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

Second technical meeting with trade on

26 March 2021

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Food and Environmental Hygiene Department

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Centre for Food Safety

Purpose

- To provide detailed testing approach to the harmful substances listed in the proposed amendments

Scopes of the Proposed Amendments

A. Harmful Substances involved

- Aflatoxins (Aflatoxins total; Aflatoxin B1; Aflatoxin M1)
- Deoxynivalenol
- Patulin
- Benzo[a]pyrene
- Glycidyl fatty acid esters (expressed as glycidol)
- Melamine
- 3-monochloropropane-1,2-diol
- Erucic acid

B. Regard partially hydrogenated oils (PHOs), the main source of industrially produced trans fatty acids (IP-TFAs), as a **prohibited substance** in food

Scopes of the Proposed Amendments

Guidelines for selection and validation of analytical method for harmful substances

- A criteria-based approach, whereby a set of **performance criteria** is established with which the analytical method used should comply, is appropriate

- Utilising this approach, laboratories would be free to use the analytical method most appropriate for their facilities and purposes. Analytical methods that are accepted by chemists internationally may be used

Aflatoxins (Performance Criteria)

Guideline - Codex Standard CXS 193-1995

Criterion	Concentration Range	Recommended Value	Maximum Permitted Value
Blanks	All	Negligible	-
Recovery-Aflatoxins Total	1 – 15 µg/kg	70 to 110%	
	> 15 µg/kg	80 to 110%	
Precision RSD_R	All	As derived from Horwitz Equation	2 x value derived from Horwitz Equation
Precision RSD_r may be calculated as 0.66 times Precision RSD_R at the concentration of interest			

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Aflatoxins (Performance Criteria)

Guideline - Commission Regulation (EC) No 401/2006

Criterion	Concentration Range	Recommended Value	Maximum permitted Value
Blanks	All	Negligible	—
Recovery — Aflatoxin M_1	0,01-0,05 µg/kg	60 to 120 %	
	> 0,05 µg/kg	70 to 110 %	
Recovery — Aflatoxins B_1, B_2, G_1, G_2	< 1,0 µg/kg	50 to 120 %	
	1-10 µg/kg	70 to 110 %	
	> 10 µg/kg	80 to 110 %	
Reproducibility RSD_R	All	As derived from Horwitz Equation (*)(**)	2 × value derived from Horwitz Equation (*)(**)

Repeatability RSD_r may be calculated as 0,66 times Reproducibility RSD_R at the concentration of interest.

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DON (Performance Criteria)

Guideline - Codex Standard CXS 193-1995

Commodity	ML (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision on HorRat	Minimum applicable range (mg/kg)	Recovery
Cereal-based foods for infants and young children	0.2	≤ 0.02	≤ 0.04	≤ 2	0.1 – 0.3	80 – 110%

DON (Performance Criteria)

Guideline - Commission Regulation (EC) No 401/2006

Level µg/kg	Deoxynivalenol		
	RSD_r %	RSD_R %	Recovery %
> 100-≤ 500	≤ 20	≤ 40	60 to 110
> 500	≤ 20	≤ 40	70 to 120

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Patulin (Performance Criteria)

Guideline - Commission Regulation (EC) No 401/2006

Level µg/kg	Patulin		
	RSD_r %	RSD_R %	Recovery %
< 20	≤ 30	≤ 40	50 to 120
20-50	≤ 20	≤ 30	70 to 105
> 50	≤ 15	≤ 25	75 to 105

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B[a]P (Performance Criteria)

Guideline - Commission Regulation (EU) No 836/2011

Parameter	Criterion
Applicability	Foods specified in Regulation (EC) No 1881/2006
Specificity	Free from matrix or spectral interferences, verification of positive detection
Repeatability (RSD_r)	$HORRAT_r$ less than 2
Reproducibility (RSD_R)	$HORRAT_R$ less than 2
Recovery	50-120 %
LOD	≤ 0,30 µg/kg for each of the four substances
LOQ	≤ 0,90 µg/kg for each of the four substances

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GE (Performance Criteria)

