs, and determination of fat content" presents a range of analytical procedures for extracting the fat portion containing the pesticide and PCB residues from different groups of fat-containing foodstuffs.

Part 3 "Clean-up methods" presents the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid/liquid partition, adsorption or gel permeation column chromatography.

Part 4 "Determination, confirmatory tests, miscellaneous" gives guidance on some recommended techniques for the determination of pesticides and PCBs in fatty foodstuffs and on confirmatory tests and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The methods selected for inclusion in this standard have been validated and are widely used throughout Europe. Any variation in the methods used should be shown to give comparable results.

EN 1528 Part 1: General considerations

1 Scope

This European Standard specifies methods for the determination of residues of pesticides and polychlorinated biphenyls (PCBs) in fatty food.

Each method described in this European Standard is suitable for identifying and quantifying a definite range of those non-polar organochlorine and/or organophosphorus pesticides which occur as residues in fats and oils as well as in the fat portion of fat-containing foodstuffs, both of either animal or vegetable origin. The PCB indicator congeners usually selected for the enforcement of maximum residue limits (MRLs) are determined along with the organochlorine pesticides.

This European Standard contains the following clean-up methods that have been subjected to interlaboratory studies and are adopted throughout Europe:

- Method A: Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil® column (AOAC) [1]
- Method B: Liquid-liquid partitioning with dimethylformamide and clean-up on a Florisil® column (Specht) [2]
- Method C: Column chromatography on activated Florisil® (AOAC) [3]
- Method D: Column chromatography on partially deactivated Florisil® (Stijve) [4]
- Method E: Column chromatography on partially deactivated aluminium oxide (Greve & Grevenstuk) [5]
- Method F: Gel permeation chromatography (GPC) (AOAC) [6]
- Method G: Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel (Specht) [7]
- Method H: High performance gel permeation chromatography (HPGPC) (MAFF) [8]

The applicability of the eight methods A to H for residue analysis of organochlorine pesticides, PCB indicator congeners, and organophosphorus pesticides, respectively, is given in table 1. Where no + sign is shown, there are no data available in literature, but this does not necessarily exclude the applicability.

Table 1: Applicability of methods A to H according to reference given in literature

Compound[2])	Method							
	A [1]	B [2]	C [3]	D [4]	E [5]	F [6]	G [7]	H [8]
Organochlorine pesticides								
aldrin (HHDN) (1)	+	+		+	+	+	+	+
cis-chlordane (12)		+		+	+	+	+	+
trans-chlordane (12)		+		+	+	+	+	+
o, p'-TDE (DDD) (21)	+			+		+	+	+
p, p'-TDE (DDD) (21)	+	+	+	+	+	+	+	+
o, p'-DDE (21)	+				+		+	+
p, p'-DDE (21)	+	+	+	+	+	+	+	+
o, p'-DDT (21)	+	+		+	+	+	+	+
p, p'-DDT (21)	+	+	+	+	+	+	+	+
dieldrin (HEOD) (1)	+	+	+	+	+	+	+	+
α-endosulfan (32)					+		+	
β-endosulfan (32)							+	
endrin (33)	+	+		+	+	+	+	+
hexachlorobenzene (HCB) (44)		+		+	+	+	+	+
α-HCH -	+	+		+	+	+	+	+
β-HCH -	+	+		+	+	+	+	+
γ-HCH (lindane) (48)	+	+		+	+	+	+	+
δ-HCH -	+	+		+			+	+
heptachlor (43)	+	+		+	+	+	+	+
heptachlor epoxide (43)	+	+		+	+		+	+
methoxychlor -	+	+		+	+	+	+	
mirex -	+					+	+	+
oxychlordane (12)		+		+	+		+	+
camphechlor (toxaphene) -				+	+	+	+	
PCB indicator congeners -	+	+	+	+	+		+	+
Organophosphorus pesticides								
Bromophos (4)								
bromophos-ethyl (5)		+		+			+	+
carbophenothion (11)							+	+
chlorfenvinphos (14)		+					+	+
chlorpyrifos (17)		+					+	+

chlorpyrifos-methyl (90)				+			+	+
crotoxyphos -								+
diazinon (22)								+
dichlorvos (25)	(+)	+					(+)	+
ethion (34)								+
famphur -	(+)			+			+	+
fenitrothion (37)								+
fenchlorphos (ronnel) (36)							+	+
fenthion (39)	(+)	+		+			+	
iodofenphos -								+
malathion (49)				+			+	+
phosmet (103)	(+)	+					+	+
pirimiphos-methyl (86)								+
parathion (58)							+	+
parathion-methyl (59)	(+)	+					+	
phenkapton -	(+)						+	
tetrachlorvinphos -				+				
								+

Key: + applicable, (+) validated for special cases, see [1]

[2]) For the full chemical names and structures, see ISO 1750 Pesticides and other agrochemicals – Common names.

Table 2: PCB indicator congeners

Chemical name	Number
1) 2, 4, 4'-trichlorobiphenyl	28
2) 2, 2', 5, 5'-tetrachlorobiphenyl	52
3) 2, 2', 4, 5, 5'-pentachlorobiphenyl	101
4) 2, 2', 3, 4, 4', 5'-hexachlorobiphenyl	138
5) 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl	153
6) 2, 2', 3, 4, 4', 5, 5'-heptachlorobiphenyl	180

2 Principle

2.1 General

The methods described in this European Standard are based on a four-stage process (in some cases two stages may be combined, in whole or in part), as described in 2.2 to 2.5.

2.2 Extraction

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residue and minimum co-extraction of any substances which can give rise to interferences in the determination.

NOTE: Methods for extraction of fat are recommended which are simultaneously applicable for the extraction and determination of fat and the residue analysis in the fat portion.

2.3 Clean-up

Maximum removal of interfering substances with minimal loss of analyte from the sample extract, so as to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

2.4 Determination

Gas chromatography (GC) with various detectors, e.g. electron-capture detector (ECD), the thermionic detector (P- or N/P- mode), the flamephotometric detector (FPD), the Hall detector or mass spectrometry (MS) as appropriate.

