

***Trans-fatty acid* assessment**

Report of the WHO expert consultation

Geneva, Switzerland, 11–12 October 2018



**World Health
Organization**

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Background

Cardiovascular disease is the leading cause of death globally, accounting for one in every three deaths. Nearly half of the deaths caused by cardiovascular disease are in lower-income countries, among people aged under 70 years. The World Health Organization (WHO) has identified the elimination of industrially produced *trans*-fatty acids (TFAs) from the food supply as a cost-effective intervention to prevent cardiovascular disease.

In May 2018, WHO called for the global elimination of industrially produced TFAs by 2023 as part of the priority targets of the WHO 13th General Programme of Work, which will guide the work of WHO in 2019–2023. WHO also released, in collaboration with Resolve to Save Lives,¹ the REPLACE² action package, including an action framework and six modules. The framework is a roadmap for countries to implement the prompt, complete and sustained elimination of industrially produced TFAs from their national food supplies.

This document is a report of a 2-day WHO expert consultation³ that was held to review and discuss the draft REPLACE “A” (“assess”) module. This module outlines key actions for assessing and monitoring TFA content in the food supply, and changes in TFA consumption in the population. The expert consultation also reviewed draft laboratory protocols, and agreed on approaches for TFA assessment in foods and blood plasma.

The objectives of the consultation were to:

- discuss and agree on surveillance approaches, including a sampling frame, for measuring TFA exposure in humans and TFA content in foods;
- review and develop laboratory protocols for measuring TFA exposure in humans and TFA content in foods;
- discuss and agree on analytical performance criteria for measuring TFA exposure in humans and TFA content in foods, and reporting formats for laboratory results; and
- identify opportunities for multisectoral collaborations to build capacity for TFA measurements in selected countries.

¹ Resolve to Save Lives is an initiative of Vital Strategies.

² REPLACE has six action areas: review (RE), promote (P), legislate (L), assess (A), create (C) and enforce (E). Six detailed REPLACE modules were developed and released in May 2019.

³ The list of participants is in the Annex.

Current experience with TFA assessment

The REPLACE action package provides a step-by-step guide for the elimination of industrially produced TFAs from the global food supply. The A module of the package focuses on the assessment and monitoring of TFA content in the food supply, and of changes in TFA consumption in the population. Although some countries may already have TFA assessment protocols, global protocols are needed to increase the accuracy and comparability of TFA data so that the impact of public health interventions can be monitored.

As part of the background documents for the expert consultation, WHO prepared laboratory protocols and surveillance tools for measuring TFA content in foods and TFA exposure in humans, building on the existing protocol developed by the United States (US) Centers for Disease Control and Prevention (CDC).

At the consultation, the REPLACE action package was introduced, and the contents and focus of the A module were reviewed, along with the online annexes that will accompany the module. The experts suggested that a food description guide will enable comparison between countries and should be included in the online resources.

The US CDC provided an overview of measuring population-level TFA intake through questionnaires and blood measurements, and explained how the plasma samples of the National Health and Nutrition Examination Survey were tested to assess the impact of US regulations on TFA labelling. A pilot study and a main study were conducted to measure the four main TFAs in samples from two surveys (10 years apart). The data indicate an overall 54% reduction in TFA intake by the population; differences were consistent for all four TFAs investigated. A presentation was given on the US CDC laboratory method for measuring TFAs in blood samples: gas chromatography–mass spectrometry (GC-MS). This method:

- enables quantification of four major TFAs and 23 other fatty acids
- has high levels of specificity, sensitivity and accuracy
- requires only 100 µL of sample
- is automated, allowing appropriate throughput for population studies
- reports results as concentration (in µM) and percentage of total fatty acids
- can be used to measure TFAs and other fatty acids in plasma, serum and red blood cells.

It was noted that the laboratory method used by the US CDC can be expensive because of the type of equipment needed for analysis. For this reason, no other country seems to have conducted the type of testing being used by the US CDC.

