GOOD Test Portions PowerPoint Transcript

Slide 1

Welcome to *GOOD Test Portions*, *Guidance on Obtaining Defensible Test Portions*

Slide 2

This is Chuck Ramsey with Envirostat. It is assumed for this presentation that you have read both *GOODSamples* and *GOOD Test Portions*. This is not a standalone presentation but a supplement to those two documents. These two documents are available free of charge from the AAFCO website and if you have not read them please take time now to read them before you continue with this presentation. We will review *GOODSamples* and discuss the impact of error on data quality. We will also discuss selection and non-selection errors, quality control, equipment selection, data assessment and inference.

Slide 3 - Introduction

GOODSamples was written for developing and assessing sampling protocols. It is a systematic approach to food investigation, which includes animal food and plant food, and can be applied to other industries that deal with similar media, for instance, environmental. Sampling error is typically larger than analytical error and therefore it must be accounted for in any investigation. Most people would not accept laboratory data without some type of analytical QC and analytical error determination. Since sampling error is larger than analytical error, it only makes sense that QC and error determination for sampling also take place in the laboratory. The role of the analyst is to define error, detect error, measure error and control error. This is exactly what these documents are for. They provide a roadmap for defensible decisions.

Slide 4 - Definitions

Definitions was one of the most difficult parts of writing *GOODSamples* and *GOOD Test Portions*. Sometimes different people use different terminology to mean the same thing, or use the same terminology to mean different things. Please make sure you understand the definitions not only on this slide but all the definitions in *GOODSamples* and *GOOD Test Portions*.

For our purposes, laboratory sampling and sub-sampling mean the same thing, and they are the combination of the selection and non-selection processes. A selection process is when a smaller amount of material is removed from a larger amount of material, and a non-selection process is when a sample is manipulated, such as comminution, or removal of extraneous material, such as moisture.

Slide 5 - Definitions

The term Global Estimation Error is very important in our discussion, as it is the sum of all errors from collection of the initial primary sample through the final measurement. Sometimes, a laboratory may say, “I cannot be responsible for everyone’s error. I can only be responsible for my error.” While this may be true in some cases, someone in your organization must make a decision from the data, and the data contains the sum of all errors.

Slide 6 – Three Subsampling Approaches

There are three approaches for sampling in the laboratory. One is to select the entire Decision Unit. If you analyze the entire laboratory sample, there is no sampling error at all since you did not take a smaller portion from a larger portion. Another type of sampling is non-probabilistic sampling where you

use your judgement to select the material. With non-probabilistic sampling, or judgement sampling, you cannot make inference or determine the sampling error. In some cases, judgment sampling may be appropriate, as in prohibitive materials, but in most cases, it is not desired. The last type of sampling is probabilistic sampling, which is the focus of this presentation.

Slide 7 - Samples

The word "sample" should always be used with a modifier so we know specifically which sample we are referring to. The sample takes many forms from the collection of the initial primary sample through the analytical sample and then to the final test portion. These modifiers allow us to keep track of where we are in the system. If the word "sample" is used without a modifier, that indicates that it's true no matter what kind of sample it is.

Slide 8 – *GOODSamples* Concepts

We are going to discuss these *GOODSamples* concepts in a little more detail. The first concept is Sample Quality Criteria, or the objectives. This is the most important part about sampling, but it is often overlooked. Material properties are the specific characteristics that make one type of material different to sample than another type of material. Sampling error impacts the confidence of the analytical results. Therefore, it is important that we understand the random errors, the systematic errors, and the blunders. The last concept we will discuss is maintaining analyte and evidentiary integrity.

Slide 9 – *GOODSamples* Concepts cont…

The Theory of Sampling allows us to identify, quantify and control sampling error. You should be familiar with these errors from your reading of *GOODSamples*. Quality Control is used to ensure that objectives are met by allowing us to measure error and compare it to the Sample Quality Criteria. Data Evaluation is interpreting the analytical results. Data evaluation is very important in every project. It is sometimes done by the analyst and sometimes done by another group. Inference is an important part of the overall measurement system. It allows us to estimate the concentration or characteristic of a larger amount of material from the testing of a smaller amount of material. We will see later that there are two types of inference that we must be familiar with.

Slide 10 – Sample Quality Criteria (SQC)

Sample Quality Criteria is the most fundamental part of sampling because it establishes the questions or the objectives the sampling is trying to answer. There are three basic parts to the SQC: what is the question, what is the decision unit, and what is the required confidence. In order to ensure that the objectives are consistent throughout the entire project, it is important that everybody involved in the project be involved in developing the SQC.

Slide 11 – What is the Question?

