BfR

Risiken erkennen – Gesundheit schützen

CEN methods.
A common approach for EU laboratories?

Lutz Alder

(Convenor of CEN TC 275/ WG3 and WG4)

Outline

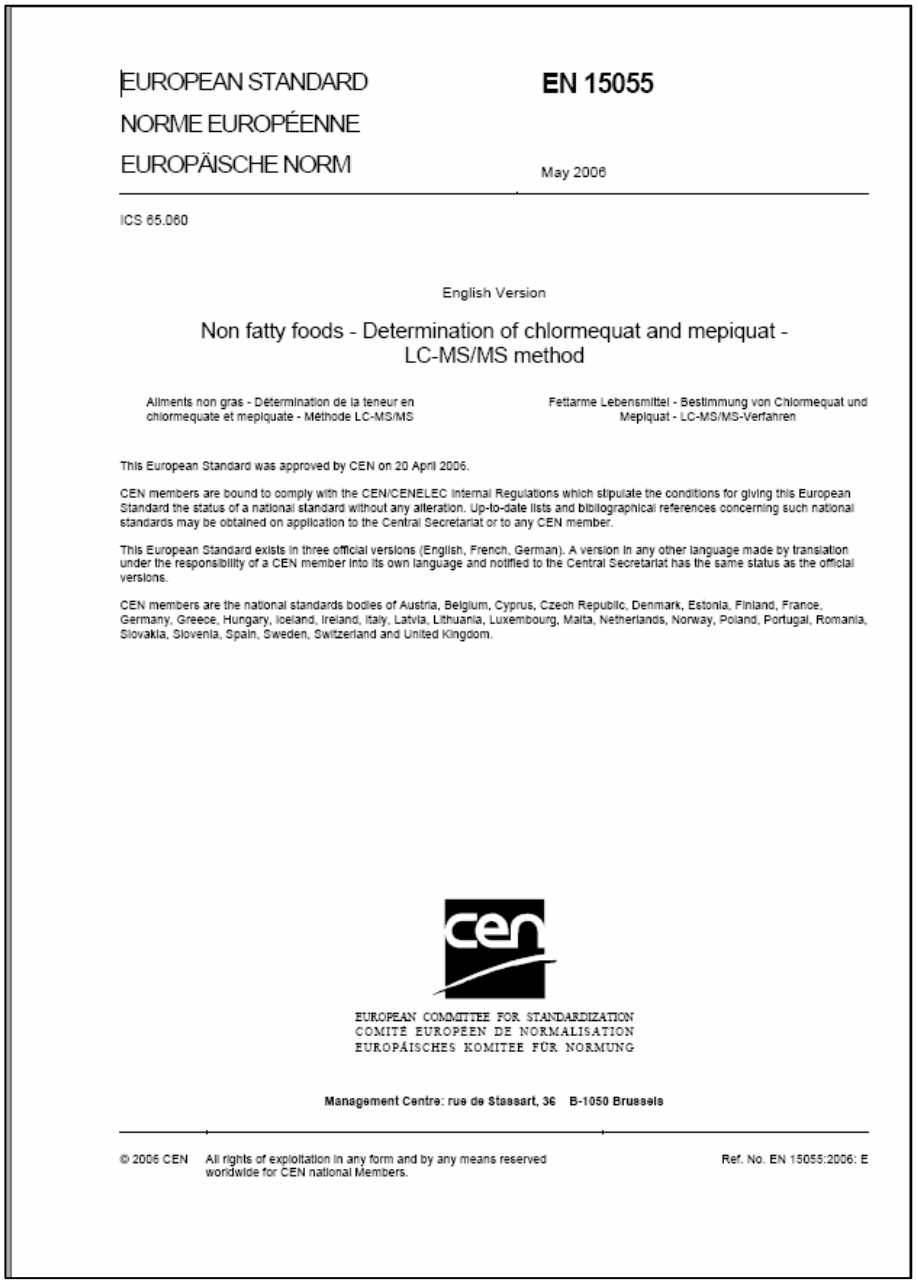
1. Which standardized methods are available?
2. Why we should have official methods?
3. Advantages of CEN methods
4. Main disadvantages of official methods
5. How do we get new standards?
6. Which methods are in preparation for foods of plant origin?
7. Which methods do we need in future?
8. Conclusion / Summary

Existing standards for foods of plant origin / example

EN15055:2006
Determination of chlormequat and mepiquat – LC-MS/MS method

- First version came from UK
- Finalized in cooperation with NL
- International validation organized by DE