2.5 Confirmation

Procedures to confirm the identity and quantity of observed residues, particularly in those cases where it would appear that the maximum residue limit has been exceeded.

3 Bibliography

[1] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA 1995, Vol. 1, Chapter 10, pp. 1-10, Method No. 970.52.

[2] Specht, W.: Organochlorine and organophosphorus pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, pp. 309-319, Method S 10.
[3] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA 1995, Vol. 1, Chapter 10, pp. 11-12, Method No. 983.21.
[4] Stijve, T.: Organochlorine and organophosphorus pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, 5, pp. 297-308, Method S 9.
[5] Greve, P.A., and Grevenstuk, W.B.F.: Meded. Fac. Landbouwwet. (Gent) 40, pp. 1115-1124 (1975), cited in: Analytical Methods for Residues in Foodstuffs, 5th edition, The Hague 1988, Vol.1, pp. 12-15, Multi-Residue Method 1, submethod 5.
[6] Cunniff, P. (Ed.): Official Methods of Analysis of AOAC INTERNATIONAL, 16th edition, Arlington VA USA l995, Vol. 1, Chapter 10, pp. 12-13, Method No. 984.21.
[7] Specht, W.: Organochlorine, organophosphorus, nitrogen-containing and other pesticides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987, Vol. 1, pp. 75-78 and pp. 383-400, Cleanup Method 6 and Method S 19.
[8] UK Ministry of Agriculture, Fisheries and Food: Analysis of pesticide residues in products of animal origin, Method FScLPest-1, (23.4.91).

EN 1528 Part 2: Extraction of fat, pesticides and PCBs and determination of fat content General considerations

1 Scope

This Part of EN 1528 specifies a range of analytical procedures for extracting the fat portion containing the pesticide and polychlorinated biphenyl (PCB) residues from different groups of fat-containing foodstuffs.

2 Principle

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residue and minimum co-extraction of any substances which can give rise to interferences in the determination. Removal of the solvents by evaporation and, optionally, determination of the fat content by weighing out the mass of the remainder.

EN 1528 Part 3: Clean-up methods

1 Scope

This Part of EN 1528 specifies the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid/liquid partition, adsorption or gel permeation column chromatography. The applicable usage of the methods A to H is given in detail in each method described.

NOTE: See also EN 1528-4 which lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

2 Principle

Removal of interfering materials from the sample extract to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

EN 1528 Part 4: Determination, confirmatory tests, Miscellaneous

1 Scope

This Part of EN 1528 gives guidance on some recommended techniques for the determination of pesticides and polychlorinated biphenyls (PCBs) in fatty foodstuffs and on confirmatory tests and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

2 Principle

The methods described in this Part of EN 1528 permit the residues present to be provisionally identified and quantified, by gas chromatographic methods using selective detectors.

All positive results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), column fractionation,

derivatization, spectral measurements, etc., are all of value. Results obtained using mass spectrometry (MS) present definitive evidence for confirmation/identification purposes.

EN 12393 Non fatty food - Multiresidue methods for the gas chromatographic determination of pesticide residues

General Introduction

This EN 12393 “Non-fatty foods - Multiresidue methods for the gas chromatographic determination of pesticide residues” is divided in three parts:

Part 1 "General considerations" provides general considerations with regard to reagents, apparatus, gas chromatography, etc., applying to each of the analytical selected methods;

Part 2 "Methods for extraction and clean-up" presents methods L to P for the extraction and clean-up using techniques such as liquid-liquid partition, adsorption column chromatography or gel permeation column chromatography, etc.;

Part 3 "Determination and confirmatory tests" gives some recommended techniques for the qualitative and quantitative measurements of residues and the confirmation of the results.

This European Standard comprises a range of multi-residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The selected methods included in this standard have been validated and/or are widely used throughout Europe.

Because these methods can be applied to the very wide range of food commodities/pesticide combinations, using different systems for determination, there are occasions when variations in equipment used, extraction, clean-up and chromatographic conditions are appropriate to improve method performance.

EN 12393 Part 1: General considerations

1 Scope

This European Standard gives general considerations for the determination of pesticide residues in non-fatty foods.

Each method described in this European Standard is suitable for identifying and quantifying a definite range of those organohalogen, and/or organophosphorus and/or organonitrogen pesticides which occur as residues in foodstuffs of plant origin.

This European Standard contains the following methods that have been subjected to interlaboratory studies and/or are adopted throughout Europe:

- Method L: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up on a silica-gel/charcoal column [1];
- Method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil® [2, 3, 4];
- Method N: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up with gel permeation and silica gel chromatography [5];
- Method O: Extraction with acetonitrile, liquid-liquid partition with light petroleum and clean-up on a Florisil column [6];
- Method P**:** Extraction of organophosphorus compounds with ethyl acetate and, if necessary, clean-up with gel permeation chromatography [7].

The applicability of the five methods L to P for residue analysis of organohalogen, organophosphorus and organonitrogen pesticides, respectively, is given for each method.

2 Principle

2.1 General

Experience has shown that errors introduced in the preparation, handling and storage of standards and standard solutions are major sources of inaccuracies. Experiences obtained by other national, European and international bodies should be observed [8], [9].

The methods described in this European Standard are based on a four-stage process (in some cases two stages may be combined, in whole or in part), as given in 2.2 to 2.5

2.2 Extraction

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residues and minimum co-extraction of any substances which can give rise to interferences in the determination.

2.3 Clean-up

Removal of interfering materials from the sample extract to obtain a solution of the extracted residue in a solvent which is suitable for determination by the selected method of determination.