There are three parts to the question: what is the analyte or characteristic of concern, what is the concentration or characteristic of concern, and how will inferences be made? Not all analytes have a concentration. Some analytes, such as odor, visual observation (say a dented can), or prohibitive material, do not have a concentration. We will call these latter ones "characteristics." The analyte of interest is important because it dictates the handling, the types of tools, the holding time, preservations, and anything else we may need to preserve the integrity of the analyte from actual field collection

through analysis. The concentration is important because not only do we have calibration in the laboratory and detections to worry about, for very low levels we have to be concerned about special sampling techniques and special types of containers that we may need to use in order to preserve the integrity of the analyte. It is important to know how the data will be used to make inference so we ensure we collect the right data to begin with. Different types of inference require different types of data, both in terms of quality and quantity. It is very frustrating for a decision-maker to plan on making one type of inference from the data, but when the data comes in, it was collected with another type of inference in mind. This frustration and confusion can be avoided by having the decision-maker be part of the SQC process to ensure they get the data they need to make the correct inference.

Slide 12 – What is the Decision Unit (DU)?

The Decision Unit is the material from which the primary sample is collected and to which the inference is made. Typically, there is one sample from a decision unit. However, in cases where there are quality control samples, there may be more than one sample from an individual decision unit. A project may have a single decision unit, such as "What is in this truckload of grain?" or a project may have many decision units, such as "What is in each of these barrels of material?" In some cases, it is very obvious what the decision unit is and in other cases it is a little more difficult to ascertain. The decision unit is something that must be determined in advance. Without a decision unit, it is impossible to go further in the sampling process or to develop the sampling protocol. Let's say we have a truckload of frozen, one-pound packages of Brussels sprouts. In this case, the decision unit could be the entire truck, the decision unit could be each individual package, or the decision unit could be each individual pallet, or maybe even something else. In other words, what scale do you want information on? It could also be that one analyte has a decision unit of a certain size, and another analyte could have a completely different-size decision unit. I cannot stress enough how important it is to decide the decision unit because it drives all sampling. In the laboratory, the laboratory sample is often as the surrogate decision unit, but in reality, the decision unit is what the primary sample was collected from.

Slide 13 – What is the Required Confidence?

When you first start applying the principles of *GOODSamples* and *GOOD Test Portions*, the decision unit is the most difficult to comprehend. With a little experience, the concept of decision unit will become very easy to understand. In fact, you will wonder how you ever completed successful projects without the concept of decision unit. The most difficult part of the SQC process is actually determining the required confidence. No matter how careful we are in our work, there is always some error. Therefore, there is always the possibility of an incorrect decision. I think this is the most difficult part of the SQC process because here is where we must admit that our decisions may not be perfect due to the presence of error. The only way to decrease the probability of making an incorrect decision is to reduce the amount of error we allow into our system. Since confidence is related to the sum of all errors, it is important that we control error in every step of the process. We cannot just ignore sampling error as it is the largest error in the measurement process.

Slide 14 – Sample Quality Criteria

It is important that all stakeholders are involved in determining the sample quality criteria. This may be as few as one or two people, or as many as five or six people, depending on the complexity of the project. At a minimum, we need the person collecting the primary sample, the person performing the

analysis, the person performing the quality control, and the person making the final decision. In some cases, this may all be the same person and in other cases, it may be four separate people. For some projects, you may need other specialties, such as a statistician or a toxicologist. It is very important that the decision maker be part of the process because we have to ensure they have all the information they need to make a defensible decision. At first, this process may seem difficult and time-consuming, but with a little practice, it becomes very easy and efficient. I find it interesting that people tell me they don't have 30 minutes to plan a project, but they have months of time after a project to try to figure out how to use the data and what the data really means. Proper planning with SQC is always the most efficient way to make decisions.

Slide 15 – Material Properties

Material properties are the unique properties of each type of material that make the sampling different. No two types of material have the same material properties – therefore, the sampling will not be the same. The two parts of material properties are the finite versus infinite element determination and an estimation or determination of the heterogeneity is the root cause of all sampling error. This will be discussed more in the next few slides.

Slide 16 – Finite vs Infinite

All materials are made up of individual elements. The elements can be classified as finite or infinite. A finite element material is a material comprised of elements that can be individually selected and individually tested. By contrast, an infinite element material is one where the elements are small, they cannot be individually identified, individually selected, or individually tested. Water would be an example of an infinite element material, where the individual elements are the molecules of water themselves. There may be some materials, such as raisins or peanuts, which could be considered as infinite or finite element materials, depending on your point of view. It is possible to randomly select raisins out of a box of raisins and test them individually – in this case, it would be a finite element material. If you have a very large container of raisins, you could collect them, groups at a time, without regard to individual raisins. In this case, you could view the raisins as infinite element material. A material that has been comminuted is typically considered an infinite element material because the elements are now too small to collect and analyze individually. Therefore, the material in its original form may be considered a finite element material, but after it is comminuted in the laboratory, it may be considered an infinite element material for selection of the test portion. The distinction between finite and infinite element material is important because Theory of Sampling must be used for infinite element material in order to control error.

Slide 17 - Heterogeneity

Compositional heterogeneity is the difference in the concentration between the individual elements that make up the material whether these items are finite or infinite. Distributional heterogeneity results from the non-random distribution of these elements. In other words, the concentration may be higher in one part of the Decision Unit and lower in another part of the Decision Unit. Since the Decision Unit has both spacial and temporal characteristics, such as in the production in an eight-hour shift, it is possible that there is a higher concentration at the beginning of a shift than at the end of a shift. Both types of heterogeneity exist in all materials. The purpose of the Theory of Sampling is to control the

impact of this heterogeneity as it relates to sampling error to ensure that we meet the Sample Quality Criteria.

