



REPLACE
TRANS FAT



**REPLACE TRANS FAT: AN ACTION PACKAGE TO ELIMINATE
INDUSTRIALLY PRODUCED TRANS-FATTY ACIDS**

MODULE 4: **ASSESS**

How-to guide for trans fat surveillance



**World Health
Organization**

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WEB RESOURCES

- › Food analysis: lab protocol
- › Background discussion paper on trans fat assessment in food: summary of analytical methods and examples of their uses
- › Blood analysis: survey protocol
- › Blood analysis: laboratory protocol
- › Background discussion paper on trans fat assessment: expert consultation on assessment of trans fat in food and human samples, meeting report
- › FEEDCITIES project protocol

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REPLACE ACTION PACKAGE

Elimination of industrially produced *trans*-fatty acids (TFA) from the global food supply by 2023 is a priority target of the World Health Organization (WHO). The REPLACE action package provides a strategic approach to eliminating industrially produced TFA from national food supplies, with the goal of global elimination by 2023. The package comprises:

- › an overarching technical document that provides a rationale and framework for this integrated approach to TFA elimination;
- › six modules; and
- › additional web resources to facilitate implementation.

The REPLACE modules provide practical, step-by-step implementation information to support governments to eliminate industrially produced TFA from their national food supplies. To achieve successful elimination, governments should implement best-practice legal measures (outlined in modules 3 and 6). Strategic actions outlined in the other modules are designed to support this goal, but it may not be necessary to implement each module.

The modules will be most useful to national governments, including policy-makers, food control or safety authorities, and subnational government bodies that advocate for, and enforce, policies relating to nutrition or food safety. Other audiences that may find these modules and accompanying web resources useful include civil society organizations, academic and research institutions, nutrition scientists and laboratories, and food industry associations and food companies.

MODULES OF THE REPLACE ACTION PACKAGE

SIX STRATEGIC ACTION AREAS		OBJECTIVE
RE	REVIEW dietary sources of industrially produced TFA and the landscape for required policy change	Introduce the REPLACE action package, and provide guidance on initial scoping activities and drafting of a country roadmap for TFA elimination. Initial scoping activities rely on information that is already known, or can be obtained through desk review or discussions with key stakeholders, with reference to other modules as needed
P	PROMOTE the replacement of industrially produced TFA with healthier oils and fats	Describe oil and fatty acid profiles, and available replacement oils and fats, including feasibility considerations and possible interventions to promote healthier replacements
L	LEGISLATE or enact regulatory actions to eliminate industrially produced TFA	Describe policy options and the current regulatory framework to eliminate industrially produced TFA. Provide guidance on assessment steps to guide policy design, and development of regulations suitable to the country context or updating of the existing legal framework to match the approach recommended by the World Health Organization
A	ASSESS and monitor TFA content in the food supply and changes in TFA consumption in the population	Describe the goals and methods for TFA assessment. Provide guidance on designing and carrying out a study of TFA in food and human samples
C	CREATE awareness of the negative health impact of TFA among policy-makers, producers, suppliers and the public	Describe approaches to advocacy and communications campaigns to support policy action. Provide guidance on key steps to design and implement effective advocacy and communications campaigns, and evaluate progress
E	ENFORCE compliance with policies and regulations	Describe TFA policy enforcement approaches, offences and roles. Provide guidance on mapping existing and creating new enforcement powers and mechanisms, public communications, penalties, funding and timelines

1. BACKGROUND

To achieve the target of eliminating industrially produced TFA from the global food supply by 2023, it is critical for countries to know how much TFA is consumed and how much is in the food supply, and to monitor the country's progress towards elimination and replacement as legislative or regulatory efforts are implemented. This module provides basic guidance to plan a TFA assessment in a country. More detailed protocols can be found in the REPLACE web resources.

2. ASSESSMENT GOAL

There are three primary purposes for a country to assess the level of TFA in the food supply and the level of TFA intake in the population:

- › to raise awareness to spur action;
- › to establish baseline TFA levels, overall and by food type; and
- › to monitor changes over time, particularly after regulations or legislation are implemented.

The assessment goal will depend on a number of factors including:

- › what is already known about TFA in the country (for example, existing studies, food databases)
- › resources available to conduct an assessment – both financial resources and human and laboratory capacity to conduct assessments involving food analysis and/or blood sample analysis
- › the level of public and policy-maker awareness of the health effects of TFA, and their level of support for action.

Module 1 provides guidance on how to gather the information above to inform the development of a country-specific roadmap for eliminating industrially produced TFA.

Box 1 provides a case study of a country's assessment of TFA in food and TFA intake.

BOX 1. USING ASSESSMENT DATA TO SPUR ACTION IN DENMARK

After a study was published in the early 1990s showing that TFA consumption increases the risk of coronary heart disease (Willett et al., 1993), the Danish Nutrition Council began to monitor TFA in food and TFA intake (Stender et al., 1995). In 2001, the Council reported that more than 50,000 Danes were at high risk of coronary heart disease based on their TFA intake. The major sources of TFA in Denmark included margarine, packaged snack products, bakery products and confectionery items (Stender & Dyerberg, 2001). These findings attracted significant media attention and led to popular support to limit industrially produced TFA. In 2003, Denmark became the first country in the world to take legal action to restrict TFA, passing a law to limit industrially produced TFA to no more than 2% of the oils and fats in products.

Table 1. TFA assessment methods

TYPE OF ASSESSMENT	ASSESSMENT METHODS	OUTPUTS
TFA content in food supply, by food type	<ul style="list-style-type: none">• Analysis of fatty acids in foods (laboratory testing)• Evaluation of food product labels, if available	<ul style="list-style-type: none">• Levels of TFA in foods, organized by food sector, category and geography• Identification of major food sources of TFA• Change in TFA (and other fatty acids) in food supply• Can support assessment of food manufacturer compliance with existing regulations (see module 6)
TFA intake	<ul style="list-style-type: none">• Analysis of fatty acids in blood samples (laboratory testing)• Dietary intake (questionnaires)	<ul style="list-style-type: none">• Estimated population levels of exposure to TFA (blood)• Estimated levels of population TFA intake, enabling comparison with WHO recommendation of less than 1% of total energy intake (dietary intake questionnaire)• Major sources of TFA intake in the population (dietary intake questionnaire)• Change in TFA and other fatty acid intake/exposure• Differences in TFA intake/exposure among subpopulations

3. ASSESSMENT TYPE

TFA can be monitored either in food or in the population. Food assessment should be the first priority in all countries. Population assessment is optional, but can be very useful to understand the impact of regulations on intake (total amount consumed) or exposure (amount in blood samples – a proxy for intake). Population assessment can be done through population surveys that include either a dietary questionnaire or a blood test for TFA exposure.