2.4 Determination

Gas chromatography (GC) with selective detectors may be used: electron-capture detection (ECD) for organohalogen, thermionic detector (NPD, P-mode or N/P mode) for organophosphorus and organonitrogen compounds and flame-photometric detector (FPD) for organophosphorus and organosulfurous pesticides. Hall detector (ECHD), atomic emission detector (AED) and mass spectrometry (MS) may also be used for a large class of pesticides.

2.5 Confirmation

Procedures to confirm the identity and quantity of observed residues, particularly in those cases where it would appear that the maximum residue limit (MRL) has been exceeded.

3 Bibliography

[1] Organohalogen, organophosphorus and triazine compounds in DFG Manual of Pesticide Residue Analysis, VCH Weinheim, Method S 8 in Vol. 1 (1987), pp. 283 and Vol. 2 (1992), pp. 313.

[2] Luke, M. A., Froberg, J.E., and Masumoto, H. T.: J. Assoc. Off. Anal. Chem. 58, 1020 - 1026, 1975.

[3] Luke, M. A., Froberg, J.E., Doose G. M., and Masumoto, H. T.: J. Assoc. Off. Anal. Chem. 64, 1187 - 1195 (1981).

[4] Pesticide Analytical Manual - Vol.I, Multiresidue methods, Section 302, 3rd Edition, 1994.

[5] Specht, W.: Organochlorine, organophosphorus, nitrogen-containing and other pesticides in DFG Manual of Pesticide Residue Analysis, VCH Weinheim Method S 19 in Vol. 1 (1987), pp. 383, and Vol. 2 (1992), pp. 317.

[6] Helrich, K. (Ed.): Organochlorine and organophosphorus pesticide residues, Method 970. 52 in A.O.A.C. Official Methods of Analysis (1990).

[7] Analytical Methods for Residues of Pesticides in Foodstuffs, Firth Edition, Rijswijk (1988).

[8] Recommended Methods of Analysis, Codex Alimentarius Commission. In: Codex Alimentarius Volume Two Pesticide residues in food - Rome; Food and Agriculture Organization of the United Nations (FAO) World Health Organization (WHO) 1993 Part 4.3, pp. 417-455, as amended by Supplement 1 to Volume 2, 1993, pp. 171-172.

[9] Horwitz, W., Kamps, L.R., and Boyer, K.W.: Quality assurance in the analysis of foods for trace constituents, J. Assoc. Off. Anal. Chem, 63, 1344 ff, (1980).

EN 12393 Part 2: Methods for extraction and clean-up

1 Scope

This European Standard specifies methods for the extraction and clean-up of non-fatty food samples for quantitative determination of pesticide residues.

Different solvents can be used for this purpose. These pesticide residues are generally associated with other co-extracted compounds which would interfere in the analysis. To purify the crude extracts to be analysed, several techniques can be used.

This European Standard specifies the details of methods L to P for the extraction and the clean-up of samples of non-fatty food. Several solvents at different volumes are used for extraction. Techniques of clean-up are listed such as liquid-liquid partition, liquid chromatography on various adsorbents and gel permeation chromatography.

A table providing the couples (matrix/pesticide) which have been submitted to collaborative studies and a list of indicative applicability of the method to different pesticides are given for each method, wherever possible.

2 Principle

As already described, in certain occasions it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall be always clearly documented and demonstrated to give valid results.

The pesticide residues are extracted from the sample by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the pesticide residues and minimum co-extracted substances which can give rise to interferences in the determination. Any interfering materials are removed from the sample extract to obtain a solution of the extracted pesticide residues in a solvent which is suitable for quantitative examination by the selected method of determination.

3 List of methods

3.1 Method L: Extraction with acetone, liquid-liquid partition with dichloromethane and clean-up on a silica gel/charcoal column

3.1.1 Principle

The chopped test portion is homogenized in acetone and the homogenate is filtered. An aliquot portion of the filtrate is diluted with water and extracted with dichloromethane. The organic phase is concentrated and chromatographed on a column of silica gel and activated charcoal. The pesticide residues are eluted with a mixture of dichloromethane, toluene and acetone. The eluate is concentrated for examination by GC.

3.1.2 Collaborative studies

Couples of matrices and pesticides which have been submitted to collaborative studies4) are presented in Table 1

Table 1

	carrot	potato	savoy cabbage	spinach	Tomato	yellow pea
Bromophos (4)	+	+			+	
Bromopropylate (70)				+	+	
Captan (7)					+	
Chlorpropham -		+				
Chlorpyrifos (17)				+	+	
Cypermethrin (118)				+		
o, p'-DDE (21)	+					
p, p'-DDE (21)	+			+		
o, p'-DDT (21)	+					
p, p'-DDT (21)	+				+	
Diazinon (22)	+		+			+
Dichlofluanid (82)	+					
Dicofol (26)				+	+	
Dieldrin (1)	+	+	+	+	+	+
α-endosulfan (32)					+	
β-endosulfan (32)				+	+	
endosulfan sulfate (32)				+	+	
Endrin (33)					+	
Ethion (34)					+	
Fenarimol (192)				+		
Fenitrothion (37)	+		+			
Fenpropathrin (185)				+		
Folpet (41)				+		
α-HCH					+	
heptachlor epoxide (43)	+		+			
Iprodione (111)				+		
lindane (γ-HCH) (48)	+	+	+	+	+	+
Malathion (49)				+		+
Mecarbam (124)				+		
Parathion (58)	+		+	+	+	
Permethrin (120)				+		
Phosalone (60)	+			+		+
pirimiphos-methyl (86)		+		+	+	+
Procymidone (136)					+	
Propham (183)		+				
Quintozene (64)				+		+
Tetradifon -					+	

4) For the collaborative studies, the activated charcoal and the silica gel 60, 63 µm to 200 µm (70 mesh to 230 mesh) from the Merck Company were used. This information is given for convenience of users of this standard and does not constitute an endorsement by CEN of these products.