Slide 18 – Heterogeneity

It is important to note that without compositional heterogeneity, there can be no distributional heterogeneity.

Slide 19 – Heterogeneity in the Laboratory

Here are some examples of heterogeneity. Whenever you pour out a solid material, the larger, less-dense material will roll to the outside and the small fines with stay in the middle. This causes distributional heterogeneity, which makes it difficult to take as scoop of this material and represent both the blue and red particles. Many times in the laboratory, people shake the sample to try to make it uniform. In some cases, shaking the material actually promotes the distributional heterogeneity. Any movement of materials will affect the distributional heterogeneity but has zero impact on the compositional heterogeneity.

Slide 20 – Mixing

Mixing is common in analytical laboratories, sometimes referred to as homogenization. Most analysts think that if they mix or homogenize a sample, everything is identical and they can remove just a small part for testing and everything is okay. However, this is not the case. Mixing has no impact on compositional heterogeneity and it may actually increase the amount of distributional heterogeneity. Mixing never creates a homogenous state. There are, however, methods to control compositional heterogeneity and methods that control distributional heterogeneity that always work and are effective.

Slide 21 – Integrity

Maintaining analyte integrity means that the identity and concentration of the analyte of interest is the same throughout the entire measurement process as it was in the Decision Unit prior to when the sample was collected. In order to maintain analyte integrity, sometimes special sampling and handling procedures may have to be implemented. In addition, in some cases, special containers may have to be used, preservations may have to be used, holding times may have to be met, etc. Analyte integrity is more of a scientific concept whereas evidentiary integrity is more of a legal concept. Evidentiary integrity includes the ability to trace an analytical result back to the specific Decision Unit it was collected from. In addition, we must be able to demonstrate that the sample was not compromised in any fashion. Integrity is maintained by a system of procedures, photos, paperwork, etc., and may be different from one organization to another. So, you need to ensure you understand the evidentiary integrity requirements for your specific organization.

Slide 22 – Sampling Error

Errors, including sampling error, can be as one of three types. The first one is random error, the second one systematic error, and the third one blunders. Random errors are sometimes called precision or (more accurately) imprecision errors, and the average of all random errors is zero. Systematic errors, sometimes called bias errors, produces data that is consistently too high or too low. Blunders, sometimes called gross errors, are mistakes. Examples are transposing numbers or switching samples. Many users do not understand the concept of error adequately. They think that the number from the

laboratory is the correct number plus or minus some error. In reality, however, error causes the analytical result to be different than the true concentration that was actually in the test portion. This leads to incorrect inferences and, in some cases, an incorrect decision.

Slide 23 – Theory of Sampling (TOS)

The Theory of Sampling gives us the ability to estimate, control and mitigate sampling error from the collection of the initial collection of the primary sample through selection of the final test portion. While the Theory of Sampling was developed in the mineral industry, it is a general theory that is applicable to all types of material, including food and feed. While it is applicable to both finite and infinite element materials, it is the area of infinite element materials where sampling theory is needed. The techniques for infinite element sampling are different for those than of finite element sampling. A problem with many sampling documents is they are based solely on finite element sampling. If finite element sampling methods are applied to infinite element materials, the most likely result will be an unrepresentative sample.

Slide 24 – Theory of Sampling cont…

In order to implement the Theory of Sampling, we need to have the Sample Quality Criteria and we need to understand the material properties. There are three basic principles in the Theory of Sampling to control error. One is selection of enough mass to control compositional heterogeneity. Another is selection of enough increments to control distributional heterogeneity, and selection of tools to control systematic error. Correct implementation of sampling theory will allow up to develop a sampling protocol.

Slide 25 – What is an Increment?

I mentioned the word “increment,” which might be a new term for some of you, so we will spend a little time discussing it. An increment is the mass or volume collected from a single operation of a sampling tool. These increments are combined together to form a sample. A sample can be made up of a single increment, sometimes called a “point sample” or a “grab sample,” or a sample can be made up of multiple increments, sometimes called a “multi-increment sample.” The more increments we collect, the better control we have on the distributional heterogeneity. Collection of a single-increment sample would not be appropriate for most solid materials. However, it may be acceptable for certain containerized liquids.

Slide 26 – Relationships

The study of the Theory of Sampling is beyond the scope of this presentation, but more information can be found in the references of *GOODSamples* and *GOOD Test Portions*. Presented here are just some basic concepts of relating error to mass and to increments. The error resulting from compositional heterogeneity is called the Fundamental Sampling Error, and it is controlled by the mass of the material we collect as the sample. The more mass we collect, the lower the Fundamental Sampling Error. The Grouping and Segregation Error results from the distributional heterogeneity. The more increments we collect, the lower the Grouping and Segregation Error. The reason we need to know the error in the SQC is so that we know how much mass and how many increments we need to collect as part of our sample. It is very difficult to have an exact estimate of the mass or the number of increments you need unless

you know a lot about the material properties. There are several examples in Appendix A of *GOOD Test Portions* on use of the Fundamental Sampling Error equation.