Guideline - Commission Regulation (EU) No 2019/2093

Parameter	Criterion
Applicability	Foods specified in point 4.2 of the Annex to Regulation (EC) No 1881/2006
Specificity	Free from matrix or spectral interferences
Repeatability (RSD_r)	0,66 times RSD_R as derived from (modified) Horwitz equation
Reproducibility (RSD_R)	as derived from (modified) Horwitz equation
Recovery	70-125 %
Limit of Detection (LOD)	Three tenths of LOQ
Limit of Quantification (LOQ) for foods specified in 4.2.1 and 4.2.2	≤ 100 µg/kg in oils and fats
Limit of Quantification (LOQ) for foods specified in 4.2.3 with a fat content < 65 % and in 4.2.4 with a fat content < 8 %	≤ two fifths of the ML
Limit of Quantification (LOQ) for foods specified in 4.2.3 with a fat content ≥ 65 % and in 4.2.4 with a fat content ≥ 8 %	≤ 31 µg/kg fat'

Edible fats and oils

Infant and follow-up formula

Melamine (Performance Criteria)

Codex recommended method: ISO TS 15495 IDF RM 230

Parameter	Criterion
Applicability	Milk, milk products and infant formulae
Specificity	Free from matrix or spectral interferences
Repeatability (RSD_r)	< 15 %
Recovery	80 – 110 %
LOD	Signal-to-noise ratio equals 3
LOQ	Lowest validated levels (≤ 1/10 of ML)

3-MCPD (Performance Criteria)

Guideline - Commission Regulation (EU) No 836/2011

Parameter	Criterion
Applicability	Foods specified in Regulation (EC) No 1881/2006
Specificity	Free from matrix or spectral interferences
Field blanks	Less than LOD
Repeatability (RSD_r)	0,66 times RSD_R as derived from (modified) Horwitz equation
Reproducibility (RSD_R)	as derived from (modified) Horwitz equation
Recovery	75-110 %
LOD	≤ 5 µg/kg (on dry matter basis)
LOQ	≤ 10 µg/kg (on dry matter basis)

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Erucic Acid (Performance Criteria)

Guideline - Commission Regulation (EU) No 2015/705

Parameter	Criterion
Applicability	Foods specified in Regulation (EC) No 1881/2006
Specificity	Free from matrix or spectral interferences
Repeatability (RSD_r)	0,66 times RSD_R as derived from (modified) Horwitz equation
Reproducibility (RSD_R)	2 × value derived from (modified) Horwitz equation
Recovery	95 — 105 %
LOD	≤ 1 g/kg
LOQ	≤ 5 g/kg

Proficiency Testing (PT)

Substance	Food / Food group	PT provider
Aflatoxins, total	Almonds; Peanuts; Pistachios; Peanut butter; Infant food; Rice	Fapas
	Nuts	LGC
Aflatoxin B1	Infant food; Rice	Fapas
Aflatoxin M1	Milk powder	Fapas & LGC
Deoxynivalenol	Infant food; Wheat flour; Maize; Maize flour	Fapas
Patulin	Apple juice	Fapas & LGC
Benzo[a]pyrene	Olive oil; Palm oil; Cocoa butter	Fapas
	Vegetable oil	LGC
Glycidyl fatty acid esters	Infant formula	Fapas

Proficiency Testing (PT)

Substance	Food / Food group	PT provider
Melamine	Infant formula; Milk powder	Fapas
3-MCPD	Soy sauce; Vegetable oil	Fapas
Erucic acid	Oil/fat	LGC
Butyric acid (BA)	Mixed fat spread; Cookie	Fapas
	Chocolate	LGC
Total trans fatty acids	Vegetable oil; Mixed fat spread; Cookie	Fapas
	Oil/fat; Mixed fat spread; Potato crisps; Tortilla chips	LGC

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
- PHOs are the primary dietary source of industrially produced trans fatty acids (IP-TFAs), containing level up to 40-50% of total fat
- Other source of dietary trans fatty acids (TFAs) included those originated from:

(i) biohydrogenation by microorganisms in the rumens of ruminant animals (R-TFAs), containing 2-8% of total fat

