

**SURVEY PROTOCOL ON *TRANS*-FATTY ACIDS  
IN HUMAN POPULATION**

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## ACKNOWLEDGMENTS

This protocol is released by World Health Organization (WHO). U.S. Centers for Disease Control and Prevention (CDC) and Resolve to Save Lives (RTSL) provided technical assistance in the development of the document.

The following are the main technical contributors:

CDC: Dr. Chaoyang Li, Dr. Hubert Vesper, Dr. Heather Kuiper, and Dr. Patricia Richter provided technical assistance on the development of the protocol. Jennifer Sabatier and Curtis Blanton reviewed and commented on the chapters related to survey design and statistical methods. Lauren Billick and Kristy Joseph reviewed and commented on the chapters related to survey plan, implementation, and dissemination.

RTSL: Dr. Laura Cobb and Lindsay Steele reviewed the entire document and provided comments and revisions.

WHO invited reviewers: Dr. Diana Doell (US FDA), Dr. Susana Pereira Casal Vicente (University of Porto, Portugal), Dr. Renuka Jayatissa (Dept. of Nutrition, Medical Research Institute, Sri Lanka), Dr. Anchalee Chittamma (Mahidol University, Thailand), and Dr. Shah Mahfuzur Rahman (Institute of Public Health, Bangladesh) reviewed and commented on the entire document.

## 1. INTRODUCTION AND BACKGROUND

Elimination of industrially-produced *trans*-fatty acids (TFA) from the global food supply has been identified as one of the priority targets of the WHO strategic plan, the 13<sup>th</sup> General Programme of Work (GPW13). The REPLACE package (<http://www.who.int/nutrition/topics/replace-transfat>) provides six strategic actions to ensure the prompt, complete, and sustained elimination of industrially-produced TFAs from the food supply:

- **RE**view dietary sources of industrially-produced TFA and the landscape for required policy change.
- **P**romote the replacement of industrially-produced TFA with healthier fats and oils.
- **L**egislate or enact regulatory actions to eliminate industrially-produced TFA.
- **A**ssess and monitor TFA content in the food supply and changes in TFA consumption in the population.
- **C**reate awareness of the negative health impact of TFA among policy makers, producers, suppliers, and the public.
- **E**nforce compliance of policies and regulations.

The overarching goal of this survey protocol is to provide general guidance on conducting TFA surveillance in human populations and ensure comparability of data across different time periods, populations, and regions. The main purposes of the survey protocol include:

- Providing step-by-step guidance
- Recommending uniform methods in analysis and reporting
- Fostering standard procedures

## **2. SURVEY DESIGN AND SAMPLE SIZE ESTIMATION**

### *2.1 Scan and Assess National or Subnational Health Examination and Nutrition Surveys*

The goal of TFA surveillance is to establish baseline TFA levels and to monitor their changes over time in the population, using stored blood specimens collected through existing or ongoing national or subnational health examination and nutrition surveys. The advantages of using existing blood specimens include cost savings, generating nationally or sub-nationally representative estimates, and linking TFA measures with other demographic, socio-behavioral, physical, or biochemical measures from the original survey. An alternative approach in countries without stored blood specimens from existing or ongoing national or subnational surveys is conducting a small-scale survey with a purposive sampling method, measuring TFA levels in a defined (usually high-risk) subpopulation with a high TFA consumption.

A random or probability sampling method is recommended as a means of sample selection whenever possible because randomization reduces biases and allows for generalizability of results to the entire population. However, when there is lack of information on TFAs in foods or in plasma, or there are no blood specimens that were stored in existing surveys or to be collected in ongoing surveys, or there is constraint in financial resources and logistics in a country, a purposive sampling (also known as judgmental sampling) method may be considered to collect a small number of blood specimens to preliminarily estimate the blood TFA levels and their variations in selected populations or groups. Since purposive sampling is a non-probability and judgmental method, the results from the purposive samples may not be representative or generalizable to the general population, but the information could potentially be useful in generating preliminary data (e.g., a pilot study) that may be needed for sample size determination when designing a national or subnational survey. A number of purposive sampling techniques may be used to select samples.<sup>2</sup>

Many countries have existing or ongoing national or subnational health examination and nutrition surveys with blood specimens collected and stored. The WHO STEPS survey has been implemented in over 100 countries or regions (<http://www.who.int/chp/steps/reports/en/>). In addition, the Demographic and Health Survey (DHS) has been conducted in 92 countries or regions (<http://dhsprogram.com/data/available-datasets.cfm>). Blood specimens were collected during certain years in some of these countries.