Most often used for chlormequat in PT-SRM



EUROPEAN STANDARD **EN 15055**

NORME EUROPÉENNE

EUROPÄISCHE NORM

May 2006

ICS 65.060

English Version

Non fatty foods - Determination of chlormequat and mepiquat - LC-MS/MS method

Aliments non gras - Détermination de la teneur en chlormequate et mepiquate - Méthode LC-MS/MS

Fettarme Lebensmittel - Bestimmung von Chlormequat und Mepiquat - LC-MS/MS-Verfahren

This European Standard was approved by CEN on 20 April 2006.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 15055:2006: E

Existing standards for foods of plant origin

- EN12396 - Determination of **dithiocarbamate** and thiuram disulfide residues (by copper complex, by headspace GC, by xanthogenate complex)
- EN13191 - Determination of **bromide** residues (total bromide or inorganic bromide)
- EN14185 - Determination of **N-methylcarbamate** residues (with post-column derivatisation)
- EN14333 - Determination of **benzimidazole** fungicides carbendazim, thiabendazole and benomyl (as carbendazim)
- EN15054 - Determination of **chlormequat and mepiquat** – LC-MS method
- EN15055 - Determination of **chlormequat and mepiquat** – LC-MS/MS method

EN12393 – (Collection of) Multiresidue methods for the gas chromatographic determination of pesticide residues

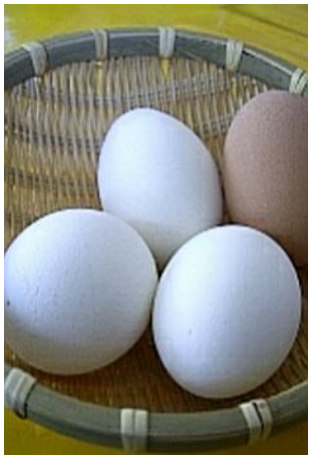
Method	Extraktion	Cleanup 1	Cleanup 2
DFG S8	Aceton	Partition into dichloromethane	Chromatography on silica-gel/charcoal column
Luke / Dutch method	Acetone	Partition into dichloromethane/ light petroleum	Chromatography on florisil
(modified) DFG S19	Acetone	Partition into dichloromethane or ethyl acetate/ cyclohexane	GPC and (if necessary) chromatography on silica-gel
Swedish method	Ethyl acetate	GPC (if necessary)	non

The standards contains methods for > 150 pesticides and GPC data for > 270 analytes.

Existing standard for foods of animal origin

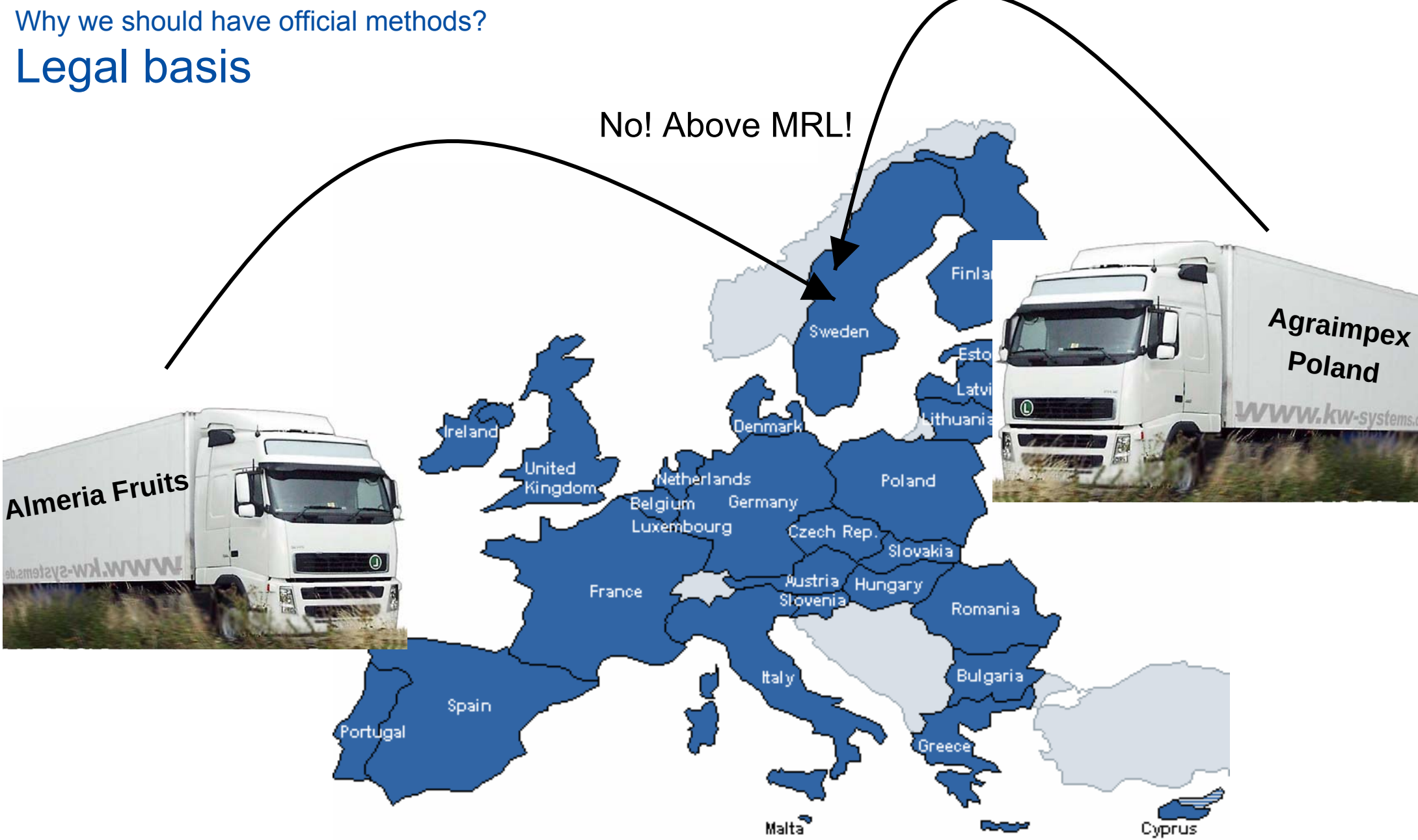
- EN14573 – Determination of 3-monochloropropane-1,2-diol by GC/MS