Both food and population assessments (Table 1) can establish a baseline value, be used to monitor change over time and raise awareness (of the level of TFA present in foods or the level of population intake of TFA). Changes after a law or regulation is passed should be monitored via food assessment and can be validated by documenting the change in exposure in population surveys. Population assessments using dietary questionnaires require data on TFA levels in foods.

4. AMOUNT OF TFA IN FOOD

TFA in food can come from three sources:

- › partially hydrogenated oils (PHO)
- › processing and heating of oils and fats
- › foods derived from ruminant animals (dairy and meat).

Most TFA restrictions exclude TFA from ruminants (usually around 2-5% of fat is TFA) (Wu et al., 2017). The TFA content of PHO (typically 25-45%) is much higher than that in ruminant-derived foods or resulting from cooking or processing (Mozaffarian & Clarke, 2009; Doell et al., 2012; Bhardwaj et al., 2016). This section, therefore, discusses assessment of TFA content in foods likely to be made with PHO or mixed foods. (Note: Levels of TFA derived from processing depend on the methods used and may be higher in some regions than others.)

Box 2 below describes the main steps involved in defining the TFA content of food.

BOX 2. KEY STEPS FOR SURVEILLANCE OF TFA CONTENT OF FOOD

1. Determine the goal of the assessment.
2. Identify probable sources of TFA in the food supply.
3. Collect food label data on saturated fatty acids (SFA) and TFA, if available.
4. Design the sampling plan.
5. Collect food samples.
6. Analyse TFA and SFA in food samples.
7. Create and maintain a database of TFA nutrient label data and analysed contents.^a
8. Assess changes over time and/or overall compliance.^a

^aDescribed in Section 6: “Publishing results”

4.1 STEP 1: DETERMINE THE GOAL OF THE ASSESSMENT

The first step is to determine the goal of the assessment so that the food sampling, analysis and data collection plans (Box 2, steps 3-6) are designed appropriately to achieve the goal with the resources available. This section focuses on TFA analyses in food where measuring TFA levels in food is the end goal; TFA food analyses to inform dietary surveys of TFA intake are addressed briefly in section 5.2: “Dietary surveys”. Possible goals include the following.

RAISING AWARENESS TO SPUR ACTION

A small study is usually sufficient to raise awareness of TFA in the food supply. Examples in the scientific literature (see Boxes 3a and 3b) including strategically selecting:

- › a small number of food categories (for example, oils and fats such as shortening, margarine and others likely to contain PHO, or specific packaged foods that can be purchased at a grocery store); or
- › a single food sector, depending on the most likely sources of TFA (for example, packaged foods only, street foods only); or
- › a single location in the country (for example, capital city only).

BOX 3A. FOCUSING ON A SINGLE FOOD SECTOR AND LOCATION

The FEEDcities project of the WHO Regional Office for Europe aims to document the street food environment in eastern Europe and central Asia, with a focus on TFA, sodium and potassium in commonly available foods. Published studies from Kyrgyzstan and Tajikistan assessed the TFA content of packaged and unpackaged foods from a representative sample of street food vendors in the capital cities. The studies found that individual food items with more than 100% of the daily maximum recommendation for TFA were readily available in both countries; they included both pre-packaged foods and freshly prepared foods (WHO Regional Office for Europe, 2017a, 2017b).

BOX 3B. FOCUSING ON A SINGLE TYPE OF FOOD

Comparisons across countries, regions or brands of a single type of food can highlight unnecessary differences in TFA levels in foods. Stender, Dyerberg & Astrup (2006) compared the TFA levels in French fries and chicken nuggets from McDonald's and KFC fast food restaurants in 20 countries. The TFA content in menu items at McDonald's ranged from 1 g in Denmark to 10 g in New York City, United States. For KFC, the TFA content ranged from 1 g in Germany to 24 g in Hungary (more than 10 times the WHO recommended level).

In 2012 and 2014, the same authors assessed levels of TFA in biscuits/cakes and wafers in six eastern European countries. The survey was narrow: researchers selected packaged foods in supermarkets with at least 15% fat content and PHO listed as an ingredient. They found an increase in the number of products with high levels of TFA over time, indicating that voluntary efforts by industry to reduce TFA were not sufficient (Stender, Astrup & Dyerberg, 2016).

UNDERTAKING BASELINE ASSESSMENT OF TFA CONTENT IN THE FOOD SUPPLY

A full-scale, nationally representative baseline study is ideal. This would include sampling:

- › all food sectors (packaged food, restaurant/bakery and street food);
- › all major contributing food categories (Step 2, below);
- › at least a few brands or types of food from each category; and
- › multiple regions, if there are regional (including urban versus rural) differences in diet or in available product types.

MONITORING CHANGE IN TFA CONTENT OF FOOD OVER TIME

If the baseline assessment was comprehensive, repeating the design every 3–5 years should capture the change in TFA levels over time. Timing should be based on whether regulations are implemented, and the timeline for implementation and compliance. Both TFA and SFA should be analysed when monitoring change over time, because the goal is to replace TFA with mono- or poly-unsaturated fatty acids.

Box 4 describes TFA assessments conducted in Denmark.

BOX 4. DENMARK BASELINE ASSESSMENT AND CHANGE OVER TIME

In Denmark, several assessments were conducted of the content of TFA in foods on the market (Ministry of Food, Agriculture and Fisheries of Denmark and the Danish Technical University, 2014). Products were selected based on existing knowledge about which food categories would typically contain industrially produced TFA. In 2002/2003, 253 products were tested to establish a baseline of TFA levels in food products before the implementation of regulations. Foods tested included chocolate, sweets, cakes, cookies, biscuits, fruit spread, microwave-oven popcorn, French fries, frozen potatoes, fast food, ice cream, catering products, margarines, shortening and fry oils.

The Danish regulations that limit industrially produced TFA to 2% of total fat in all foods were introduced in January 2004. To monitor compliance with the regulations, 148 samples were tested in 2004/2005, 45 in 2006/2007, 96 in 2010, and 95 in 2012/2013. Fewer products were tested in later assessments because findings showed higher compliance rates.

MONITORING COMPLIANCE WITH REGULATION OR LEGISLATION ON TFA CONTENT OF FOOD

To monitor compliance with a regulation in a way that aids enforcement, food testing needs to be more comprehensive. All major brands should be tested in key categories, and smaller brands should also be randomly sampled.

