

**3rd International Workshop on Harmonisation
of GMO Detection and Analysis for Central
and South America, Cartagena, Colombia,
3 - 4 July 2012**

Update on ISO activities

Lutz Grohmann, Marco Marzarra, Hermann Broll

International Organization for Standardization (ISO)

- ✓ The International Organization for Standardization widely known as ISO, is an international-standard-setting body composed of representatives from various national standards organizations;
- ✓ ISO is a non-governmental organization;
- ✓ It is setting standards that often become law, either through treaties or national standards;
- ✓ Therefore it makes it more powerful than most non-governmental organizations.
- ✓ Standards, technical specifications etc are usually developed together with experts from governmental organisations, company representatives and other interested stakeholders.

International Organization for Standardization
Organisation internationale de normalisation
Международная организация по стандартизации ^[1]



International Organization for Standardization

English language logo of the ISO

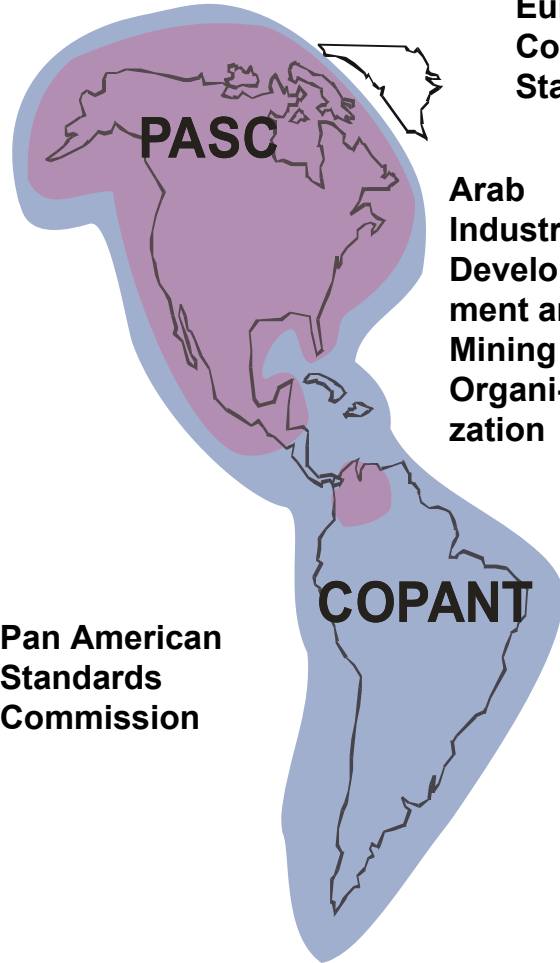


List of members

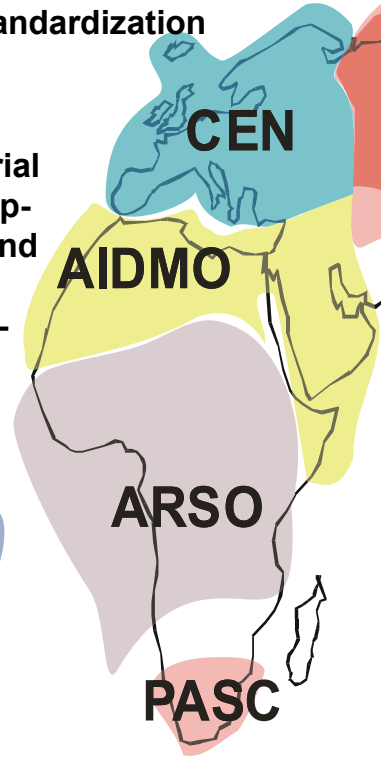
Formation	23 February 1947
Type	NGO
Purpose/focus	International standardization
Headquarters	Geneva, Switzerland
Membership	163 members ^[2]
Official languages	English, French and Russian
Website	www.iso.org 