Slide 27 – In the Laboratory

Selecting increments at random in the laboratory to control Grouping and Segregation Error is not that difficult since you have access to the entire sample. This can be much more difficult when collecting a primary sample from a ship or from a rail car where access is impeded. Obtaining sufficient mass to control Fundamental Sampling Error is more of a challenge in the laboratory, mainly due to the small sample size or test portion size used by many of the analytical methods. People in charge of laboratories like smaller test portions – this means fewer reagents and smaller vessels, it means higher throughput. But all this comes at the cost of representativeness. Controlling Fundamental Sampling Error can only be done two ways. One is through increased mass, as we saw on the previous slide, and the other is through comminution, which you should remember from your reading of *GOODSamples* and *GOOD Test Portions.*

Slide 28 - Comminution

As we can see from the Fundamental Sampling Error equation, Fundamental Sampling Error can be controlled through mass or through controlling the particle size of the material. Reduction in particle size, called comminution, is a very effective way to control the Fundamental Sampling Error, especially in cases where it is difficult to increase the sample mass, such as the laboratory. Comminution helps with other sampling errors, as well. If a material is ground to a consistent size and shape, there is less chance of segregation from the effects of gravity. It is also easier to maintain sample correctness with smaller particles that are uniform in nature. When performing comminution, it is important that the entire sample be comminuted or at least a representative portion. In some cases, people take a small amount of material that is unrepresentive to begin with and then perform comminution. This will not yield a representative portion. Not all materials can be comminuted – analyte integrity is one of the reasons why. For instance, you may lose volatile material through the agitation of the comminution process, or you may have some materials that degrade due to the heat buildup in comminution. Not all materials can be comminuted with the same type of equipment. Therefore, a laboratory may have several types of equipment in the comminution laboratory.

Slide 29 – Sampling Errors

In the laboratory, there are two major types of sampling errors, those from nonselection processes and those from selection processes. We will investigate these sampling errors and investigate the relationship to mass, increments and sample correctness.

Slide 30

This chart from *GOOD Test Portions* illustrates all the errors and their relationship to the Global Estimation Error. The Global Estimation Error is the sum total of the Total Sampling Error and the Total Analytical Error. In this graph, we are only focusing on the Total Sampling Error. The Total Sampling Error is the sum of the error from non-selection processes and from selection processes. Non-selection processes are those processes that manipulate the sample but do not perform any type of mass reduction. An example would be comminution. Errors from non-selection processes are all systematic errors. Examples of all these errors can be found in the *GOOD Test Portions* document. Errors from the

selection processes can be random or systematic. The random errors include the Fundamental Sampling Error and the Grouping and Segregation Error. The systematic errors in the selection process are also referred to as sample correctness. They include: increment delimitation error, increment extraction error, and increment weighing error. Examples of all these errors can be found in *GOOD Test Portions*. Blunders are not listed here but can happen in any of the selection or non-selection processes. Take some time to review this chart and be sure you understand the basic types of error before proceeding.

Slide 31 – Random Errors – Selection Process

There are two random errors in sampling, both associated with the selection process. They are the Fundamental Sampling Error and the Grouping and Segregation Error. The magnitude of these errors is controlled by mass and increments. In the case of Fundamental Sampling Error, comminution is also a tool that we can use to control the error.

Slide 32 – Controlling Random Errors

When controlling Fundamental Sampling Error through comminution, it is important to realize that some types of comminution are better than others. Our goal is to produce a consistent-sized material after comminution. If there is large variation in particle size after comminution, it increases the chance of segregation and thus increases the Grouping and Segregation Error. Sometimes a laboratory purchases just one type of comminution equipment and then tries to put every type of material through it. That is a mistake. A well-equipped laboratory may have a half-dozen or more pieces of comminution equipment. Grouping and Segregation Error can be controlled by increasing the number of increments or by controlling the distributional heterogeneity. Sometimes, an analyst thinks that if they mix the sample, they have made the material uniform and therefore controlled the distributional heterogeneity. While this may be true in some cases, mixing typically changes the distributional heterogeneity but it does not eliminate the distributional heterogeneity. It is important to remember the change in the distributional heterogeneity does not change the compositional heterogeneity and the Fundamental Sampling Error still exists.

Slide 33 – Systematic Errors – Selection Process

There are several types of systematic errors in the selection process. Unlike systematic errors in the analytical process, it is impossible to estimate the magnitude of systematic errors in the sampling process. It is therefore critical that all systematic errors in sampling be controlled.

Slide 34 – Sample Correctness

Systematic errors in sampling are collectively referred to as sample correctness. In technical terms, sample correctness is the equiprobable selection of all the elements at the increment location. There are many causes of the non-equiprobable selection of elements. One common problem is that the opening of the sampling tool is too small to accept the largest particles. Another common problem is small particles sticking to the sampling tool. The magnitude of errors from sample correctness can be quite large, so it is very important that we understand and control these errors.