The easiest assessment would be to repeat the baseline assessment, using the same foods. If resources allow, it could be expanded to include brands that were not included in the original baseline assessment. If not included in the baseline assessment, foods packaged for sale in the wholesale market, or foods sold in restaurants or by street vendors could be included at this time. If independent research has verified the accuracy of food labels, expanded monitoring of packaged food can be done without additional testing. (See module 6 for more information.)

In many cases, national laws are enforced also at the regional or local level. In this case, testing of foods for compliance should be done at that level; this is particularly important when the food supply varies substantially by region.

Box 5 describes Argentina's compliance monitoring program.

BOX 5. ARGENTINA'S COMPLIANCE MONITORING PROGRAM

(Kakisu et al., 2018)

Argentina implemented a multisectoral approach to monitoring the reduction in TFA in response to national food regulations. In 2010, Argentina enacted regulatory maximum limits of 2% industrially produced TFA as a proportion of total fat in margarines and vegetable oils, and 5% of total fat in other foods. The compliance period for manufacturers to achieve these limits was two years for margarines and vegetable oils (2012), and four years for foods (2014).

The nationwide monitoring strategy was developed with local authorities with jurisdiction over food control. The national sampling plan involved domestic and imported foods registered by the local food control authorities, and packaged and pre-packaged foods. Foods were sampled from retail outlets (regular markets and supermarkets), and imported foods were analysed when they entered the country. Food samples were collected and analysed in 2014 (before the compliance deadline) and in 2015 (after the deadline). Certain food categories were tested again in 2016. Label data were also recorded. The monitoring results were regularly shared with food manufacturers and suppliers to enforce the food code norms.

Before the compliance deadline (in 2014), the first assessment found that 73% of foods met the limits. After the compliance period (in 2015), the second assessment found that 93% of foods complied with the regulations.

4.2 STEP 2: IDENTIFY PROBABLE SOURCES OF TFA IN THE FOOD SUPPLY

To determine which foods to include in the assessment, identify the food categories that are likely to contain TFA (for example, frying oils, cookies, French fries/chips) and key food sectors likely to sell or serve food high in TFA (for example, packaged food, restaurant food, street food). Ideally the assessment would include all food categories identified, but priority should be given to categories that are expected to make the largest contribution to TFA intake. These are likely to be those that either contain high levels of TFA or contain moderate levels of TFA but are consumed in large quantities.

Partially hydrogenated oils, the source of industrially produced TFA, have specific functional properties and are therefore most likely to be used in similar types of food across countries. Food products that contain high levels include:

- › oils and fats¹
 - vegetable fats that are solid at room temperature, including margarines, shortenings and Vanaspati ghee
 - frying and cooking oils
- › foods that are cooked or made with fat
 - baked goods such as biscuits, cookies, cakes and doughnuts
- › foods that are fried in oil or fat, such as French fries/chips, samosas, potato chips/ crisps, fried snacks and fried rice.

For many countries, food categories that commonly contain TFA have been identified; review both published and grey literature to find existing TFA data. Nongovernmental organizations, academic researchers, industry and the government may also be sources of TFA data. Other sources of information on foods likely to contain TFA include:

- › labels – see Step 3
- › food industry – food manufacturers, edible oil refiners and their associations will know where PHO are being used in the food supply
- › other countries – food categories identified as having TFA in neighbouring countries can be a good starting point if no other sources are available. (See Annex 1 for example categories tested in a selection of countries.). In addition, global resources such as the International Network of Food Data Systems (INFOODS) and the food composition databases of the Food and Agriculture Organization of the United Nations (FAO) may be a place to start
- › national nutrition or food composition databases – even if national food consumption surveys do not contain data on TFA, they can be used to identify high-fat foods.

Once food categories likely to contain TFA are identified, they should be prioritized based on population purchasing habits. Data sources for this include national nutrition or household consumption surveys, sales data/databases, industry sources (for example, distributors, grocery store owners, market vendors), and visits to stores or markets to understand what is for sale. If pre-packaged food is not a major part of the food supply in the country, focusing on oils and fats may be the best place to start because they are purchased by both consumers and restaurants/street vendors.

4.3 STEP 3. COLLECT DATA ON TFA AND SFA FROM NUTRITION LABELS, IF AVAILABLE

Laboratory testing is the gold standard of TFA assessment studies; however, in some countries, nutrition labels are required to include TFA content in packaged food. In these countries, a hybrid data collection model is possible: a broad cross-section of food types is listed on the basis of the review of nutrition labels, and then a smaller subset of foods is tested in a laboratory. This only works for packaged food (and for chain restaurants that provide verified nutrition information). If labels are accurate, a smaller number of foods can be tested. (Health Canada eventually stopped testing food because it determined that labels were generally accurate; the average difference was 0.08 g of trans fat per serving; Pantazopoulos et al., 2011.)

¹ In addition to vegetable oil, fish oils can be partially hydrogenated. Laboratory methods exist to measure TFA in fish products; these are not discussed in this module.

Box 6 describes a sampling strategy in the United States that uses data from nutrition labels.

BOX 6. UNITED STATES DEPARTMENT OF AGRICULTURE'S SENTINEL FOOD SURVEILLANCE FOR SODIUM

To monitor changes over time in the sodium content of foods in the United States, the United States Department of Agriculture collects 125 sentinel foods at stores and restaurants, and tests their sodium levels using laboratory analyses. To ensure that they have a more comprehensive sample, they also collect sodium data every two years on a larger sample of about 1,100 products, using only nutrition label information (USDA, 2018).

Labels can be used to identify either foods that contain any TFA or foods that have PHO listed as an ingredient. Labels can be accessed by visiting a supermarket and checking the labels of popular foods, by using existing nutrition databases (often created by either commercial firms or nongovernmental organizations), or on company websites.

When sampling products for label review only, the products do not need to be purchased. Data can be collected by (ideally) scanning the label, taking pictures of the label and product information, or (last resort) entering the information into a tablet or other data collection tool. Collected data should include:

- › product name
- › brand and manufacturer
- › unique identifier (ISBN number, bar code, etc.)
- › package size
- › serving size
- › nutrition information, including: SFA, TFA, total fat and ideally all other available macronutrients
- › ingredient list (at least if PHO are an ingredient)
- › date collected
- › nutrition and health claims relating to trans fat content.