The following table provides examples of national or subnational surveys:

**Table 2.1.1 Examples of National or Subnational Health Examination or Nutrition Surveys**

Surveys	National or subnational	Examples (year; country)	Cycle	Blood specimens collected and stored
<b>National Nutrition or Health Examination Survey</b>	National or subnational	<ul style="list-style-type: none"> <li>• National Health and Nutrition Examination Survey (every 2 years; United States)</li> <li>• National Nutrition Monitoring Bureau (NNMB) Surveys (every 5 years since 1975-1979 2011-2012; in 10 states of India)</li> <li>• National Health Examination Survey (NHES-5, 2013; Thailand)</li> <li>• National Nutrition Survey (2011; Pakistan)</li> <li>• Mexico National Survey of Health and Nutrition (2012; ENSANUT2012; Mexico)</li> <li>• National Health Survey (2013; Brazil)</li> <li>• National Turkey Nutrition and Health Survey (2016; Turkey)</li> </ul>	Every 2-5 years	In some surveys
<b>STEPS†</b>	National or subnational	In 122 countries or provinces (states)	Every 3-5 years	In some countries or provinces (states)
<b>DHS‡</b>	National	In 92 countries	Every 5 years	In some countries or years

† WHO STEPS: <http://www.who.int/chp/steps/reports/en/>.

‡ DHS: <http://dhsprogram.com/data/available-datasets.cfm>.

A survey scan (i.e., systematic literature review, web search, or expert consultation) can be performed to identify existing or ongoing national or subnational health examination or nutrition surveys in a country. The following methods could be considered for the survey scan:

- Google, Google Scholar, or similar search engines in different countries
- PubMed search or other literature search tools for published and unpublished work or similar search tools in different countries
- WHO website
- Expert consultations

Key words for the survey scan may include “nutrition”, “health examination”, “survey”, “surveillance”, “study”, “biochemical”, and “blood (plasma, serum, or erythrocyte membrane)” or “adipocyte membrane”.

## *2.2 Sampling Methods and Sample Size Estimation*

A subsample can be randomly selected from the total blood specimens collected in existing or ongoing national or subnational surveys to conduct a pilot study, a full-scale baseline survey, or both. The information of the original survey design and de-identified information of the total stored blood specimens must be used when selecting the subsamples.

In many countries, data on the population standard deviations (SD) for TFAs in blood and effect size (ES) for the change in TFA levels are unavailable. Estimation of sample size could be made based on available data from previous studies, published reports in literature, or a pilot study. For example, information from the National Health and Nutrition Examination Survey (NHANES;

<https://wwwn.cdc.gov/nchs/nhanes/>), a program of surveys conducted by the U.S. CDC that uses a statistically representative sample of the noninstitutionalized, civilian U.S. population, can be used to estimate sample size required for TFAs.

In general, the standard statistical formulas for sample size estimations are based on the assumption of a normal distribution of the outcome.<sup>3</sup> Data from the NHANES surveys show that the distributions of the plasma TFA levels are skewed to the right; therefore, a natural logarithm transformation of plasma TFA measures is recommended to approximate a normal distribution.

To estimate the required sample size, the following information is needed:

- Target population: general population (nationally or sub-nationally representative sample), or restricted high-risk subpopulation (representative or purposive sample).
- Mean plasma levels of TFAs in the target population: average levels of TFA consumption from all food sources.
- Standard deviation or variance of plasma TFA measures in the population: variability or heterogeneity of plasma TFA levels in the target population.
- Statistical significance level ( $\alpha$ ) or confidence level: two-sided  $\alpha = 0.05$ , 95% confidence level ( $Z_{1-\frac{0.05}{2}} = 1.96$ ).
- Margin of errors: critical value of a predetermined confidence level (1.96 for 95% confidence)  $\times$  standard error (SE).
- Statistical power ( $1 - \beta$ ): 80% ( $Z_{1-0.20} = 0.84$ ).
- Effect size: small (0.2), medium (0.5), or large (0.8).

Based on the NHANES 2009-2010 data, the mean level of the sum of four TFAs in natural log scale in the US adult population was 3.6 (95% confidence interval [CI]: 3.5-3.7; geometric mean: 37.1; 95% CI: 35.8-38.4). The population SD of sum of the four TFAs in natural log scale among the US adults was 0.5, ranging between 0.4 and 0.6. The margin of errors (MOE) ranged between 0.02 and 0.18. The relative standard errors ranged between 1.0% and 9.0%. The ES ranged between 1.2 and 1.8, with a relative reduction of 54% in plasma TFA levels in 10 years (from 1999-2000 to 2009-2010).<sup>1</sup> The population estimates of the total sample in NHANES (n=1,854 in 1999-2000, n=2,623 in 2009-2010) and three subsets selected from the total sample using a simple random sampling (SRS) method (n=160, 320, 480, respectively) on plasma TFA levels are similar. In the NHANES 1999-2000 survey, percentage of the missing data for the four TFAs was ranging from 9.0% to 15.0%, whereas in the NHANES 2009-2010 survey, the percentage of missing data for the four TFAs was ranging from 1.0% to 7.0% in the total sample and three SRS subsamples.

### 2.2.1 Sample size estimation for descriptive studies

For descriptive measures, sample size estimation will be based on the population SD and desired MOE using the following formula:<sup>4</sup>

$$n = \left( \frac{Z_{1-\frac{\alpha}{2}} \sigma}{MOE} \right)^2 \approx 4(\sigma^2 / MOE^2), \text{ where } \sigma \text{ is the population SD, } MOE = Z_{1-\frac{0.05}{2}} \times SE \\ = 1.96 \times SE, \text{ is the margin of error corresponding to the 95\% confidence level.}$$

Table 2.2.1.1 provides sample size estimation for plasma TFA measures based on three assumptions for population SDs and four desired levels for MOEs:

**Table 2.2.1.1 Sample size estimation based on population SD ( $\sigma$ ) and MOEs**

Population estimate of SD in natural log scale, $\sigma$	Sample size estimation				Adjusting for missing data (20%)			
	MOE				MOE			
	0.1	0.08	0.06	0.05	0.1	0.08	0.06	0.05
0.4	64	100	178	256	80	125	223	320
0.5	100	156	269	400	125	195	336	500
0.6	144	225	400	576	180	281	500	720

MOE = margin of error. SD = standard deviation.