(ii) Refined oils containing TFAs less than 2% of total fat

(iii) Cooking and heating of oils containing TFAs less than 3% of total fat

- Laboratory analysis methods exist for TFAs but do not exist for PHOs

Partially Hydrogenated Oils (PHOs)

- Testing approach:

i) For **Non-blended oils and fats***

- Iodine value of greater than 4 to indicate unsaturation possibly due to presence of PHOs
- Iodine value of less than or equal 4 to define hydrogenated oils (FHOs)

* *with documentary proof that the oil/fat has been hydrogenated*

ii) For **blended oils and fats and food products**

- Check level of IP-TFAs in blended oils and fats and food products

Laboratory approach for estimation of PHOs for Non-blended oils/fats

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

  R-CH=CH-R' + I_2 R-CHI-CHI-R'

- **Definition:** mass of halogen, expressed as iodine, absorbed by the test portion, divided by the mass of the test portion
  - Glycerides of the unsaturated fatty acids present react with halogen and hence a measure of the degree of unsaturation
  - Test portion dissolved in solvent followed by addition of Wijs reagent (iodine monochloride in acetic acid). After a specified time, KI and water was added, and liberated iodine titrated with sodium thiosulfate solution.

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **IP-TFA method for oils/pre-packaged food 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=

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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 (BA) (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

Unique occurrence in ruminant fats

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

Definition of TFAs

- 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)

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- Subjected to a proposed decision making algorithm to determine whether the food contains:

  - less than the threshold limit for TFA;
  - only IP-TFA in amounts exceeding the limit;
  - only R-TFA in amounts exceeding the limit;
  - a mixture of IP-TFA and R-TFA in amount exceeding the limit

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Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