If this information is available for a large number of products in a nutrition database or on company websites, in-store sampling may not be needed. But it should be noted that the database must be regularly updated, with the date of the update available.

4.4 STEP 4: DESIGN THE SAMPLING PLAN

The precise design of the sampling plan will depend on:

- › the food supply
- › demographics
- › the distribution chain
- › other factors unique to a given population or country and the goal of the assessment
- › the resources that are available.

The sampling plan methodology should describe the plan and be made public. This section describes some of the decisions and key considerations.

SCALE OF THE ASSESSMENT

First, determine the number of samples that can be tested by a laboratory, given the budget and time frame. TFA laboratory analyses are expensive and time-consuming. The cost includes collection and preparation of the foods, storage and shipping (if needed), and laboratory costs to analyse TFA and SFA.

If resources are limited, strategic sampling (single categories, single location) may be most appropriate, at least as a first step. Using an existing survey that collects food data may also reduce costs. Finally, if funds are very limited, samples of the same food can be mixed together in a composite, regardless of where they were procured; this results in the mean TFA level for foods in the category, but not the range of TFA (not generally recommended).

LEVELS OF SAMPLING

Regardless of the scale of the study, multiple aspects have to be considered, including geography, food sectors, food categories, food outlets and specific food items. Below are some considerations for these factors. Choices at every level should be defensible with respect to the goal of the assessment.

Table 2 provides definitions and examples of levels of sampling.

Table 2. Levels of sampling: definitions and examples

SAMPLING LEVEL	DEFINITION	EXAMPLES
Location	City, town or village where testing will occur	Capital city
Food sector	Part of the food industry/ environment	Packaged food, restaurant food
Food category	Foods that are usually grouped together and are somewhat similar	Biscuits, fried street food
Outlet	Individual supermarket, restaurant or street food vendor	Supermarket X on street Y, fast food restaurant Z on street Y
Sample	Total number of sample units of a specific item from a single outlet	Three packages of biscuits, brand X from store Y
Sampling unit	Package of food or serving of restaurant food	One package of biscuits, brand X

Geography

One of the first decisions is where to conduct the survey. Key considerations include the following.

- › Number of locations: If there is evidence of differences in food availability and diet by either region or level of urbanization, multiple locations may need to be sampled to truly understand TFA in the food supply. Some assessments have included multiple regions; others have included both urban and rural areas (Gupta et al., 2016).

- › Specific locations: Once regions are chosen, there are multiple ways to choose specific cities, towns and villages in the study:
 - Purposive sample: Sites are chosen based on evidence that TFA may be more common in foods there. (This sampling method does not represent a national average.)
 - Random sample: Major cities in the region are listed, and one is chosen at random, ideally with the selection weighted so that larger cities are more likely to be chosen.
 - Convenience sample: If only one location is chosen, it can be where it is easy to conduct the study. For example, the FEEDcities studies use the capital cities, where the government, researchers and possibly the laboratories are located, and the widest range of foods is available.

Box 7 illustrates the potential importance of geography.

BOX 7. WHEN GEOGRAPHY MATTERS

In India, a major source of TFA intake is Vanaspati ghee, a vegetable shortening used as a cheaper substitute for traditional ghee. Historical data from the Vanaspati industry shows that Vanaspati consumption is twice as high in Punjab as in any other Indian state and more than 10 times as high as in most states (Frost & Sullivan, 2009). This means that it is critical that any assessment of TFA in India includes Punjab.

Food sector

Choose what sectors of the food environment (for example, packaged food, restaurant food, street food, institutional food) to focus on.

- › Comprehensive surveys should include all sectors; however, this is often not possible because of resource constraints.
- › Priority should be given to the sector that is thought to contribute the most TFA to the diet. If this is unknown, population purchasing habits should be a guide.
- › If sufficient resources are available, institutional food (for example, schools, hospitals) is an important sector to include because the populations served are often vulnerable and have little control over their diet.

Food categories

Choose key food categories for each food sector.

- › Identify specific food categories based on the main contributors to TFA intake identified in Step 2. Give preference to either the most popular categories or those likely to have the highest TFA levels based on previous assessments (locally or, if not available, in other countries).
- › Identify the number of food categories based on the resources available. Prioritize more complete assessments of food categories over partial assessments of multiple categories.

Box 8 describes sampling of packaged foods for TFA analysis in Argentina.

BOX 8. ARGENTINA'S SAMPLING OF PACKAGED FOODS IN RETAIL OUTLETS

(Kakisu et al., 2018)

In 2014 and 2015, Argentina's Ministry of Health, with cooperation from the provincial food control authorities, conducted the first nationally representative sampling of key food categories to assess their levels of TFA. To create the sampling frame, categories of foods that could contain industrially produced TFA were selected according to previous data on TFA in foods in Latin America. As a next step, food products were collected if labels listed hydrogenated or partially hydrogenated fat, vegetable oils, margarines, or mixtures of vegetable oils and ruminant fats. Samples were also chosen when a high TFA content might be presumed. During 2014 and 2015, the fatty acid profiles of 565 commercial foods across 19 food categories (see Annex I) were analysed in different regions of the country.

Food outlets

Once the food sector and food categories are established, choose food outlets.

- › Type of food outlet:
 - If packaged food is included, sample grocery stores, supermarkets or market stalls, as appropriate. (Ensure that the type of outlet selected is one that serves most of the population, not only the wealthy.)
 - If oils and fats are included, consider also including restaurant supply stores or wholesalers.
 - If the restaurant/street food sector is included, choose restaurants and street food vendors that are likely to serve selected food categories.
- › Number of food outlets:
 - The number of food outlets depends on the types that are being surveyed and how much they differ from each other. Restaurants and street food vendors often sell a narrower range of foods than grocery stores, so more food outlets may be needed if a large number of food categories is included.
- › Specific food outlets:
 - Random sample or stratified random sample
 - Purchase or acquire a list of outlets. In many places, databases and lists of commercial enterprises can be purchased. Food outlets can be randomly selected based on the list.
 - Randomly select a neighbourhood from a list (or by convenience). List the food outlets in the neighbourhood through key informant interviews, mapping locations or a purchased list. If food availability differs by income, stratify neighbourhoods by income or other key characteristics. Randomly select food outlets.
 - Randomly select a neighbourhood and choose a central point. Predetermine walking directions for all neighbourhoods. Select the first X number of outlets (or every other outlet) until you get to the needed number.
 - Convenience sample:
 - Select food outlets that meet criteria and are within easy reach of surveyors. This makes sense for grocery stores, since available products are likely to be similar between stores.