### 2.2.2 Sample size estimation for comparative studies or hypothesis testing

For the comparative studies or hypothesis testing, differences between two sample means will be tested. Therefore, the sample size estimation will be based on statistical accuracy ( $\alpha = 0.05$ , 95% confidence level, 1.96), statistical power ( $1 - \beta = 80\%$ , 0.84) and expected ES using the following formula:<sup>4</sup>

$$n = 2 \left( \frac{Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}}{ES} \right)^2 = 2 \left[ \frac{(1.96 + 0.84)^2}{ES^2} \right] \approx \frac{16}{ES^2}, \text{ where } ES = \frac{|\mu_1 - \mu_0|}{\sigma}, \\ |\mu_1 - \mu_0| \text{ is the difference between two population means, and } \sigma \text{ is the population SD.}$$

With available data in previous studies or published literature, the difference between two sample means of the TFA measures,  $|\bar{y}_2 - \bar{y}_1|$ , and the pooled SD ( $SD_{pooled}$ ) of the TFA measures in two samples can be used to estimate the ES:

$$SD_{pooled} = \sqrt{\frac{SD_1^2(n_1-1) + SD_2^2(n_2-1)}{n_1 + n_2 - 2}},$$

where  $SD_1$  and  $SD_2$  are the standard deviations of the TFA measure in sample 1 and sample 2;  $n_1$  and  $n_2$  are the sample sizes of sample 1 and sample 2.

It has been proposed that an ES of 0.2, 0.5, and 0.8 be small, medium, and large respectively for two-group comparisons.<sup>5</sup>

Table 2.2.2.1 provides sample size estimation for plasma TFA measures based on assumptions for confidence level (95%), statistical power (80%), and four ES:

**Table 2.2.2.1 Sample size estimation based on 95% confidence, 80% statistical power, and ES in natural log scale**

	ES			
	0.4	0.3	0.25	0.2
Sample size	100	180	256	400
Adjusting for missing data (20%)	125	220	320	500

### 2.2.3 An illustration of subsample selection based on the simple random sampling method

The above sample size estimations provide minimum sample size requirement for a TFA survey based on a simple random sampling design and the information can be used to select a random subsample from the entire stored blood specimens. In most of the national or subnational surveys, a complex survey design (e.g., multistage, probability sampling design, with stratification or clustering) may be used. Therefore, the information of the complex survey design (e.g., strata, cluster, and sample weights of the participants) assigned in the original surveys should be accounted for when selecting the subsamples.

The following demonstrates an example on how a random subsample can be generated using a random number table or a computer program based on the existing total blood specimens. Here are the major steps:

- 1) Determine the sampling frame: this could be all the blood specimens collected in the existing or ongoing survey.
- 2) Stratify the total blood specimens by relevant factors such as geographic location, socioeconomic status, age, and sex to ensure the balance of sample distribution, or probability proportional to size according to the stratification in the original survey.
- 3) Based on the total sample size estimation, allocate number of samples for each stratum. Oversampling may be determined by assigning a higher proportion of samples according to available information or a priori assumption.
- 4) List of sequence numbers of all blood specimens in each stratum and randomly select the specimens needed using the random number table or a computer program.

Table 2.2.3.1 demonstrates an example of selecting 100 samples from a total of 1,000 stored blood specimens stratified by SES, age, and sex in a hypothetical region.



**Table 2.2.3.1 An example of explicit stratified random sampling with oversampling**

Within each region	Socioeconomic status (SES)	Age group	Sex	Total stored specimens	% samples <sup>†</sup>	Number of samples
	High SES	18-44 y	Male	125	15.0%	15
			Female	125	15.0%	15
		45+y	Male	125	10.0%	10
			Female	125	10.0%	10
	Low SES	18-44 y	Male	125	15.0%	15
			Female	125	15.0%	15
		45+y	Male	125	10.0%	10
			Female	125	10.0%	10
Total samples in each region				1,000	100.0%	100
Grand total for the pilot study (3 regions)						300

<sup>†</sup> Proportion of samples to be selected, with oversampling in the age group 18-44y.

### 3. DEVELOPMENT OF PROPOSAL AND IMPLEMENTATION PLANS

#### 3.1 Preparation of Project Proposal

After a survey with stored blood specimens has been identified and meets ethical rules and regulations, a project proposal should be developed by an implementation agency, with help from national or international collaborators or partners. A proposal will be prepared, competed, and submitted to the corresponding country governmental agencies for review and clearance, with technical assistance from collaborators or partners (e.g., CDC, WHO) during preparation and drafting of the TFA surveillance proposal. Refer to Annex A for a proposal template.