- EN1528 – Determination of pesticides and polychlorinated biphenyls (PCBs)
  Collection of 8 methods for 49 pesticides:
  - AOAC method 970.52 (16th ed., 1990)
  - DFG S 10
  - AOAC method 983.21 (16th ed., 1990)
  - DFG S 9
  - Dutch MRM 1, submethod 5
  - AOAC method 984.21 (16th ed., 1990)
  - DFG S19
  - UK method FScLPest-1 (1991)



Note: 2nd part of EN1528 offers special extraction methods for different kinds of food of animal origin (butter, milk, fat, cheese, meat, eggs).

Legal basis



Free trade, but 27 different competent authorities with 27 NRLs! Therefore…

Directive 85/591/EEC: Introduction of Community methods of sampling and analysis

Regulation 882/2004: Official control of foodstuffs (until 31.12.05 →Dir. 89/397/EEC)

Legal basis

Directive 85/591/EEC:
COUNCIL DIRECTIVE … concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption

Preamble:

„Whereas the methods of sampling and analysis used for this purpose can have direct repercussions on the establishment and functioning of the common market; whereas they should, therefore, be harmonized…“

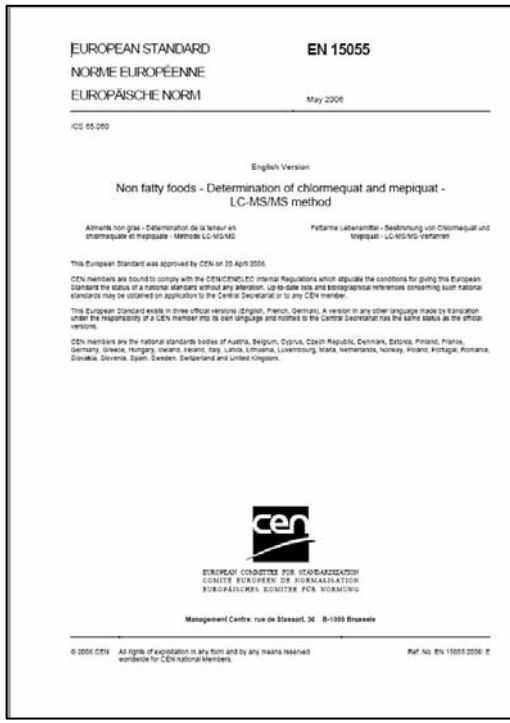
Article 2

3. The introduction of the measures provided for in Article 1 (1) shall not preclude Member States from using other tested and scientifically valid methods provided that this does not hinder the free movement of products recognized as complying with the rules by virtue of Community methods. However, in the event of differences in the interpretation of results, those obtained by the use of Community methods shall be determinant.