Regional standardization bodies

Pacific Area
Standards Congress

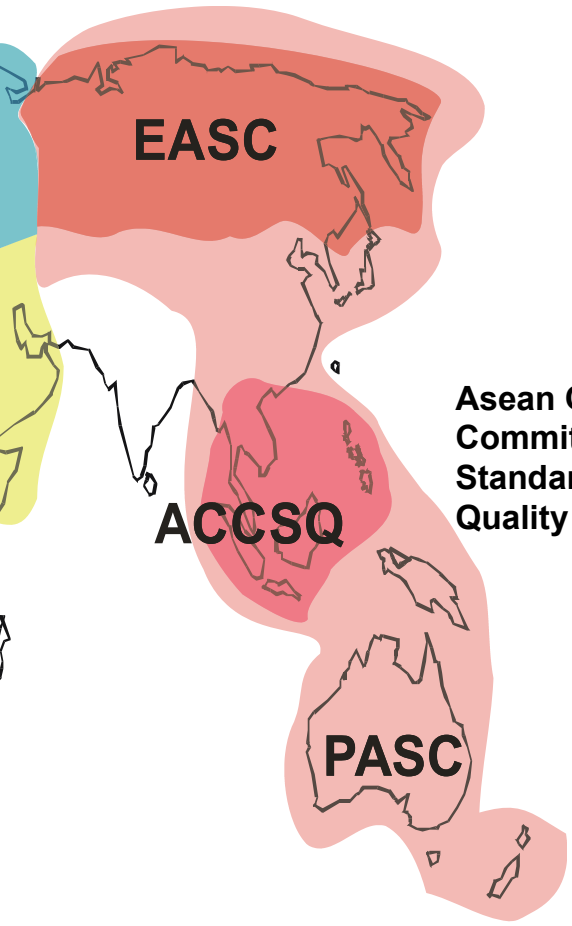


European
Committee for
Standardization



Arab
Industrial
Development
and
Mining
Organi-
zation

Euro-Asian Council for
Standardization, Metrology
and Certification



Asean Consultative
Committee for
Standards and
Quality

Pan American
Standards
Commission

African Regional
Organization for
Standardization

MO/LDE9606B.CDR
2003-03-19

CEN TC 275 Food analysis - Horizontal methods

WG 11: Genetically modified foodstuffs

Convener: Dr. Marianna Schauzu, Federal Institute for Risk Assessment, Berlin
 Secretary: Carola Seiler, DIN, Germany

EN ISO	Topic	Stage	Details
21572	Foodstuffs - Methods for the detection of genetically modified organisms and derived products - Protein based method	Standard ratified in November 2003	Corrigendum to change the status of the Annex from "normative" into "informative" has been published by ISO and is under way in CMC
21571	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction	Standard ratified in February 2005	
21569	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods	Standard ratified in June 2005	
24276	Foodstuffs - Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions	Standard ratified in January 2006	
21570	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods	Standard ratified in October 2005	
21568	Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products – Sampling	European Technical Standard 2006	No agreement within ISO

**Sampling CEN/TS 15568
consignment → lab sample**

DNA based methods

Extraction
EN ISO 21571
test sample → DNA

GMO detection
DNA → test result

Screening
EN ISO 21569
Qualitative PCR

Identification
EN ISO 21569
Qualitative PCR

Quantitation
EN ISO 21570
Quantitative PCR

**Protein based methods EN ISO
21572**

Extraction
test sample → Protein

GMO detection
Protein → test result

Screening

Identification

Quantitation

CEN → ISO

2008

“According to the Vienna Agreement, the secretariat for the future work on those EN/ISO standards concerning GMO, which were elaborated under CEN/TC 275/WG 11 (CEN-Lead), has been transferred to ISO. The responsible expert group is ISO/TC 34/SC 16 which had its first meeting in Chicago from 11th to 13th November 2008.”