#### 3.2 Pilot Study

A pilot study can play an important role before conducting a full-scale project. A pilot study aims to assess the feasibility of methods and procedures and predict an appropriate sample size for the full-scale survey.

The objectives of the pilot study for the TFA survey are:

- To build or enhance laboratory capacity and quality assurance for the TFA testing.
- To obtain preliminary data on statistical measurements (e.g., means, SDs, SEs, MOR, or RSE) and the patterns of missing data for the four major TFAs in order to improve sample size estimation.
- To raise awareness to spur action.

The pilot study is descriptive, thus the sample size estimation could be referred to Table 2.2.1.1. The sample size for a pilot study could range between 80 and 720, depending upon the

heterogeneity of TFA consumption across demographic and socioeconomic subgroups or geographic locations, accuracy of estimates, and financial or logistic constraints.

### *3.3 Full-Scale Baseline Survey*

The objectives of the full-scale baseline survey are:

- To obtain population estimates of the plasma/serum TFA levels overall and by sex, age, SES, and regions.
- To establish a baseline for monitoring the change in plasma/serum TFA levels in repeated surveys (e.g., 5-year or 10-year interval).

Baseline survey data will be used to estimate the TFA consumption in the population and for monitoring the change in TFA consumption in the future; thus the sample size estimation can be referred to Table 2.2.2.1. Based on the available data in NHANES,<sup>1,6</sup> the ES was large between 1999-2000 and 2009-2010 (10 years apart). However, most other national or subnational surveys may be conducted every 5 years, therefore, the ES is expected to be between small and medium. Therefore, the sample size for the baseline survey can vary between 125 and 500, depending upon expected ES and financial or logistic constraints, given 95% confidence and 80% statistical power.

### *3.4 Ethical and Technical Review and Clearance*

The proposal must be reviewed and approved by an Institutional Review Board (IRB), or ethical review board (ERB) to ensure human subject's rights and welfare are protected. The meetings of IRB or ERB in an institute or agency are typically held regularly (e.g., every 2 or 3 months) to review agency-lead studies. In addition, administrative or technical review of the proposal should be conducted by the government in many countries to ensure priority and sustainability.

### *3.5 Proposal Submission, Review and Timelines*

An application package needs to be prepared according to the requirement of the agencies that review and make a decision on the proposal. The major documents in the application package may include: project proposal, IRB or ERB review approval, forms, and supporting documents. Setting up timelines for the preparation and submission of the application package will ensure the application be received, reviewed, and approved by the governmental agencies in a timely manner and avoid unnecessary delays.

### *3.6 Integration of TFA Measures in National Health and Nutrition Surveys*

It can be cost-effective, feasible, and a sustainable approach to integrate the TFA component or module into the ongoing national or subnational health and nutrition surveys for assessing the plasma/serum TFA levels in the general population at baseline and monitor the trends over time.

## 4. COLLECTION, TRANSPORTATION, AND STORAGE OF BLOOD SPECIMENS

### *4.1 Stored Specimens of Existing Surveys*

TFAs in blood can be analyzed using frozen plasma, serum or red blood cells. Specimens can be kept refrigerated for 3 days. For long-term storage, specimens are stored at -70 °C. Studies have shown that storage of fatty acids in all lipid classes at -60 °C resulted in negligible changes in concentration.<sup>7</sup> Specimens are stable for TFA analysis at least 5 years, if stored at -70 °C.<sup>8</sup> Note polyunsaturated fatty acids may require different storage conditions or may have shorter storage stability. This needs to be considered when analyzing regular fatty acids along with TFAs.

The amount of specimen required for TFA analysis depends on the sensitivity of the instrumentation used for analysis. Typically, a specimen volume between 100 – 200 µL is needed for analysis. To allow for repeat analysis, a specimen volume of 0.5 mL is preferable. Hemolized specimens should be avoided as TFAs in red blood cell and serum or plasma reflect different periods of intake and may make interpretation of findings difficult.

### *4.2 Collection, Transportation, and Storage of New Blood Specimens*

Fasting specimens (i.e., specimens collected in the morning after at least 8 hours overnight fast) are recommended to minimize variability caused by recent food consumption (<https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf>). Collection of serum using serum separator tubes should be avoided as the gel used in these tubes may contain fatty acids. Specimens should be transported frozen or refrigerated. The specimen should be transported in 2.0-mL cryogenic vial with external screw-caps. These cryovials should be labeled in accordance with relevant policies and regulations.

For the details on the requirements, room set up, equipment, and supplies for collecting blood specimens, refer to WHO STEPS surveillance manual Step 3 ([http://www.who.int/ncds/surveillance/steps/STEPS\\_Manual.pdf?ua=1](http://www.who.int/ncds/surveillance/steps/STEPS_Manual.pdf?ua=1)).

## 5. LABORATORY CAPACITY BUILDING

### *5.1 Assessment of Laboratory Capacity*

Analysis of blood specimens should be performed in a biosafety level 2 laboratory. The laboratory needs to have space and power supplies to accommodate analytical equipment. It should have a chemical fume hood and have the infrastructure in place to store, handle and dispose of hazardous chemicals. For examples of chemicals, instrumentation and supplies used for analyzing TFAs, see the laboratory protocol in Annex B.