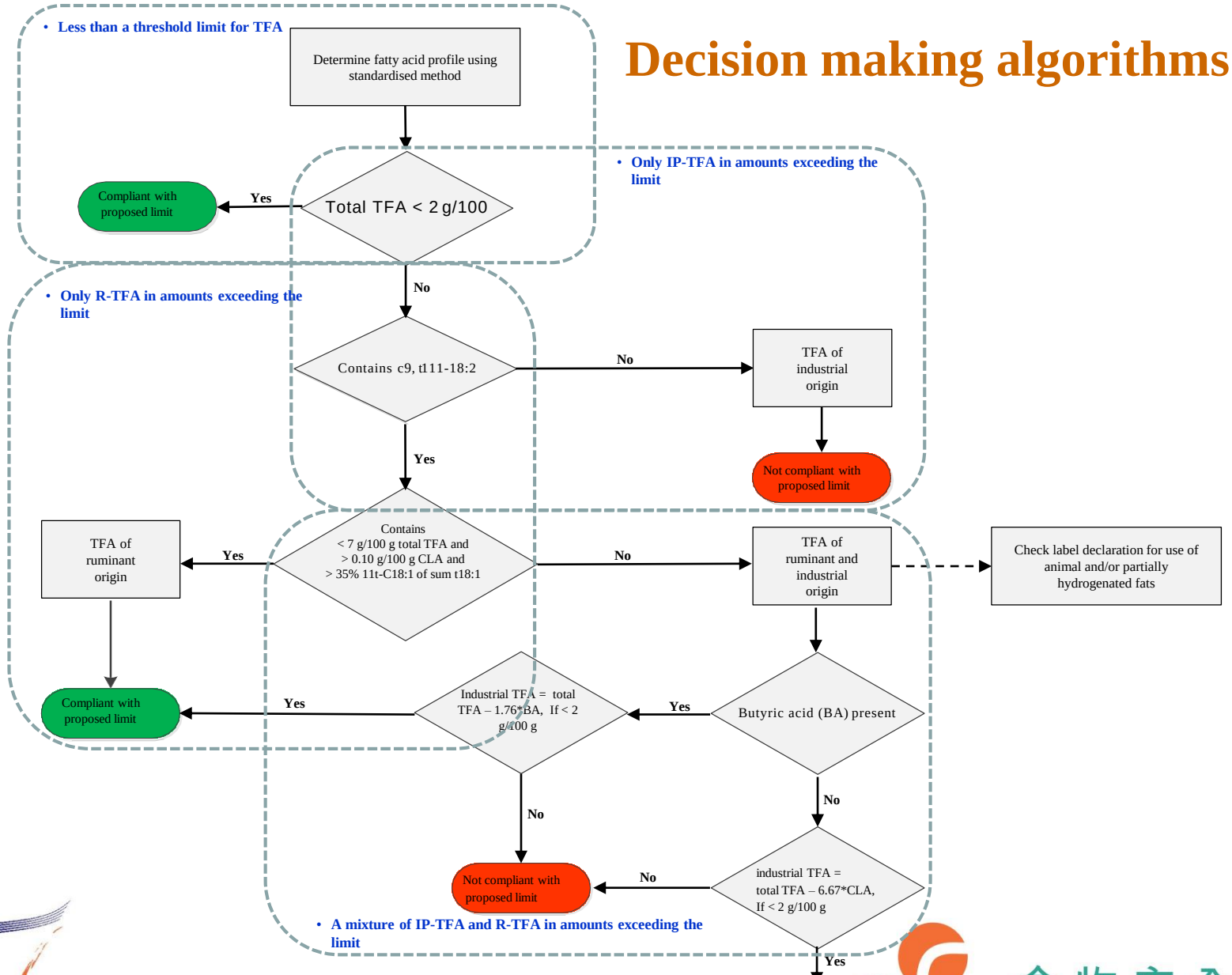
- Decision making algorithm

  - Difference in TFAs profiles between PHOs and ruminant fat related primarily to:

    - distribution of positional isomers of trans octadecenoic acid C18:1, and
    - exclusive presence of conjugated linoleic acid, CLA (*c*9,t11-18:2)

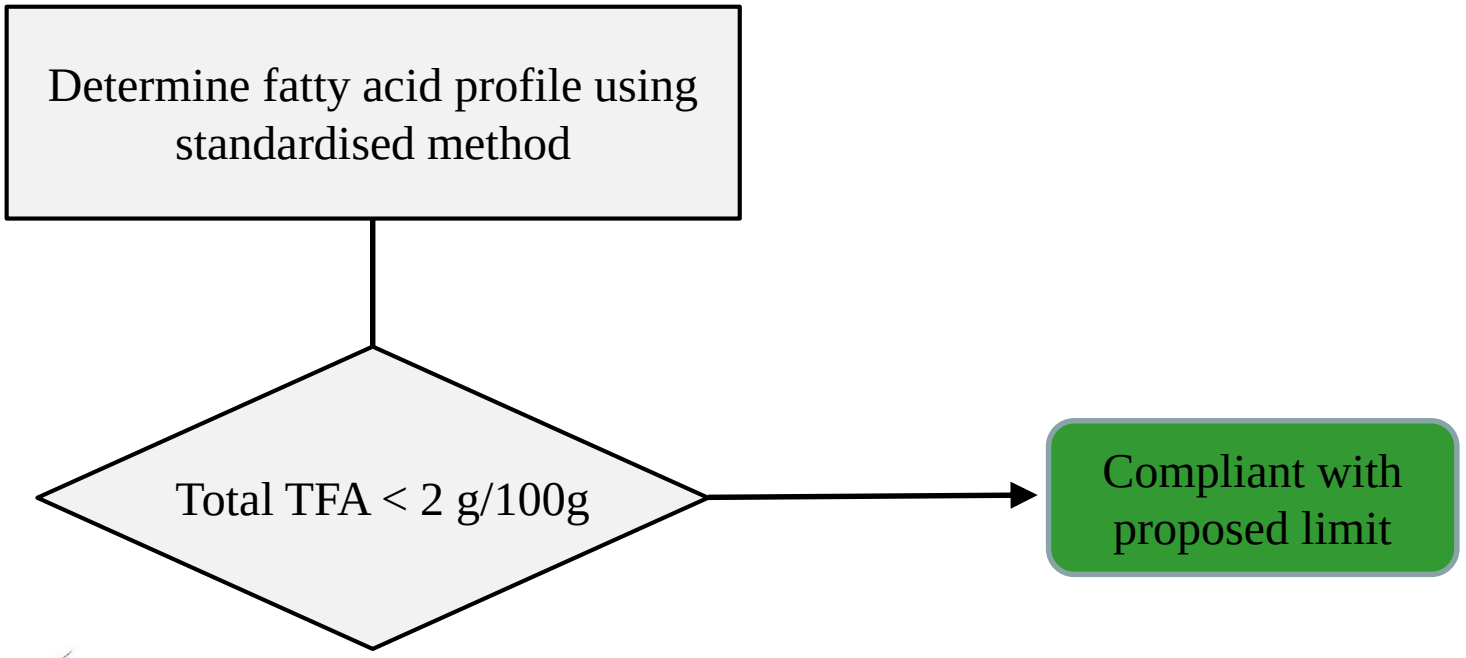
  - Trans-vaccenic acid (*t*11-18:1) is the predominant monounsaturated fatty acid of ruminant fats, accounting from 35 – 55 % of total trans-C18:1

