

Proposed Amendments to the Harmful Substances in Food Regulations (Cap. 132AF)

**First technical meeting with trade on
27 January 2021**

Purpose

- ❁ **To provide technical guidance on testing of harmful substances in food**
 - ❁ **Introduction of harmful substances covered and reference methods of analysis**
 - ❁ **Criteria for selection of testing methods**
 - ❁ **Guide to selection of laboratory validation methods**

Harmful substances covered in amendment

- ✿ **3 types of mycotoxins in food**
 - **Aflatoxins, total; Aflatoxin B1; Aflatoxin M1**
 - **Deoxynivalenol (DON)**
 - **Patulin**
- ✿ **3 types of harmful substances in edible fats and oils and condiments**
 - **Benzo[a]pyrene (B[a]P)**
 - **Erucic acid**
 - **3-monochloropropane-1,2-diol (3-MCPD)**

Harmful substances covered in amendment

✿ 3 types of harmful substances in formula products intended for infants

- Benzo[a]pyrene (B[a]P)

- Glycidyl fatty acid esters (expressed as glycidol) (GE)

- Melamine

✿ Partially Hydrogenated Oils (PHOs)

1. Aflatoxins

(“Aflatoxins, total (i.e. “aflatoxins B1+B2+G1+G2”))

Food / Food group	Proposed ML
Non-ready-to-eat peanuts, almonds, Brazil nuts, hazelnuts and pistachios	15 µg/kg
Non-ready-to-eat products of the above food	
Spices	
Ready-to-eat peanuts, almonds, Brazil nuts, hazelnuts and pistachios	10 µg/kg
Ready-to-eat products of the above food	
Dried figs	
Any other food	5 µg/kg

1. Aflatoxins

“Aflatoxin B1”

Food / Food group	Proposed ML
Any food intended to be consumed principally by persons under the age of 36 months	0.1µg/kg

“Aflatoxin M1”

Food / Food group	Proposed ML
Infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months	0.025µg/kg
Any other milk and dried milk	0.5µg/kg

Test Methods – Aflatoxins

✿ Reference international standards

✓(Aflatoxins, total)

- BS EN ISO 16050:2011 (Cereals, nuts and derived products) (LC-FLD)
- BS EN ISO 14123:2007 (Nuts and figs) (LC-FLD)
- AOAC 999.07 (Nut derived products) (LC-FLD)
- AOAC 994.08 (Nuts) (LC-FLD)
- BS EN ISO 17424:2020 (Spices) (LC-FLD)

✓(Aflatoxin B1)

- BS EN 15851:2010 (HPLC-FLD)
- AOAC 2000.16 (HPLC-FLD)

✓(Aflatoxin M1)

- BS EN ISO 14501:2007 (LC-FLD)
- AOAC 986.16 (LC-FLD)

2. Deoxynivalenol (DON)

Food / Food group	Proposed ML
Any food containing cereal intended to be consumed principally by persons under the age of 36 months	200 μ g/kg

☀ Reference international standard

✓ BS EN 17280:2019 (LC-MS/MS)

2. Test Methods – Deoxynivalenol (DON)

✓ BS EN 17280:2019 (LC-MS/MS)

- ✿ DON are extracted from homogenized sample with a mixture of ACN and H₂O by mechanical shaking for 60 min
- ✿ The extract is filtered and evaporated to dryness at *ca.* 40°C under N₂/air
- ✿ The residue is dissolved with a mixture of MeOH and H₂O and applied into a SPE column
- ✿ After purification, the column eluate is evaporated to dryness and reconstituted with injection solvent for LC-MS/MS analysis

3. Patulin

Food / Food Group	Proposed ML
Apple juice and other beverages to which apple juice has been added	50 µg/kg

✿ Reference international standards

- ✓ BS EN ISO 15890:2010 (HPLC-UV)
- ✓ AOAC 995.10 (HPLC-UV)

3. Test Methods – Patulin

✓ BS EN ISO 15890:2010 (HPLC-UV)

- ✿ Patulin is extracted with a mixture of ethyl acetate and hexane in the presence of Na_2SO_4 and NaHCO_3
- ✿ Extract is purified by solid-phase extraction and evaporated
- ✿ The residue is re-dissolved in water of pH4 and patulin is determined by reverse phase HPLC-UV

4. Benzo[a]pyrene (B[a]P) (Edible oils and fats)

Food / Food group	Proposed ML
Any oil or fat or any mixture of oil and fat	5 µg/kg

