Taking Defensible Samples - The basis of nutritional decisions

Tri-State Dairy Nutrition Conference
April 17, 2018
Fort Wayne, Indiana

by
Charles Ramsey
EnviroStat, Inc.

ENVIROSTAT

Why are Samples Collected?

- Because we cannot and do not want to analyze all the material we are interested in
- To verify conditions, concentration, blend uniformity etc.
- Regulatory requirements
- Supplier certification
- Incoming raw material verification
- Process control

…to make decisions

ENVIROSTAT

Decisions

- We make decisions every day
- Some decisions are based on analytical results
- Analytical results are based on analysis of a very small amount of material
- If the small amount of material (hundreds of grams) collected in the field and the even smaller amount of material (grams or portions of grams) analyzed in the laboratory do not represent the entire material of interest…

Your decisions are only as good as your samples

ENVIROSTAT

Decisions

ENVIROSTAT

Previous Papers

TMR Sampling: Valuable Exercise or a Random Number Generator?

Bill Weiss[1,2], Peihua Zhang[2], John Goeser[3], and Normand St-Pierre[2]
[2]Department of Animal Sciences, The Ohio State University
[3]Rock River Laboratory, Inc. and
Department of Dairy Science, University of Wisconsin

Proper Sampling and Sample Scheduling Can Prevent Reduced Milk Yields

William P. Weiss[1,2], Christopher Hill[3], and Normand St-Pierre[2]
Department of Animal Science, The Ohio State University[2]
Poulin Grain, Inc., Newport VT [3]

Optimizing Performance of TMR Mixers

Dennis Buckmaster[1]
Department of Agricultural and Biological Engineering
Purdue University

ENVIROSTAT

How are Samples Collected?

- Follow a protocol from some "reputable" source
- Just fill up a bag - how hard can that be!
- Unknown person with unknown procedure
- How we have always done it

But different objectives have different sampling protocols!

ENVIROSTAT

What Should the Criteria be for Sampling?

- Representative - but what does that mean?
- If analyze everything we obtain the "truth"
- We get the same answer we would have gotten if we had analyzed all the material we are interested in (we will refer to that as the Decision Unit)
- How do we accomplish that?
- How close do we need to be to the "truth?"

It is error that causes the analytical results to be different than the truth

ENVIROSTAT

Error

- Is what makes the analytical result from the laboratory different than the actual concentration of the material sampled
- Without knowledge of the magnitude of the error, it is impossible to have confidence in the resulting decision
- The quantification of the amount of error in the analytical result is therefore necessary for defensible decisions
- A methodology to incorporate tolerable error into the sampling protocol is therefore a necessary requirement
- The amount of error tolerable is different from project to project

Error

- Many think that the number from the analytical laboratory is still the right number, even if there is some error (Just give me the number—and only one number)
- WRONG!
- Error makes the number from the laboratory incorrect
- Only through knowledge of the error can we estimate how wrong the result may be

5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5

Mean = 5, Error = 0

5	4	4	5	4	5	5	4	6	5
6	5	5	5	5	5	5	5	5	5
5	4	6	5	5	4	5	4	7	5
6	6	4	4	4	5	5	5	5	5
5	5	5	4	5	5	5	6	4	5
5	5	6	5	4	5	5	5	5	5
5	6	5	4	5	4	5	5	5	5
5	4	6	5	6	5	5	5	5	5
5	4	5	6	4	4	5	5	5	4
5	5	5	5	5	5	6	5	5	4

Mean = 5, RSD = 10%

ENVIROSTAT

4	7	6	5	7	5	5	7	6	6
5	3	5	5	6	6	5	5	6	5
6	5	7	7	3	5	6	5	6	5
5	6	5	3	5	5	4	5	6	6
6	5	4	4	3	4	6	4	5	5
7	4	5	3	5	5	4	6	5	5
4	5	4	6	4	7	5	2	5	4
4	5	5	7	6	5	4	5	4	5
4	4	7	7	5	3	4	4	7	5
5	7	6	5	4	4	3	5	6	4