Staff performing the laboratory tasks need to have appropriate training for safely handling blood specimens, hazardous chemicals, and must be familiar with the laboratory protocol. Furthermore, staff need to have training on laboratory equipment operation from qualified trainers or staff.

### *5.2 Training and Technical Assistance*

Staff analyzing TFAs in blood need to obtain training from qualified and experienced staff. For example, staff operating the TFA laboratory procedure described in Annex B can obtain training through CDC or the regional laboratories certified by CDC or WHO for processing specimens, analyzing measurement data, and quality control procedures. In addition, staff need to obtain special training from the manufacturer of the laboratory equipment on appropriate operation and maintenance of the equipment. Furthermore, staff need to obtain training on handling chemicals and blood specimens in compliance with local regulations.

To ensure measurement quality, the laboratory needs to have a quality system in place. The quality system may include, but is not limited to, the following:

- documentation of all steps in the specimen handling process (from receiving specimens to reporting specimen results)
- performance of tests and their documentation to ensure instrumentation and reagents perform as needed
- use of quality control specimens analyzed with each set of specimens<sup>9</sup>
- participation in external quality assurance schemes

### *5.3 Preparation of Laboratory Equipment and Materials*

Laboratory equipment needs to be prepared according to manufacturer's recommendations. Additional tests may be required to ensure the instrumentation is performing as intended for TFA analysis. Materials such as solutions and reagents need to be prepared as outlined in the laboratory procedure used by the laboratory. Annex B includes procedures for preparing materials used for this laboratory procedure.

### *5.4 Multisectoral Collaborations and Sharing of Resources*

TFA surveillance in human blood and in foods may involve multiple governmental sectors or academic institutions. A multisectoral coordination committee or working group may help facilitating the TFA surveillance activities in a country to:

- Identify possible governmental sectors or academic institutions with interest or laboratory capacities for TFA testing.
- Share resources such as equipment, technology, expertise, information or data, and staff on TFA testing in human blood or foods, if possible.

## **6. LABORATORY STANDARD OPERATING PROCEDURES**

Depending on the instrumentation and materials available in the laboratory, different standard operating procedures (SOPs) are needed. SOPs need to be customized to the laboratory where the SOP is used. The SOP needs to include detailed information on all relevant tasks performed in the laboratory for analyzing TFAs including quality control procedures. Furthermore, the SOP may need to be compliant with local policies and regulations.

Annex B provides an example for an SOP that has been reviewed by an expert panel.

### *6.1 Method Performance*

Analytical method performance depends on the TFAs being analyzed and the SOP being used. The analytical performance of the method needs to be documented in the SOP. The SOP shown in Annex B provides information on analytical performance.

### *6.2 Quality Assurance, Proficiency Testing/External Quality Assessment (PT/EQA)*

The quality of the measurement result is assured by maintaining the method performance at the level needed for the intended use. Method performance needs to be assessed periodically through internal assessments and participation in PT/EQA programs. This includes assessment of individual components used in this method (i.e., accuracy of pipettes needs to be assessed periodically). If changes in analytical performance occur, then the reasons for these changes need to be investigated and corrective actions may need to be performed.

The laboratory needs to participate in regular EQA schemes to ensure the quality and consistency of measurement results. In this scheme, a set of unknown specimens are analyzed by the laboratory and results are reported to the PT/EQA provider where results are analyzed and findings are reported back to the laboratory. CDC is one organization providing PT/EQA schemes for analyzing TFAs.

### *6.3 Test Result Analysis and Reporting System*

Test results should be reported as requested by the organization or principal investigator. For transparency, documentation should be in place to trace test results back to the original instrument data. Preferred reporting format as concentrations on the four individual TFAs and the sum of four TFAs. If reported as a percentage of total, then the fatty acids measured should be mentioned so that it can be reproducible.

## **7. SURVEY DATA ANALYSIS**

### *7.1 General Guidelines on Survey Data Analysis*

The national or subnational surveys generally use a complex sampling design to generate representative samples; therefore, analysis must account for stratification, clustering, and unequal selection probabilities to obtain valid point estimates, SEs, confidence intervals, and tests of hypotheses. The variance may be either underestimated or overestimated if the sampling design of a survey is not properly accounted for. Since the subsample for the TFA testing is randomly selected from the total blood specimens of the original survey, all information of the survey design (strata, cluster, sample weights) should be included in data analyses and reports. The sample weights may be recalculated for the subsample, particularly when oversampling is used.