Box 9 provides details of sampling methods used in the FEEDCities project.

BOX 9. FEEDCITIES SAMPLING

(WHO Regional Office for Europe, 2017b)

In Kyrgystan, FEEDCities investigators worked with local authorities to identify 19 markets in the capital city. Ten markets were randomly chosen from the list to represent more than half of the available markets. A 500-metre circle was drawn around markets, and investigators canvassed each market, identifying all street food vendors in the study area. Through observation, foods sold at the street vendors were identified and classified into categories to identify the most available home-made foods (20 foods) and foods produced by the food industry (10 foods). Four samples of each of these foods were selected; which foods were selected at a specific market on a given day was based on a randomization scheme. Specific food vendors in a market were chosen through random selection of a starting point; investigators proceeded north and then in a clockwise direction until they found vendors selling the sampled foods. Only one food item was sampled at each vendor, and the most common foods were sampled first.

Specific food items

Once outlets are identified, create a mechanism to choose individual food items (often called samples).

- › Determine the number of food items to be collected per category at each outlet; this will depend on the total number of items to be analysed and the number of outlets sampled.
- › Choose the most popular items at selected outlets (preferred); determine the most popular items based on
 - sales data (preferred)
 - key informant interviews with outlet staff
 - products with the most shelf space.
- › Choose randomly selected products in key categories at selected outlets
 - Once outlets are selected, visit and list the products available. Randomly select the products based on the list.
 - Preferentially test food items that have some PHO based on label or ingredient information. This will not provide a representative sample. Ideally, note the number of foods without PHO that do not qualify for testing.

4.5 STEP 5: COLLECT FOOD SAMPLES

For packaged food, collect three consumer-sized packages for each food sampled. For food from restaurants or street vendors, collect at least three portions. Foods must be purchased, stored and transported in a way that maintains the integrity of the food, including refrigeration or freezing, if necessary. Foods should be tested within four months of purchase. Foods such as cookie dough and instant soups may need to be prepared before testing if the goal is to determine nutrition information of foods as consumed, not as purchased. More details on sample collection are included in the laboratory protocol.

Table 3 lists the information to be recorded about each food.

Table 3: Data to include in a log (database or spreadsheet) from sampling

- › Stock keeping unit (SKU) or other unique identifier
- › Product name and brand
- › Label information as available (ingredients, nutrition information)
- › Lot number, if applicable
- › Place of collection
- › Date of collection
- › Laboratory name
- › Sample ID (assigned for study)
- › TFA, SFA, mono-unsaturated fatty acid, poly-unsaturated fatty acid, total fat (from labels, if available)
- › Picture of sample
- › Name of person who collected the item
- › TFA, SFA, mono-unsaturated fatty acid, poly-unsaturated fatty acid and total fat (from laboratory analysis; entered after testing)

4.6 STEP 6: ANALYSE TFA AND SFA IN FOOD SAMPLES

The main method of assessing the levels of TFA and SFA in a food is testing the food in a laboratory. Laboratory analysis includes homogenizing (mixing) foods, extracting fatty acids, and then analysing them using gas chromatography–flame ionization (GC-FID). Additionally, laboratory testing can be used to distinguish between ruminant and industrially produced TFA because one fatty acid, butyric acid, only occurs in milk fat (Bysted, 2015). Laboratory analysis can be slow and costly, and care and attention are needed to identify a laboratory that can conduct high-quality analyses. Details of laboratory analysis for TFA in food can be found in the protocol in the web materials. Criteria for selecting a laboratory can be found in Annex 2.

5. POPULATION TFA INTAKE

5.1 TFA ASSESSMENT IN BLOOD OR PLASMA

Blood assays can be used to assess the levels of TFA exposure and provide a proxy for TFA intake. This type of monitoring has been pioneered by the United States Centers for Disease Control and Prevention using adult blood samples collected as part of the National Health and Nutrition Examination Survey surveillance system (see Box 10). The analysis found that TFA intake declined between 2000 and 2010, when TFA content was added to the nutrition information on packaged foods and some cities restricted TFA in restaurants (Vesper et al., 2017). In Canada, a decline in TFA exposure was measured in samples of breast milk (Ratnayake et al., 2014); however, this can only be done in lactating women. TFA testing of blood can confirm changes in TFA intake in a population.

BOX 10. USING TFA IN BLOOD TO MONITOR CHANGES IN TFA EXPOSURE IN THE UNITED STATES

In 2006, the United States Food and Drug Administration (FDA) required that TFA content (in grams) be included in the Nutrition Facts label (FDA, 2003). As a result, many food manufacturers reduced the amount of industrially produced TFA in foods; as well, some jurisdictions regulated the content of TFA allowed in restaurant foods and ingredients.

To measure the impact of these measures, the United States Centers for Disease Control and Prevention used stored blood samples from the National Health and Nutrition Examination Survey to determine levels of the four most common individual TFAs in the 2000 and 2010 surveys. They found that TFA levels decreased by 54% during that period, confirming the drop in TFA exposure (Vesper et al., 2017). From 18 June 2018, the FDA effectively banned partially hydrogenated oils in processed foods, which will further decrease exposure to industrially produced TFA.

The benefit of measuring TFA in blood is that it does not rely on either dietary assessment or food testing of TFA. In addition, it can be done on stored blood samples (but not on blood spots) that have been collected for another purpose and stored at a low enough temperature. Downsides are that it is a relatively new process, few laboratories have the expertise and capacity to do it, it only includes the most common TFAs, and it is time-consuming and expensive (70 minutes per sample).

A brief summary of the steps involved is provided below. More detailed protocols for both sampling and laboratory analysis can be found in the web materials.

STEP 1: IDENTIFY A POPULATION SURVEY

Representative health surveys that include blood samples are fairly common in many countries (for example, National Health and Examination Surveys, STEPS surveys, Demographic and Health Surveys). Start by identifying a recently completed health survey or one that will be completed soon. If such surveys do not exist (or if a blood draw cannot be added for a subsample of people in an ongoing survey), it may not be worth pursuing this method.

STEP 2: DETERMINE THE SUBSAMPLE

The number of people that need to be included in the sample will depend on:

- › how many samples need to be tested to give adequate statistical accuracy and power
- › how many samples can be analysed given the budget and time frame.

Subsamples can be either representative or purposive. Representative samples provide a complete picture of the intake of the population. However, given the cost and time-consuming nature of the test, the population tested should be likely to consume TFA if the test is to have adequate power – that is, purposive sampling. The results in the case of purposive sampling would no longer be average TFA exposure; instead, they would reflect the TFA exposure among specific segments of the population. Once the population of interest is identified, a random sample should be drawn from the existing survey or subpopulations of the survey.