Mean = 5, RSD = 20%

ENVIROSTAT

7	6	5	6	1	6	4	5	4	4
5	3	8	2	3	4	6	8	6	4
6	4	3	4	3	8	7	5	3	5
7	8	5	4	4	5	2	4	3	4
2	4	6	4	6	5	4	5	8	4
5	5	6	5	8	7	5	4	4	5
3	6	6	5	6	5	6	4	6	3
9	5	4	4	6	5	4	5	7	10
9	4	5	5	6	5	6	6	6	5
4	5	5	5	3	5	5	3	5	3

Mean = 5, RSD = 35%

ENVIROSTAT

7	5	5	4	4	6	5	3	7	2
-1	5	5	2	5	2	8	5	6	4
4	1	2	5	3	5	2	4	8	6
-1	0	7	4	8	-2	5	6	3	6
3	3	6	6	6	6	8	7	5	7
4	4	11	8	5	0	6	10	5	1
5	7	7	3	1	7	7	0	7	1
4	10	4	6	8	6	1	7	5	8
5	6	3	6	7	-2	4	4	2	8
4	3	0	4	10	3	2	6	0	6

Mean = 5, RSD = 50%

ENVIROSTAT

9	7	7	4	9	-2	11	-3	1	8
1	8	3	12	9	-1	5	5	8	5
4	4	6	10	6	11	9	5	6	8
7	0	7	7	6	3	3	5	5	6
5	4	6	7	3	8	14	5	5	9
8	0	3	6	11	9	-4	7	-1	7
3	4	9	2	1	6	4	7	6	1
8	6	1	4	3	2	1	4	5	11
6	0	9	1	7	5	-1	4	6	1
5	8	5	11	-1	1	2	5	6	7

Mean = 5, RSD = 75%

ENVIROSTAT

3	4	2	9	-7	4	-2	4	5	5
6	11	1	5	-5	8	7	6	8	1
12	-4	9	4	3	-2	7	9	7	3
6	18	12	13	3	4	10	17	-2	-1
6	16	9	6	4	10	-1	7	-1	4
10	5	9	11	6	3	5	6	4	0
11	12	6	-5	13	6	9	6	12	5
4	8	13	1	10	1	2	7	-1	6
6	1	7	-1	-5	6	0	8	3	-8
2	1	11	13	6	5	9	3	-1	11

Mean = 5, RSD = 100%

ENVIROSTAT

2	9	-6	12	-6	9	4	18	-8	1
14	5	8	8	14	-10	4	2	15	4
-17	5	-1	6	-3	5	6	9	-6	-6
4	-1	2	21	-11	9	7	10	5	5
6	3	3	2	4	11	-1	3	13	4
1	-3	-6	13	0	1	-9	-11	38	-2
-6	7	-9	8	-4	10	9	4	12	0
-1	10	6	2	11	-9	-8	1	8	19
-3	13	2	16	-7	-2	16	8	1	11
12	15	0	-1	3	-3	12	15	13	22

Mean = 5, RSD = 160%

ENVIROSTAT

15	-16	26	18	9	15	-6	5	10	31
-9	-3	-7	4	-6	7	14	1	22	7
10	12	5	6	-8	-6	16	5	-3	8
20	8	6	12	-20	14	13	0	11	-15
4	23	-16	17	-13	5	17	7	-7	21
16	8	11	2	17	0	14	3	16	3
2	11	24	-16	1	18	1	3	5	-3
-2	17	11	6	16	9	7	9	6	-1
-1	12	18	10	-4	-10	4	0	35	-10
20	9	1	1	14	19	-8	-6	5	1

Mean = 5, RSD = 200%

ENVIROSTAT

Error Causes

- Different people (and even the same person) to get different results from the same material
- One, or all, may be wrong
- Wasted time and money
- Improper animal care or field management
- Think there is a problem when there isn't
- Think there is not a problem when there is

ENVIROSTAT

What Determines Tolerable Error?

- Consequences of incorrect decision
- The higher the consequence, the less the tolerable error
- The tolerable error is determined during the Sample Quality Criteria (SQC) process
- The actual error is quantified through the use of Quality Control (QC)
- The tolerable error is different for different projects

When Error is More of an Issue (or Larger)

- Low concentrations of the analyte of interest (e.g., drugs)
- Concentrated additives (e.g., minerals)
- High confidence needed (economic, safety, legal, etc.)
- Clumping or natural segregation (e.g., mycotoxins)
- Different shape, size, and density of ingredients (e.g., TMR)