✿ Reference international standards

- ✓ BS EN ISO 15302:2017 (HPLC-FLD)
- ✓ BS EN ISO 15753:2016 (HPLC-FLD)
- ✓ BS EN ISO 22959:2009 (HPLC-FLD)

4. Benzo[a]pyrene (B[a]P) – Edible oils and fats

✓ ISO 15302:2017 (HPLC-FLD)

- ✿ A test portion is dissolved in petroleum ether/n-hexane
- ✿ The sample solution is applied into an alumina SPE column
- ✿ After purification, the column eluate is concentrated to 0.5 - 1 mL in water bath at 65°C
- ✿ Continue the evaporation to dryness at 35°C under N₂ and reconstituted with injection solvent for HPLC-FLD analysis

5. Erucic acid

Food / Food group	Proposed ML
Low erucic acid rapeseed oil	2 per centum by weight of their fatty acid content

✿ Reference international standards

- ✓ ISO 12966-4:2015 (GC-FID)
- ✓ AOAC 985.20 (GC-FID)

6. 3-Monochloropropane-1,2-diol (3-MCPD)

Food / Food group	Proposed ML
Solid condiments	1 mg/kg
Any other condiments	0.4 mg/kg

☀ Reference international standard

✓ BS EN ISO 14573:2004 (GC-MS)

6. Test Methods – 3-Monochloropropane-1,2-diol (3-MCPD)

✓ BS EN ISO 14573:2004 (GC-MS)

- ✿ The sample is mixed with sodium chloride solution. The mixture is first extracted with a mixture of n-hexane and diethyl ether
- ✿ 3-MCPD is eluted with diethyl ether, the eluate is derivatised using heptafluorobutyrylimidazole. The solution is analysed by GC-MS

7. Benzo[a]pyrene (B[a]P) (Infant and follow-up formula)

Food / Food group	Proposed ML
Infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months	1 µg/kg

✿ Reference international standards

- ✓ BS EN ISO 16619:2015 (GC-MS)
- ✓ CEN/TS 16621:2014 (HPLC-FLD)

7. Benzo[a]pyrene (B[a]P) – Infant and follow-up formula

✓ BS EN 16619:2015 (GC-MS)

- ✿ Sample extraction is performed by pressurized liquid extraction (PLE) or Soxhlet extraction with n-hexane/cyclohexane
- ✿ The extract is concentrated to ca. 5 mL
- ✿ The sample extract is then purified by size exclusion chromatography (SEC) and followed by SPE with activated silica
- ✿ After purification, the column eluate is concentrated to a appropriate volume for GC-MS analysis

7. Benzo[a]pyrene (B[a]P) – Infant and follow-up formula

✓ CEN/TS 16621 (HPLC-FLD)

- ✿ A test portion is extracted by dichloromethane
- ✿ The sample extract is then purified by size exclusion chromatography (SEC) cleanup
- ✿ After purification, the column eluate is evaporated to dryness at 27-28°C under N₂
- ✿ The residue is reconstituted with injection solvent for HPLC-FLD analysis

8. Glycidyl fatty acid esters (GE) (expressed as glycidol)

Food / Food group	Proposed ML
Powdered infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months	50 µg/kg
Liquid infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months	6 µg/kg

✿ Reference international standards

✓ AOAC 2018:03 (GC-MS/MS)

8. Test Methods – Glycidyl fatty acid esters (GE) (expressed as glycidol)

✓ AOAC 2018.03 (GC-MS/MS)

- ✿ Fat is extracted from infant or follow-up formula with ethyl acetate by liquid-liquid extraction.
- ✿ Mono- and di-glyceride content is removed by a aminopropyl SPE column.
- ✿ GEs are converted to 3-MBPD esters in an acid solution containing bromide. 3-MBPD esters are then converted to free 3-MBPD in an acidic methanol.
- ✿ 3-MBPD is derivatized with PBA prior to GC-MS/MS analysis

9. Melamine

Food / Food group	Proposed ML
Liquid infant formula and follow-up formula intended to be consumed principally by persons under the age of 12 months	0.15 mg/kg

✿ Codex suggested test method

✓ DD ISO/TS 15495:2010 (infant formula) (LC-MS/MS)

✿ Other reference international standard

✓ BS EN ISO 16858:2017 (foodstuffs) (LC-MS/MS)

10. Partially Hydrogenated Oils (PHOs)

- PHOs are edible fats and oils that have undergone the process of hydrogenation but are not fully saturated as a result of that process

Partially Hydrogenated Oils (PHOs)