tolclofos-methyl (191)				+		
Vinclozoline (159)	+	+		+	+	

3.1.3 Applicability

The following pesticides can be analysed by this method**:**

Aldrin (1)
Ametryn
Atrazine
Azinphos-ethyl (68)
Azinphos-methyl (2)
Aziprotryne
Bifenthrin (178)
Bromacil
Bromophos (4)
Bromophos-ethyl (5)
Bromopropylate (70)
Bupirimate
Captafol (6)
Captan (7)
Carbophenothion (11)
Chlorbenside
Chlorfenson
Chlorfenvinphos (14)
Chlorflurenol
Chlorpropham
Chlorobenzilate (16)
Chloropropylate
Chlorpyrifos (17)
Chlorpyrifos-methy (90)l
Chlorthal
Chlorthiophos
Cyanazine
Cyanofenphos (91)
Cyanophos
Cyfluthrin (157)
λ-Cyhalothrin (146)
Cypermethrin (118)
p,p'-DDD (21)
o,p'-DDE (21)
p,p'-DDE (21)
o,p'-DDT (21)
p,p'-DDT (21)
Deltamethrin (135)
Desmetryn
Dialifos (98)
Diazinon (22)
Dichlobenil
Dichlofenthion
Dichlofluanid (82)
Dichlorvos (25)
Dicofol (26)
Dieldrin
Dimethachlor
Dimethoate
Dioxathion
Disulfoton
Ditalimfos
α-Endosulfan (32)
ß-Endosulfan(32)
Endosulfan sulfate (32)
Ethion (34)
Ethoprophos (149)
Etrimfos (123)
Fenamiphos (85)
Fenarimol (192)
Fenchlorphos (36)
Fenitrothion (37)
Fenpropathrin (185)
Fenson
Fensulfothion (38)
Fenthion (39)
Fenvalerate (119)
Fluchloralin
Flucythrinate (152)
Fluorodifen
Fluvalinate
Folpet (41)
Fonofos
Formothion (42)
α-HCH
ß-HCH
Heptachlor (43)
Heptachlor epoxide (43)
Heptenophos
Iodofenphos
Iprodione (111)
Isofenphos (131)
Lindane (48)
Malaoxon
Malathion (49)
Mecarbam (124)
Metalaxyl (138)
Metazachlor
Methidathion (51)
Methoprotryne
Methoxychlor
Metolachlor
Metribuzin
Mevinphos (53)
Naled
Nitrofen (140)
Paraoxon
Parathion (58)
Parathion-methyl (59)
Pendimethalin
Permethrin (120)
Perthane
Phenkapton
Phorate (112)
Phosalone (60)
Pirimiphos-methyl (86)
Procymidone (136)
Profenofos (171)
Profluralin
Prometryn
Propazine
Propham (183)
Propyzamide
Prothiofos
Pyrazophos
Pyrethrum (63)
Quinalphos
Quintozene (63)
Simazine
Sulfotep
Tecnazene (115)
Terbacil
Terbufos (167)
Terbutryn
Tetrachlorvinphos
Tetradifon
Tetramethrin
Tetrasul
Thionazin
Tolclofos-methyl (191)
Tolylfluanid (162)
Triadimefon (133)
Tri-allate
Triazophos (143)
Trichloronat
Trifluralin
Vinclozolin (159)

Crops and foods on which the method was tested**:**

Apples
Apricots
Aubergines
Grapes
Head cabbage
Honey
Pineapples
Plums
Potatoes

Beans	Kohlrabi	Radishes (large and small types)
Carrots	Leeks	Red cabbage
Celeriac	Lettuce	Savoy cabbage
Cherries	Mandarin oranges	Spinach
Chillies	Mushrooms	Strawberries
Chinese cabbage	Oranges	Sweet peppers
Corn salad	Parsley	Tomatoes
Cucumbers	Peaches	Witloof chicory
Dandelion	Pears	
Endives	Peas	

3.2 Method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum, if necessary clean-up on Florisil®

Principle

The chopped test portion is homogenized in acetone and the homogenate is filtered. An aliquot portion of the filtrate is extracted with a mixture of light petroleum and dichloromethane and then with dichloromethane. The organic phase can be injected directly without clean up into a gas chromatograph with an appropriate detector or purified on a Florisil® column. The eluates are concentrated for examination by GC.

3.3 Method N: Extraction with acetone, liquid-liquid partition with dichloromethane, clean-up with gel permeation and silica gel chromatography

3.3.1 Principle

The chopped test portion is homogenized in acetone, after addition of water, depending on the natural water content of the sample, in order to ensure an acetone/water ratio of 2/1 (V/V). The homogenate is filtered. An aliquot portion of the filtrate is saturated with sodium chloride and diluted with dichloromethane, resulting in separation of excess water.

The organic phase is concentrated and cleaned up by gel permeation chromatography (GPC) on BioBeads S-X3® (polystyrene gel) using a mixture of cyclohexane and ethyl acetate as eluant. The residue-containing fraction is concentrated, and analyzed directly by gas chromatography using a phosphorus or nitrogen selective detector. For analysis by electron capture and in some cases also by nitrogen-selective detection, a supplemental clean-up on a small silica gel column may be necessary. In this clean-up step, the pesticides are separated in several fractions thus providing additional leads for identification.