STEP 3: UNDERTAKE LABORATORY TESTING

The fatty acids are separated by capillary gas chromatography and detected by mass spectrometry. See the detailed protocol in the web materials.

5.2 DIETARY SURVEYS

Dietary surveys are currently the most common way to determine TFA intake in a country. In countries such as Canada and Denmark, data on TFA intake were critical to spurring action.

Although useful, dietary surveys require that a country has an existing population survey that includes a detailed dietary surveillance instrument – for example, a 24-hour dietary recall or a three-day diet diary coupled with a nutrition database that includes TFA content in food. Another consideration is that self-reported dietary intake often underestimates energy intake, which is likely to lead to underestimation of TFA intake. As well, nutrition databases often draw on old information, so TFA content may not be accurate.

Consider investing in dietary surveys to measure TFA intake in the following situations.

- › The country has a system of dietary surveys and a nutrition database with TFA content information.
- › The country has a system of dietary surveys and a nutrition database, but that database lacks information on TFA. In this case, assessment of TFA in food could be incorporated into the nutrition database and generate estimates of TFA intake.
- › The country would like to invest in dietary surveys in general (not just for TFA). In this case, build TFA assessment into the project.

If food assessment will be done to inform a population-based survey of TFA intake, some aspects of sampling for food analysis differ from the information presented above.

- › Random sampling: Within identified food categories, it is much more important that random selections of food should be sampled to obtain a representative sample of the category (sales-weighted random selection is even better).
- › Using composites: Because the goal is to obtain average values of TFA for foods in different categories, it is not necessary to separately analyse every single food sample within a category. Instead, composites can be made for each category. (For instance, the United Kingdom's most recent assessment of TFA intake measured TFA in 62 samples, each of which was a composite of 5–12 subsamples; Roe et al., 2013.)

Resources on creation of national nutrition and food composition databases exist, including INFOODS.

Box 11 summarizes the use of surveys by a food safety authority to estimate TFA intake.

BOX 11. ESTIMATION OF TFA INTAKE FROM DIETARY SURVEYS IN AUSTRALIA AND NEW ZEALAND (FSANZ, 2009)

Food Standards Australia New Zealand (FSANZ) undertook a review in 2009 to assess the impact of voluntary measures to reduce TFA intake in Australia and New Zealand. FSANZ used the most recent national food consumption data (four surveys spanning 1995–2007) and the results of a 2008/2009 survey on TFA concentrations in foods to determine that industrially produced TFA intake declined by 25–45% between 2007 and 2009.

The following model was used: Dietary intake of TFA in an individual = Σ all foods (food consumption \times TFA concentration)

The 2008/2009 TFA concentration data were from a survey conducted in New Zealand and three states of Australia that sampled 456 takeaway and processed foods. TFA concentration data for other foods (for example, beef, lamb, dairy, eggs and other minimally processed foods) were obtained from data tables of national food composition from Australia and New Zealand. These data were used as inputs in a model to provide estimates of TFA concentration for all foods consumed.

6. PUBLISHING RESULTS

Regardless of the type of study, the end goal should be to publish and publicize the results so that they can be used to facilitate regulatory or policy decision-making, or for advocacy.

For food data, use collected data to create a database of TFA in food (steps 3 and 7), which is ideally public or accessible to researchers. Regularly report on the results, particularly TFA and SFA content overall, by food category and by brand (if relevant). When a new regulation is in place, describe the level of compliance, overall and by food sector. In addition, if multiple studies have been done, report on change over time to document whether TFA is decreasing in the food supply (Box 2, step 8).

For population intake studies, the methodology and results should be made available either as a report on a website or in a journal.

In all cases, publishing the information with an accompanying press release may help ensure media attention. Further, coordinating with advocates in the country is critical to ensure that the data can help advance policy goals (see module 5).

Box 12 describes the practice of the Canadian Government to make data from Canada's trans fat monitoring program publicly available.

BOX 12. CANADA'S TRANS FAT MONITORING PROGRAM

Between 2007 and 2009, Canada put in place a trans fat monitoring program to assess whether companies were complying with the voluntary guidelines put out by Health Canada to limit TFA in oils to less than 2% and in foods to less than 5% of fat. Every six months, Health Canada released a new set of data, including the company, the product name, the date of sampling, the fat content, and the percentage of total fat that was TFA, SFA and TFA + SFA. These detailed data tables are still available online (Government of Canada, 2009).

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ANNEX 1.

FOOD CATEGORIES TESTED IN EARLIER STUDIES

^a Assessments were designed to monitor either voluntary initiatives (Canada) or regulations (Argentina). Categories were based on earlier data sources indicating that the categories had contained trans fats.

^b Assessments were designed to understand where TFA was in the food supply, not to monitor reduction.

CANADA: TRANS FAT MONITORING PROGRAM, 2007–2009 (Government of Canada, 2009)^a

Packaged food
Brownies
Cakes
Coffee whiteners/creamers
Cookies
Crackers
Croissants
Danishes
Doughnuts
Frozen appetizers
Frozen dinners/entrees
Frozen potato products
Frozen chicken strips/nuggets
Frozen pizzas
Frozen baked desserts
Garlic breads
Garlic spreads
Granola bars
Instant noodles
Lard
Margarines – hard
Margarines – soft
Muffins
Pies/tarts
Popcorn
Shortening
Snack puddings

Fast food, restaurants, bakeries
Chicken strips/nuggets
Doughnuts
Fish products
French fries
Miscellaneous
Muffins
Onion rings
Pizzas
Pizza dipping sauces

Cafeterias in institutions
French fries
Cookies
Muffins
Chicken products
Onion rings and fish
Bakery products and desserts
Miscellaneous
Margarines
Popcorns and snacks

ARGENTINA: TRANS FAT MONITORING, 2014–2016 (Kakisu et al., 2018)^a

Margarines (n = 16)
Soybean- and sunflower-oil mixtures (n = 4)
Pastry coatings (n = 31)
Pastes for filling (n = 13)
Crackers (n = 60)
Sweet biscuits (n = 90)
Cereal bars and breakfast cereals (n = 44)
Potato and corn crisps (n = 58)
French fries and croquettes (n = 4)
Croissants (n = 33)
Pastries and doughnuts (n = 40)
Muffins and puddings (n = 32)
Chewy candies (n = 8)
Chocolate confections (n = 23)
Alfajores (n = 42)
Pie doughs (n = 39)
Ice creams and whipping creams (n = 13)
Coffee whiteners and cappuccino powders (n = 13)
Soups (n = 10)