Slide 35 – Sample Correctness

Sample correctness is a function of a correct tool design and correct tool usage. There is more information on sample correctness in *GOOD Test Portions* and in the reference materials at the end of

GOOD Test Portions. IWE, or increment weighting error, occurs when the increments are not of the approximately same mass. An increment that is larger over-represents that area, and an increment that is small under-represents that area. Sometimes, an analyst will take a few larger increments to begin with to get close to the target sample mass, and then they take a few small increments at the end to try to hit the target mass exactly.

Slide 36 – Sample Correctness

This is an example of sample correctness when selecting a final test portion. In this example, the particles or element vary in particle size from the small red ones to the larger green ones. When using a flat spatula, the largest particles roll off the spatula and there is a bias towards the small, finer particles – in this case, the red ones. A rounded scoop may be good at collecting the larger green particles, but it will be biased against the smaller, red particles. Only a square scoop with tall sides would allow equiprobable selection of both the large and fine particles. Hopefully you can see from the illustration that if the material was ground to a consistent size, this problem probably would not happen. It is very difficult to collect a representative test portion when there is a large variation of particle sizes. This would apply to a flat spatula and to a rounded scoop.

Slide 37 – Increment Extraction Error

Increment Extraction Error comes from incorrect or sloppy tool use. Sometimes, extra material is collected, and sometimes material is lost. Contamination introduction error can come from tools, from the environment, from the containers, and even from the people collecting the samples. This is especially critical when sampling low-level analytes and for aseptic sampling. Analyte integrity must be maintained at all times. In addition, materials that may act as an interferent during analysis also need to be controlled.

Slide 38 – Increment Weighting Error

Here is an example of increment weighting error. If the sampling tool is sized at approximately the increment mass you are trying to collect, it makes it much easier to control the increment weighting error. You can see that it is easier to get consistent increment mass with the tool on the bottom as opposed to the tool shown on the top.

Slide 39 – Systematic Errors – Nonselection Process

Two of the systematic errors for the nonselection process are the same as for the selection process, and there are two additional errors. We still have the contamination introduction error and the analyte integrity error, but we no longer have the increment delimitation error and the increment extraction error. Instead, we have the extraneous material error and the mass recovery error. Sometimes, when the primary sample is collected, extraneous material is included in the sample. An example would be seeds or skins that are collected as part of the primary sample but are not part of the decision unit and would need to be removed before the test portion is selected. In some cases, moisture would be considered an extraneous material. If the extraneous material ends up in the test portion, it causes an extraneous material error. Common examples of mass recovery error are spillage and material left behind in the sample processing equipment. When evaluating sample processing equipment, it is always important to look at how much material is left over inside the equipment after the sample has been processed. Once again, these are systematic errors, and while we may be able to detect that such an

error exists, there is no way that we can determine the magnitude of the error and its impact on the final analytical results.

Slide 40 - Blunders

Blunders are mistakes or accidents in the laboratory that cause loss of analyte or evidentiary integrity. Laboratories typically have systems in place to prevent blunders during analysis. We need to ensure that we also have systems in place to prevent blunders during sampling. Blunders are not considered as part of the global estimation error because their magnitude cannot be estimated. In addition, it is impossible to even know in some cases whether a blunder has occurred or not.

Slide 41

In the laboratory, you should have a plan to define, detect, measure and control each of these errors.

Slide 42 – Example of Increment Delimination Error

Here is an example of sample correctness. In the picture on the left, we are sampling a cylinder with a pipette. You will notice that in the pipette, we can get some of everything that was in the cylinder and in the same proportions it was in the cylinder. In the picture on the right, we are sampling a flask with the same pipette. If you look carefully at the picture, you will notice that we are over-representing the top phase and under-representing the bottom phase. In other words, while we have some of each phase, we do not have the phases in the correct proportion.

Slide 43 – Quality Assurance and Quality Control

Quality assurance and quality control are very important parts of the analytical laboratory. They can be used for sampling protocols just like for analytical protocols. For instance, they can be used for validation and verification of laboratory sampling protocols. In addition to quality assurance for overall protocols, we can perform specific quality control on individual samples. We can measure random error directly through replication at any stage of the measurement process, and in some cases, we can detect the presence of systematic error.

Slide 44 – Two Types of Inference

There are two types of inference we can make, Type I and Type II. In most cases, there will always be a Type I inference and in some cases there will be a Type II inference also. A Type I inference is when we tie an analytical result back to a specific decision unit. In any project, there may be only one decision unit or several decision units. A Type II inference is when there are more decision units than can be sampled. In this case, we sample some of the decision units and make a Type II inference to the decision units that we could not sample. The sampling requirements for each of these types of inference is different, so we need to make sure we understand exactly which type of inference we're trying to perform. Not understanding the difference between the types of inference is a huge source of confusion when it comes to data evaluation. Many of the currently available sampling protocols are incorrect because they confuse these two types of inference.

