CODEX ALIMENTARIUS COMMISSION

E

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 6 **CX/MAS 16/37/6**
January 2016

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
37th Session
Budapest, Hungary, 22 - 26 February 2016

DISCUSSION PAPER ON CRITERIA FOR ENDORSEMENT OF BIOLOGICAL METHODS USED TO DETECT CHEMICALS OF CONCERN

(Prepared by the Electronic Working Group led by Chile and France)

BACKGROUND

1. At the 35th session of the Codex Committee on Methods of Analysis and Sampling (CCMAS35) there was extensive discussion about the typing of the methods used to quantify marine toxins: biological and chemical, and therefore about their possible endorsement in Codex standards.

2. This debate about biology and chemistry is not new because it can also take place in other assays like the determination of vitamins. However, for toxins assays the use of living animals to test the samples can be considered as a great concern by some countries. The list of existing biological methods was also not well defined. Biological methods, with the same principle, are often classified differently without a precise reason.

3. CCMAS35 agreed to establish an electronic Working Group on criteria for endorsement of biological methods to detect chemicals of concern, led by Chile, and co-chaired by France, and working in English only.

4. The eWG was tasked with preparing a discussion paper on the development of criteria for endorsement of biological methods used to detect chemicals of concern.

5. For the purpose of this working group biological methods were considered to be those methods of analysis, which use whole or parts of organisms as analytical indicators excluding PCR, enzymatic and ELISA methods. This work has also excluded the methods used for food hygiene assessment under the CCFH mandate.

6. The Working Group was challenged to:

i.) Classify biological methods according to the nature, principles, characteristics, etc…

ii.) Identify for which classes of the methods the criteria approach applies.

iii.) Recommend criteria to endorse each class of biological methods defined in step (i)

7. The eWG had over 47 participants. The list of participants and affiliations are listed at the end of the document.

PRELIMINARY SCHEDULE

8. A first document was drafted and sent to all the members at the end of July 2015. It was composed of a list of the existing methods and of a list of definitions. Biological methods currently formalized in the Codex Alimentarius are listed as below. The definitions are listed in annex II:

Commodity	Provision	Method	Principle	Type
Margarine	Vitamin D	AOAC 936.14	Bioassay	II
Minarine	Vitamin D	AOAC 936.14	Bioassay	II
Special foods	Folic acid	AOAC 944.12	Microbioassay	II

Special foods	Nicotinamide for milk-based foods	AOAC 944.13	Microbioassay	II
Special foods	Pantothenic acid / enriched foods	AOAC 945.74	Microbioassay	II
Special foods	Pantothenic acid / non-enriched foods	The Analyst 89 (1964):1, 3-6, ibid. 232	Microbioassay	IV
		US Dept Agr., Agr. Handbook 97 (1965)		
Special foods	Protein efficiency ratio (PER)	AOAC 960.48	Rat bioassay	I
Special foods	Vitamin B12	AOAC 952,20	Microbioassay	II
Special foods	Vitamin B6	AOAC 961.15	Microbioassay	II
Special foods	Vitamin D	AOAC 936,14	Rat bioassay	IV
Follow-up formula	Pantothenic acid	AOAC 992.07 Measures total pantothenate : free pantothenic acid + bounded forms (ACP and CoA)	Microbioassay	II
Infant formula	Folic acid	AOAC 992.05 *Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid* EN 14131:2003 *Total folate (free + bound), aggregated and measured as folic acid*	Microbioassay	II
Infant formula	Niacin	AOAC 985.34 *Niacine (preformed) and nicotinamide*		III
Infant formula	Vitamin B6	AOAC 985.32	Microbioassay	III
Infant formula	Vitamin B6	EN 14166 *(Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine, measured as pyridoxine)*	Microbioassay	III

1.

9. Some comments about the definitions were received and Annex 2 was modified.

10. Some propositions to add other method references were received. Most of them were methods for microbiology (out of the scope) and were not introduced in the list.