Why and when we should use standardized methods

Why?

- Methods are based on widely accepted methods with sufficient validation data.
- Standards are available in three languages (EN, DE and FR).
- Clear description with all details including calibration and calculation.
- Checked by experts from many member states.
- More easy to convince accreditation bodies.



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Main problems in standardization of methods

- Validation requirements not easily to fulfill.
- Editorial process very laborious, because many comments have to be considered.
- Official character of “old” methods may hinder analytical progress

  Conclusion to the 3rd point:

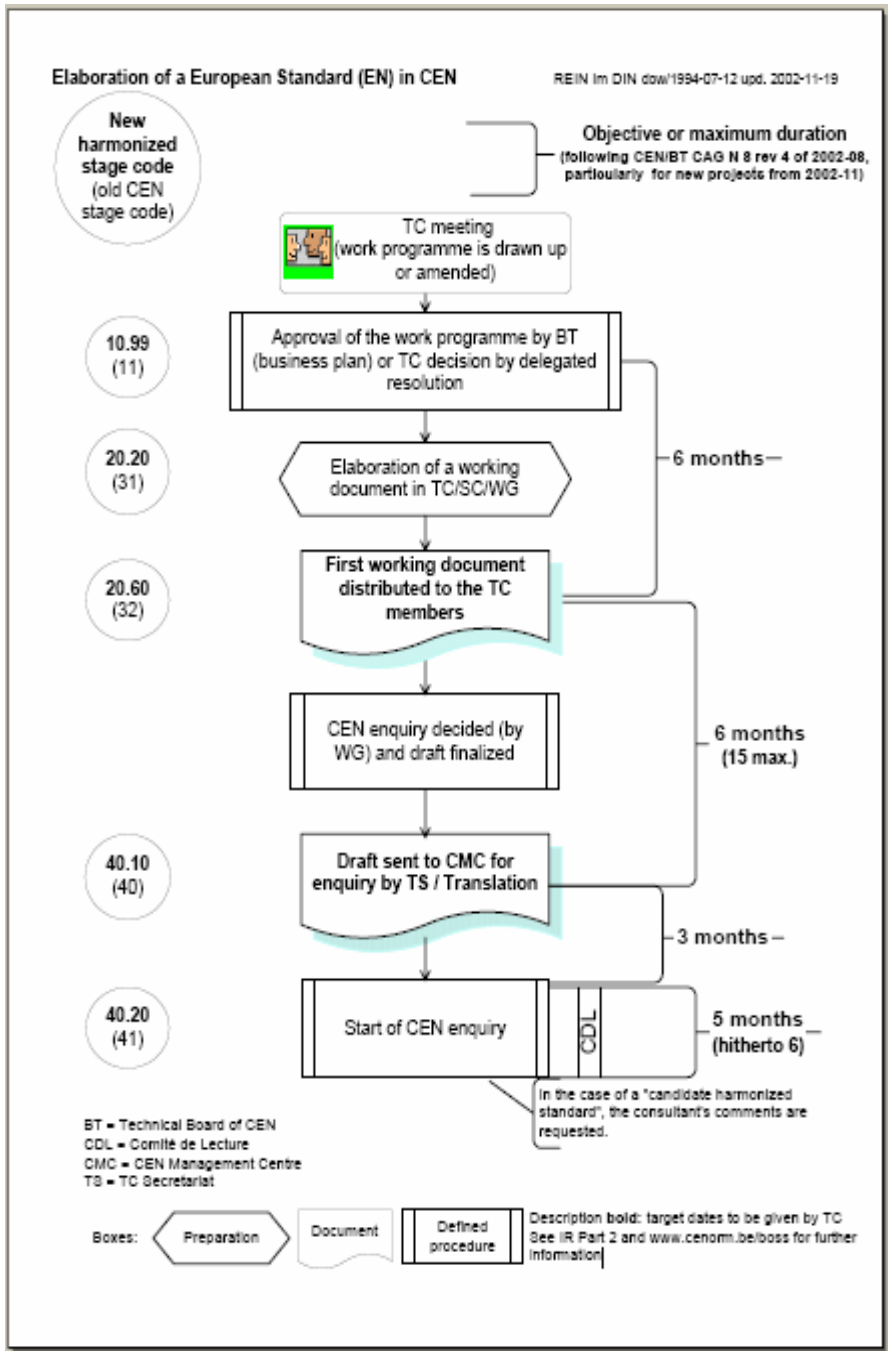
  > Whenever possible, standardized methods should offer the flexibility to apply methods in a changing “analytical world”, e.g. with improved instruments or for new analytes.