ISO/TC 034/SC 16 "Horizontal methods for molecular biomarker analysis"



TC 34/SC 16 - Horizontal methods for molecular biomarker analysis

Participating countries: 21
Observing countries: 11

Chairmanship: USDA

Secretariat: AOCS

Secretariat:

USA (ANSI)

Participating Countries

Argentina (IRAM)

Canada (SCC)

China (SAC)

Denmark (DS)

Egypt (EOS)

France (AFNOR)

Germany (DIN)

India (BIS)

Iran, Islamic Republic of (ISIRI)

Ireland (NSAI)

Japan (JISC)

Korea, Republic of (KATS)

Namibia (NSI)

Netherlands (NEN)

Norway (SN)

Russian Federation (GOST R)

Sweden (SIS)

Switzerland (SNV)

Thailand (TISI)

United Kingdom (BSI)

Observing Countries

Austria (ASI)

Belgium (NBN)

Croatia (HZN)

Cyprus (CYS)

Czech Republic (UNMZ)

Italy (UNI)

Poland (PKN)

Romania (ASRO)

Serbia (ISS)

Slovakia (SUTN)

Spain (AENOR)

Organizations in liaison:

[AOAC](#), [CAC](#), [EC](#), [IDF](#), [IH&RA](#), [IRMM](#), [IUPAC](#), [NMKL](#), [OIE](#), [SSAFE](#), [UNECE](#), [UNIDO](#), [WHO](#)

Items to be displayed:

Published standards

Standards under development

Withdrawn standards

Subcommittees

◆ Subcommittee	◆ Subcommittee Title
TC 34/SC 2	Oleaginous seeds and fruits and oilseed meals
TC 34/SC 3	Fruit and vegetable products
TC 34/SC 4	Cereals and pulses
TC 34/SC 5	Milk and milk products
TC 34/SC 6	Meat, poultry, fish, eggs and their products
TC 34/SC 7	Spices, culinary herbs and condiments
TC 34/SC 8	Tea
TC 34/SC 9	Microbiology
TC 34/SC 10	Animal feeding stuffs
TC 34/SC 11	Animal and vegetable fats and oils
TC 34/SC 12	Sensory analysis
TC 34/SC 14	Fresh, dry and dried fruits and vegetables
TC 34/SC 15	Coffee
TC 34/SC 16	Horizontal methods for molecular biomarker analysis
TC 34/SC 17	Management systems for food safety



Scope and composition of SC16

Scope: Horizontal methods for molecular biomarker analysis

Standardization of biomolecular testing methods applied to: foods; feeds; seeds and other propagules of food and feed crops. The scope includes methods that analyze nucleic acids [e.g., polymerase chain reaction (PCR), genotypic analysis and sequencing], proteins [e.g. enzyme linked immunosorbent assay (ELISA)], and other suitable methods. The scope also includes variety identification and detection of plant pathogens. The scope does not include food microbiological methods.

Standards under development

- ISO/CD 13484: General requirements for molecular biology using analysis for detection and identification of pathogenic and destructive organisms of the plants and derived products.
- ISO/CD 13495: Principles of selection and criteria of validation for the varietal identification methods using specific nucleic acid analysis.
- ISO/WD 16383 on Qualitative methods (performance criteria)
- ISO 16578 on the use of microarray detection of specific nucleic acid sequences
- ISO 16577 Terms and Definitions

Summary of meeting resolutions

Res. 44/11

The Committee debated the classification of documents ..and agreed that reclassification or confirmation of current classification will be decided on a case-by-case basis, especially for TS

- Flax FP967 method NWIP was accepted as TS, CD draft now, few comments, will be quickly adopted as standard method
- Meetings every 18 months; Next meeting will be in Europe in spring 2013 most probably in London!!