- USA

- > Defines PHOs as those fats and oils that have been hydrogenated, but not to complete or near complete saturation, and have an iodine value (IV) of greater than 4, as determined by a method that is suitable for this analysis (e.g., ISO 3961 or equivalent)
- > Defines Fully Hydrogenated Oils (FHOs) as fats and oils that have been hydrogenated to complete or near complete saturation, and have an IV of 4 or less, as determined by a method that is suitable for this analysis (e.g., ISO 3961 or equivalent)

<https://www.federalregister.gov/documents/2015/06/17/2015-14883/final-determination-regarding-partially-hydrogenated-oils>

Partially Hydrogenated Oils (PHOs)

- Canada

- > Defines PHOs as those fats and oils that have been hydrogenated, and have an iodine value of greater than 4
- > Defines FHOs as those fats and oils that have been hydrogenated, and have an iodine value of 4 or less

<https://www.canada.ca/en/health-canada/services/food-nutrition/public-involvement-partnerships/modification-prohibiting-use-partially-hydrogenated-oils-in-foods/information-document.html>

Laboratory approach for estimation of PHOs

✿ Iodine value

(ISO 3961, AOAC 993.20 & AOCS Cd 1d-1992)

✿ Method principle (titration)

Glycerides of the unsaturated fatty acids present react with halogen and hence a measure of the degree of unsaturation

✿ Applicability

Suitable for non-blended oil/fat

Laboratory approach for estimation of IP-TFAs

- ✿ Identifying the amount of R-TFA and IP-TFA in a product is not available as they composed of similar isomers with similar fatty acid profiles
- ✿ Estimation of IP-TFA could be done by calculation methods, e.g.:
 - **Deduction method as proposed by Denmark**
(Analysis of trans fatty acids in Denmark, industrially produced versus ruminant trans fatty acids)
<https://orbit.dtu.dk/en/publications/analysis-of-trans-fatty-acids-in-denmark-industrially-produced-ve>
 - **IP-TFA method for oils/pre-packaged food as proposed by EU**
(Analytical approach for checking the compliance of fats and oils against a possible regulated limit for IP-TFA)
https://ec.europa.eu/growth/tools-databases/tbt/en/search/?tbtaction=get.comment&Country_ID=EU&num=602&dspLang=EN&comment_num=5&lang_id=EN&basdatedeb=&basdatefin=&baspays=HUN&baspays2=HUN&basnotifnum=30&basnotifnum2=&bastypepays=&baskeywords=

Denmark's Deduction Method

- Based on distinguishing IP-TFA and R-TFA, it relied on the presumptions of content of C4:0 in milk fat is 3.6 g/100 g fat and TFA in milk fat from ruminant is 6 g/100 g fat
- The content of the milk fat in the product and content of R-TFA is calculated based on the above presumptions
- Finally, the content of IP-TFA = Total trans fat – R-TFA

Denmark's Deduction Method

✿ Definitions of TFAs

- Defined as the sum of all fatty acid isomers with 14, 16, 18, 20 or 22 carbon atoms and one or more trans double bonds, i.e. C14:1, C16:1, C18:1, C18:2, C18:3, C20:1, C20:2, C22:1, C22:2 fatty acid trans isomers, but only polyunsaturated fatty acids with methylene interrupted double bonds

Denmark's Deduction Method

✿ Reference international standards for measuring fatty acids

- AOAC 996.06 (GC-FID)
- AOCS Ce 1h-05 (GC-FID)

