



# Sampling Protocol

## for Rapid Assessment of *trans*-Fatty Acids in Fats and Oils

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This document outlines the key procedural steps to design a study and collect fats and oils samples to assess the levels of *trans*-fatty acids (TFA) in main sources of industrially produced TFA (iTFA) intake in a specific population. The goal of the study is to raise awareness and support policy action for elimination of iTFA or to monitor compliance with existing regulations. It is not possible or necessary to analyze every available package or serving of a food. A narrow and rapid assessment of fats and oils is a low-cost way to raise awareness and spur policy action.

Use this document to guide study design and sample collection of fats and oils for laboratory analysis of TFA. Reasons for conducting the assessment in fats and oils (vs. in foods) include the following:

- Cooking/frying oils or fats used for baking/binding have been identified as a probable primary source of iTFA intake in the population;
- Restaurants, bakeries or other food preparers have been identified as major contributors of iTFA intake in the population (it may be more efficient to sample the fewer fats and oils used vs. sampling many different food items prepared using those fats and oils);
- A small number of fats and oils manufacturers have been identified in the country and comprise a large share of the market;
- It is logistically simple and will be quick to test fats and oils and document the presence of iTFA; or
- There are political or policy reasons for testing fats and oils.

## Background

The World Health Organization (WHO) recommends that all individuals consume TFA less than 1% of total daily energy. To achieve this, countries should implement best practice policies—either a mandatory limit on iTFA to 2% of total fat in all fats, oils and foods, or ban on the use and sale of partially hydrogenated oils (PHO). PHO is the main source of iTFA intake in most countries. Lower levels of iTFA can also be formed during oil refinement processes using high temperatures. Preliminary research can focus on fats and oils only to help estimate the amount of iTFA present in the food supply; follow-up research can, if necessary, include other food products.

## Research Objective

Collect fats and oils samples from [PHO manufacturing facilities; distributing facilities; or retail outlets and food preparers] in [geography] for TFA laboratory analysis.

## Study Design

- 1 Food Sector**  
The assessment should focus on fats/oils sold business to business (B2B). If including B2B fats/oils is not possible, packaged fats/oils sold to consumers (retail) can be included.
- 2 Outlet Types**  
Conduct a simple desk review or stakeholder interviews to determine whether PHOs used in the country are primarily imported or manufactured domestically.

- a. If PHOs are primarily manufactured domestically, the assessment should focus on domestic PHO manufacturing facilities. If PHO manufacturing facilities are not centralized or if it is difficult to access the facilities, the assessment should focus on PHO distributing facilities.
- b. If PHOs are primarily imported, the assessment should focus on PHO distributing facilities.
- c. If sampling from distributing facilities is not possible, restaurants and bakeries (B2B) and supermarkets/markets (retail) should be included in the assessment. If sampling from restaurants and bakeries is not possible, focus sampling on supermarkets/markets. If there are other major outlet types where consumers or food preparers purchase fats/oils, include that outlet type.

### ③ Geography Selection

Select a geography (e.g., city, state) within the country for the assessment.

- a. If PHO manufacturing facilities are included in the assessment select 1 geography within the country where PHO manufacturers are concentrated. This information can be obtained from the Bureau of Commerce (or similar agency) or industry.
- b. If PHO distributing facilities are included in the assessment select 1 geography within the country where PHO distributors are concentrated. This information can be obtained from the Bureau of Commerce (or similar agency) or industry.
- c. If restaurants, bakeries and/or supermarkets/Markets are included in the assessment select 1 geography within the country. Give preference to the most populous geography or geography with political influence.

### ④ Fats and Oils Categories

Cooking/frying oils, baking fats (e.g., shortening), and spreads (e.g., margarine) should be included in the assessment. If there are other categories of fats/oils that are a probable major source of iTFA consumption in the population, include that category. Table 1 provides examples of fats/oils categories by sector that may contain TFA.