Actual projects on ISO

Reference	Document title	Reg. date	Crnt stage	Stage date	Limit date	VA	Time frame	Committee	Project leader
ISO/DIS 13484	Foodstuffs -- General requirements for molecular biology analysis for detection and identification of destructive organisms in plants and derived products	2009-03-23	40.60	2012-03-12	2012-09-23 (Cancellation date: 2013-03-23)		48	ISO/TC 34/SC 16/WG 4	Bois, Frédéric M.
ISO/DIS 13495	Foodstuffs -- Principles of selection and criteria of validation for the varietal identification methods using specific nucleic acid analysis	2009-03-23	40.99	2012-03-28	2013-06-23		Extended	ISO/TC 34/SC 16/WG 3	Forzy, Christelle Mme
ISO/NP 16393	Molecular biomarker analysis -- Qualitative methods -- Determination of the performance characteristics and method validation	2011-03-23	10.99	2011-03-23	2013-03-23		48	ISO/TC 34/SC 16/WG 5	Shillito, Ray Mr.
ISO/WD 16577	Molecular biomarker analysis -- Terms and definitions	2011-03-23	20.20 (Start date: 2011-03-24)	2011-03-24	2012-09-23 (Cancellation date: 2013-03-23)		36	ISO/TC 34/SC 16	Jenkins, Ron Dr.
ISO/DIS 16578	Molecular biomarker analysis -- General definitions and requirements for microarray detection of specific nucleic acid sequences	2011-03-23	40.20 (Start date: 2012-06-20 End date: 2012-11-21)	2012-06-20	2014-09-23		48	ISO/TC 34/SC 16	Akiyama, Hideo Dr.
ISO/WD TR 17622	Molecular biomarker analysis -- SSR analysis of sunflower	2011-10-21	20.20 (Start date: 2011-10-21)	2011-10-21	2013-04-21		24	ISO/TC 34/SC 16/WG 3	Forzy, Christelle Mme
ISO/WD TR 17623	Molecular biomarker analysis -- SSR analysis of maize	2011-10-21	20.20 (Start date: 2011-10-21)	2011-10-21	2013-04-21		24	ISO/TC 34/SC 16/WG 3	Forzy, Christelle Mme
ISO/WD TS 21098	Foodstuffs -- Nucleic acid based methods of analysis of genetically modified organisms and derived products -- Information to be supplied and procedure for the addition of methods to ISO 21569, ISO 21570 or ISO 21571	2008-11-13	20.98	2011-10-27			36	ISO/TC 34/SC 16/WG 2	Shillito, Ray Mr.
ISO 21569:2005/DAmD 1	Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Qualitative nucleic acid based methods -- Amendment 1	2008-11-13	40.20 (Start date: 2012-02-09 End date: 2012-07-10)	2012-02-09	2012-11-13 (Cancellation date: 2013-05-13)	ISO lead	Extended	ISO/TC 34/SC 16	Schulze, Manuela Dr.
ISO/PRF TS 21569-2	Horizontal methods for molecular biomarker analysis -- Methods of analysis for the detection of genetically modified organisms and derived products -- Part 2: Construct-specific real-time PCR method for detection of event FP967 in linseed and linseed products	2011-10-21	50.00	2012-06-19			24	ISO/TC 34/SC 16	Grohmann, Lutz Dr.
ISO 21570:2005/DAmD 1	Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Quantitative nucleic acid based methods -- Amendment 1	2008-11-13	40.20 (Start date: 2012-04-30 End date: 2012-10-01)	2012-04-30	2012-05-13	ISO lead	Extended	ISO/TC 34/SC 16	Bois, Frédéric M.
ISO 21571:2005/DAmD 1	Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- Nucleic acid extraction -- Amendment 1	2008-11-13	40.60	2012-04-19	2012-11-13 (Cancellation date: 2013-05-13)	ISO lead	Extended	ISO/TC 34/SC 16	Grohmann, Lutz Dr.
ISO/DIS 21572.2	Foodstuffs -- Molecular biomarker analysis -- Protein-based methods	2007-12-20	40.99	2012-03-28	2011-06-20	ISO lead	Extended	ISO/TC 34/SC 16	Grothaus, G. David Dr
ISO 24276:2006/DAmD 1	Foodstuffs -- Methods of analysis for the detection of genetically modified organisms and derived products -- General requirements and definitions -- Amendment 1	2009-07-03	40.99	2012-03-28	2013-01-03 (Cancellation date: 2013-07-03)	ISO lead	48	ISO/TC 34/SC 16	Lelong, Baptiste M.