Major Sources of Error in the Field

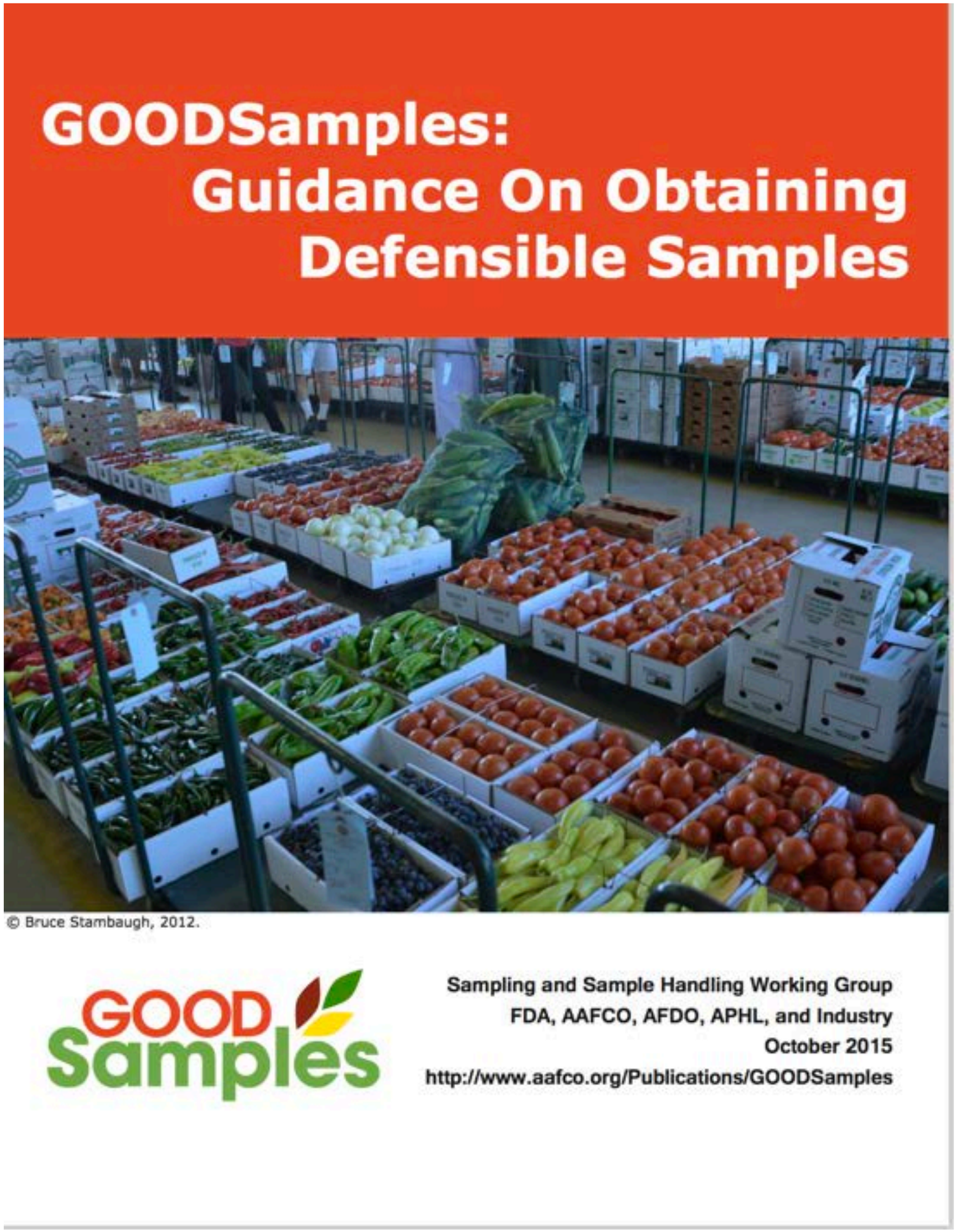
- Access to entire Decision Unit
- Incorrect tools
- Not enough mass
- Not enough increments

Guidance on Obtaining Defensible Samples—GOODSamples (2015)

- Development started about 5 years ago
- Committee of state regulators, industry, FDA
- AAFCO, AFDO, APHL
- Original objective was the "perfect" protocol
- Protocol for protocols

What is GOODSamples?