### ⑤ Neighborhood selection

*(ONLY RELEVANT if restaurants, bakeries or supermarkets/markets are included in the assessment.)*

For the selected geography, identify and list neighborhoods. Categorize the neighborhoods into 2 socio-economic status (SES) groups (e.g., high/middle - low-income) based on available data (e.g., rent prices, census data). Remove any neighborhoods that are not generally representative (e.g., sparsely populated). Create numbered lists of all included neighborhoods for each SES group. Use a random number generator to select 2 neighborhoods to be visited for each SES group. Table 2 is a template that can be used. Skip this step if the assessment focuses on PHO manufacturing or distributing facilities.

### ⑥ Specific Outlets Selection

- a. If PHO manufacturing facilities are included in the assessment: Include up to 10 PHO manufacturing facilities within the selected geography in the assessment. If there are fewer than 10 facilities, include all in the assessment. If there are more than 10 facilities, include the 10 facilities with the largest PHO production. Production size can be determined from sales data, number of products in products lines, size of facilities, and interviews with facilities' owners or employees.

To identify all facilities in the selected geography that produce PHOs, procure a list of all fats/oils manufacturers in the geography and identify manufacturers of PHOs. This list can be obtained from the Bureau of Commerce or similar agency. If needed, conduct site visits or interviews with facility owners or employees to determine whether partial hydrogenation is currently used. Partial hydrogenation towers are large and easily identifiable.

- b. If PHO distributing facilities are included in the assessment: Include up to 5 PHO distributing facilities within the selected geography in the assessment. If there are 5 or fewer facilities, include all in the assessment. If there are more than 5 facilities, include the 5 facilities with the largest distribution of PHOs. Distribution size can be determined from sales data, number of brands and products carried, size of facilities, and interviews with facilities' owners or employees. To identify all distributing facilities in the selected geography that sell

PHOs, procure a list fats and oils distributors in the geography and identify distributors of PHOs. This list can be obtained from the Bureau of Commerce or similar agency. If needed, conduct site visits or interviews with facility owners or employees to determine whether products contain PHOs.

- c. If restaurants, bakeries and supermarkets/markets are included in the assessment: Within the selected neighborhoods, select 1 popular supermarket or market where consumers frequently purchase cooking/frying oils, baking fats, spreads, and 2 popular restaurants and 2 popular bakeries where consumers frequently purchase foods prepared using fats and oils. Use key informant interviews (e.g., local guide, municipal council members, local health officials) or review media/social media to get information on most popular outlets. If more than 2 outlets are popular (visited by large number of consumers), create a list and choose 2 outlets randomly.

## ⑦ Fats and oils items and brands selection

- a. For manufacturing facilities: Select all liquid and all solid PHOs from each facility.
- b. For distributing facilities: Select all liquid and all solid PHOs from each facility.
- c. For restaurants: Select all cooking/frying oils from each outlet. If the restaurant also has baking fats in the kitchen, select all baking fats. If the kitchen also has fat spreads in the kitchen, select all spreads.
- d. For bakeries: Select all baking fats from each outlet.
- e. For markets/supermarkets: Select the 3 most popular items for each fats/oils category (cooking/frying oils, baking fats, and spreads) from each outlet. If ingredients lists are available on labels, select items with “partially hydrogenated oil”, “hydrogenated oils” or similar on the lists.

This step of selecting specific items can be done by the sample collector at the time of sample collection.

### Note

You may need to reduce the number of items in the assessment based on available budget. Once you know the total budget, determine the total number of samples that can be collected and analyzed.<sup>1</sup> If you need to reduce the total number of samples in the assessment, remove smaller/less popular outlets or less popular items.

Based on steps ① – ⑦, create a sample collection framework that data collectors will use to visit outlets and collect fats/oils samples. (Table 3a is a template for sampling from manufacturing facilities; Table 3b is a template for sampling from distributing facilities; Table 3c is a template for sampling from restaurants, bakeries and retail outlets). The total number of samples collected will be determined by the number of facilities/outlets visited and the number of PHO products available at each sampling facility/outlet.