3.3.2 Collaborative studies

Couples of matrices and pesticides which have been submitted to collaborative studies are presented in table 2:

Table 2

	carrot	potato	savoy cabbage	spinach	tomato	yellow pea
Acephate (95)				+		
Bromophos (4)	+	+			+	
Bromopropylate (70)				+	+	
Captan (7)					+	
Chlorothalonil -				+		
Chlorpropham -		+				
Chlorpyrifos (17)				+	+	
o, p'-DDE (21)	+					
p, p'-DDE (21)	+			+		
o, p'- DDT (21)	+					
p, p'-DDT (21)	+				+	
Diazinon (22)	+		+			+
Dichlofluanid (82)	+					
Dicloran (83)	+					
Dicofol (26)				+	+	
Dieldrin (1)	+	+	+	+	+	+
α-endosulfan (32)					+	

β-endosulfan (32)				+	+	
endosulfan sulfate (32)				+	+	
Endrin (33)					+	
Ethion (34)					+	
Fenarimol (192)				+		
Fenitrothion (37)	+		+			
Fenpropathrin -				+		
Folpet (41)				+		
HCB (44)	+	+		+	+	+
α-HCH					+	
lindane (γ-HCH) (48)	+	+	+	+	+	+
heptachlor epoxide (43)	+		+			
Iprodione (111)				+		
Malathion (49)				+		+
Mecarbam (124)				+		
Parathion (58)	+		+	+	+	
Permethrin (120)				+		
Phosalone (60)	+			+		+
pirimiphos-methyl (86)		+		+	+	+
Procymidone (136)					+	
Propham (183)		+				
Quintozene (64)				+		+
Tetradifon-					+	
tolclofos-methyl (191)				+		
Vinclozolin (159)	+	+		+	+	

3.3.3 Applicability

The pesticides that can be analysed by this method are listed in table 3

Table 3:Crops and foods on which the method was tested are the following:

Apples	Currants, red	Peas
Bananas	Grapes	Pineapples
Beans	Head cabbage	Plums
Beer	Hops	Potatoes
Carrots	Kohlrabi	Savoy cabbage
Cauliflower	Lettuce	Spices
Cereals	Melons	Spinach
Cherries	Must	Strawberries
Citrus fruit	Nuts	Sugar beets
Cacao products	Onions	Sweet peppers
Coffee, raw	Peaches	Tea and tea-like products
Cucumbers	Peanuts	Tobacco
Curly kale	Pears	Tomatoes
		Wine

3.4 Method O: Extraction with acetonitrile, liquid-liquid partition with light petroleum and clean-up on a Florisil® column

3.4.1 Principle

The chopped test portion is homogenized in acetonitrile or in a water/acetonitrile mixture and the extract is filtered. The filtrate is extracted with light petroleum. This extract is purified on a Florisil® column. The organohalogen pesticides are eluted with diethyl ether/light petroleum mixtures. The eluates are concentrated for examination by GC.

3.4.2 Collaborative studies

All couples of the following matrices and pesticides have been submitted to collaborative studies:

apples, apricots, cauliflower, endives, lettuce, potatoes,	Aldrin (1), DDE (21), diazinon (22), dieldrin (1), endrin (33), ethion (34), fenchlorphos (36), HCB (44), heptachlor (43), heptachlor epoxide

strawberries	(43), lindane (48), malathion (49), methoxychlor, o,p'-DDT (21), p,p'-DDT (21), parathion (58), parathion-methyl (59), perthane, TDE (210

3.4.3 Applicability

Pesticides and crops on which the method was tested are the following:

Aldrin(1), BHC, DDE (21), o,p'-DDT (21), p,p'-DDT (21), dieldrin (1), endrin (33), heptachlor (43), heptachlor epoxide(43), lindane (48), methoxychlor, mirex, perthane, TDE (21).	apples, apricots, barley, beets, bell pepper, broccoli, cabbage, cantaloupes, cauliflower, celery, collard greens, corn meal, cucumbers, eggplant, endive, grapes, green beans, kale, lettuce, oats, peaches, pears, peas, plums, popcorn, poptatoes, radishes, spinach, squash, strawberries, sugar beets, sweet potatoes, tomatoes, turnips, wheat.
Diazinon (22), ethion (34), fenchlorphos(36), malathion (49), parathion (58), parathion methyl(59).	apples, apricots, barley, broccoli, cabbage,carrots, cauliflower, cucumbers, endives, grapes, green pepper, kale, lettuce, oats, potatoes, squash, strawberries, tomatoes, turnips, wheat.

3.5 Method P: Extraction of organophosphorus compounds with ethyl acetate, and if necessary, clean-up by gel permeation chromatography

3.5.1 Principle

The chopped test portion is homogenized with sodium sulfate in ethyl acetate and the homogenate is filtered. An aliquot portion of the filtrate is concentrated and can be injected directly without clean-up into a gas chromatograph with a phosphorus selective detector, or, if necessary, purified by gel permeation chromatography on BioBeads® S-X3 using a mixture of cyclohexane and ethyl acetate as eluant. The eluate is concentrated for examination by GC.

3.5.2 Applicability

The following pesticides can be analyzed in fruits and vegetables by this method:

Acephate (95)	Dichlofenthion	Heptenophos	Phosmet ((103)
azinphos-ethyl (68)	Dichlorvos (25)	Isofenphos (131)	Phoxim (141)
azinphos-methyl (2)	Dimethoate (27)	malathion (49)	pirimiphos-ethyl
bromophos(4)	Dioxathion (28)	methamidophos (100)	pirimiphos-methyl (86_
bromophos-ethyl (5)	Disulfoton(74)	methidathion (51)	prothoate
carbophenothion (11)	Ditalimfos	menazon	pyrazophos (153)
chlorfenvinfos (14)	Ethion (34)	mevinphos (53)	sulfotep
chlorpyrifos (17)	Ethoprophos (149)	monocrotophos (54)	temephos
chlorpyrifos-methyl (90)	etrimfos (123)	naled	TEPP
chlorthiophos	fenamiphos (85)	omethoate (55)	Tetrachlorvinphos
coumaphos(18)	fenchlorphos (36)	oxydemeton-methyl (166)	Thiometon (76)
cyanofenphos (91)	fenitrothion(37)	parathion (58)	tolclofos-methyl (191)
demeton-S-methyl (73)	fensulfothion (38)	parathion-methyl (59)	triamiphos
demeton-S-methyl sulfone (164)	fenthion (39)	phorate (112)	triazophos (143)
dialifos ((98)	fonofos	phosalone (66)	trichlorfon (66)
diazinon (22)	formothion (42)	phosphamidon (61	trichloronate
			vamidothion (78_

EN 12393 Part 3:Determination and confirmatory tests

1 Scope

This European Standard gives guidance on some recommended techniques for the determination of pesticide residues in non-fatty foods and on confirmatory tests.