- Publication of a method as CEN standard requires a very long time

Elaboration of an European Standard (EN) in CEN – part 1

Individual first steps
(typically ≥ 20 month):

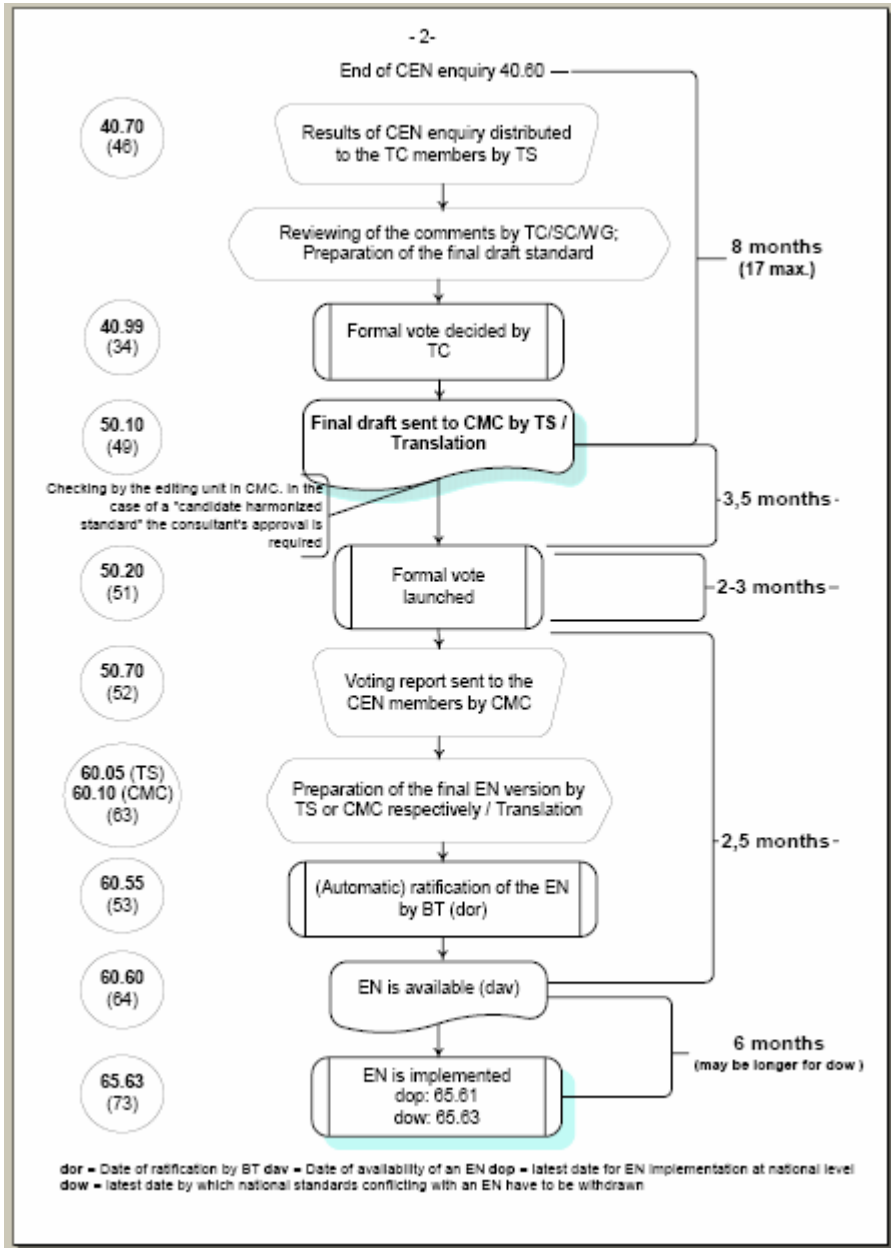
1. Proposal of a new method in the Working Group
2. Acceptance of the new working item by the Technical Committee 275
3. Preparation of the first working document in CEN format
4. Discussion of first working document in the Working Group
5. Preparation of the final draft
6. Preparation of German and French version
7. Circulation of the first draft in all EU countries (CEN enquiry)



Elaboration of an European Standard (EN) in CEN – part 2

…and final steps
(typically ≥ 20 month):

8. Collection of comments from all EU countries
9. Reviewing of comments by TC
10. Preparation of final draft
11. Final translation
12. Formal vote of all EU standardization bodies
13. Ratification and publication



Who prepares the methods for new standards?