11. The two methods for the determination of marine biotoxins were received and added.

Commodity	**Provision**	**Method**	**Principle**	**Type**
Determination of marine biotoxins	PSP	AOAC 959.08	Mouse bioassay	IV
	PSP	AOAC 2011.27	Mouse bioassay	IV

12. Most of the methods identified are methods to quantify vitamins and more exactly vitamin activities transformed in amounts of provision. Most of the commodities concerned are special foods or infant formula.

It is well known that the vitamins are now quantified by chromatographic methods. Will these methods be kept endorsed in the coming years by the CCMAS?

13. It seems that there is a high probability that most of them would not be kept as endorsed by the CCMAS. In that case the CCMAS is requested to consider whether there is a real need to establish criteria for endorsement of biological methods.

14. The EWG established 2 other issues to address, which could not be discussed for reasons of time, and that are essential for work to develop:

- Identify to which classes of methods the criteria approach applies
- Recommend criteria to endorse each class of biological methods defined

RECOMMENDATIONS

15. The eWG recommends CCMAS to re-evaluate the list of biological methods and to consider their endorsement in the future before proceeding.

16. IChile and France as the lead countries of the eWG considers it necessary to continue the work of the electronic working group with the aim to further discuss the topics suggested: Identify to which classes of methods the criteria approach applies & recommend criteria to endorse each class of biological methods defined.

ANNEX 1

ELECTRONIC WORKING GROUP ON CRITERIA FOR ENDORSEMENT OF BIOLOGICAL METHODS USED TO DETECT CHEMICAL OF CONCERN

N°	Name		Organization	Country	email
1	Codex Argentina			Argentina	codex@minagri.gob.ar
2	Codex Australia			Australia	codex.contact@agriculture.gov.au
3	EU Codex Contact point			UE	codex@ec.europa.eu
4	Korea Contact Point		Ministry of Food and Drug Safety	Korea	codexkorea@korea.kr
5	Acevedo	Irma	Servicio Agricola Ganadero	Chile	irma.acevedo@sag.gob.cl
6	Alzamora	Claudia	Instituto nacional de defensa de la competencia y de la protección de la propiedad intelectual - Indecopi	Perú	calzamora@indecopi.gob.pe
7	Arpad	Ambrus	CCMAS Chair	Hungary	ambrusadr@yahoo.co.uk
8	Behnisch	Peter	BioDetection Systems b.v.	Germany	peter.behnisch@bds.nl
9	Choi	Eun-Jin	Ministry of Food and Drug Safety	Korea	cej1@korea.kr
10	Coghlan	Richard	National Measurement Institute - Department of Industry and Science	Australia	richard.coghlan@measurement.gov.au
11	de Vreeze	Marcel	AgroFood & Consument Standardization consultant Agrofood NEN and ISO	Netherlands	marcel.devreeze@nen.nl
12	Denisse	Canouet	SERNAPESCA	Chile	dcanouet@sernapesca.cl
13	Donders	Mauricio	Universidad Tecnologica Metropolitana	Chile	mdonders@utem.cl
14	Dubois-Lozier	Aurélie	IDF	IDF	adubois@fil-idf.org
15	Flores	Laura	Laboratorio Tecnológico del Uruguay, LATU	Uruguay	lflores@latu.org.uy
16	Fodor	Andrea	National Food Chain Safety Office Food and Feed Safety Directorate	Hungary	fodora@nebih.gov.hu
17	Franklin Pinto	Rosane Maria		Brasil	alimentos@anvisa.gov.br
18	Gálvez González	César Omar	Commission for Analytic Control and Expansion of Coverage Federal Commission for Protection against Health Risks	México	cgalvez@cofepris.gob.mx czargg@gmail.com
19	Geetanjali		Central Food Laboratory, Kolkata, Food Saftey and Standards Authority of India.	India	geetanjali.sharma.cfl@gmail.com
20	Guiim	Moon	National Institute of Food and Drug Safety Evaluation	Korea	luna@korea.kr