## Sample Collection, Storage and Shipping

Recruit and train local field researchers to collect, store and transport the fats/oils samples to the laboratory. Sample collection, storage and shipping procedures:

1. For manufacturing or distributing facilities, travel to the first facility. For restaurants, bakeries and markets/supermarkets, travel to the first neighborhood and go to the first outlet.
2. Once inside the facility/outlet, look for and purchase/collect each fat/oil item on the sample collection list. If selection of items for sampling is being done at the time of sample collection, see instructions in step 7 of the study design. Note: For sampling being done at restaurants or bakeries, sample collectors will need to ask outlet proprietors or staff for samples of the fats and oils used in food preparation since they are not available for retail purchase.
  - a. Collect a minimum of 100mL of each fat/oil item. Split each item into 2 sterile and shatter proof bottles with at least

<sup>1</sup> Laboratory testing of fatty acids generally costs approximately 100 USD per sample. Costs for purchasing, storing and shipping samples as well as human resources for sample collection should be considered, as relevant.

50mL of the item in each bottle.<sup>2</sup> If it is not feasible to split the fats/oils into bottles at the site of sample collection, this step can be done back in the office. Place both bottles of each item in 1 Ziploc bag with a unique ID.

- b. While in facilities/outlets, use a sticker to record the brand name, item name and price, and place the sticker on each item. There will be identical stickers on each pair of bottles that contain the same item. Take a photo of each item sampled that shows the brand name, item name, manufacturer name and location, printed expiration date, nutrition information (including SFA, TFA and total fat if available), and ingredient list. Restaurants, bakeries and manufacturers may or may not charge for collecting samples of fats/oils used in their kitchens/facilities. If charged, negotiate based on pricing of similar products purchased from retail outlets.
- c. Place each item purchased/collected from 1 facility/outlet in 1 bag and label the bag with the city, outlet type, SES, neighborhood, date, and total number of items purchased/collected.
- d. Place all the perishable samples in an icebox with icepacks. Perishable items are anything stored in a refrigerator (e.g., margarines). Non-perishable items do not need to be transported or stored in cold chain.

#### Note

For markets/supermarkets, if the outlet does not have all the items in each category, purchase what is available then go to any nearby market/supermarket that meets the SES/vendor criteria and purchase the remaining items from the list. If the second outlet does not have the remaining items available, try a third outlet. If the remaining items still cannot be found at a third outlet, purchase the next similar item and move on to the next outlet on the list.

3. Travel to the second facility/outlet and follow the same steps.
4. Complete sample collection for all remaining facilities/outlets by following the same steps.
5. Once back in the office, label each product with a unique identifier, and log information about each product in an Excel database. The following details for each product should be documented in Excel:

- |                                   |  |                            |
|-----------------------------------|--|----------------------------|
| a. Unique identifier              | f. Nutrition information, including SFA, TFA, total fat ( <i>if available on nutrition label</i> )         | i. Product quantity        |
| b. Food category                  | g. Ingredient list ( <i>if PHO, shortening, hydrogenated oil, or similar, is listed as an ingredient</i> ) | j. Product price           |
| c. Fat/oil item name              | h. Product weight (grams)  | k. Printed expiration date |
| d. Brand name                     |  | l. Purchase city           |
| e. Manufacturer name and location |  | m. SES group               |
|                                   |  | n. Vendor outlet name      |
|                                   |  | o. Date purchased          |

6. Deliver or ship 1 bottle of each sample to the laboratory for analysis. Retain the remaining samples for future re-analysis, if needed. Perishable samples should be shipped on dry ice. If shipping time is greater than 3 days, dry ice should be changed every 3 days. This is to avoid deterioration of the samples. Non-perishable samples should be shipped without the cold chain.