- NOT the working groups of the Technical Committee 275
- Each national standardization body can propose new methods!
- The Working Groups 3 and 4 decide, if presented methods should be proposed as new working items
- Main problem: Sufficient validation data must be available or must be produced
- The working groups help to find participants for validation

However …

eventually best way in future:
CEN gets the proposals from the CRLs!

AENOR
AFNOR
BDS
BSI
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EVS
IPQ
IST
MSZT
NBN
NSAI
ON
SFS
SUTN

Actual working items

- EN15662: QuEChERS method (GC-MS and LC-MS)

  In EU-PT 9 used by ≥22 Laboratories (most often used method)

- EN15637: ChemElut method (LC-MS/MS only)

  In EU-PT 7 used by 14 laboratories

  78% were in category A / good (mean 38%)

- TR15641: Collection of tandem mass spectrometry parameters

  Necessary for both methods above

EN15662: QuEChERS method

Use a test portion of 10 g in a 50mL teflon tube

Add 10 mL Acetonitrile

Shake 1 min

Add 4 g $MgSO_4$ und 1 g NaCl

Shake 1 min

Add internal standards

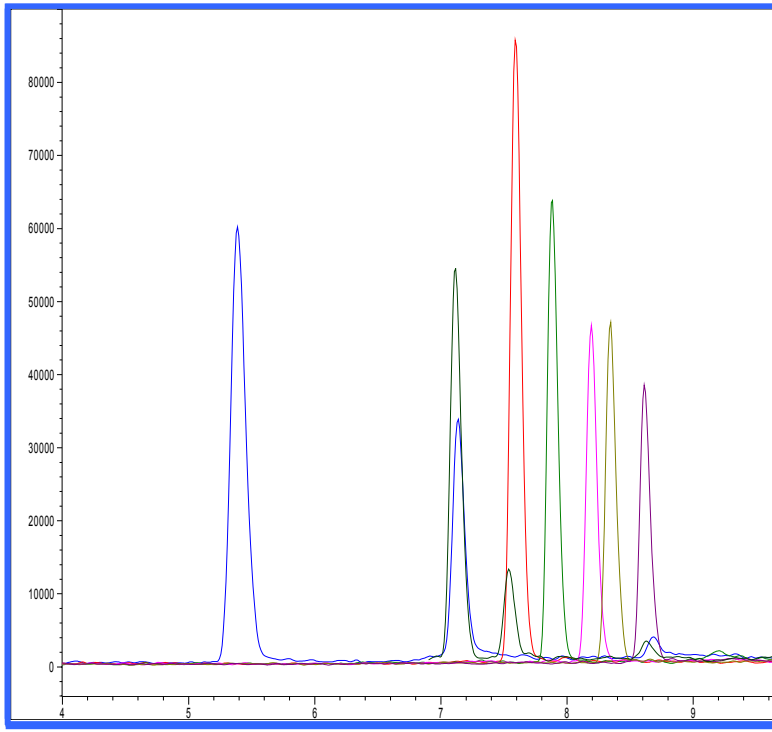
Shake 30 seconds | **Centrifuge**

Add $MgSO_4$ and Aminophase (“dispersive SPE”)

Shake 30 seconds | **Centrifuge**

Add HAc (0,1%) and “Analyte Protectants” (GC only)

GC-MS und LC-MS/MS



Anastassiades et al.
J. AOAC Int.
86 (2) 412-431

I thank Dr. Michelangelo Anastassiades for the permission to use this slide.

EN15637: ChemElut method (LC-MS/MS only)

Add water to 10 g sample (sum 10 ml)

↓

Homogenize with 20 ml methanol;
if necessesary filtration or centrifugation

↓

Add to an aliquot NaCl and soak
ChemElut

↓

Elution with dichloromethane;
evaporate to dryness

↓

Reconstitute in methanol/water
(final sample concentration 1 g/ml)