- It is not a set of protocols
- It is a methodology
  - develop objectives
  - understand error and consequences
  - integrate objectives into a defensible sampling protocol
  - verify that objectives were achieved
- Integrates objectives into a scientifically based, defendable protocol providing data for decision making



GOOD Test Portions

Coming soon

ENVIROSTAT

Philosophy of GOODSamples

- Provide a sound scientific basis for all decisions
- Provide a systematic process for development and evaluation of sampling protocols
- Meet FSMA requirements and your requirements
- Flexible enough to address
  - emerging contaminants
  - changing priorities
  - new products, expansion of programs
  - unanticipated field conditions
  - outbreak response

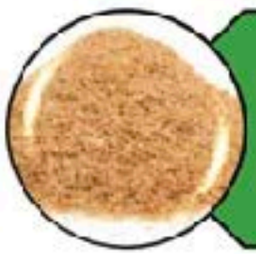
Systematic flexibility!

Decision Unit

- Truck load of grain

Increments

- Individual probes of grain



Primary Sample

- All increments combined

Laboratory Sample

- Package sent to/received by laboratory

Analytical Sample

- Grain prepared for testing

Test Portion

- Mass taken for analytical test

ENVIROSTAT

GOODSamples: Guidance on Obtaining Defensible Samples

Sampling and Sample Handling Working Group
FDA, AAFCO AFDO, APHL,

Sample Quality Criteria

Material Properties

↓ ↓

Theory of Sampling

↓

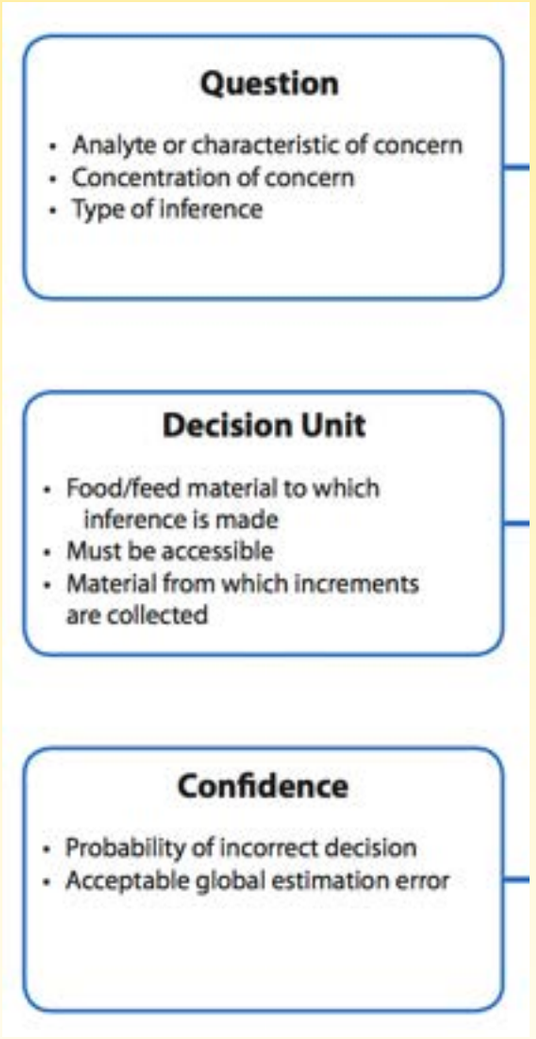
Sampling Protocol → Representative Samples → Correct and Defensible Decisions

Key Elements

- Sample Quality Criteria
  - question
  - Decision Unit
  - confidence
- Material Properties (Nature of Material)
  - nature of the elements
  - nature of the heterogeneity
- Theory of Sampling (TOS)
  - mass
  - increments
  - tools