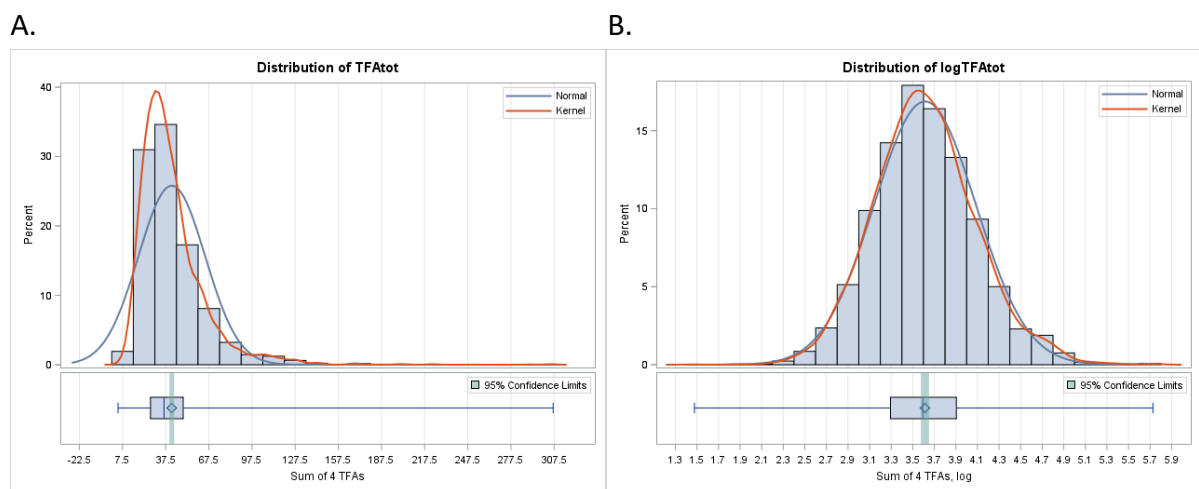
It is suggested to report the weighted point estimate along with the upper and lower bound 95% confidence interval (95% CI). The 95% CI can be calculated based on the point estimates and their SEs (i.e., lower bound = point estimate – 1.96 × SE; upper bound = point estimate +

1.96 × SE) using appropriate methods for variance estimation of complex survey data. The commonly used variance estimation methods include Taylor Series Linearization (TSL), Jackknife Repeated Replication (JRR) or Balanced Repeated Replication (BRR). TSL is recommended to ensure comparability among Country Reports.

Currently, several major statistical packages (e.g., SAS®, SUDAAN®, SPSS®, STATA, and R) provide procedures or modules for analyzing data with complex survey design. It is recommended to use statistical tests to determine differences between subgroups. Differences between point estimates should be considered statistically significant if  $p < 0.05$  or the 95% confidence interval does not contain zero. For details on survey data analysis, refer to the book “Analysis of Health Surveys”.<sup>10</sup>

### 7.2 Estimation of Mean TFA Levels in Plasma/Serum

In general, the means, standard deviations, and standard errors of continuous outcome variables are estimated with assumption of a normal distribution in most of the standard statistical software programs. Because the distributions of plasma TFA levels are skewed to the right (Figure 7.2.1 A), a natural log-transformation can be used to approximate a normal distribution (Figure 7.2.1 B) for the plasma TFA measures.



**Figure 7.2.1. Distribution of sum of the four TFAs in plasma in the original metric scale (A) and in natural log-transformed scale (B), NHANES 2009-2010.**

The following summarizes the major steps of estimation of mean TFA levels in blood:

- Check the frequencies and distributions of the TFA variables.
- Handle missing data: with large sample size and adequate statistical power or data are missing at random or missing completely at random, a listwise deletion may be used. With low proportion of missing data ( $\leq 5\%$ ), impute values below the limits of detection (LOD) with  $\text{LOD}/\sqrt{2}$ . When the proportion of missing data is high ( $> 5\%$ ), specific statistical methods may be used to impute the missing data (e.g., multiple imputation or extrapolation).<sup>11,12</sup>
- Log-transformation and re-check the distribution of log-transformed variables.

- Use of the standard statistical procedures or modules for survey data to obtain estimates of mean TFA levels, their SEs, and the 95% confidence intervals of log-transformed variables.
- Take exponentiation of the final results (logarithmic means and 95% confidence intervals) of log-transformed variables to convert the log-transformed values back to their original metric values (geometric means and 95% confidence interval).

Alternatively, geometric means and the 95% confidence intervals of the data with original scale could be directly estimated when such procedures for survey data are available in the statistical software package.

In addition, demographic characteristics of the participants can be reported, along with the sample size, geometric means, and 95% confidence intervals, by sex, age, socio-economic status, and regions. Refer to Table 1 in Annex C for the example that can be adapted to present additional information.

### 7.3 Assessment of Change: Methods for Testing Differences

In addition to estimation of descriptive TFA measures at a time point (e.g., baseline), difference between two time points (e.g., before or after the implementation of TFA policy) will also be estimated. Reporting the estimates at two surveys (e.g.,  $y_1$  and  $y_2$ ) and their relative percentage difference between the two estimated measures are recommended. Refer to Table 2 in Annex C for the example that can be adapted to present additional information.

The difference of two estimates,  $D = y_2 - y_1$ , assesses the extent of difference or change between two time points. The relative percentage difference,  $RD$ , can be interpreted as the percentage of the difference in estimate in time 2 as it decreases or increases compared to time 1, i.e.,

$$RD = (y_2 - y_1)/y_1 \times 100\%.$$

The following provides the formulas for estimating relative percent difference and its 95% confidence interval.