What is Codex Alimentarius?

- ✓ Codex Alimentarius (Latin for "food book") is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety;
- ✓ Codex Alimentarius was established in 1963 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO);
- ✓ In 2006, 99 % of the world's population were represented in Codex Alimentarius Commission through 174 member countries and one Member Organization (European Union);
- ✓ Commission's main aims are stated as being to protect the health of consumers and ensure fair practices in the international food trade;
- ✓ The Codex Alimentarius is recognized by the World Trade Organization (WTO) as an international reference point for the resolution of disputes concerning food safety and consumer protection;
- ✓ Codex Alimentarius covers all foods, whether processed, semi-processed or raw, but far more attention has been given to foods that are marketed directly to consumers;
- ✓ It contains general standards covering matters such as food labeling, food hygiene, food additives and pesticide residues, and procedures for assessing the safety of foods derived from modern biotechnology;
- ✓ It also contains guidelines for the management of official (i.e., governmental) import and export inspection and certification systems for foods;
- ✓ The Codex Alimentarius is published in Arabic, Chinese, English, French and Spanish.

Codex Alimentarius – scientific sounds

- *“The food standards, guidelines and other recommendations of the Codex Alimentarius shall be based on the principle of sound scientific analysis ...”;*
- *Codex Alimentarius work is dedicated to member countries;*
- *However, since its beginning, the Commission has welcomed the participation of consumers, whose organizations have been represented at its sessions;*
- *Decisions are made based on consensus; there is normally no voting procedure established;*
- *Member countries are encouraged to adopted Codex standards, guidelines etc in national regulations.*

Codex Alimentarius is quoted in trade agreements

World Trade Organization (WTO)

- ✓ WTO is an organization that intends to supervise and liberalize international trade.
- ✓ Existing since 1995 (before GATT)
- ✓ The organization deals with regulation of trade between participating countries
- ✓ The WTO has 153 members, representing more than 97% of total world trade
- ✓ Decision making process is generally by consensus, and relative market size is the primary source of bargaining power

World Trade Organization (English)
Organisation mondiale du commerce (French)
Organización Mundial del Comercio (Spanish)

WORLD TRADE ORGANIZATION 



■ WTO founder members (January 1, 1995)
■ WTO subsequent members

Formation	January 1, 1995
Headquarters	Centre William Rappard, Geneva, Switzerland
Membership	153 member states
Official languages	English, French, Spanish ^[1]
Director-General	Pascal Lamy
Budget	189 million Swiss francs (approx. 182 million USD) in 2009. ^[2]
Staff	625 ^[3]
Website	www.wto.org 

wikipedia

Relevance of Codex Alimentarius – Fostering consumer protection worldwide

In 1985 United Nations General Assembly

Guidelines for consumer protection

Stated that:

“When formulating national policies and plans with regard to food, Governments should take into account the need of all consumers for food security and should support and, as far as possible, adopt standards from the Food and Agriculture Organization’s ... and the World Health Organization’s Codex Alimentarius ...”.

In 1995 Agreement on the Application of Sanitary and Phytosanitary Measures (SPSS) and Agreement on Technical Barriers to Trade (TBT) of the World Trade Organization (WTO).

Formally recognized:

International standards, guidelines and recommendations, including the Codex Alimentarius, as reference points for facilitating international trade and resolving trade disputes in international law.

Codex Alimentarius is quoted in trade agreements (WTO)

SPS Agreement:

Agreement on the Application of Sanitary and Phytosanitary Measures

Article 2.2 of the SPS Agreement states:

“Members shall ensure that any sanitary and phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence ...”.

“The SPS Agreement has identified and chosen the standards, guidelines and recommendations established by the Codex Alimentarius Commission for food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling, and codes and guidelines of hygienic practice. This means that Codex standards are considered scientifically justified and are accepted as the benchmarks against which national measures and regulations are evaluated.”