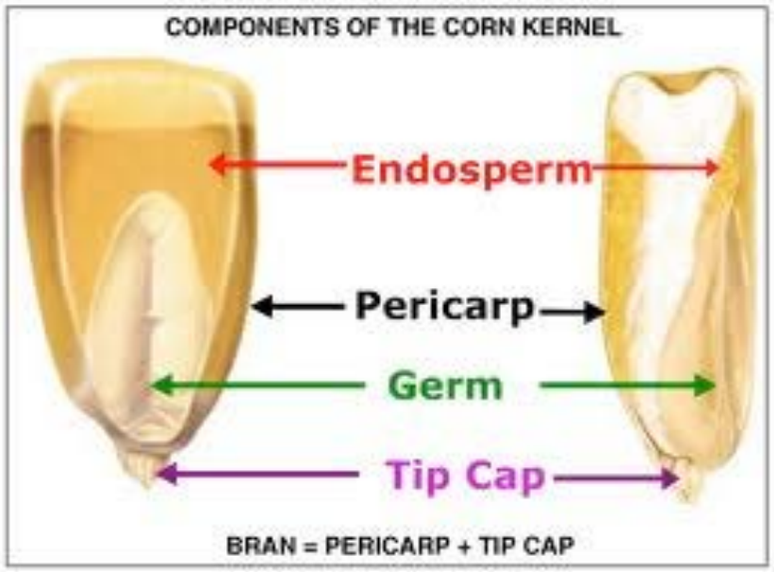
Sample Quality Criteria

- Question
  - What is the analyte(s) of interest?
  - How is the data going to be used to make a decision?
- Decision Unit
  - the specific material the question is referring to
  - the specific material inference is made to (scale of information)
- Confidence
  - the probability the decision is correct
  - related to the total error in the entire measurement process



Just give me one number!

ENVIROSTAT



What needs to be in compliance?

Material Properties

- Nature of the elements
  - finite
  - infinite
- Nature of the heterogeneity
  - compositional
  - distributional

This granola illustrates a solid infinite element material with both compositional and distributional heterogeneity.

ENVIROSTAT

Theory of Sampling (TOS)

- Critical for the sampling of infinite element materials
- Relates the concept of mass to sampling error
- Relates the concept of increments to sampling error
- Relates the concept of sample correctness (tool design and use) to sampling error
- Provides the ability to measure and mitigate error to meet specific objectives

What is the one phenomenon that causes ALL sampling error?

Heterogeneity

Compositional

Distributional

What is Compositional Heterogeneity?

- Difference in composition of the particles (or molecules) that make up the Decision Unit
- Heterogeneity only applies to the analyte(s) of interest

If different particles (or molecules) have different concentration/ mass of the analyte of interest, then each of them needs to be properly represented in the sample.