Decision making algorithms



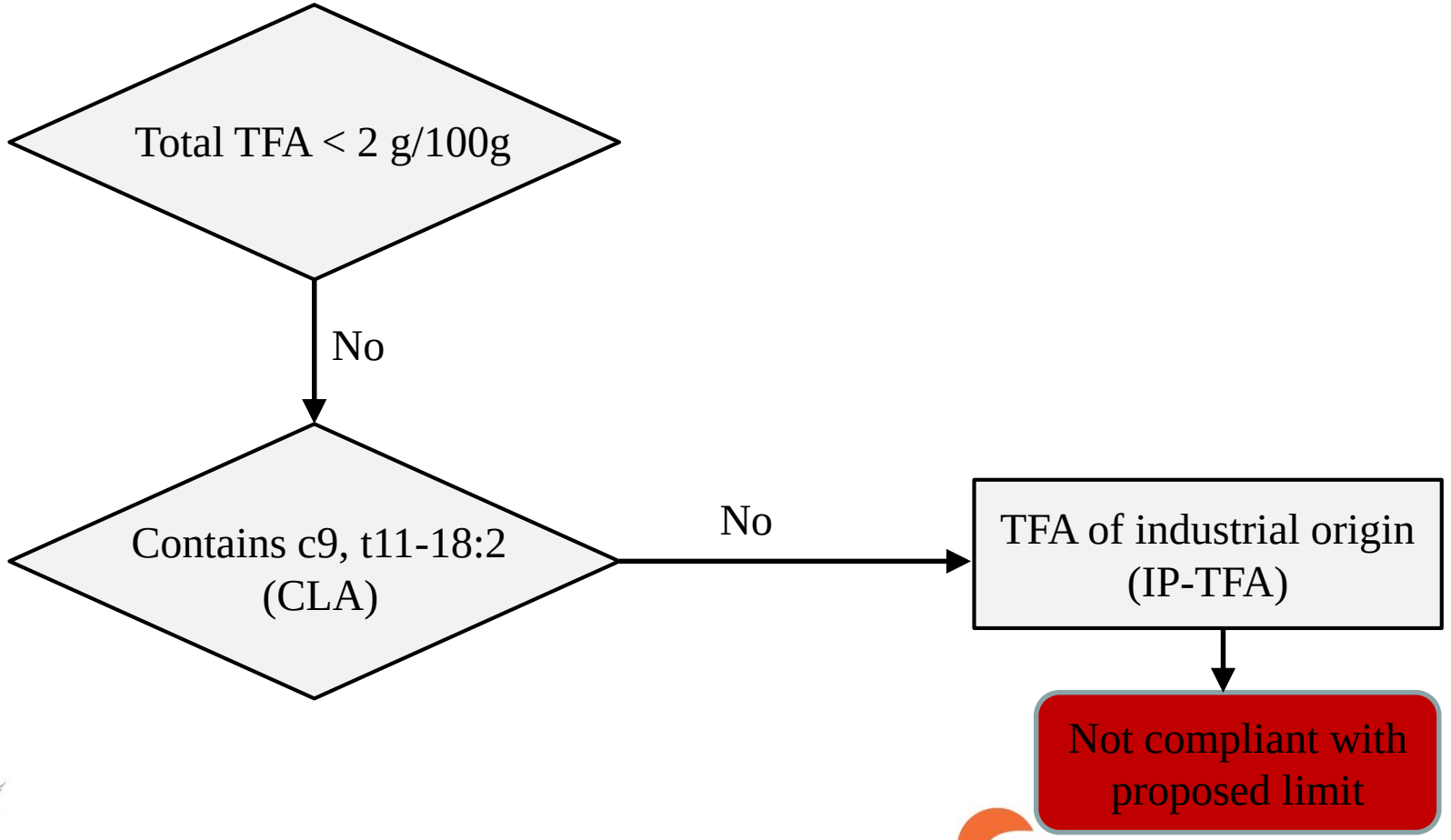
Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