↓

Pass through a 0,45 µm syringe filter

↓

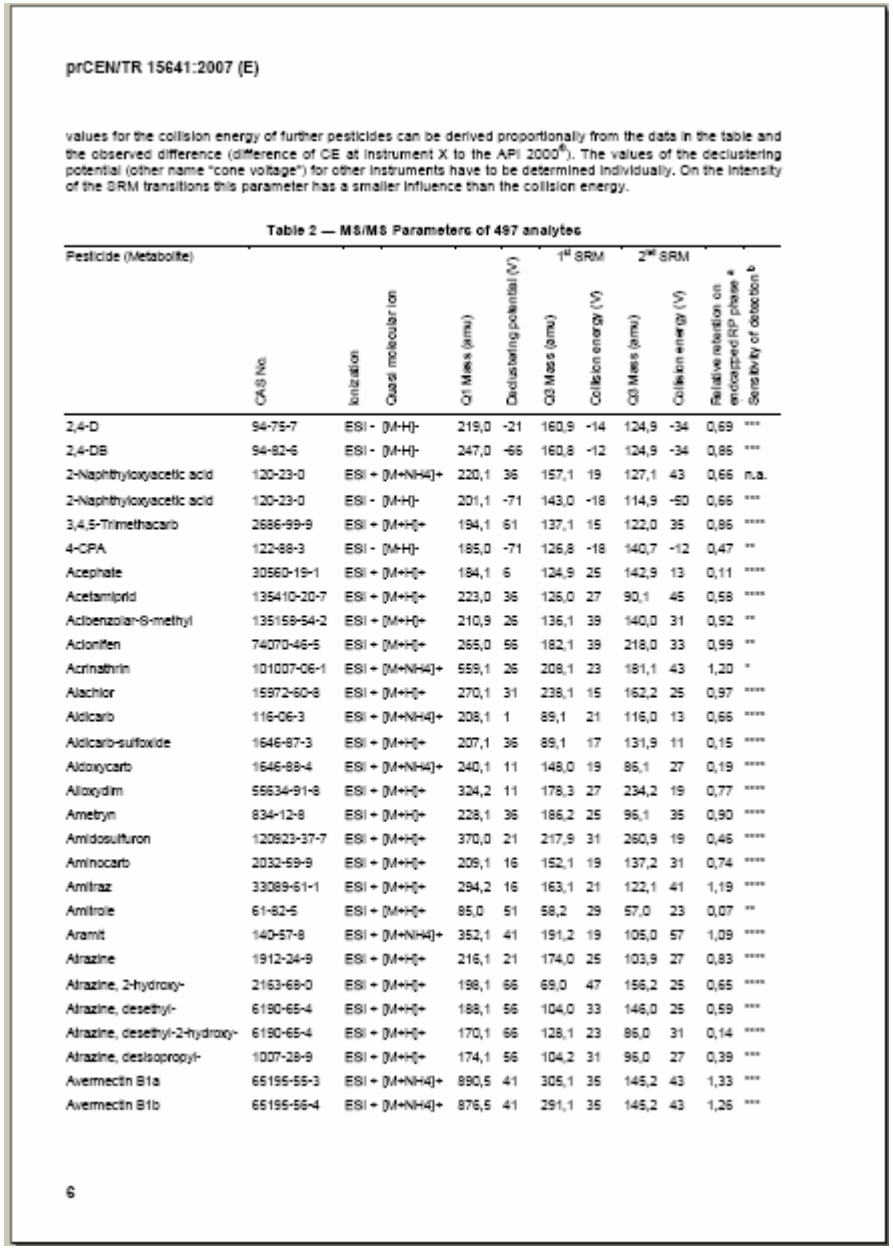
chromatography on 5 cm x 2 mm
RP18-column, LC-MS/MS in MRM mode

productivity:
≥10 samples / person and day plus
0,5 days evaluation of results

TR15641: Collection of tandem mass spectrometry parameters

Contains for approx. 500 pesticides:

- CAS-Number
- Ionization method
- Structure of quasimolecular ion
- Mass of parent ion
- Declustering potential
- Mass of two main fragments
- Appropriate collision energies
- Relative retention times
- Classification of response



prCEN/TR 15641:2007 (E)

values for the collision energy of further pesticides can be derived proportionally from the data in the table and the observed difference (difference of CE at instrument X to the API 2000®). The values of the declustering potential (other name "cone voltage") for other instruments have to be determined individually. On the intensity of the SRM transitions this parameter has a smaller influence than the collision energy.