The identity of any observed pesticide residue is confirmed, particularly in those cases in which it would appear that the maximum residue limit has been exceeded.

2 General

The methods described in this European Standard permit the residues present provisionally to be identified and quantified, by gas chromatographic methods using selective detectors.

All positive results require confirmation of identity and quantity.
The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, thin-layer-chromatography (TLC), high-performance liquid chromatography (HPLC), column fractionation, derivatization, spectral measurements, etc. are all of value.
Results obtained using mass spectrometry (MS) present the most definitive evidence for confirmation/identification purpose.
As already described in the introduction, in certain occasions it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall be always clearly documented and demonstrated to give valid results.

EN 12396 Non fatty food - Determination of dithiocarbamate (105) and thiuram disulfide residues
General Introduction
This European Standard EN 12396 „Non-fatty foods - Determination of dithiocarbamate (105) and thiuram disulfide residues“ consists of three parts:
Part 1: Spectrometric method
Part 2: Gas chromatographic method
Part 3: Xanthogenate method

EN 12396 Part 1: Spectrometric method
1 Scope
This European Standard specifies a spectrometric method for the determination of residues of dithiocarbamates and thiuram disulfides, which release carbon disulfide under the described conditions (e.g. mancozeb, maneb, propineb, thiram, zineb). It is applicable to such compounds in and on fruits and many vegetables and also in and on cereals and other foodstuffs of plant origin.
Only the quantification of the whole group is possible using this method but not the identification of individual compounds. Generally the maximum residue limits (MRLs) are expressed in terms of carbon disulfide.
2 Principle
The sample is heated with hydrochloric acid and tin(II)chloride to release carbon disulfide from any dithiocarbamates and/or thiuram disulfide present. The carbon disulfide is separated and purified by distillation and collected in an ethanolic solution of copper(II)acetate and diethanolamine.
With copper(II)acetate and diethanolamine, the carbon disulfide forms two yellow copper(II)-N,N-bis(2-hydroxy-ethyl)-dithiocarbamate complexes with the molar ratio
Cu:CS_2 = 1:1 and 1:2. The absorption of these reaction products is measured in a spectrometer at a wavelength of 435 nm and the concentration of dithiocarbamate and/or thiuram disulfide residues is calculated and expressed in terms of milligrams of carbon disulfide per kilogram of foodstuff. For further information on the principle of this method, see [1] to [4].

3 Precision data
In accordance with ISO 5725:1986 [5], the following parameters have been defined in an inter-laboratory test. The test was conducted by the Arbeitsgruppe "Pestizide", Lebensmittelchemische Gesellschaft, division of the "Gesellschaft Deutscher Chemiker", Frankfurt, Germany, [1], [6].
For the inter-laboratory tests the tin(II)-hydrochloric acid solution (4.11) was heated until boiling before it was added to the decomposition apparatus (5.2). To remove interfering substances, concentrated sulfuric acid (4.6) was used in the first adsorption tube prior to the sodium hydroxide solution (4.7) in the second tube.
Table A.1

Sample	**Apple**	**Witloof chicory**
Year of inter-laboratory test:	1993	1994
number of samples:	1	1
number of laboratories:	7	16
Number of laboratories retained after elimination	6	15
Number of eliminated laboratories	1	1
Number of accepted results	35	80
Mean value $\bar{x}$ mg/kg	2,15	0,24
Repeatability standard deviation s_r mg/kg	0,17	0,023
Repeatability relative standard deviation RSD_r	8,0 %	9,7 %

Repeatability limit *r* mg/kg	0,49	0,066
Reproducibility standard deviation s_R mg/kg	0,28	0,041
Reproducibility relative standard deviation RSD_R	13,0 %	16,9 %
Reproducibility limit *R* mg/kg	0,79	0,12
Horrat value H_{OR}	0,93	0,89

4 Bibliography

[1] Working Group on Pesticides, Food and Forensic Chemistry Division of the Gesellschaft Deutscher Chemiker (German Chemical Society): Dithiocarbamate and thiuram disulfide fungicides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim, 1987, Vol. 1, pp 353 - 360 method S15.

[2] EEC document: 1729/VI/80 - final 1.

[3] Cullen, T. E.: Spectrophotometric determination of dithiocarbamate residues on food crops. Anal.Chem., 1964, 36, 221-224.

[4] Keppel, G.E.: Modification of the carbon disulfide evolution method for dithiocarbamate residues. J. Assoc. Off. Anal. Chem., 1969, 52, 162-167.

[5] ISO 5725:1986 Precision of test methods – Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests

[6] Gilsbach W.:Ringversuche der Arbeitsgruppe "Pestizide" zur Ermittlung von Präzisionsdaten bei der Bestimmung von Dithiocarbamaten und Thiuramdisulfiden. Dtsch. Lebensm. Rdsch., 1996, 92, pp. 351-353.

EN 12396 Part 2: Gaschromatographic method

1 Scope

This European Standard specifies a gas chromatographic method for the determination of residues of dithiocarbamates and thiuram disulfides, which release carbon disulfide under the described conditions (e. g. mancozeb, maneb, propineb, thiram, zineb). It is applicable to such compounds in and on fruits and some vegetables but also in and on cereals and other foodstuffs of plant origin.

Only the quantification of the whole group is possible using this method not the identification of individual compounds. Generally the maximum residue limits (MRLs) are expressed in terms of carbon disulfide.

2 Principle

The sample is heated with hydrochloric acid and tin(II)chloride in a gas-tight flask to release carbon disulfide from any dithiocarbamates and/or thiuram disulfide present. The quantity of carbon disulfide collecting in the headspace of the flask is determined by gas chromatography (GC) with an electron capture detector (ECD) or with a flame-photometric detector (FPD) in the sulfur mode. For further information on the principle of this method, see [1] to [4].