KYRGYZSTAN: FEEDCITIES STREET FOOD SURVEY, 2016 (WHO Regional Office for Europe, 2017b)^b

Industrial foods
Biscuits*
Buns
Chalap
Crisps
Chocolate*
Corn snacks
Dried breadcrumbs
Maksym
Sweet pastries
Wafers**

Freshly made
Ashlyamfu
Belyashi*
Bread
Buns
Cake*
Chebureki*
Corn
Hamburger*
Hot dog*
Keksi
Kompot
Kurut
Lagman*
Manty**
Piroshky*
Porridge
Salad
Samsa*
Sandwich*
Sausage roll

* >10% of TFA daily limit
** >100% of TFA daily limit

TAJIKISTAN: FEEDCITIES STREET FOOD SURVEY, 2016 (WHO Regional Office for Europe, 2017a)^b

Industrial foods
Biscuits (cookies)
Biscuit rolls
Bread*
Crisps*
Chocolate
Corn snacks
Dried bread*
Sunflower seeds*
Sweet pastries*
Wafers**

Freshly made
Baklava*
Belyashi
Bread (chapati)
Bread (e.g. buckwheat)
Breads (fatir, girdacha, kulcha, lepyoshka, samarkand)
Buns
Cakes*
Chebureki
Fried fish
Fried potatoes
Hamburgers
Hot dogs
Ice cream
Kurut
Pioshky
Plov*
Sambusa
Sausage buns
Shawarma
Soup*
Sweet pastries*

* >10% of TFA daily limit
** >100% of TFA daily limit

INDIA, STREET FOOD SURVEY, VILLAGE AND SLUM, 2012–2013 (Gupta et al., 2016)^b

Packaged unlabelled snacks
Bhindi (wheat puffs)
Bun*
Chakri
Fun pop
Kachri
Sweet biscuits
Salted biscuits
Rusk
Pao (bread buns)
Fan (pastry puffs)**

Open snacks
Besan Ladoo
Besan barfi**
Maida barfi
Biscuits
Bhujia
Mixture
Mathri**
Soan papdi*
Moong dal
Cream roll*
Pastry
Patty**
Muffin

Freshly made
Samosa**
Kachodi**
Bread pakora
Bhatura
Namakpara*
Pakora
Aloo tikki
Boondi ladoo

Fats/oils
Vanaspati**
Desi ghee (home-made)
Desi ghee (market)**
Mustard oil*

* In at least one location, TFA >2% of total fat
** In at least one location, TFA >10% of total fat

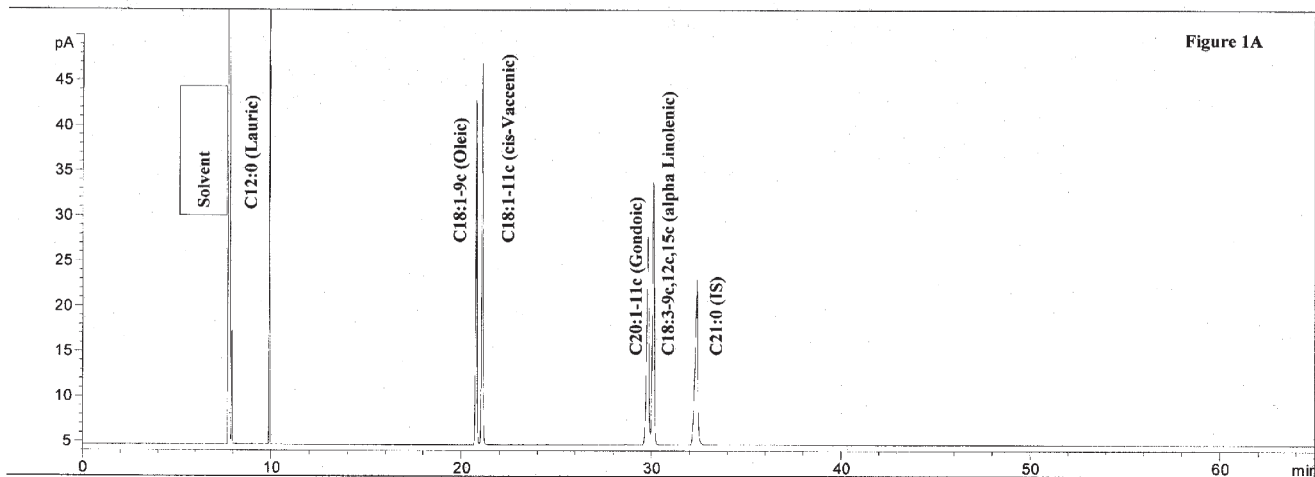
ANNEX 2.

CRITERIA FOR SELECTING A LABORATORY TO CONDUCT TFA ANALYSIS

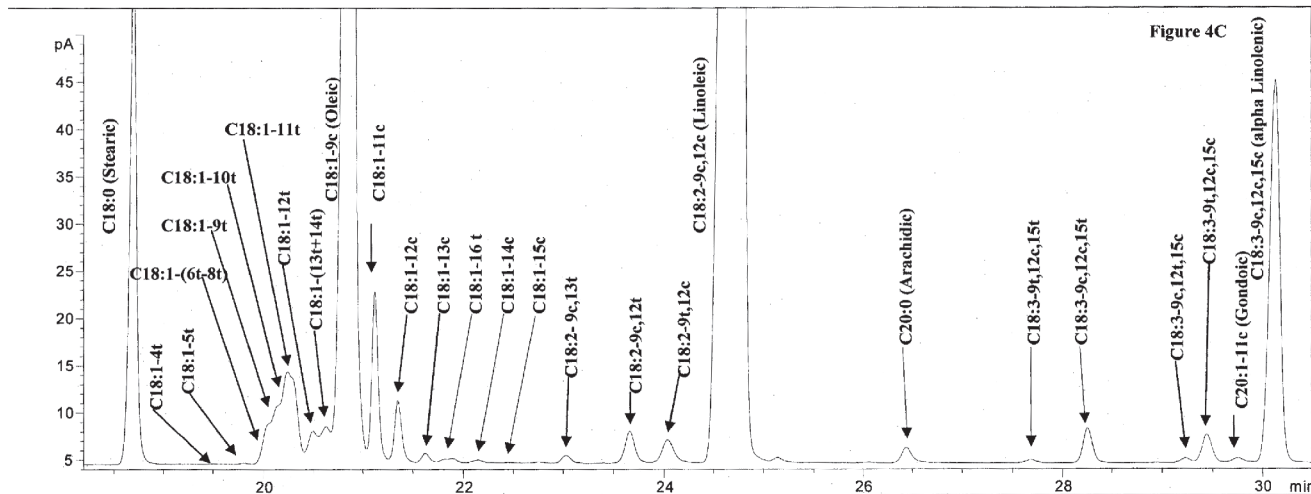
In selecting a suitable laboratory for performing TFA analysis, the following criteria should be taken into consideration.