Codex Alimentarius is quoted in trade agreements (WTO)

TBT Agreement: Agreement on Technical Barriers to Trade

- ✓ **It exists to ensure that technical regulations, standards, testing, and certification procedures do not create unnecessary obstacles to trade;**
- ✓ **The TBT agreement strongly encourages countries to recognize the results of other countries' conformity assessment tests – the tests that determine whether a product conforms to a given standard;**
- ✓ **It also promotes the development of international standards and provides governments and inter-governmental bodies with guidance on how to best develop such standards. TBT members are strongly encouraged to adopt international standards as their technical requirements whenever possible.**

Article 2.6 of the TBT Agreement states:

“With a view to harmonizing technical regulations on as wide a basis as possible, Members shall play a full part, within the limits of their resources, in the preparation by appropriate international standardizing bodies of international standards for products for which they have either adopted, or expect to adopt, technical regulations.”

Codex Alimentarius is quoted in trade agreements

Codex and its work have been quoted in many bilateral and multilateral trade agreements, including:

- Mexico–Bolivia, 1995
- Baltic Area Free Trade Agreement, 1996
- Chile–Mexico, 1997
- Bulgaria–Turkey, 1998
- Central America–Chile, 1999
- Association of Southeast Asian Nations (ASEAN), 2000
- Turkey–Bosnia and Herzegovina, 2002
- Australia–Thailand, 2005
- United States of America–Australia, 2005

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CRITERIA AND VALIDATION OF METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS IN FOODS*

- ✓ Criteria are applicable to protein and DNA-based methods;
- ✓ Criteria approach, it is foreseen not to endorse individual methods;
- ✓ Scope includes applications such as ‘food derived from modern biotechnology’, food authentication, food speciation and other purposes;
- ✓ Contains the reference to ‘food derived from modern biotechnology’ twice;
- ✓ Title was modified to reflect the scope; ‘foods derived from modern biotechnology’ is still in the footnote to the title;
- ✓ Text was modified extensively during the sessions, however major parts are derived from the ENGL document ‘Definition of minimum performance requirements for analytical methods of GMO testing’;
- ✓ Modular Approach to Method Validation is included;
- ✓ On several places thresholds are indicated.

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CRITERIA AND VALIDATION OF METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS IN FOODS*

Requirements with defined thresholds in the document I:

Repeatability standard deviation (RSDr)

21. The relative repeatability standard deviation for the PCR step should **be $\leq 25\%$** over the whole dynamic range of the method.

Reproducibility standard deviation (RSDR)

22. The relative reproducibility standard deviation for the PCR step should be **below 35%** over the majority of the dynamic range, except at the limit of quantification, where the RSDR could be higher.

Robustness

23. Robustness is a measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Examples of such variations include: reaction volumes (e.g., 29 vs. 30 μ l), annealing temperature (e.g., +/- 1oC) and/or other relevant variations. The experiments need to be performed at least in triplicate. The response of an assay with respect to these small changes **should not deviate more than $\pm 35\%$** in reproducibility experiments from the response obtained under the original conditions.

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CRITERIA AND VALIDATION OF METHODS FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF SPECIFIC DNA SEQUENCES AND SPECIFIC PROTEINS IN FOODS*

Requirements with defined thresholds in the document II:

Sensitivity

25. For a quantitative PCR method, a linear relationship of the Ct as a function of the logarithm of the template concentration should be obtained across the range of the method. The correlation coefficient, y-intercept and slope of the regression line should be reported. **The % of residual for each of the calibrators should preferably be $\leq 30\%$.**

30. For assays selective for the target DNA. Experimental evidence of selectivity for the target DNA should include:

Two replicates should be analyzed for each DNA sample, which shall **give results within a Ct-value of 0.5.**

32. For assays on taxon-specific DNA sequences. Experimental evidence of taxon selectivity should include:

Two replicates should be analyzed for each DNA sample, which shall **give results within a Ct-value of 0.5.**