Less than a threshold limit for TFAs



Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **Only IP-TFAs in amounts exceeding the limit**



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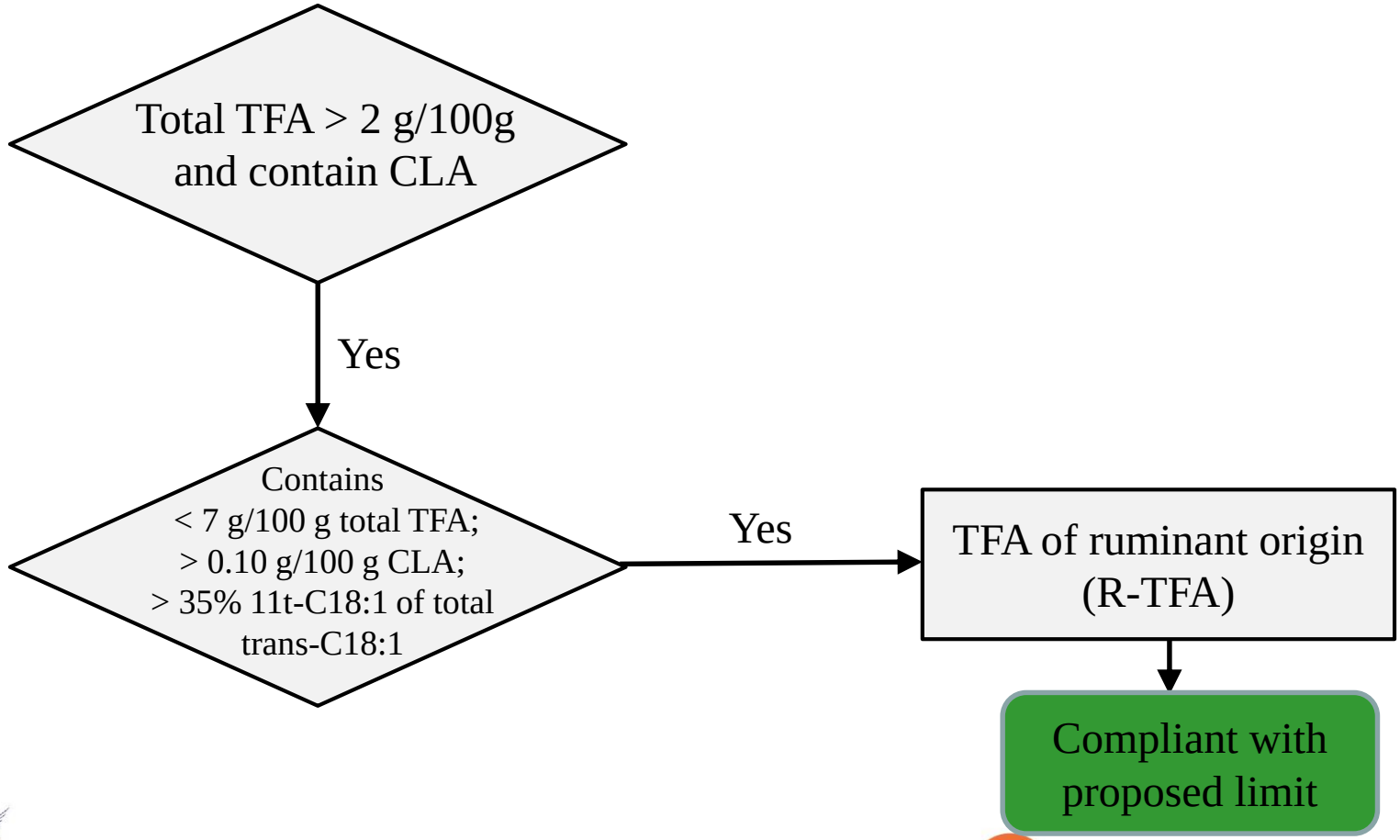
Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **Only R-TFAs in amounts exceeding the limit**