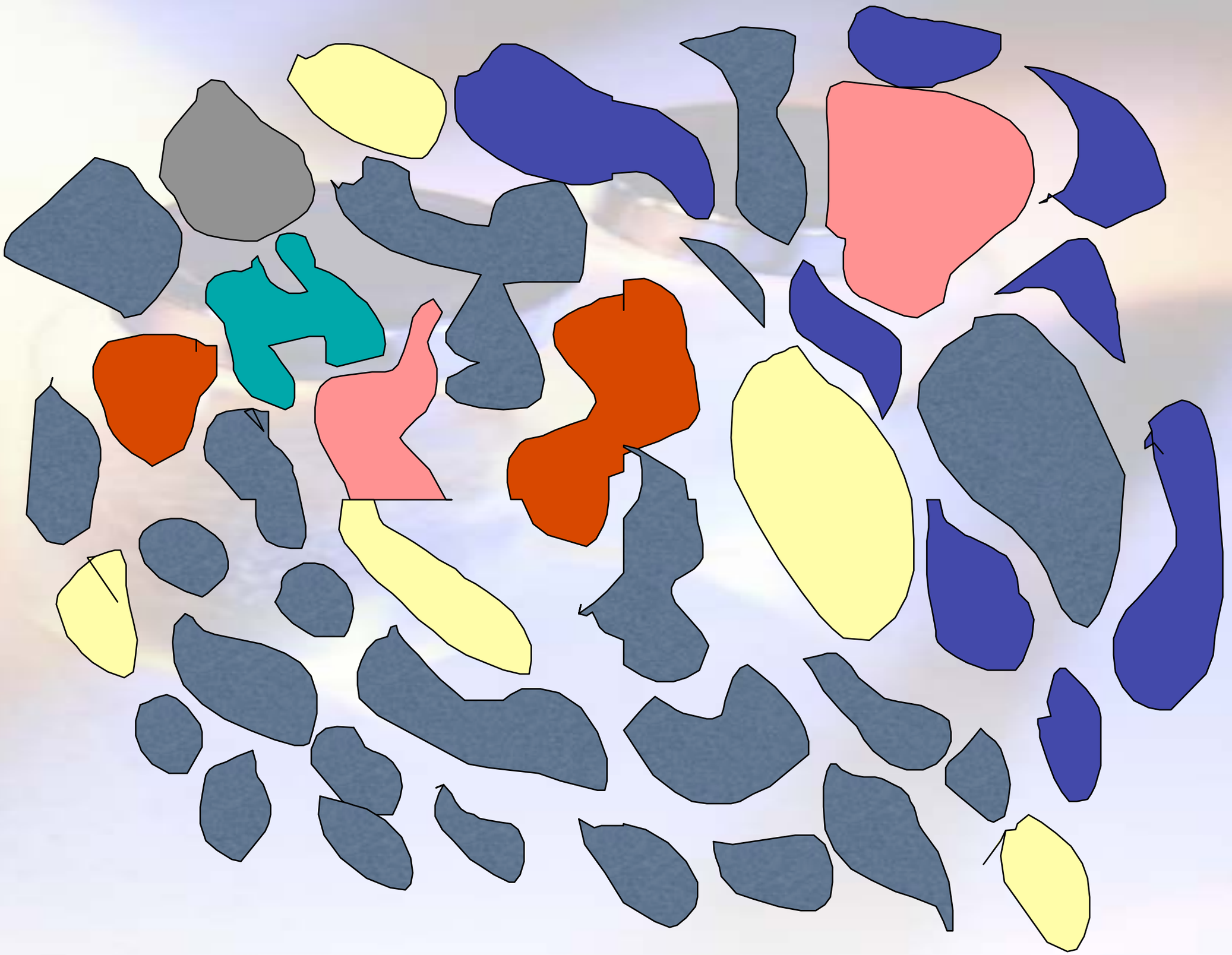
ENVIROSTAT

What is Distributional Heterogeneity?

- Difference in composition of the increments (or groups of elements) that make up the Decision Unit
- Heterogeneity only applies to the analyte(s) of interest
- Non-random distribution of particles or molecules
  - time
  - space

If the particles (or molecules) are different in different locations or times, we still need to represent them. How?

ENVIROSTAT



The concept of homogeneity does not exist!

How is Heterogeneity Controlled?

- Compositional heterogeneity leads to fundamental sampling error (FSE) which is controlled by sample **mass/volume**
- Distributional heterogeneity leads to grouping and segregation error (GSE) which is controlled by collecting many random **increments**

Mass and increments must be collected correctly.

ENVIROSTAT

Representative Sampling

- What is in the Decision Unit is in the primary sample
  - some of everything
  - same proportion
- The integrity of the analyte is not compromised
- What is in the primary sample is in the test portion
  - some of everything
  - same proportion
- We can demonstrate that it is so
  - documentation
  - quality control