21	Gulyás	Márta	National Food Chain Safety Office	Hungary	GulyasM@nebih.gov.hu
22	Józwiák	Ákos	National Food Chain Safety Office	Hungary	jozwiaka@nebih.gov.hu
23	Katikou	Panagiota	National Reference Laboratory for Marine Biotoxins Department of Aquatic Organisms Pathology, Control of Marine Biotoxins and Toxins in Other Waters Directorate of Veterinary Centre of Thessaloniki Ministry of Productive Reconstruction, Environment and Energy	Greece	biotoxin@otenet.gr
24	Khudhair Abas	Sumeia	Central Organization for standardization and quality control (COSQC)	Iraq	sumeiak.abas37@yahoo.com
25	Krishnan	Anoop	Export Inspection Agency (EIA), Kolkata	India	eia-kolkatalab@eicindia.gov.in
26	Lee	Barbara	Health Products and Food Branch Health Canada	Canada	Barbara.Lee@hc-sc.gc.ca
27	Lindner Schreiner	Ligia		Brasil	ligia.schreiner@anvisa.gov.br
28	Maratos	Marie	U.S. Codex Office Food Safety and Inspection Service	USA	Marie.Maratos@fsis.usda.gov
29	Máthé	Endre	University of Debrecen Faculty of Agricultural and Food Sciences and Environmental Management	Hungary	endre.mathe64@gmail.com
30	Morris	Susan	Ministry for Primary Industries	New Zealand	susan.morris@mpi.govt.nz
31	Nagy	Attila	National Food Chain Safety Office	Hungary	nagyattila@nebih.gov.hu
32	Noonan	Gregory	Division of Analytical Chemistry Center for Food Safety and Applied Nutrition U.S. Food and Drug Administration	USA	gregory.noonan@fda.hhs.gov
33	Norden	Timothy	Technology & Science Division Grain Inspection, Packers and Stockyards Administration U. S. Department of Agriculture	USA	timothy.d.norden@usda.gov
34	Pellegrino	Marcelo	SENASA	Argentina	mpellegr@senasa.gob.ar
35	Salhi	Maria	Laboratorio de Análisis de Productos Pesqueros, Departamento de Industria Pesquera, Dirección Nacional de Recursos Acuáticos (DINARA)	Uruguay	msalhi@dinara.gub.uy
36	Soto	Marcelo	ISPCH	Chile	msoto@ispch.cl

37	Sri Heruwati	Endang	Researcher, Ministry of Marine Affairs and Fisheries (past)	Indonesia	endang_heruwati@yahoo.com endang_heruwati@gmail.com rina@bsn.go.id codex_indonesia@bsn.go.id
38	Suárez Brito	Pamela	Department of International Operation Federal Commission for Protection against Health Risks	México	codex@cofepris.gob.mx psuarez@cofepris.gob.mx
39	Takanori	Ukena	Food Safety and Consumer Policy Division Food Safety and Consumer Affairs Bureau Ministry of Agriculture, Forestry and Fisheries	Japan	takanori_ukena@nm.maff.go.jp codex_maff@nm.maff.go.jp
40	Torres	Véronica	SENASA	Argentina	vtorres@senasa.gob.ar
41	van der Schee	Henk	Wetenschappelijk medewerker Nederlandse Voedsel- en Warenautoriteit	Netherlands	h.a.vanderschee@nvwa.nl
42	Verger	Philippe	Department of Food Safety and Zoonoses World Health Organization	WHO	vergerp@who.int
43	Watanabe	Takahiro	National Institute of Health Sciences Ministry of Health, Labour and Welfare	Japan	codexj@mhlw.go.jp
44	Weesepoel	Yannick	RIKILT	Netherlands	yannick.weesepoel@wur.nl
45	Zentai	Andrea	Vice Chair CCMAS	Hungary	zentaia@nebih.gov.hu

ANNEX 2

DEFINITIONS LINKED TO BIOLOGICAL METHODS

1. **Bioassay:** Method in which power or potency of a substance is measured by the response of living organisms or living systems. (c)

2. **Bioassays classification based on nature:**

   2.1. Animal-based biological assays, which measure an organism's biological response to the product; a)

   2.2. Cell culture-based biological assays, which measure biochemical or physiological esponse at the cellular level; a)

   2.3. Biochemical assays, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions. a)

3. **Classification based bioassays type:**

   3.1. Qualitative bioassays are those that do not generate a measurable graduated response, obtaining an absolute answer to the test unit. The bioassay gives a negative or positive response based on a specified concentration threshold.