Table 2 — MS/MS Parameters of 497 analytes

Pesticide (Metabolite)	CAS No.	Ionization	Quasi molecular ion	Q1 Mass (amu)	Declustering potential (V)	1st SRM Q3 Mass (amu)	1st SRM Collision energy (V)	2nd SRM Q3 Mass (amu)	2nd SRM Collision energy (V)	Relative retention on endcapped RP phase a	Sensitivity of detection b
2,4-D	94-75-7	ESI -	[M-H]-	219,0	-21	160,9	-14	124,9	-34	0,69	***
2,4-DB	94-82-6	ESI -	[M-H]-	247,0	-66	160,8	-12	124,9	-34	0,86	***
2-Naphthyloxyacetic acid	120-23-0	ESI +	[M+NH4]+	220,1	36	157,1	19	127,1	43	0,66	n.a.
2-Naphthyloxyacetic acid	120-23-0	ESI -	[M-H]-	201,1	-71	143,0	-18	114,9	-60	0,66	***
3,4,5-Trimethacarb	2686-99-9	ESI +	[M+H]+	194,1	61	137,1	15	122,0	35	0,86	****
4-CPA	122-88-3	ESI -	[M-H]-	185,0	-71	126,8	-18	140,7	-12	0,47	**
Acephate	30560-19-1	ESI +	[M+H]+	184,1	6	124,9	25	142,9	13	0,11	****
Acetamiprid	135410-20-7	ESI +	[M+H]+	223,0	36	126,0	27	90,1	45	0,58	****
Acibenzolar-S-methyl	135158-54-2	ESI +	[M+H]+	210,9	26	136,1	39	140,0	31	0,92	**
Aclonifen	74070-46-5	ESI +	[M+H]+	265,0	66	182,1	39	218,0	33	0,99	**
Acrinathrin	101007-06-1	ESI +	[M+NH4]+	559,1	26	208,1	23	181,1	43	1,20	*
Alachlor	15972-60-8	ESI +	[M+H]+	270,1	31	238,1	15	162,2	25	0,97	****
Aldicarb	116-06-3	ESI +	[M+NH4]+	208,1	1	89,1	21	116,0	13	0,66	****
Aldicarb-sulfoxide	1646-87-3	ESI +	[M+H]+	207,1	36	89,1	17	131,9	11	0,15	****
Aldoxycarb	1646-88-4	ESI +	[M+NH4]+	240,1	11	148,0	19	86,1	27	0,19	****
Alloxydim	55634-91-8	ESI +	[M+H]+	324,2	11	178,3	27	234,2	19	0,77	****
Ametryn	834-12-8	ESI +	[M+H]+	228,1	36	186,2	25	96,1	35	0,90	****
Amidosulfuron	120923-37-7	ESI +	[M+H]+	370,0	21	217,9	31	260,9	19	0,46	****
Aminocarb	2032-59-9	ESI +	[M+H]+	209,1	16	152,1	19	137,2	31	0,74	****
Amitraz	33089-61-1	ESI +	[M+H]+	294,2	16	163,1	21	122,1	41	1,19	****
Amitrole	61-82-5	ESI +	[M+H]+	85,0	51	58,2	29	57,0	23	0,07	**
Aramit	140-57-8	ESI +	[M+NH4]+	352,1	41	191,2	19	105,0	57	1,09	****
Atrazine	1912-24-9	ESI +	[M+H]+	216,1	21	174,0	25	103,9	27	0,83	****
Atrazine, 2-hydroxy-	2163-68-0	ESI +	[M+H]+	198,1	66	69,0	47	156,2	25	0,65	****
Atrazine, desethyl-	6190-65-4	ESI +	[M+H]+	188,1	56	104,0	33	146,0	25	0,59	***
Atrazine, desethyl-2-hydroxy-	6190-65-4	ESI +	[M+H]+	170,1	66	128,1	23	86,0	31	0,14	****
Atrazine, desisopropyl-	1007-28-9	ESI +	[M+H]+	174,1	56	104,2	31	96,0	27	0,39	***
Avermectin B1a	65195-55-3	ESI +	[M+NH4]+	890,5	41	305,1	35	145,2	43	1,33	***
Avermectin B1b	65195-56-4	ESI +	[M+NH4]+	876,5	41	291,1	35	145,2	43	1,26	***

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How to identify the next candidates for CEN methods?

- Based on regulation 396/2005 on maximum residue levels of pesticides
  - Less important: pesticides evaluated and registered in the EU (270)
  - More important: other (provisional) MRLs (approx. 250)
  - List published in spring 2007 by EFSA (REASONED OPINION ON THE POTENTIAL CHRONIC AND ACUTE RISK …FROM PROPOSED TEMPORARY EU MRLS: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620776373.htm)
  - fungicides and insecticides more important than herbicides

- My other personal favorites
  - Dithiocarbamates without CS_2
  - Multi-residue method (or variant) for acidic pesticides
  - Extreme polar pesticides (ethephon, glyphosate etc)

Yesterday also proposed by Mette Poulsen!

(Use of) CEN methods. A “must” for EU laboratories?

1. No, ...
2. ... but nice to have an agreement, which methods are good,
3. ... and methods should be available in all labs!
4. Nevertheless: Don‘t forget to search for better methods.

FEDERAL INSTITUTE FOR RISK ASSESSMENT

Thank you for your attention

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