Estimated relative percentage difference:

$$\widehat{RD} = (\hat{y}_2 - \hat{y}_1)/\hat{y}_1 \times 100\% = (\frac{\hat{y}_2}{\hat{y}_1} - 1) \times 100\%$$

$$SE \text{ of } \widehat{RD} (SE_{\widehat{RD}}) \approx \frac{\hat{y}_2}{\hat{y}_1} \times \sqrt{\frac{\hat{V}(\hat{y}_2)}{\hat{y}_2^2} + \frac{\hat{V}(\hat{y}_1)}{\hat{y}_1^2}}, \text{ where } \hat{V}(\hat{y}_1), \hat{V}(\hat{y}_2) \text{ are variance estimates for } \hat{y}_1, \hat{y}_2, \text{ respectively.}^{13}$$

$$95\% \text{ confidence interval for } \widehat{RD} = \widehat{RD} \pm 1.96 \times SE_{\widehat{RD}}$$

#### 7.4 Age Adjustment

Age compositions may differ among various subpopulations, across different geographic regions, and over time, and TFA consumptions may also differ across age groups. Therefore, age adjustment is needed when comparing the estimates of mean plasma TFA levels at two or more time points in a population. By definition, age adjustment is to apply observed age-specific estimates of an indicator in the survey population to age composition of a hypothetical 'standard' population to eliminate differences in crude estimates that result from differences in the age composition.

The direct age-adjusted estimates are generally used when two estimates at different time periods are compared. Age adjustment follows four basic steps:

- 1) Choose a standard population. In general, the standard population can be a single population, a pooled population, or an external population. For the global NCD mobile phone survey, it is recommended that WHO's world standard population be used for calculating age-adjusted rates to facilitate comparison across different countries.<sup>14</sup>
- 2) Apply the age-specific estimate (rate or mean) in the survey population to the age composition of the standard population. This is typically done in 5- or 10-year age groups. The age-adjusted estimate is obtained by summing the products of the age-specific estimate ( $\hat{x}_i$ ) in the survey population and the proportion of people in the age group ( $w_i$ ) of the standard population.

$$\text{Age-adjusted estimate (AAE)}^{15} = \sum_{i=1}^k \hat{y}_i \times w_i$$

- 3) Compute variance estimate for the age-adjusted estimate. The variance of the age-adjusted estimate is computed by summing the products of the variance of the age-specific estimate ( $V_{\hat{y}_i}$ ) and the square of proportion of people in the age group ( $w_i^2$ ) of the standard population.

$$\text{Variance of age-adjusted estimate (V}_{AAE}) = \sum_{i=1}^k V_{\hat{y}_i} \times w_i^2$$

- 4) Compute the 95% CI based on the age-adjusted estimate and its SE:

$$95\% \text{ CI} = \text{AAE} \pm 1.96 \times \sqrt{V_{AAE}}$$

Note that the identical standard population and age composition should be used consistently once they are chosen for age adjustment. Because an age-adjusted estimate is a hypothetical measure, it should be reported only for the purpose of comparisons, assuming that both groups have identical age compositions. If age compositions are similar or there is no significant difference in mean plasma TFA levels, unadjusted (or crude) estimates should be used or reported. The unadjusted mean plasma TFA levels can also be important because it reflects the actual consumption in the population. Refer to Table 3 in Annex C for the calculation of age-adjusted estimates.



## 8. DISSEMINATION AND REPORTING

The data or findings should be made available to a scientific audience through survey reports and peer reviewed journal articles and to the general public through fact sheets and website articles. Dissemination materials should be developed in partnership with countries, in order to ensure effective utilization of the results of the TFA surveys.

### *8.1 Survey Reports*

Survey reports should be in a format that allows comparison to other surveys. Refer to Annex C for example tables that can be adapted to present additional information. The purpose of the survey reports is to summarize the design, implementation, results, and recommendation of the TFA survey. The report can be used by stakeholders to develop intervention programs.

The following components are recommended when writing a survey report for TFA surveillance:

- Executive Summary: objectives, survey design, key findings, and conclusions.
- Introduction: burden of TFAs and related CHD, current policies on TFAs, and objectives of the TFA Survey
- Methodology: study population, sampling design, blood specimen collection, transportation and storage, laboratory testing, and statistical analysis
- Results: sample and population characteristics, mean plasma (serum) levels of TFAs, change in TFA levels between [TIME 1] and [TIME 2] (if two surveys were conducted)
- Conclusion
- Recommendation
- References
- Appendices

Refer to Annex D for recommended format of a survey report.

### *8.2 Factsheets*

One-page fact sheets can be developed to disseminate information in an abbreviated fashion to present study findings for a specific target audience. Below are elements that can be included:

- Objectives – list 1-2 main objectives of the TFA surveillance
- Methods – describe survey design, sampling method, sources and collection of blood specimens, laboratory testing, and statistical analysis
- Highlights – demonstrate plasma TFA levels overall and by sex, age, SES, and region
- Table(s) – present 1 or 2 tables for the main results and findings
- Charts(s) – present 1 or 2 figures
- Conclusions and implications – summarize major findings, public health implications, and recommendation

### *8.3 Scientific Articles*

Larger dissemination of the findings can be published as scientific articles in peer reviewed journals. The following would be journals that could publish this information including American Journal of Clinical Nutrition, Public Health Nutrition, or other similar journals. Journals for more general medical and health professional or journals with policy implications may also be considered.