   3.2. Quantitive bioassays produce a graduated response that generates a numeric value. These tests can be used for statistical power calculation methods, such as the model of parallel lines.

4. **Classification of time-based bioassays:**

   4.1. Short term de toxicity test: 24- 48 h.

   4.2 Intermediate term toxicity test: 10 to 90 days.

   4.3 Long term toxicity test: exposition extending. (b)

Other term in relation:

5. **Biological Activity:** The specific ability or capacity of the product to achieve a defined biological effect. Potency is the quantitative measure of the biological activity. **(a)**

6. **Potency:** The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties. **(b)**

7. **No-Observed-Effect-Concentration (NOEC):** in a full- or partial –life-cycle test, the highest toxicant concentration in which the values for the measured response are not statistically significantly different from those in the control. **(b)**

8. **Lowest-Observed-Effect-Concentration (LOEC):** in a full- or partial- life cycle test, the lowest toxicant concentration in which the values for the measured response are stastistical significantlly different from those in the control. **(b)**

9. **Median lethal concentration (LC_{50}):** Statistically derived concentration of a substance in an environmental medium expected to kill 50% of organisms in a given population under a defined set of conditions. **(e)**

10. **Asymptomatic LC_{50}:** Toxicant concentration at which LC50 approaches a constant for a prolonged exposure time. **(b)**

11. **Lethal Concentration (LC):** Concentration of a potentially toxic substance in an environmental medium that causes death following a certain period of exposure. **(e)**

12. **Exposure time:** The time a test organism is exposed to test. **(b)**

13. **Dose:** Amount of toxicant that enters the organism. Dose and concentration are not interchangeable. **(b)**

14. **Toxicity:** Capacity to cause injury to a living organism defined with reference to the quantity of substance administered or absorbed, the way in which the substance is administered and distributed in time (single or repeated doses), the type and severity of injury, the time needed to produce the injury, the nature of the organism(s) affected and other relevant conditions. **(d)**

15. **Acute toxicity:** *Adverse effects* of finite duration occurring within a short time (up to 14 d) after

administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures), usually within 24 h of a starting point (which may be exposure to the toxicant, or loss of reserve capacity, or developmental change etc.). Ability of a substance to cause adverse effects within a short time of dosing or exposure. **(d)**

16. **Chronic toxicity:** Adverse effects following chronic exposure. Effects which persist over a long period of time whether or not they occur immediately upon exposure or are delayed. **(d)**

17. **Toxicity equivalency factor (TEF, *f*):** Ratio of the toxicity of a chemical to that of another structurally related chemical (or index compound) chosen as a reference. **(d)**

18. **Toxicity equivalent (TEQ), T_{xe}:** Contribution of a specified component (or components) to the toxicity of a mixture of related substances. **(d)**

    Note 1: The amount-of-substance (or substance) concentration of total toxicity equivalent is the sum of that for the components B, C … N.

    Note 2: Toxicity equivalent is most commonly used in relation to the reference toxicant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [2,3,7,8-tetrachlorooxanthrene] by means of the TEF which is 1 for the reference substance. f1-Toxic equivalent factor assigned. Hence, where c is the amount-of-substance concentration:

$$T_{xe} = \sum_{i=B}^{N} f_1 c_i$$

Bibliography:

(a) ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological / Biological Products
(b) Standard Methods for Examination of Water and wastewater. 22nd Edition. APHA.AWWA.WEF.
(c) Ensayos toxicologicos y métodos de evalaución de calidad de aguas. Estandarización, intercalibración, resultados y aplicaciones. Morales, Gabriela. IMTA. México. 2004.
(d) IUPAC glossary of terms used in toxicology, 2nd edition - Iupac recommendations 2007. Published in Pure Appl. Chem., Vol. 79, No. 7, pp. 1153-1344, 2007
(e) IUPAC Gold Book. 2003.