Chapter 18
Addressing Uncertainty and Variability in Total Diet Studies

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Introduction

Total diet studies (TDSs) are powerful tools for collecting data on the concentration of chemicals in food, estimating dietary exposure and undertaking risk assessments for these chemicals for population groups of interest. As with all scientific studies, uncertainty and variability that are encountered when conducting a TDS need to be considered when reporting and interpreting the findings.

Uncertainty in a TDS arises when there is insufficient information available to accurately determine the value of a particular parameter being investigated [1]. Uncertainty can, in principle, be reduced through additional research and more accurate data [2]. *Variability* in a TDS refers to the inherent variation in the parameters being investigated; it contributes to total uncertainty in an exposure assessment. Variability cannot be reduced through further research but can be better understood [1, 3]. It is important to document both the uncertainty and the variability in the data sources used in a TDS and to make some judgment regarding their impact on the dietary exposure estimates and overall risk assessment associated with the study.

The specific consideration of uncertainty in diet-related risk assessments is a developing science. Some recent publications in this area provide detailed discussion on uncertainty and variability in exposure assessments [1–3]. This chapter does not aim to summarize the comprehensive information provided in these publications

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or to discuss every area where uncertainty may be encountered. Instead, it aims to provide a brief consideration of the main areas of uncertainty for the purpose of conducting a TDS.

Three key principles have been identified for the consideration of uncertainty in exposure assessments [2]:

- Uncertainty analysis should be an integral part of risk assessments including dietary exposure assessments associated with a TDS.
- The level of detail of the uncertainty analysis should be based on a tiered approach and consistent with the overall scope and purpose of the assessment.
- Sources of uncertainty and variability should be systematically identified and evaluated in the risk assessment.

Where Do Uncertainty and Variability Occur in Total Diet Studies?

Uncertainty and variability can affect every aspect of a TDS, starting with the formulation of the objectives through to the characterization of the risk. In practice, when planning a TDS, the project manager needs to focus carefully on the sampling, measurement and dietary exposure assessment phases of a TDS, assuming that they are already clear on the objectives of the study and how the proposed survey design will achieve these objectives. The project manager should also consider, at the planning stage, how to incorporate detailed checking and review processes throughout the project to minimize errors, such as calculation and programming mistakes.

One of the challenges of conducting a TDS is to minimize uncertainty as far as possible within practical limits and to understand the major areas of expected variability. Acknowledging the limitations of the study and identifying and addressing areas where uncertainty and variability exist is seen as good practice in reporting TDSs. It also provides risk managers with important information to assist with the interpretation of the study's findings [4].

One of the key outputs of a TDS is the risk characterization. The risk characterization allows the comparison of exposure estimates with relevant established health-based guidance values, such as the Acceptable Daily Intake (ADI), Recommended Dietary Intake (RDI), Provisional Tolerable Weekly Intake (PTWI), acute Reference Doses (ARfD), etc. In comparing exposure assessments with health-based guidance values, it is important to note that there is also uncertainty in the establishment of the guidance value including the use of safety factors to account for inter- and intra-species variability. This chapter does not explore the uncertainty of health-based guidance values in detail; however a number of documents are available which address this area [5, 6].

Variability

Variability is an inherent property of biological systems. Levels of chemicals in foods can be highly variable, even within the same type of food. Many factors affect this variation including season, location of sample, soil types, agricultural practices, breed or cultivar, maturity of plants or animals, production and cooking methods, batch-to-batch variation in processed foods, and many others [7]. Due to the limited number of samples usually collected for analysis, a TDS can never capture all the variability that occurs in foods, but understanding the sources of variation can help in the design of the sampling frame and in the interpretation of results. Uncertainty associated with variability is likely to be most significant in the sampling phase and therefore a well-designed sampling plan is vital to capture the most representative sample of foods practicable.

Sampling Considerations Related to Variability

A key component of TDSs is the identification and collection of foods upon which analytical measurements are undertaken. Representative foods are first identified via a food list (see Chap. 6 – Preparing a Food List for a Total Diet Study). Each of the foods selected as being representative of total diet patterns will vary in chemical concentration over time. In addition, the pattern of variation in food chemical levels may differ according to the chemical being investigated. Therefore, before planning sampling, it is important to research the factors that may affect variation in levels of the chemicals being investigated in your TDS. Then the sampling plan can be designed to at least take account of the major sources of variation.

For example, for an unprocessed food, such as lettuce, it may be important to ensure that more than one variety of lettuce is collected, that samples cover the major growing practices (e.g. hydroponic production as well as traditional 'in ground' production), that samples were collected from different production regions (where relevant) and that samples are collected at different times of the year. For a manufactured food such as breakfast cereal, purchase location may be less relevant than for a fresh product, as there may be a limited range of producers who distribute their product nationwide. In this case consideration may be given to sampling products with varying formulations, with different batch numbers and/or packaged in different materials.