3 Bibliography

[1] McLeod, H.A., and McCully, C.A.: Head space gas chromatographic procedure for screening food samples for dithiocarbamate pesticide residues, J. Assoc. Off. Anal. Chem, **52**, pp.1226-1230, 1969.

[2] Panel on determination of dithiocarbamate residues of the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs of the Ministry of Agriculture, Fisheries and Food: Determination of residues of dithiocarbamate pesticides in foodstuffs by a headspace method, Analyst, **106**, pp. 782-787, 1981.

[3] EEC document: 1729/VI/80 - final 1.

[4] Greve, P.A. (editor): Analytical methods for residues of pesticides in foodstuffs: Part II, Method Dithiocarbamates, 5th edition, SDU publishers, 1988, Den Hague.

12396 Part 3: UV-spectrometric xanthogenate method

1 Scope

This European Standard specifies a UV spectrometric method for the determination of low-level residues of dithiocarbamate and thiuram disulfide fungicides as xanthogenate. Dithiocarbamate and thiuram disulfide fungicides release carbon disulfide under specified conditions (e.g. mancozeb, maneb, propineb, thiram, zineb). It is applicable to such compounds especially in and on those foodstuffs of plant origin for which low maximum residue levels have been set.

Only the quantification of the whole group is possible using this method and not the identification of individual compounds. Generally the maximum residue levels (MRLs) are expressed in terms of carbon disulfide.

2 Principle

The sample is heated with hydrochloric acid and tin(II)chloride to release carbon disulfide from any dithiocarbamates and/or thiram disulfides present. The carbon disulfide is separated and purified by distillation and collected in a methanolic potassium hydroxide solution. Under these conditions, carbon disulfide forms potassium xanthogenate. The absorption of this reaction product is measured spectrometrically at a wavelength of 302 nm with base line correction at wavelengths of 272 nm and 332 nm. The mass fraction of dithiocarbamate and/or thiuram disulfide residues is calculated and expressed in terms of milligrams of carbon disulfide per kilogram of foodstuff. For further information on this method, see [1], [2], [3].

3 Precision Data

In accordance with ISO 5725 : 1986 [4], the following parameters have been defined in an inter-laboratory test. The tests were conducted by the Arbeitsgruppe "Pestizide", Lebensmittelchemische Gesellschaft, division of the "Gesellschaft Deutscher Chemiker", Frankfurt, Germany [3],[5].

Sample	Witloof chicory	baby food carrot juice	baby food apple + banana	baby food spinach
Year of inter-laboratory test	1994	1995	1995	1995
Number of samples	1	1	1	1
Number of laboratories	12	11	13	11
Number of laboratories retained after eliminating outliers	12	10	13	11
Number of outliers (laboratories)	0	1	0	0
Number of accepted results	57	50	63	53
Mean value $\bar{x}$ mg/kg	0,26	0,010	0,020	0,033
Repeatability standard deviation s_r mg/kg	0,024	<0,001	0,002	0,002
Repeatability relative standard deviation RSD_r %	9,4	8,5	10,8	7,0
Repeatability limit r mg/kg	0,068	0,002	0,006	0,007
Reproducibility standard deviation s_R mg/kg	0,037	0,002	0,007	0,006
Reproducibility relative standard deviation RSD_R %	14,1	23,2	35,7	17,1
Reproducibility limit R mg/kg	0,102	0,006	0,020	0,016
Horrat value (RSD_R observed / RSD_R predicted)	0,72	0,73	1,23	0,63

4 Bibliography

[1] Working Group on Pesticides, Food and Forensic Chemistry Division of the Gesellschaft Deutscher Chemiker (German Chemical Society): Dithiocarbamate and thiuram disulfide fungicides. In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim 1987,Vol. 1, pp. 353 - 360, method S 15.

[2] EEC document: 1729/VI/80 - final 1.

[3] Gilsbach, W.: Ringversuche der Arbeitsgruppe "Pestizide" zur Ermittlung von Präzisionsdaten bei der Bestimmung von Dithiocarbamaten und Thiuramdisulfiden, 2. Mitteilung: Validierung einer Xanthogenat-Methode. Dtsch. Lebensm. Rundsch. **93** , pp. 39 - 44, (1997).

[4] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory test.

[5] Gilsbach, W.: Ringversuche der Arbeitsgruppe "Pestizide" zur Ermittlung von Präzisionsdaten bei der Bestimmung von Dithiocarbamaten und Thiuramdisulfiden, 1. Mitteilung: Validierung der DFG-Methode S 15. Dtsch. Lebensm. Rundsch. **92**, pp. 351 - 353 (1996).

EN 12393 Non fatty food - Determination of bromide residues -
Part 1: Determination of total bromide as inorganic bromide

1 Scope

This European Standard specifies a gas chromatographic (GC) method for the determination of bromide residues (including some organic bromine present) as inorganic bromide in non-fatty foods.
Generally, the maximum residue levels are expressed in terms of bromide ion from all sources but not including covalently bound bromine.
The method is applicable to beets, carrots, chicory, endives, cereal grains, lettuce, potatoes, spinach, strawberries and tomato. It has been validated in an interlaboratory test on lettuce [1].

2 Principle

An aqueous ethanolic extract of the test portion is evaporated to dryness and the residue is ashed in the presence of sodium hydroxide. The ash is solubilized with sulfuric acid and the solution is treated with ethylene oxide in di-isopropyl ether. Inorganic bromide is converted to 2-bromoethanol, which is analyzed by gas chromatography with electron-capture detection [2].

3 Precision data

In accordance with ISO 5725:1986 [3] the following parameters have been defined in an interlaboratory test. The test was conducted by the Working Group "Development and Improvement of Residue-analytical Methods" in the Netherlands.