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## 10. ANNEXES

### *Annex A: Proposal Template*

#### **Trans-Fatty Acid Surveillance in [Country]**

1. Project Title
2. Contact Person (Principle investigator for the project)
3. Project Abstract Summary (No more than 1 page)
4. Background
5. Approach
  - 5.1. Objectives
  - 5.2. Expected Outcomes
  - 5.3. Strategies and Activities
    - 5.3.1. Survey Design
    - 5.3.2. Specimen Source
    - 5.3.3. Sampling Method and Sample Size Estimation
    - 5.3.4. Ethical Considerations
    - 5.3.5. Laboratory Testing, Quality Control, and Data Management
    - 5.3.6. Data Analysis and Dissemination
6. Work Plan (Activities and timelines, performance measures, data sources and target population(s), staff and administrative roles and functions, monitoring and evaluation plan)
7. Budget
8. Organizational Capacity of Applicants to Implement the Approach
9. Public Health System Impact Statement
10. PI's Biographical Sketch(es) with Job Description(s)
11. References

*Annex B: Laboratory Protocol - Analysis of Total Trans-Fatty Acids in Plasma and Serum by GC-NCI-MS*

The World Health Organization global laboratory protocol for analysis of total trans-fatty acids in plasma and serum by Gas Chromatography-Negative Chemical Ionization-Mass Spectrometry (GC-NCI-MS) was developed in collaboration with the US Centers for Disease Control and Prevention, National Centers for Environmental Health, Division of Laboratory Sciences.

The laboratory protocol can be accessed on the WHO REPLACE action package website:  
<https://www.who.int/nutrition/topics/replace-transfat/>

## Annex C: Example of Tables

**Table 1. Demographic characteristics and mean plasma TFA levels ( $\mu\text{mol/L}$ ), [year], [country]**

Characteristics	n	Weighted %	Palmitelaidic acid	Elaidic acid	Vaccenic acid	Linoelaidic acid	Sum of 4 TFAs
			Geomean (95% CI)	Geomean (95% CI)	Geomean (95% CI)	Geomean (95% CI)	Geomean (95% CI)
Overall							
Sex							
Male							
Female							
Age							
18-44y							
45-64y							
65+y							
SES							
High							
Low							
Region							
East							
South							
West							
North							

**Table 2. Change in mean plasma TFA levels ( $\mu\text{mol/L}$ ), from [time 1] to [time 2], [country]**

TFAs	TFA Survey at Time 1			TFA Survey at Time 2			Change from Time 1 to Time 2	
	n	Geometric Mean, $\mu\text{mol/L}$	95% CI	n	Geometric Mean, $\mu\text{mol/L}$	95% CI	Difference in Geometric Mean, $\mu\text{mol/L}$	% Change
Palmitelaidic acid								
Elaidic acid								
Vaccenic acid								
Linoelaidic acid								
Sum of 4 TFAs								

**Table 3. Computation of direct age-adjusted estimate of plasma TFA levels between two time points or regions, [Country], [Year]**

Time 1			Time 2							
Age (y)	Estimate of the Survey Population		Standard Population Age-Adjustment Weight *	A × C	B <sup>2</sup> × C <sup>2</sup>	Estimate of the Survey Population	Standard Population Age-Adjustment Weight		F × H	G <sup>2</sup> × H <sup>2</sup>
	Mean TFA level	S.E.				Mean TFA level	S.E.			
	A	B	C	D	E	F	G	H	I	J
18-24			0.1684					0.1684		
25-34			0.2259					0.2259		
35-44			0.1998					0.1998		
45-54			0.1659					0.1659		
55-64			0.1202					0.1202		
65+			0.1197					0.1197		
Sum			1.00	Age-adjusted estimate at Time 1	Variance of age-adjusted estimate at Time 1			1.00	Age-adjusted estimate at Time 2	Variance of age-adjusted estimate at Time 2

\* Based on WHO World Standard Population Distribution (%), world average population between 2000-2025 (Ahmad OB, Boschi-Pinto C, Lopez AD, Murray CJL, Lozano R, Inoue M. Age standardization of rates: A new WHO standard. GPE Discussion Paper Series: No. 31. EIP/GPE/EBD World Health Organization 2001. Available at <http://www.who.int/healthinfo/paper31.pdf>).

**Recommended Template for Country Survey Report on Trans-Fatty Acid Surveillance**

Preface

Acknowledgements

Executive Summary

1. Introduction

1.1. Burden of Trans-Fatty Acids (TFAs) and Related Coronary Heart Disease in [Country]

1.2. Current Policies on TFAs in [Country]

1.3. Objectives of the TFA Survey

2. Methodology

2.1. Study Population

2.2. Sampling Design

2.3. Blood Specimens: Collection, Transportation, and Storage

2.4. Laboratory Testing

2.5. Statistical Analysis

3. Results

3.1. Sample and Population Characteristics

3.1. Mean Plasma/Serum Levels of TFAs

3.2. Change in TFA Levels between [TIME 1] and [TIME 2] (for repeated surveys)

4. Conclusion

5. Recommendation

6. References

7. Annexes

Annex A: Sample Design

Annex B: Blood Specimens

Annex C: Technical and Survey Staff

Annex D: Glossary of Terms