The US CDC has provided some capacity-building training on the use of the laboratory method to the National Institute of Nutrition in India and Mahidol University in Thailand in recent years. Mahidol University provided an overview of its experiences with building

capacity to measure TFAs in stored blood samples. The aim is to begin testing of human blood samples in its laboratories in 2019.

An overview of current best-practice methods for measuring TFA levels in food, including laboratory and sampling approaches, was presented by Dr Nimal Ratnayake, who supported the drafting of a WHO laboratory protocol for assessing TFA content in foods. The University of Porto and the National Institute of Health in Portugal also shared their experience with assessing TFAs in foods. Their sampling approach was based on a desk review and product ingredient lists. Foods were collected from supermarkets, smaller retailers and local pastry shops for (laboratory) testing of the TFA content. The laboratory testing method was selected for its simplicity, sufficient accuracy, low environmental impacts, speed and cost. Portugal observed a decrease in TFAs in foods following voluntary recommendations for industry; however, replacement of TFAs has been primarily with saturated fatty acids.

Through the country presentations and group discussion, the following challenges with TFA testing in blood and foods, and potential solutions were identified.

- **Cost:** Cost could be high for both food and blood, but particularly for blood.
- **Capacity:** Knowledge and expertise to carry out food and blood testing are limited in many countries.
- **Quality assurance:** Internal standards and calibrators are unavailable in many countries and are expensive.

An overview of an interlaboratory comparison study (“ring trial”) was provided. The US CDC, WHO, Virginia Tech and Resolve to Save Lives are co-investigators in the study. The ring trial will address gaps in the information that is needed to compare TFA measurements in foods that are used to assess status and progress of TFA interventions. Up to 10 laboratories around the world will be invited to participate, and findings will be an input to the global TFA food surveillance standards discussed during the consultation.

Review of draft protocols

Experts were organized into two working groups to review and discuss sampling and laboratory methods for food and human testing. The experts reached consensus on protocols after some revisions had been agreed upon. The agreed revisions will be made to the food and human surveillance documents, and the REPLACE A module will be revised accordingly.

During working group sessions, experts addressed the global challenges of cost, capacity and quality assurance. They agreed on levels of accuracy, specificity and other performance metrics that would give laboratories the flexibility to use alternative (less expensive) methods to generate comparable data. Other recommendations to reduce cost included starting with a food study to determine what or where the problem is, doing a pilot blood study before a full study and using composite samples. Options to reduce the cost of measuring TFAs in food included starting with just testing fats

and oils, using composite samples, and using distributors' sales data or surveying food distribution sites (rather than purchasing expensive market data reports). Leveraging regional hubs, where available, and multisectoral collaborations may offer solutions to the challenges of capacity and quality assurance.

The findings of the reviews of the various protocols and the REPLACE A module can be summarized as follows.

- Review of the human sampling protocol. It was agreed that:
 - sections of the document need to be revised, to improve the flow and readability;
 - a statistician should review the sampling section; and
 - a section should be added on the intent and intended audience of the protocol.
- Review of the human laboratory protocol. It was agreed that:
 - aspects of the document should be revised to make it more relevant to various countries; and
 - tables should be reordered to group TFAs separately from other fatty acids.
- Review of the food sampling guide in the REPLACE A module. It was agreed that:
 - the A module needs to be revised to include country examples of food categories that are to be included in an assessment; and
 - the food sampling protocol should include clear steps that take into account feasibility in different contexts (e.g. where resources are limited); guidance on number and unit size of samples for collection; guidance on storage and handling of samples; and guidance on timelines for analysis.
- Review of the food laboratory protocol. It was agreed that:
 - sections of the document need to be revised – for example, to clarify the key performance criteria for separation of *cis* and *trans* isomers, and to include concentration requirements; and
 - the protocol should include a tool to assess the suitability of a laboratory (e.g. a questionnaire about laboratory methods and experience), and a reporting template (e.g. an Excel file with all fatty acids, measurements and statistics).