Slide 45 – Example Type I Inference

In this example, there are seven truckloads of grain and each truckload is a separate decision unit. A sample is collected from each truck and sent to the laboratory. The laboratory analyzes each of these

seven different samples, and makes seven Type I inferences. In another situation, all seven trucks could be considered as a single decision unit. In that example, a single sample comprised on increments of grain across all seven trucks would be sent as an individual sample to the laboratory and a single Type I inference would be made from the analytical results to all of the seven truckloads of grain.

Slide 46 – Example Type II Inference

In this example, there are 7,000 trucks of grain. If each truck is a decision unit, there are 7,000 decision units. If we desire to make a Type I inference to all the 7,000 trucks, we would need to collect 7,000 samples and perform 7,000 analyses. However, this project does not warrant the resources to collect and analyze 7,000 samples. Therefore, only some of the 7,000 trucks will be sampled, and a Type II inference will be made from the sampled trucks to those that are not sampled. Let's say we decide to sample 59 trucks. We select, at random, 59 trucks from the 7,000 and collect a sample from each of the 59 trucks. A Type I inference is made for each of the 59 trucks that were sampled. A Type II inference is made from the 59 trucks that were sampled to the 6,941 trucks that were not sampled. While we have more information on the 59 trucks that were sampled, we can still make inference to the trucks that were not sampled. This is a trade-off between the amount of information we obtain on all 7,000 trucks and the sampling effort. As part of the SQC process, you need to determine the confidence you need to this type of inference, which will drive the number of trucks that need to be sampled.

Slide 47

Here is an example of a Type I inference, where we have a single decision unit and we collect increments from inside that decision unit, combine them for analysis, and we have one analysis that we can use to make a Type I inference to this specific decision unit.

Slide 48

This is an example of both a Type I inference and a Type II inference. There are 80 decision units in total, and we are going to sample and perform a Type I inference on six of these. Each of these six decision units, which have been selected at random, will be sampled in exactly the same way as in the precious slide. We will make a Type I inference for each of the sampled decision units. From the results of the six sampled decision units, we can make a Type II inference to the 74 decision units that were not sampled. In this scenario, we have both Type I inference and Type II inference. While we can have only a Type I inference in some cases, we cannot have a Type II inference without a Type I inference. In this example, if the confidence must be really high, it is possible that the decision maker wants a Type I inference for each of the 80 decision units. In other words, 80 samples will be collected and analyzed, and 80 Type I inferences will be made. There would be no Type II inference in that case.

Slide 49 - Inference

This diagram illustrates the differences between Type I and Type II inferences. For Type I inference, we are concerned about mass, increments, and sample correctness. For Type II inference, we're only concerned about the number of decision units that are sampled. The number of decision units for Type II inference is based on the confidence you wish to achieve. There is more information on inference in the paper entitled, "Consideration for inference to decision units," referenced in the back of *GOOD Test Portions*.

Slide 50 – Data Assessment

Data assessment is a very important, yet often overlooked, part of making defensible decisions. As part of data assessment, we evaluate what we have done and compare it with what we said we were going to do. We evaluate the sample quality criteria, we evaluate the quality control data, estimate the total error, the global estimation error, and then we can perform inference and eventually make decisions. Inference is only one part of the decision-making process. Decision-making also includes economics, politics, and many other things that are beyond the scope of *GOOD Test Portions*.

Slide 51 – Data Assessment

In order to estimate the global estimation error, we need the data from our quality control events. This includes replication for random errors and checks for systematic errors where possible. Once we have the global estimation error, we can compare that to the error that was allowed in the SQC. If the global estimation error is less than the error allowed in the SQC, then the data is acceptable for decision-making at the specified confidence. If, however, the global estimation error is greater than that allowed by the SQC, we have a problem, as the data is not sufficient for decision-making. In this situation, we need to identify the source of the error or errors and correct them. Once the errors are corrected, you can re-perform the sampling and analysis. Many people mistakenly think that if you don't meet your objectives, you just go back and lower the objectives in the SQC. This is incorrect. The objectives in the SQC are the minimum objectives you need to make a defensible decision.

Slide 52 - Workflow

This flowchart shows the interaction of all the selection and nonselection processes that may be required. It is important to note that there could be several selection and nonselection processes as part of laboratory subsampling.

Slide 53 – Laboratory Sampling Processes

A few more comments on laboratory sampling before we go to equipment design. Be sure and ensure that you have the correct amount of increments and that these increments are collected at random throughout the material. Do not attempt to obtain an exact weight at the balance. This can be a source of sampling error. Trying to add a small mass of material or take away a small mass of material at the scale leads to an increment weighting error because that increment is not the same mass as the other ones that make up that sample. Be sure you use the correct sampling tools in the laboratory. Sometimes, even a visual observation is sufficient to tell you whether the sampling tool is biased towards or against one type of particle or another. Do not attempt to scale down the test portion mass without validating the impact on the fundamental sampling error. Increased variation when validating a smaller test portion mass may be solely a function of the smaller mass and have nothing to do with the analysis.