#### Note on Laboratory Selection

Laboratories must be able to perform GC-FID with a 100-meter GC column. Full guidance on how to select a laboratory to conduct analysis of the food samples is available on the WHO REPLACE webpage (see Annex 2 of the [REPLACE Module 4](#)).

#### Note on Shipping Samples

Preference should be given to acceptable domestic laboratories to avoid international shipping challenges. If

<sup>2</sup> Sterile and shatter-proof bottles with minimum 60mL capacity should be used, e.g. [Thermo Scientific Nalgene IP2 Bottles](#) (Fisher Scientific Catalog # 01-288-36; Manufacturer # 3420890004).

samples must be shipped internationally to a laboratory, customs requirements will depend on the receiving country and contents of the shipment. For example, some countries do not permit dry ice into their countries; some countries have lengthy customs review/approval processes for animal products or research subjects. The customs logistics process should be verified before the sample collection process begins. Customs agents may be able to aide in verifying the requirements and process. If feasible to transport items with a traveler, this may be recommended.

**Note on Perishable Items**

If shipping logistics are complicated by the inclusion of perishable items and the need for dry ice, it may be advisable to remove these items from the sample set.

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**Table 1. Fats and Oils Sampling Framework**

① **Geography** \_\_\_\_\_

<p>② <b>Food Sector</b> <i>check box for sectors</i></p>	<p><input type="checkbox"/> Business to business (B2B)</p>	<p><input type="checkbox"/> Retail packaged fats/oils</p>
<p>③ <b>Food Outlet Type</b> <i>check box for outlet types within selected sectors</i></p>	<p><input type="checkbox"/> Bakeries</p> <p><input type="checkbox"/> PHO manufacturers or distributors</p> <p><input type="checkbox"/> Restaurants</p> <p><input type="checkbox"/> Other: _____</p>	<p><input type="checkbox"/> Supermarkets</p> <p><input type="checkbox"/> Markets</p> <p><input type="checkbox"/> Other: _____</p>
<p>④ <b>Fats and Oils Category</b> <i>check box for categories within selected sectors</i></p>	<p><input type="checkbox"/> Baking fats (e.g., shortening)</p> <p><input type="checkbox"/> Cooking/frying oil</p> <p><input type="checkbox"/> Spreads (e.g., margarine)</p> <p><input type="checkbox"/> Other: _____</p>	

### Table 2. Locations Sampling Framework for Retail Outlets, Restaurants and Bakeries

*Skip this step if the assessment focuses on fats/oils manufacturing or distributing facilities.*

<p><b>5</b> Select neighborhoods using random number</p> <p><b>6</b> Select Specific Outlets using key informant interviews</p>	<b>SES group</b>	<b>High Income (HI)</b>	<b>Low Income (LI)</b>
	<b>Random number</b>	_____ , _____	_____ , _____
	<b>Neighborhood 1</b>	HI-N1 _____	LI-N1 _____
	Bakery Outlet 1	HI-N1-BO1 _____	LI-N1-BO1 _____
	Bakery Outlet 2	HI-N1-BO2 _____	LI-N1-BO2 _____
	Restaurant Outlet 1	HI-N1-RO1 _____	LI-N1-RO1 _____
	Restaurant Outlet 2	HI-N1-RO2 _____	LI-N1-RO2 _____
	Market Outlet 1	HI-N1-MO1 _____	LI-N1-MO1 _____
	<b>Neighborhood 2</b>	HI-N2 _____	LI-N2 _____
	Bakery Outlet 1	HI-N2-BO1 _____	LI-N2-BO1 _____
	Bakery Outlet 2	HI-N2-BO2 _____	LI-N2-BO2 _____
	Restaurant Outlet 1	HI-N2-RO1 _____	LI-N2-RO1 _____
	Restaurant Outlet 2	HI-N2-RO2 _____	LI-N2-RO2 _____
	Market Outlet 1	HI-N2-MO1 _____	LI-N2-MO1 _____