- The laboratory is reputable and follows the Workplace Hazardous Materials Information System (WHMIS) or Globally Harmonized System of Classification and Labelling of Chemicals (GHS) safety standards (or equivalent safety standards).
- The laboratory is equipped with the following laboratory equipment, glassware, reagents, chemicals, authentic fatty acid standards and certified reference food materials. These are essential for analysis of food samples, and obtaining reliable and accurate data on their fat content and fatty acid composition.
 - **Gas chromatograph (GC):** Suitable for use with a capillary column, equipped with hydrogen flame ionization detector (FID), temperature controlled split mode injector unit, oven chamber for the capillary column capable of maintaining the desired temperature to ± 1 °C, monitor, and chromatographic software capable of manipulating the GC controls.
 - **Capillary column:** Fused silica capillary column 100 m, and 0.25 mm internal diameter, coated with either SP-2560 or CP-Sil 88 stationary phase. Capillary columns providing resolution of fatty acids similar to those of SP-2560 and CP-Sil 88 may also be used.
 - **Microsyringe** for GC 10 μ L delivery, with a hardened needle or an equivalent.
 - **Carrier gas:** Hydrogen or helium, 99.999% pure or better, GC quality, dried, and oxygen removed by suitable filters.
 - **Flame ionization detector gases:** Hydrogen and air, GC quality.
 - **Standard laboratory glassware and equipment:** Volumetric flasks, glass volumetric pipettes, disposable pipettes, heating block (hot water bath for methylation), glass test tubes, glass vials, electronic weighing scale for measuring milligram quantities of material, liquid nitrogen, nitrogen cylinder, teflon-lined screw caps, vortex mixer, rotor–evaporator for removing solvents.
 - **Chemicals and reagents:** Hydrochloric acid (12 molar, analytical grade), ammonium hydroxide (58% w/w, analytical grade), hexane (analytical grade), diethyl ether (anhydrous, purity \geq 99.7%, containing 1 ppm BHT inhibitor, packed in aluminium containers), ethanol (95% v/v), toluene (nano-grade), chloroform or methylene chloride (reagent grade), sodium sulfate (anhydrous), 14% boron trifluoride-methanol reagent (14% BF₃-methanol), acetone (reagent grade), ready supply of distilled water (very often prepared in the laboratory).
 - **Authentic fatty acid methyl ester reference standards:** Saturated fatty acids of different chain lengths, *cis*-monounsaturated fatty acids, *trans*-monounsaturated fatty acids, natural *cis*-polyunsaturated fatty acids (e.g. linoleic and alpha-linolenic acid), *trans*-polyunsaturated fatty acids (especially *trans*-linoleic and *trans*-linolenic). These are required for identification of the fatty acid profile of dietary fats of food samples.

- **Wide ranges of highly purified (>99%) FAME standards**, both as individual standards and as model mixtures – these are readily available from a number of commercial sources (e.g. Alltech Associates, Inc., Deerfield, IL; Nu-Chek-Prep, Elysian, MN; Supelco Inc., Bellefonte, PA; Sigma Chemical Co., St. Louis, MO; MilliporeSigma Canada Co., Oakville, ON, Canada). The American Oil Chemist’s Society (AOCS, 2710 S. Boulder, Urbana, IL 61802-6996, USA; email general@aocs.org) also offers a series of quality reference materials (QRMs) for analysts to gain practice with calibrating equipment and testing new methods. The QRMs include non-hydrogenated soybean oil and hydrogenated soybean oil for TFA determination, and come with GC chromatograms.
 - **Quality reference materials (QRMs):** These are standard reference materials (SRMs) produced by reputable international organizations. For example, a meat homogenate (Standard Reference Material 1546a) is available from the National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. This SRM is intended primarily for validation of methods or determining fatty acids and several other nutrients.
- › The laboratory team consists of experienced technicians who are familiar with capillary GC procedures to analyse fatty acid composition of dietary fats. The laboratory has checked GC performance using reference standards and AOCS QRMs described above.
- There is a baseline separation between 9c-18:1 and 11c-18:1 (as shown in Fig. 1).
 - The *trans* isomers from 4t-18:1 to 12t-18:1 are readily separated from all the *cis*-18:1 isomers (as shown in Fig. 2).
 - There is a good partial separation between 13t + 14t-18:1 and 9c-18:1 (as shown in Fig. 2).
 - There is a near baseline separation between 16t-18:1 from 13c-18:1 and 14c-18:1 (as shown in Fig. 2).
 - There is near baseline separation among 11c-20:1, 9c,12c,15c-18:3 and 9t,12c,15c-18:3 (as shown in Fig. 2).
 - Any GC runs showing poor resolution of the above critical FAMEs are not acceptable.
- › The analysts have previous experience and training relating to the analysis of dietary fatty acid composition as follows:
- general laboratory safety training;
 - hazardous chemical waste management training;
 - records management training;
 - training on the specific GC instrumentation and software (such as Agilent ChemStation) used with this measurement procedure from a qualified laboratory or from the instrument manufacturer;
 - training to correctly calculate and interpret results obtained, and transfer data to an appropriate database; and
 - experience in the analytical procedure described here by participating in the analysis of SRMs produced by reputable international organizations.
- › The analysts have the knowledge and skills to understand and successfully adapt standard analytical procedures, such as the TFA analysis procedure described here.



▲ **Fig. 1.** GC profile of some FAME standards on SP-2560 capillary column (180 °C isothermal, H₂ at 1.0 mL/min)
 Source: adapted from AOCS Ce 1h-05, 2017 revised (AOCS, 2017)



▲ **Fig. 2.** GC profile of a low-trans margarine FAME on SP-2560 100 m capillary column (180 °C isothermal, H₂ at 1.0 mL/min)
 Source: adapted from AOCS Ce 1h-05, 2017 revised (AOCS, 2017)



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