Slide 54 – Equipment Design

Equipment must be very robust, otherwise it will break and sit in the corner, unused. Equipment must also be inert – in other words, it does not contain the analytes you're looking for. This may not be a concern when you're analyzing for protein, but if you're looking for trace metals, especially chrome, this may be an issue. Safety is always a concern, especially in comminution equipment where the machine

cycles at high rates of speed. If the equipment is not easy to clean and maintain, it will be neither cleaned nor maintained. Maintenance is important for proper operation, and cleaning is important to prevent carryover from one sample to the next. It is critical that all equipment meet the criteria of the theory of sampling.

Slide 55 – Equipment Design

Other criteria for equipment design include the flowability of the material you are working with. The capacity of the equipment needs to be adjusted to the size of the samples you typically deal with. Heat generation is also a consideration, especially if you have analytes that are sensitive or degrade in the presence of heat. Material hardness is a very important design criteria. There are many types of equipment designed to handle material that is very soft up to material that is very hard. Particle size is also important – not only the size of the feed material, but the particle size you desire to end up with after comminution. There is a wide variety of equipment available, and you can always find something to suite your individual needs.

Slide 56 - Equipment

In *GOOD Test Portions*, there is a description of the types of equipment used for selection and nonselection processes.

Slide 57

Here is a picture of squash that has been comminuted with a blender. One of the important criteria for comminution is that the final material has the same particle size. In this case, you will see that we have small and large pieces of the skin from the squash. If the analyte we are concerned about is associated with the skin of the squash, the analytical result may depend on whether we get that one, large piece of squash skin or miss it. This would be a source of variation (or random error), specifically, the fundamental sampling error. In this case, we would need more mass than if we had been able to comminute everything to a small, uniform particle size. Blenders are typically not very good comminution devices. However, if the test portion is large enough, they can work adequately. The presence of a few large particles of material in a comminuted sample can cause serious sampling errors.

Slide 58 – Mass Reduction Technique (Selection Process)

There are many types of mass reduction techniques, and we will discuss four of them: stationary riffling, rotary riffling, alternate shoveling and slab cake. Sometimes, an analyst will just grab a single portion of material and call that “mass reduction,” or potentially they may pour out the sample, divide it in half, and take half for mass reduction. These are not adequate mass reduction techniques except in a few very rare cases.

Slide 59 - Splitting

Splitting is a very common, and in some cases a very good, mass reduction technique. Stationary splitters, also called “Jones splitters,” are common in analytical laboratories. However, they are not recommended for splitting samples. One reason is they do not provide enough increments to properly control the grouping and segregation error. In addition, they are very prone to operator error. I have personally seen this type of splitter used hundreds of times, and not in one case have I seen it used correctly. Even with proper training, it is difficult because people get in a hurry and cut corners. The best

way to split a sample is with a rotary splitter. Rotary splitters allow for the collection of hundreds of increments. The two pictured here are the Retch PT 100 and the QuantaChrome spinning microriffler. If a material is not flowable or a rotary splitter is not available, there is another method called alternate shoveling which can be done manually. An advantage of alternate shoveling is that you can split a sample into two, three, four or more splits. While alternative shoveling is not as good as rotary splitting, it is good enough to meet most sample quality criteria.

Slide 60 – Two Dimensional Slab Cake

Slab caking is another mass reduction technique. One dimensional slab cakes are typically not used in our industry due to the large amount of material that we typically deal with in the primary sample. A two dimensional slab cake, however, has limited application. This method typically works well with fine, uniformly-sized particles. In other words, it would work fine for flour but would not perform well for unground soil. To implement this method, you pour out the material on a solid surface and flatten the pile to a thin, uniform height. Then you collect random increments as shown in the figure. No matter how careful you are, you will always collect more material from the top of the pile than the bottom of the pile. A material with a uniform particle size will not have as much segregation as a material that has different particle sizes. With a uniform particle size, it is less important that we collect more material from the top of the pile than from the bottom of the pile. You cannot use this method for an unground material with varying particle sizes, such as soil. In addition, if you have an analyte that preferentially sticks to the solid material the pile is resting on, you will underestimate the analyte concentration. Once again, this method is only appropriate for a material of very uniform particle size. See *GOOD Test Portions* for more information on slab cake sampling.

Slide 61 – Composites

There is a lot of confusion on the term “composite sample,” so we should spend a few minutes and discuss it. A composite sample is a combination of individual samples that are combined solely for the purpose of analytical efficiency. The word “composite” has nothing to do with sample representativeness, even though sometimes it is used that way. Combining increments to form a sample is not the same thing as a composite sample. Compositing in the truest sense of the word has been around in agriculture since the late 1800s.

Slide 62 – One of the First Composites

This is one of the earliest references to composite samples that I am aware of. The dairy industry is one of the few examples where the term composite is used correctly.

Slide 63 – Example of Composite

This is an example of a composite. In this example, five different representative samples are put together to form a composite sample. These individual samples have the correct number of increments, have the correct mass, and were taken using correct sampling tools. A composite sample is nothing more than a representative portion from each of the five individual samples. It is much easier to composite liquid samples than it is solid samples. This example would be valid for both chemistry and for micro.