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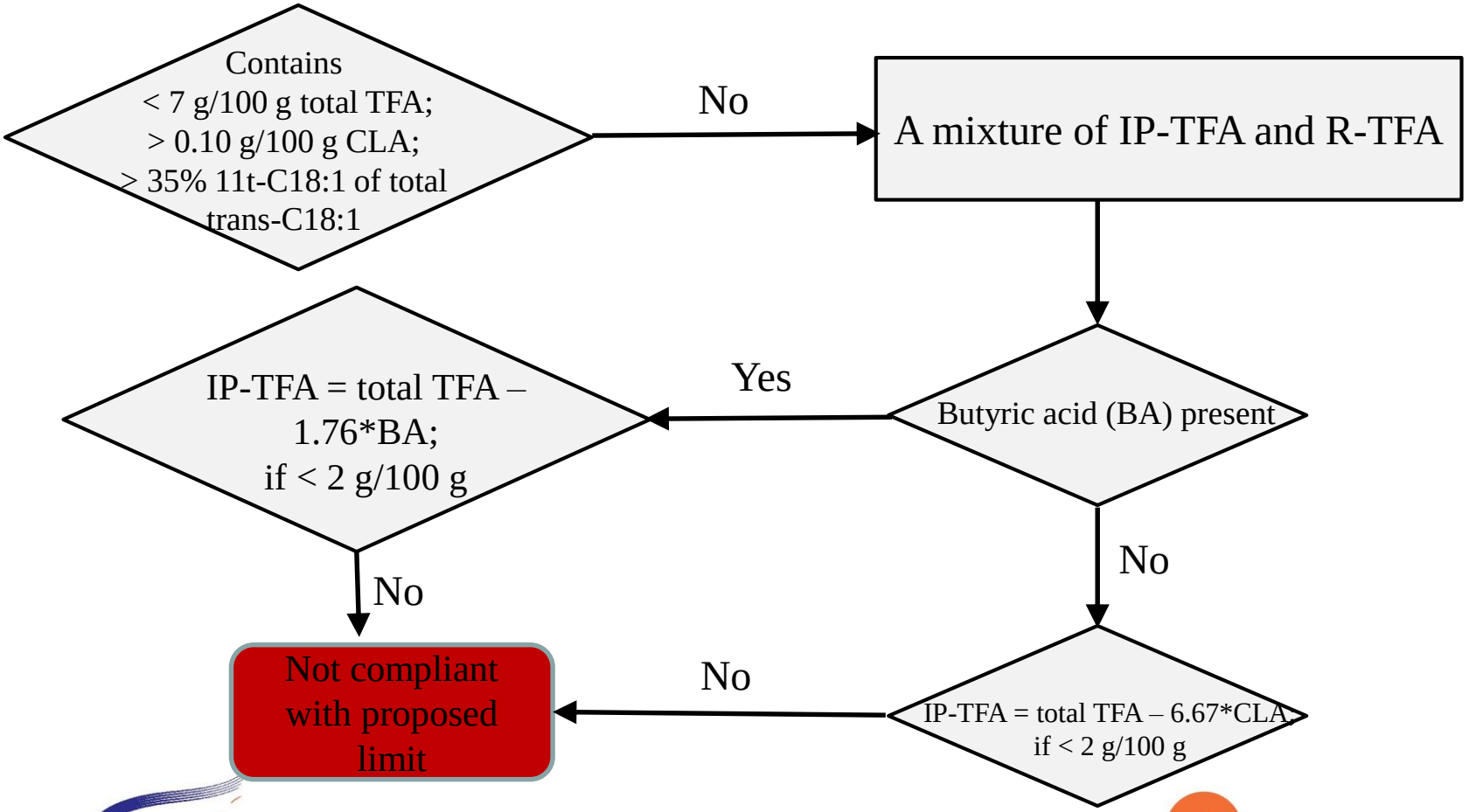
Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **A mixture of IP-TFAs and R-TFAs in amounts exceeding the limit**



Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **Estimation of IP-TFAs in mixtures of ruminant and industrial fats**

  IP-TFA = total TFA – 1.76*BA

  - **The amount of BA is for approximate the amount of milk fat (MF) in the mixture and to amount of R-TFA originating from MF**

    R-TFA [g/100 fat] = (BA [g/100g fat] x 29.4 x 6)/100 = 1.76*BA

  - **The factor 29.4 for converting the amount of BA to MF based on an average content of 3.4 g BA/100 g MF**

  - **Assumed that MF contains 6 g TFA/100 g fat**

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

Presumptions

IP-TFA = total TFA – 6.67*CLA

- **The amount of *c*9, *t*11-18:2 is used to approximate the amount of R-TFA originating from bovine fat in mixture in the event that BA is not present.**

R-TFA [g/100 fat] = (c9, t11-18:2 [g/100 g fat])/0.15 = 6.67*CLA

- **Based on the TFAs concentration in bovine fat is similar to MF, a factor of 0.15, which approximates the relation of total TFA to *c*9, *t*11-18:2 for MF is applied to estimate the amount of total TFAs in bovine fat**

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

- **Reference international standards for measuring fatty acids**
  - AOAC 996.06
  - AOAC 2012.13 / BS EN ISO 16958:2020
  - other suitable methods

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

	AOAC 996.06	AOAC 2012.13 / BS EN ISO 16958:2020
Scope	Foods	Milk, Milk products, infant formula and adult nutritionals
Detection Method	GC-FID	GC-FID
Prior Fat Extraction	Yes (by hydrolysis)	No (fat extraction can be done if products containing < 1.5% fat)
Transesterification (Methylation)	Boron trifluoride (BF_3) in methanol	Methanolic sodium methoxide solution
Quantitation	All interesting trans fatty acid can be identified and quantified by reference standards	Intended to quantify all TFA, within the specified region of the chromatogram

Laboratory approach for estimation of IP-TFAs for blended oils and fats and food products

	AOAC 996.06	AOAC 2012.13 / BS EN ISO 16958:2020
$C_{18:1}$ TFA	$C_{18:1T}$(total) is sum of $C_{18:1T}$(6-*t*), $C_{18:1T}$(9-*t*) and $C_{18:1T}$(11-*t*)	Sum of $C_{18:1}$ with all 4-*t* to 16-*t*
$C_{18:2}$ TFA	$C_{18:2T}$(9-*c*, 12-*t*), $C_{18:2T}$(9-*t*, 12-*c*) and $C_{18:2TT}$ (9, 12-*t*)	Sum of $C_{18:2}$ with All (9-*t*, 12-*t*), (9-*c*, 12-*t*), (9-*t*, 12-*c*), (9-*c*, 13-*t*), *(8*-*t*, 12-*c*) and (11-*t*, 15-*c*)
$C_{18:3}$ TFA	NA	Sum of $C_{18:3}$ (All *trans*-9,12,15) - $C_{18:3T}$(9-*c*,12-*c*,15-*t*), - $C_{18:3T}$(9-*c*, 12-*t*, 15-*c*), - $C_{18:3TT}$(9-*c*, 12-*t*, 15-*t*), - $C_{18:3T}$(9-*t*, 12-*c*, 15-*c*), - $C_{18:3TT}$(9-*t*, 12-*c*, 15-*t*), - $C_{18:3TT}$(9-*t*, 12-*t*, 15-*c*), - $C_{18:3TTT}$(9-*t*, 12-*t*, 15-*t*).

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- END –
(Thank you)