Sample	lettuce (from untreated soil)	Lettuce (from Treated soil)
Year of inter-laboratory test	1975	1975
Number of laboratories	8	8
Number of laboratories retained after eliminating outliers	8	8
Number of outlying laboratories	0	0
Number of samples	1	1
Number of accepted results	64	64
Mean value, x*x* mg/kg	13,3	209
Repeatability standard deviation s_r mg/kg	1,28	12,5
Repeatability relative standard deviation RSD_r %	9,6	6,0
Repeatability limit *r* mg/kg	3,6	35,0
Reproducibility standard deviation s_R mg/kg	2,46	26,8
Reproducibility relative standard deviation RSD_R %	18,4	12,8
Reproducibility limit *R* mg/kg	6,9	75,0
Horrat value (RSD_R observed / RSD_R predicted)	1,7	1,8

4 Bibliography

[1] Greve, P.A., and Grevenstuk, W.B.F: Gas-liquid chromatographic determination of bromide ion in lettuce: Interlaboratory Studies. J. Assoc. Off. Anal. Chem. 62, 1155-1159 (1979)
[2] Greve, P.A., and Grevenstuk, W.B.F: Optimisation studies on the determination of bromide residues in lettuce. Meded. Fac. Landbouww. Rijksuniv. Gent 41, 1371-1381 (1976)
[3] ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory test

EN 12393 Non fatty food - Determination of bromide residues –
Part 2: Determination of bromide (47)

1 Scope

This European Standard specifies a gas chromatographic method for the determination of inorganic bromide residues in non-fatty foods.
Generally, the maximum residue levels are expressed in terms of bromide ion from all sources but not including covalently bound bromine.

The method is applicable to cereals, dried fruit, dried vegetables, dried mushrooms, fresh fruit and vegetables. It has been validated in interlaboratory studies on maize flour, carrot flakes, lettuce, potatoes, cereal flour and hazelnuts [1], [2].

2 Principle

The test portion is suspended in an aqueous solution of propylene oxide acidified with sulfuric acid whereupon inorganic bromide is extracted simultaneously and converted to a mixture of 1-bromo-2-propanol and 2-bromo-1-propanol [3] (derivatization A). The derivatives are partitioned into ethyl acetate and determined by gas chromatography with electron-capture detection [4], [5].

As an alternative, ethylene oxide which is more difficult to handle and which is somewhat more toxic, can be used instead of propylene oxide. In this case (derivatization B) inorganic bromide is converted to 2-bromoethanol [4], [5].

3 Precision data

In accordance with ISO 5725 : 1986 [6], the following parameters have been defined in interlaboratory tests. The tests were conducted by the Arbeitsgruppe "Pestizide", Lebensmittelchemische Gesellschaft, division of the "Gesellschaft Deutscher Chemiker", Frankfurt/Main, Germany.

Table 1: Derivatization with propylene oxide

Sample	lettuce	potatoes	flour	hazelnuts
Year of inter-laboratory test	1995	1995	1995	1995
Number of samples	1	1	1	1
Number of laboratories	18	18	18	18
Number of laboratories retained after eliminating outliers	15	15	18	17
Number of outlying laboratories	3	3	-	1
Number of accepted results	75	75	90	85
Mean value $\bar{x}$ mg/kg	49,2	4,3	23,5	120,0
Repeatability standard deviation s_r mg/kg	2,6	0,29	1,0	5,7
Repeatability relative standard deviation RSD_r %	5,3	6,7	4,3	4,8
Repeatability limit r mg/kg	7,4	0,83	2,83	16,1
Reproducibility standard deviation s_R mg/kg	6,9	0,63	2,5	16,1
Reproducibility relative standard deviation RSD_R %	14,0	14,5	10,7	13,5
Reproducibility limit R mg/kg	19,5	1,79	7,13	45,9
Horrat value (RSD_R observed / RSD_R predicted)	1,6	1,1	1,1	1,7

Table 2: Derivatization with ethylene oxide

Sample	maize flour	carrot flakes
Year of inter-laboratory test	1980	1980
Number of samples	1	1
Number of laboratories	12	12
Number of laboratories retained after eliminating outliers	12	12
Number of outlying laboratories	0	0
Number of accepted results	42	42
Mean value $\bar{x}$ mg/kg	42,0	26,1
Repeatability standard deviation s_r mg/kg	1,61	1,11
Repeatability relative standard deviation RSD_r %	3,8	4,3
Repeatability limit r mg/kg	4,5	3,1
Reproducibility standard deviation s_R mg/kg	2,45	3,33
Reproducibility relative standard deviation RSD_R %	5,9	12,8
Reproducibility limit R mg/kg	7,0	9,4
Horrat value (RSD_R observed / RSD_R predicted)	0,7	1,3

4 Bibliography

[1] Arbeitsgruppe "Pestizide": 8. Mitteilung. Überprüfung einer gaschromatographischen Analysenmethode für Bromidrückstände. Lebensmittelchem. gerichtl. Chem. 35, 49 (1981)

[2] Gilsbach, W., and Diserens, H.: Ringuntersuchungen zur Validierung einer gaschromatographischen Methode zur Bestimmung von Bromidrückständen in pflanzlichen Lebensmitteln. Lebensmittelchem. 50, 123-126 (1996)

[3] Stijve, T.: Inorganic bromide - a simple method for the confirmation of residue identity, Dtsch. Lebensm. Rundsch. 81, 321-322 (1985)
[4] Stijve, T.: Gas chromatographic determination of inorganic bromide residues - a simplified procedure, Dtsch. Lebensm. Rundsch. 77, 99-101 (1981)
[5] Stijve, T., and Thier, H.-P.: Bromine containing fumigants (as total inorganic bromide). In: Deutsche Forschungsgemeinschaft, Manual of Pesticide Residue Analysis, VCH Verlagsgesellschaft Weinheim, 1987, Vol I, 377-381, Method S 18
[6] ISO 5725 : 1986. Precision of test methods - Determination of repeatability and reproducibility for a standard test method by interlaboratory test