EU's Approach for Estimating IP-TFA in Oils and Pre-packaged Foods


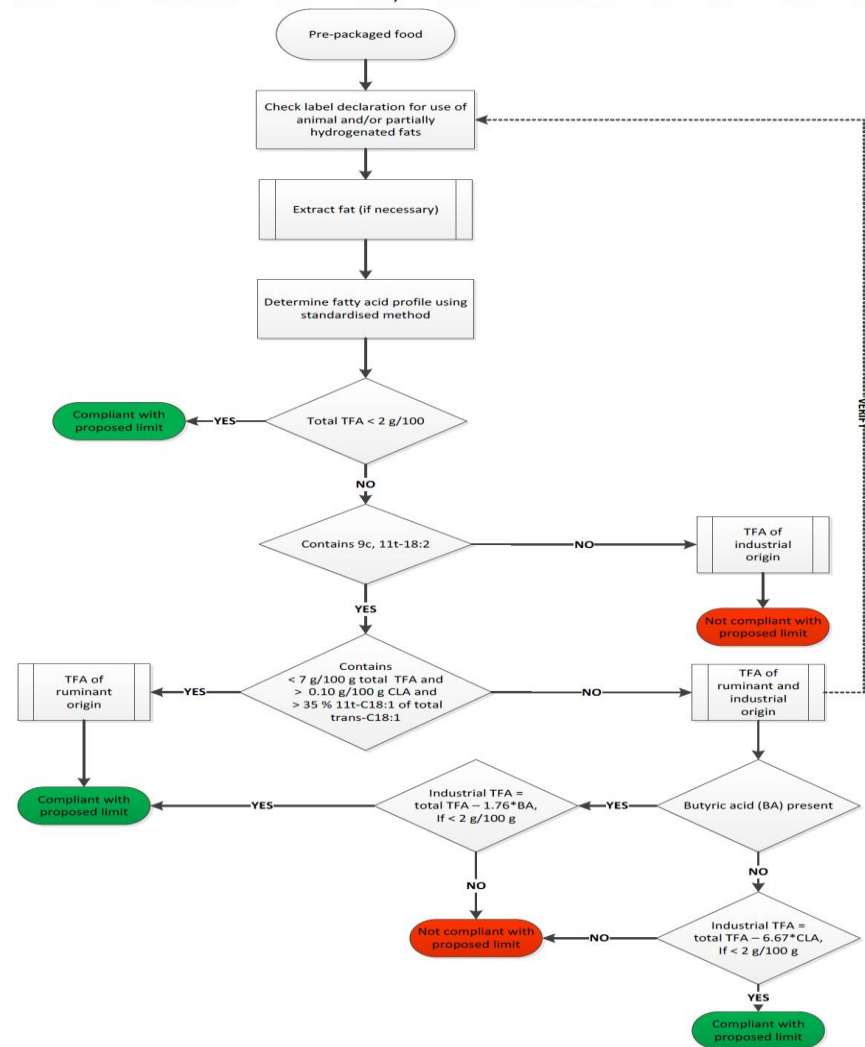

 The proposed approach is based on a **decision making algorithms**

Figure 1: Work-flow for checking the compliance of fats and oils against a possible regulated limit of industrial trans fatty acids contained in oils and fats



EU's Approach for Estimating IP-TFA in Oils and Pre-packaged Foods

- ✿ The proposed approach builds on the determination of:
 - the amounts (g/100 g fat) of butyric acid (4:0),
 - the amounts (g/100 g fat) of total TFA (sum of fatty acids with at least one non-conjugated carbon-carbon double bond in the trans configuration), usually trans-isomers of:
 - hexadecenoic acid (*t*16:1)
 - octadecenoic acid (*t*18:1)
 - octadecadienoic acid (*t*18:2)
 - octadecatrienoic acid (*t*18:3)
 - the amounts (g/100 g fat) of conjugated linoleic acid (CLA) (*c*9, *t*11-18:2)
 - the proportion (%) of trans-vaccenic acid (*t*11-18:1) relative to the sum of *t*18:1

EU's Approach for Estimating IP-TFA in Oils and Pre-packaged Foods

✿ Reference international standards for measuring fatty acids

- (i) AOAC 2012.13 (GC-FID)
- (ii) BS EN ISO 16958:2015 (GC-FID)
- (iii) Other standards such as AOAC 996.06 and AOCS Ce 1j-07 provided that both methods deliver equivalent results to (i) and (ii)

Methods of Analysis

- ✿ **Internationally recognised standards are preferred**
- ✿ **Practicability for routine use**
- ✿ **Preference given to methods with established reliability**

Criteria for Selection of Methods

- *Performance Characteristics can include, but are not limited to:*

- ✿ **Applicability**
- ✿ **Minimum applicable range**
- ✿ **Accuracy**
- ✿ **Limit of Detection (LOD)**
- ✿ **Limit of Quantification (LOQ)**
- ✿ **Precision**
- ✿ **Recovery**
- ✿ **Trueness**

Guide to Laboratory Validation Methods

- ✿ **Validated according to an internationally recognized protocol, e.g.**
 - ✦ **Harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis**
 - ✦ **EURACHEM Guide ‘The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics**
 - ✦ **ISO 11843-2 ‘Capability of Detection-Part 2: Methodology in the Linear Calibration Case**
 - ✦ **AOAC INTERNATIONAL ‘AOAC Peer-verified Methods Program-Manual on Policies and Procedures**

Guide to Laboratory Validation Methods

- ✿ **Embedded in a quality system in compliance with ISO/IEC 17025**
- ✿ **Demonstrated with data on accuracy**
 - ✦ **Participation in proficiency tests**
 - ✦ **Calibration using certified reference materials**
 - ✦ **Study on recoveries**
 - ✦ **Verified with other method(s), etc.**

- END -
(Thank you)