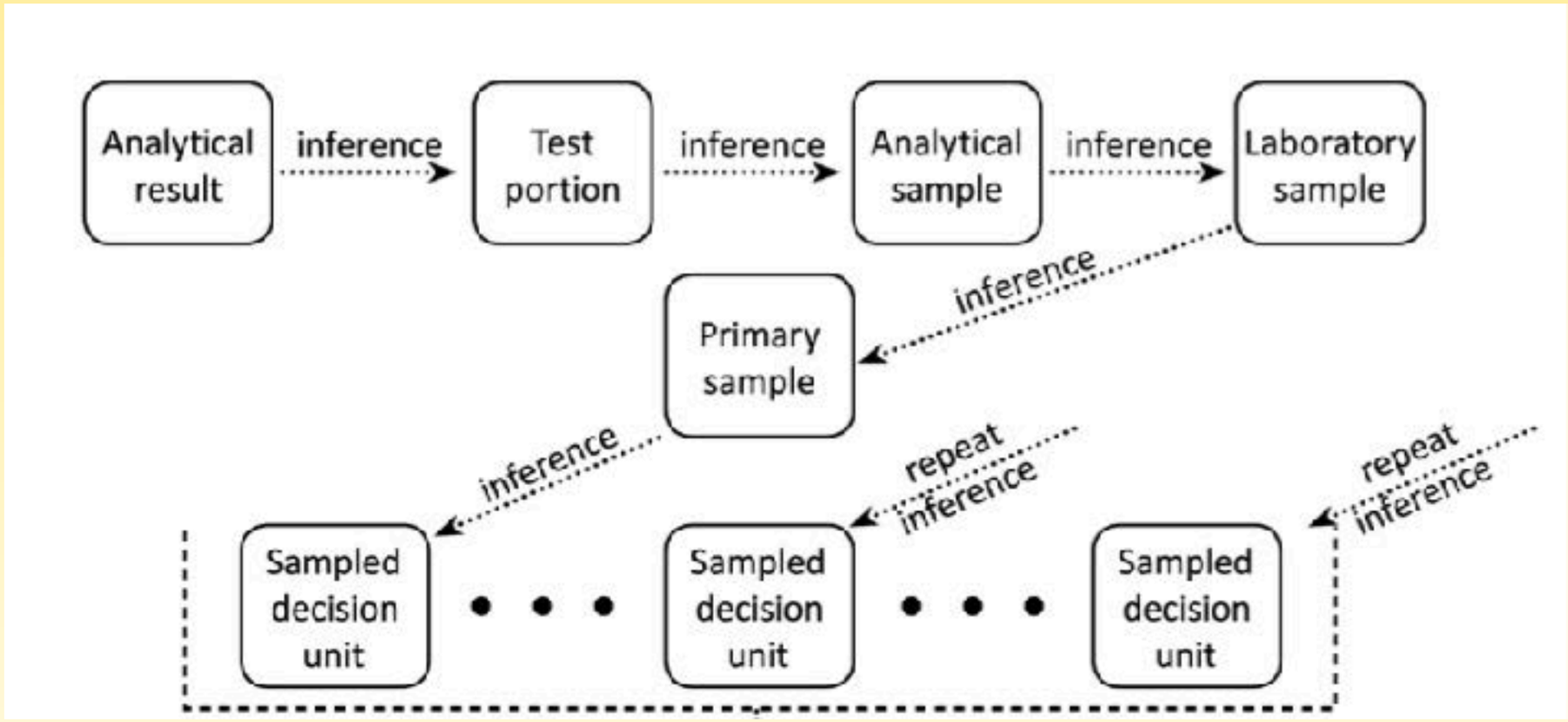
Increments at Random

- Without randomness there is no ability to make inference
  - simple
  - stratified
  - systematic
  - host of others
- Some random schemes are easier to implement than others
- If material is selected because it is different
  - What can be stated about the Decision Unit?
  - Does that meet the SQC?
- If a material is selected because it is convenient

Integrity

- What is concentration in the Decision Unit?
  - sampling did not change it
  - packing and transportation did not change it
  - laboratory processing did not change it
- Multiple analytes (behave differently)
- Many factors
  - environmental factors
    - humidity
    - temperature
  - time
    - holding time
    - preservatives