When levels of a food chemical are highly variable, it is preferable to draw on a large primary sample to generate a more robust estimate of the mean concentration of the chemicals in question. In addition, analysis of as many individual samples as possible will allow a better understanding of the magnitude of the variation in chemical levels around the mean. However where different samples are aggregated or composited prior to analysis, as a means of reducing costs and analysis time, additional uncertainty may arise because information on sample variability is reduced.

Sample Preparation and Variability

A key element of a TDS is the preparation of samples to a 'as consumed' state before analysis is undertaken. The preparation steps will vary according to the type of food and should reflect food preparation practices within each country. There is considerable variation in how people prepare foods, such as variation in cooking time, storage practices before cooking, cooking equipment used, etc. While the aim of preparation practices is to use what is assumed to be the most common cooking method, the chosen method will not cover all possibilities and therefore will not reflect the full variation in preparation techniques.

Variability and Selection of an Analytical Aliquot

Adequate homogenization of analytical samples is required to ensure that aliquots removed for analysis will be representative of the original samples. This may be difficult to achieve when deal with large bulk samples. For example, aflatoxin sampling plans require an initial 20 kg sample be ground to a fine powder.

Variability in Food Consumption Data Used to Estimate Dietary Exposure

Dietary exposure assessments conducted as part of a TDS require not only the chemical concentration data measured in the study, but also representative food consumption data for the population being studied. In the same way that a sample of foods can never capture the full variability of the food supply, a survey of food consumption will not cover the full variability in consumption patterns that an individual, group or population might follow, particularly where consumption data are collected over a short period of time (typically 24 h). However if a large, well designed food consumption survey is used as a basis for estimating dietary exposure to the chemicals measured in a TDS, this source of variability is likely to be minimized, at least in terms of the mean amounts of each food consumed in a population.

Uncertainty

In this section, sources of uncertainty are considered, aside from considerations associated with the innate variability of foods and food consumption patterns.

Uncertainty Associated with Sampling

Sampling uncertainty or error can arise for a number of reasons, such as when the wrong samples have been purchased or the samples have not been prepared or stored correctly. Each sampling step can introduce errors from a range of mechanisms, such as loss of analyte, contamination of samples within and/or from containers, spoilage of samples or inadequate detail to identify samples for analysis. To address these areas of uncertainty it is important to develop robust sampling plans and provide clear instructions on the collection, packing, recording and transportation associated with the foods being collected as part of the TDS. It is important to recognize that sampling protocols can never describe the action required by the sampler for every eventuality that may arise in the real world of selecting samples [8]. However protocols should be clear and concise to reduce the sampling uncertainty, as explained in Chap. 8 – Preparing a Procedures Manual for a Total Diet Study.

Measurement Uncertainty

There are many excellent references on measurement uncertainty and approaches to estimating it for any given analysis [1], for those readers who need more detailed information than that provided here. Analytical measurement uncertainty is an important consideration in TDSs. Measurement uncertainty may arise from many possible sources including sample preparation, matrix effects and interferences, environmental conditions, uncertainties of masses and volumetric equipment, reference values, approximations, instrument maintenance and calibration, experience of the analyst and assumptions incorporated in the measurement method and procedure. To address measurement uncertainty in TDSs, it is important to ensure that the laboratory selected to undertake the analysis work is accredited or to ensure that analytical methods are validated.

Random errors are present in all measurements and cause replicate results to fall on either side of the mean value. The random error of a measurement cannot be compensated for, but increasing the number of observations may reduce the magnitude of such errors. Systematic errors occur in most experiments. The sum of all the systematic errors in an experiment is referred to as the bias. They may go undetected unless appropriate precautions (e.g. validating the analytical method by use of standard reference materials) are taken [2]. Both random and systematic errors will affect measurement uncertainty.

The selection of instrument and method validation is an important consideration in a TDS. Measurement uncertainty can arise in analytical results if the instrument and method selected are not suitable to the analyte of interest. In practice the fitness for purpose of analytical methods applied for routine testing is most commonly assessed through method validation studies [1]. Laboratories should have established the measurement uncertainty associated with each analyte for the methods of analysis they are using and be able to report this uncertainty with the analytical results.

Dealing with Non-detects

Within analytical data sets there may be concentrations of a food chemical that are shown as 'not detected' or are below the Limit of Quantification (LOQ) or Reporting (LOR) for the analytical method. For the purposes of the dietary exposure assessment, a numerical value needs to be assigned to these. There are a number of techniques for doing this, but whatever method is chosen there will be associated uncertainty. For example, if a 'worst case' approach is taken of assigning the LOQ to non-detect values, dietary exposure is likely to be overestimated, particularly where a large proportion of the analytical results were non-detect results (see Chap. 16 – Reporting and Modeling of Results Below the Limit of Detection and Chap. 17 – Dietary Exposure Assessment in a Total Diet Study). Conversely, assigning a zero value could underestimate dietary exposure, particularly for food chemicals such as contaminants that are not intentionally added to foods but are naturally occurring and therefore likely to be present, albeit below the limit of detection. It is important to document any assumptions made in the treatment of non-detect values, including noting the likely direction of the uncertainty. Other techniques are available for the treatment of non-detects [9].