Assessment of TFAs in blood

The blood plasma laboratory protocol developed, based on the protocol used by the US CDC, analyses four TFAs (palmitelaidic, elaidic, vaccenic and linolelaidic acids) to assess the impact of TFA regulation. The aim is to examine associations between fatty acids and health outcomes.

Although it is not possible to investigate all foods, an assessment of TFAs in blood plasma at the population level can provide information on the results of efforts to eliminate or reduce TFA in foods. Globally, few population-level TFA assessments have been undertaken, apart from the one being conducted in the US. Results of a population blood plasma analysis conducted using an alternative protocol should reflect the level of specificity, precision and accuracy described in section 9 of the laboratory protocol.

The preferred reporting format is concentration of TFAs; however, results can be calculated as percentage of TFAs, if necessary.

To identify laboratories that can conduct blood plasma assessments, it is important to have a checklist that includes laboratory capacity, availability of equipment and other resources, political commitment and previous experience with human samples. Other important considerations are:

- shipment of human samples, when establishing regional hubs;
- consent to use samples in analysis, if using existing blood samples; and
- that procuring GC-MS equipment and strengthening capacity for this type of analysis open up options for conducting other types of laboratory assessments on the population.

As more countries implement policies to reduce or eliminate TFAs, it will be important to evaluate the impact of such policies. This can be done by measuring cholesterol biomarkers in human blood plasma.

Assessment of TFAs in foods

The first step in planning a food assessment should be to determine the goal of the assessment, because this will affect the sampling approach used. Two possible goals are measuring compliance with TFA policies and measuring population intake of TFAs. The sampling approach used will also depend on the resources available, because testing requires time and money. The recommendations given below are for assessments that aim to measure compliance.

- Food categories to include in the assessment include oils and fats (partially hydrogenated oils, fully hydrogenated oils and refined edible oils), and foods made with oils and fats, including mixed foods (which may contain TFAs from industrial and ruminant sources).

- Food categories to exclude include foods that exclusively contain TFA from ruminant sources. There is a recommendation to add a footnote stating that the protocol may not be appropriate for fish products, and that appropriate methods for such products are available.
- Depending on the country situation (e.g. limited resources or food often cooked at home), testing can start with oils and fats that are used in products plus edible oils. If resources allow, testing can also include food products that are major potential sources of industrially produced TFAs.
- Approaches to selecting food categories include:
 - conducting a background assessment of the food supply (i.e. making use of existing data);
 - purchasing market data to determine popular food categories; where this is not possible, researchers can contact food distributors or conduct a survey of outlets;
 - using examples from other countries;
 - reviewing nutrition labels or ingredient lists, where available; and
 - using existing resources, such as food balance sheets from the Food and Agriculture Organization of the United Nations; food composition databases for different regions may also provide some information.
- Countries will need to determine where to collect samples (in terms of both geographical location and food outlet types). Where people buy food depends on the country and on the food category. The assessment should include institutions such as schools, homes for the elderly and hospitals (as a second-tier setting for sampling when resources are available), because the populations of such institutions are vulnerable and are often overlooked. Some countries may have different risk levels in rural and urban areas, based on differences in behaviour and available foods; also, regional recipes should be taken into consideration. If geographical differences are suspected, composite samples are not appropriate.
- At least three samples of each food type should be collected for testing. Where variation across lots is expected (e.g. street foods), it is recommended that samples be collected from different lots and at several time points. Samples should be homogenized within vendors. Where variation within foods is not expected (e.g. foods from large establishments or supermarkets), it is sufficient to collect samples from the same lot and then homogenize them. It is important not to create composites of different brands. It is also important to include products from large, medium and small enterprises.
- The sampling unit should be one regular portion size (with three samples), based on needing at least 100 mg of fat for reliable analysis.
- The analysis must be done within 1 month of sample collection; hence, the protocols should include information on storage and handling of samples.
- If countries have standard operating procedures (SOPs) that are in general agreement with the principles outlined here, it is acceptable to use these

procedures, which should include reference to the International Network of Food Data Systems (INFOODS).¹ SOPs can be adapted to meet the principles.