⑦

**Table 3a. Sample Collection Framework – PHO Manufacturing Facilities**

\*Total # of samples will depend on # of PHO manufacturers and # of PHO items available at each facility

Facility	Category	Item/brand	# of samples	Total # of samples*
M1	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
M2	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	

M3	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
M4	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	



M5	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
M6	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	

M7	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
M8	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	

M9	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
M10	Liquid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
	Solid PHOs	1	1	6
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
<b>TOTAL</b>				<b>120</b>

⑦

**Table 3b. Sample Collection Framework – PHO Distributing Facilities**

\*Total # of samples will depend on # of PHO distributors and # of PHO items available at each facility

Facility	Category	Item/brand	# of samples	Total # of samples*
D1	Liquid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
	Solid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	

D2	Liquid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
	Solid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	

D3	Liquid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
	Solid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	

D4	Liquid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
	Solid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	

D5	Liquid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
	Solid PHOs	1	1	10
		2	1	
		3	1	
		4	1	
		5	1	
		6	1	
		7	1	
		8	1	
		9	1	
		10	1	
<b>TOTAL</b>				<b>100</b>



⑦

**Table 3c. Sample Collection Framework – Bakeries, Restaurants and Retail Outlets**

\*the total # of samples will depend on the # of PHO items available at each outlet

**Bakeries**

Outlet	Category	Item/brand	# of samples	Total # of samples*
HI-N1-BO1	Bakery fats	1	1	3
		2	1	
		3	1	
HI-N1-BO2	Bakery fats	1	1	3
		2	1	
		3	1	
HI-N2-BO1	Bakery fats	1	1	3
		2	1	
		3	1	
HI-N2-BO2	Bakery fats	1	1	3
		2	1	
		3	1	
LI -N1-BO1	Bakery fats	1	1	3
		2	1	
		3	1	
LI -N1-BO2	Bakery fats	1	1	3
		2	1	
		3	1	

LI -N2-BO1	Bakery fats	1	1	3
		2	1	
		3	1	
LI -N2-BO2	Bakery fats	1	1	3
		2	1	
		3	1	
<b>Subtotal, Bakery fats and oils</b>				<b>24</b>

**Restaurants**

Outlet	Category	Item/brand	# of samples	Total # of samples*
HI-N1-RO1	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	

HI-N1-RO2	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	
HI-N2-RO1	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	

HI-N2-RO2	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	
LI -N1-RO1	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	

LI -N1-RO2	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	
LI -N2-RO1	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	

LI -N2-RO2	Cooking/ Frying oils	1	1	3-9
		2	1	
		3	1	
	Bakery fats (if available)	1	1	
		2	1	
		3	1	
	Spreads (if available)	1	1	
		2	1	
		3	1	
<b>Subtotal, Restaurant Fats and Oils</b>				<b>28-96</b>

## Markets/Supermarkets

Outlet	Category	Item/brand	# of samples	Total # of samples*
HI-N1-MO1	Cooking/ Frying oils	1	1	9
		2	1	
		3	1	
	Bakery fats	1	1	
		2	1	
		3	1	
	Spreads	1	1	
		2	1	
		3	1	
HI-N2-MO1	Cooking/ Frying oils	1	1	9
		2	1	
		3	1	
	Bakery fats	1	1	
		2	1	
		3	1	
	Spreads	1	1	
		2	1	
		3	1	

LI-N1-MO1	Cooking/ Frying oils	1	1	9
		2	1	
		3	1	
	Bakery fats	1	1	
		2	1	
		3	1	
	Spreads	1	1	
		2	1	
		3	1	
LI-N2-MO1	Cooking/ Frying oils	1	1	9
		2	1	
		3	1	
	Bakery fats	1	1	
		2	1	
		3	1	
	Spreads	1	1	
		2	1	
		3	1	
<b>Subtotal, Retail Fats and Oils</b>				<b>36</b>
<b>TOTAL, Bakery, Restaurant and Retail Fats and Oils</b>				<b>88-156</b>