Trueness

35. A trueness value of **$\pm 25\%$, in regards to the PCR step**, should be acceptable over the whole dynamic range.

Codex Alimentarius

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

codex alimentarius commission



FOOD AND AGRICULTURE
ORGANIZATION
OF THE UNITED NATIONS

WORLD
HEALTH
ORGANIZATION



JOINT OFFICE: Viale delle Terme di Caracalla 00153 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

ALINORM 10/33/23

http://www.codexalimentarius.net/download/report/738/al33_23e.pdf

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX ALIMENTARIUS COMMISSION

Thirty-third Session
Geneva, Switzerland, 5-9 July 2010

REPORT OF THE THIRTY-FIRST SESSION OF THE
CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Budapest, Hungary
8-12 March 2010

Developments within Codex Alimentarius regarding genetically modified foods

Codex Committee for Food Labelling (CCFL)

- **In 1999 a WG was established to draft a text regarding GMO labelling**
- **WG comprised Japan, Thailand, India, Brazil, USA, Canada, European Community**
- **Text was drafted «Proposed draft recommendations for the Labelling of Food and Food Ingredients derived from Biotechnology»**
- **Options were given for method of production labelling as well as labelling only for significant changes in composition incl. nutrition**

Detection methods were mentioned for the first time in Codex system

Developments within Codex Alimentarius regarding genetically modified foods

Codex Committee for Food Labeling (CCFL)

Proposed draft Recommendations for the labelling of foods and food ingredients obtained through certain techniques of genetic modification/genetic engineering (at Step 3)

From „**Mandatory Labelling Provisions**“



From „**Voluntary Labelling Provisions**“

There is still no consensus reached; consequently no global standard for gm food labelling is existing

Developments within Codex Alimentarius also dedicated to genetically modified foods

Draft Revised Guidelines on Measurement Uncertainty

At step 6 of the Codex procedure

To be included as an Annex to the Guidelines on Measurement Uncertainty (CAC/GL 54-2004)

Explanatory Notes to the Codex Guidelines on Measurement Uncertainty

Users of validation data should note that sources of uncertainty that are not covered by validation studies include:

- Sampling
- Pre-treatment
- Method bias
- Variation in conditions
- Changes in sample matrix

4 different scenarios are described in the guideline (above maximum level incl. MU up to incl. MU less than maximum level)

Useful References

Developments within Codex Alimentarius also dedicated to genetically modified foods

GUIDELINES FOR SETTLING DISPUTES OVER ANALYTICAL (TEST) RESULTS (CAC/GL 70-2009)

1. SCOPE

These guidelines provide guidance to governments on the procedures to resolve disputes which arise between food control authorities about the status of a food consignment, when the assessment based on test results made in the importing country disagrees with the assessment made by the exporting country on the same lot.

These guidelines only address disputes related to methods of analysis or laboratory performance and do **not** address questions of **sampling**. The procedure examines only the validity of the importing country's results on which non-compliance is alleged. It is recognised that disputes may arise from other cause(s), which should also be investigated.

These guidelines do not cover microbiological test results!

Developments within Codex Alimentarius also dedicated to genetically modified foods

GUIDELINES FOR SETTLING DISPUTES OVER ANALYTICAL (TEST) RESULTS (CAC/GL 70-2009)

PREREQUISITES/ASSUMPTIONS for applying these guidelines are:

- both countries agree on using this guideline;
- laboratories comply with quality assurance provisions;
- at least one representative sample⁵ from the same food lot has been taken;
- laboratories report quantitative analytical results in the form of “ $a \pm 2u$ ” or “ $a \pm U$ ”
- laboratories use specific methods of analysis, which have been endorsed by the Codex Alimentarius

THE RESULTS AND PROCEDURES OF THE LABORATORY OF THE EXPORTING COUNTRY AND ITS COUNTERPART IN THE IMPORTING COUNTRY ARE COMPARED

ANALYSING RESERVE SAMPLE

ANALYSIS OF REMAINING RESERVE SAMPLE by third party (importing country can select a laboratory)

The Commission adopted the Draft Guidelines in *Thirty-Second Session in 2009*

Thank you for your attention

Hermann Broll

Department of Food Safety

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