Slide 64 – Composite Example

For the chemistry composite sample, we will assume that there is a regulatory limit or concentration of concern at 10 parts per million (ppm). In this case, five samples are composited into one sample. If the concentration in the composite is below 2 ppm, then none of the original samples could have had a concentration above 10 ppm. A worst-case example would be to assume that one sample had a concentration of 10 and the other four samples had a concentration of 0. In this case, the concentration of the composite would be 2. If any result of the composite sample is less than 2, then none of the original 5 samples could have a concentration of 10 or higher. If we get a value above 2, say 3, we don't know if one sample had 15 and the other four had 0 or if all five samples had 3. So for any value 2 or above, we would have to go back and analyze all five original samples to see if any of them had a value at 10 or above. This strategy is only going to work when we get values below 2 a certain percentage of the time. If every time we get a value above 2, we would have to abandon this strategy as it would take more analytical resources than just analyzing the samples individually to begin with. The biological sample is also very interesting. Many times for microbiology we are looking for a pathogen, and we have a violation if any pathogen is detected. If a pathogen is detected in the composite sample, we know at least one of the original samples must have had a pathogen in it. If there is no detection of a pathogen in the composite sample, it is not necessary to analyze any of the five original samples. Compositing is a very powerful technique to increase the confidence by analyzing more samples without the extra cost of the analysis.

Slide 65 - Composite

A few words of caution on composite samples. We need to make sure that we can still detect the analyte of concern in the composite sample. In the chemistry example, if our detection limit was 5 ppm, we could measure the analyte in the original sample but we could not measure the analyte in the composite sample. For composite samples, we have to be concerned about detection limits. The same is true for biological samples. As long as we can detect the pathogen in the composite sample, we are okay. But if we ever dilute something to the point where we cannot detect a pathogen, we will have a problem. In biological samples, the pathogens are typically replicated – therefore, this is usually not an issue. Composite samples allow for the analysis of many samples without increased analytical cost. This is a great alternative for an outbreak response where we want to process many samples in a short amount of time. Composites are also very useful when the tests are very expensive or we have a limited number of tests available to us. Once again, we must not confuse composite samples, which are from different decision units, with increments, which are from the same decision unit.

Slide 66 - Training

Training is critical for the implementation of the principles in *GOOD Test Portions*. The proper PPE and safety practices may be much different for comminution equipment than for analytical equipment in the laboratory, and the analyst may be unfamiliar with the equipment and techniques that are required. Training on SQC, Theory of Sampling, data assessment, equipment selection and use, is also important. It is also important that the entire organization be trained, from management through technicians. Agencies that have successfully implemented the principles of *GOOD Test Portions* have had training throughout all levels of the organization.

Slide 67 – Effects of Poor Laboratory Sampling

Poor laboratory sampling means that more error is incorporated into the analytical results – both random error and systematic error. Excessive error always has undesirable results. Sometimes people confuse outliers with samples that just contained too much error. This may lead to rejection of data or other negative consequences. Retesting of samples due to excessive error is a very common outcome. The cost associated with retesting, resampling, performing root cause investigations, or making incorrect decisions is typically greater than the cost of controlling the error from the beginning. Many times, a laboratory will retest a sample until they get a result they are comfortable with. This is not a scientific approach to analysis.

Slide 68 – Achieving Defensible Sampling

There are the basic concepts that are outlined in *GOOD Test Portions* that allow you to achieve defensible sampling.

Slide 69 – Steps in Developing Defensible Sampling Protocol

If we are developing a sampling protocol or reviewing a sampling protocol, we need the following elements. First, sample quality criteria. Without a well-defined sample quality criteria, it is impossible to develop a sampling protocol or to evaluate an existing protocol to see if it meets the needs of the current project. It is critically important that the decision unit be very well defined. It is also critical that we know how we are going to use the data to make inference so we can ensure that we collect the correct data to begin with.

Slide 70 – Steps in Developing Defensible Sampling Protocol cont…

Once the sample quality criteria are established, we need to define the material properties. As previously stated, once a material becomes comminuted, it becomes an infinite element material. With all infinite element materials, Theory of Sampling must be implemented. This includes mass, increments, and sample correctness.

Slide 71 – Steps in Developing Defensible Sampling Protocol cont…

The Theory of Sampling is critical for the control of errors. Without the Theory of Sampling, there is no way to estimate the sampling error nor to control the sampling error.

Slide 72- Steps in Developing Defensible Sampling Protocol cont…

Once we understand the required mass, increments and tools, then we can begin to develop a defensible sampling protocol. This protocol should incorporate everything from the initial primary sampling through the selection of the final test portion and include appropriate quality control.

Slide 73 - Summary

This has only been an overview of *GOOD Test Portions.* Further study and training is required in order for you to successfully implement these principles. Thank you for listening to this presentation. If you have any questions or comments, please contact me, and good luck on your endeavor to reduce sampling error.

Slide 74 – Work Group Members

[no audio]

GOOD Test Portions PowerPoint Transcript

Slide 75 – EnviroStat Contact Information

[no audio]

Slide 76- Funding

[no audio]