Assigning Measured Concentrations to Other Foods

Another source of uncertainty is the extrapolation of concentration data measured in one food to individual foods reported as consumed in the population being studied. For example, the chemicals that are the subject of the study may have been measured in wheat-based bread; these values may also be applied to rye- and maize-based breads and flatbreads if there are no analytical data for these breads. Clearly this introduces further uncertainty. It is difficult to quantify the magnitude of this uncertainty but it is reduced by a well-designed sampling plan that includes the most important foods for your population. It is also reduced through careful extrapolation by trained staff by a 'mapping' process that assigns concentration levels to a wider number of foods than that analyzed (see Chap. 45 – Food Mapping in a Total Diet Study).

Uncertainty in Food Consumption Data

The quality of the food consumption data is an important aspect to consider in undertaking dietary exposure assessments for the purposes of a TDS and other assessments [10]. Uncertainty exists in food consumption data due to the methods used to collect, collate and report those data.

In addition to variability, uncertainty occurs in the collection and reporting of food consumption data. This uncertainty may include factors such as reporting errors (under- or overreporting consumption of foods) and errors in estimation of

portion size, food categorization and data entry. There is additional uncertainty for some obscure or occasionally consumed foods where there may not be sufficient consumers of the food in a survey population to enable a robust estimate of the amount of food consumed to be made [2].

Using short-term food consumption surveys may capture an unusual eating occasion for an individual that does not describe how they normally eat. This could potentially over- or underestimate their typical food consumption and in turn exaggerates the reported extremes of food consumption across the survey group. The distribution of food consumption amounts for a survey of one 24-h duration is much broader than that of two or more days. Therefore, the number of days of food consumption data affects the predicted high food consumption amount [9]. This in turn affects estimated high consumer dietary exposure (typically represented by the 90th percentile of exposure where only 1 day of food consumption data are available) particularly for food chemicals in occasionally consumed foods. Uncertainty in the estimates of dietary exposure for high consumers will be greater than for the population mean dietary exposure.

In many countries, specific 'model diets' are developed to represent usual patterns of consumption for each population sub-group of interest; these may be derived from individual dietary records or other sources of information (see Chap. 17 – Dietary Exposure Assessment in a Total Diet Study). There will be uncertainties in the food consumption amounts in 'model diets' due to the assumptions made in formulating the 'model diet'.

Documenting Sources of Variability and Uncertainty

Even though it is challenging to quantify uncertainty associated with a TDS, it is generally possible to make a qualitative assessment of the major sources of uncertainty (including that originating from variability). It is important to note both the significance of the uncertainty and its direction (i.e. whether it would be likely to lead to an over- or underestimation of dietary exposure). In some cases a degree of overestimation of exposure is preferred so as to provide a 'worst case' scenario and to ensure that risk is not underestimated. However in relation to dietary exposure to nutrients, where a minimum intake (exposure) is required to assess the risk of nutritional inadequacy, underestimation is preferred as this will better identify areas for further work in relation to public health objectives including meeting relevant recommended dietary intakes. An example of a way to report uncertainty is provided in Table 18.1; in these examples, the assessments of the direction and magnitude were made for specific investigations being conducted and may differ in other assessments.

Depending on the circumstances of the assessment, a quantitative or semi- quantitative assessment of uncertainty may be important for interpreting results in a particular situation, especially if considering risk management options to address an apparent problem, for example if estimated population dietary exposure to a chemical is close to a health-based reference value, but there is considerable measurement or sampling uncertainty that could have led to a conservative assessment of risk.

Table 18.1 Examples of the impact of uncertainties and variability on dietary exposure assessment for a food chemical

Sources of uncertainty and variability	Direction and magnitude
Measurement uncertainty in analytical results, particularly at low analyte concentrations (may be possible to quantify this for some or all analytes)	+++/−−−
Number of sub-samples collected for each food	++/−−
Size and variability of analytical data set	++/−−
Influence of non-detects in analysis	from +/− to +++/−−−
Uncertainty in assigning foods to concentration categories and developing analyte concentrations for mixed foods	++/−
Use of 24-h food consumption recall data to assess usual food consumption amounts and subsequent food chemical dietary exposure	++/−
Overall evaluation of total uncertainty	*+ (whole population)*

+, ++, +++ represent uncertainty with potential to cause small, medium or large overestimation of dietary exposure to food chemical

−, −−, −−− represent uncertainty with potential to cause small, medium or large under-estimation of analyte intake

Conclusion

Significant areas of uncertainty and variability exist in the sampling, measurement and dietary exposure estimate phases of a TDS. In undertaking a TDS, it is necessary to recognize areas of uncertainty and variability and to address these where possible. It is also important to clearly document the uncertainties associated with a TDS as this assists in interpreting any risk assessment outcomes and the development of risk management options if required.

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