- If the goal is to monitor population intake, the recommendation is to start with the national dietary survey, if one exists. If databases or food composition tables exist, TFAs should be included. It is not worth establishing these systems for TFAs alone.

Analytical methodology for TFA assessment in foods

The draft protocol for the analytical methodology for TFA assessment in foods was developed based on Health Canada's protocols. It has been validated, and generates comparable data across different laboratories. Other suitable methods may give comparable results, and laboratories may use such other methods, provided that they meet the defined performance criteria. For example, some laboratories may find it easier to implement International Organization for Standardization (ISO) (European) standards. The idea of referencing ISO in the protocols was discussed, but the experts did not reach consensus on this point.

Ensuring separation of *cis* and *trans* isomers is a key performance criterion that should be emphasized in the protocol. Key performance criteria include:

- the *trans* isomers from 4t-18:1 to 12t-18:1 – these should be readily separated from all the *cis* 18:1 isomers;
- good partial separation of 13t-18:1 and 14t-18:1 from 9c-18:1;
- near-baseline separation of 16t-18:1 from 13c-18:1 and 14c-18:1; 16t-18:1 should be separated from 13c-18:1 and 14c-18:1; and
- near-baseline separation among 11c-20:1, 9c,12c,15c-18:3 and 9t,12c,15c-18:3.

If the key performance criteria are not met, small, incremental adjustments should be made to the sample size, sample concentration and column oven temperature to achieve the critical separations.

It may be appropriate to use manual (rather than automatic) injection.

The concentration should be 15–20 mg/mL, and 100 mg or 200 mg of sample should be diluted to obtain this concentration. In terms of volume, 5 mL of sample is needed per 100 mg (10 mL of sample per 200 mg).

At a minimum, general classes of TFAs must be reported (e.g. 18:1, 18:2 and 18:3 regions). Reporting on individual TFAs will be left up to the researcher. For acceptable accuracy, the assessment must be able to meet a limit of quantification of 0.1% of total fatty acids.

¹ See <http://www.fao.org/infoods/infoods/en/>

All identified peaks and all fatty acids in the GC profile should be reported. Table 1 in the draft protocols will be updated to include all fatty acids; 0.5% of total fatty acids is the cut-off point for including unidentified fatty acids. Reporting should include mean, standard deviation, range, *n* and a description of the sample.

Fatty acids should be reported in two formats: percentage of total fatty acids and g per 100 g food. The percentage expression is more user-friendly, whereas the g/100 g food expression is more useful for nutritionists. What is most useful may depend on the regulations in place; regulations are often based on the percentage of fatty acids.

Multisectoral collaboration and regional hubs

Multisectoral collaborations could reduce many barriers for countries, such as identifying and procuring standards and equipment, and having access to a support structure for maintaining the instruments. Potential solutions include:

- making key suppliers aware of the WHO global call for the elimination of industrially produced TFAs so that they can take necessary actions, including increasing availability of equipment and services in underresourced areas;
- making WHO Regional and Country Offices aware, providing guidance to governments and increasing the level of commitment at the national level;
- adding information on key suppliers (including contacts) and internal standards to the WHO website, to reduce delays in procurement; this was relevant to Thailand, where having information in a central location might have limited delays in implementing the programme;
- encouraging collaboration with academia to obtain scientific support; and
- convening meetings at a global or regional level to exchange experiences, share and compare results, and discuss ways to overcome barriers.

To build the capacity of laboratories to set up and carry out TFA assessment (in both food and human samples), training and technical assistance may be needed. Regional laboratories with necessary capacities and experience may be able to fill gaps in capacities. WHO will therefore follow up with experts participating in the consultation, to solicit names and contact information for laboratories that may be qualified to serve as regional hubs.

Annex

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