Inference



The laboratory and the field are equally important

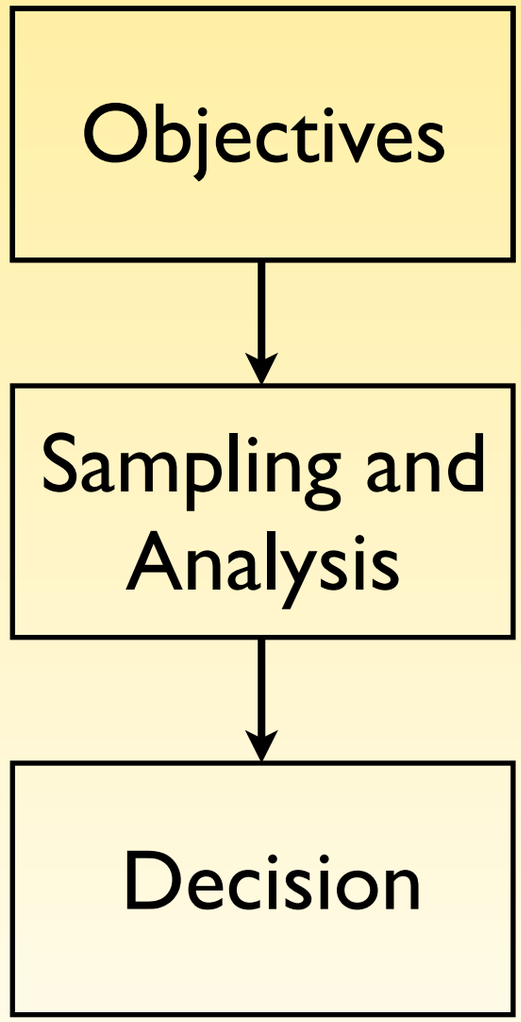
ENVIROSTAT

Sampling Logic

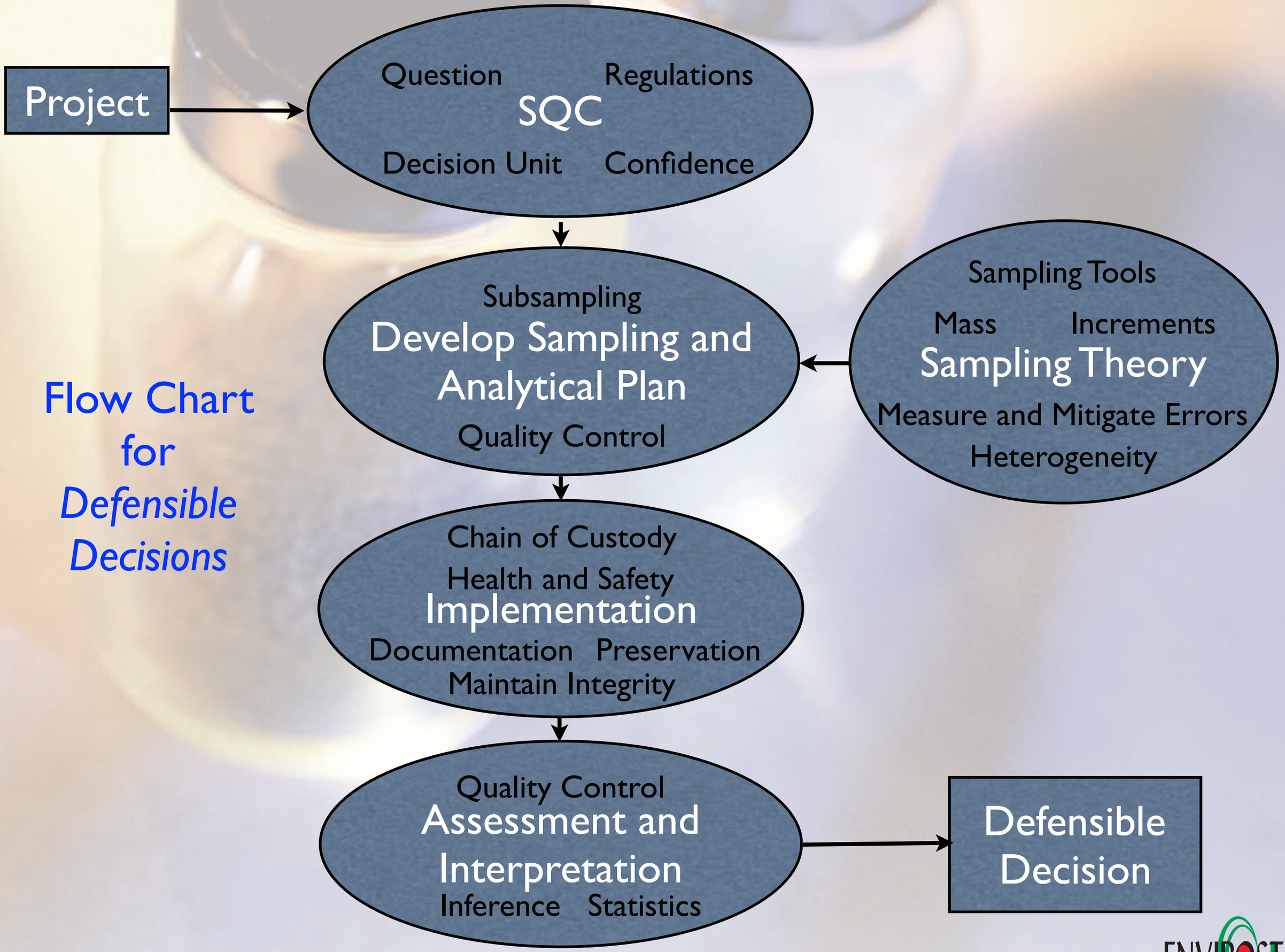
- What is the material in question (Decision Unit)?
- Does the Decision Unit fit in the sample jar?
- If so, collect it all
- If not, collect a sample to get the same answer you would get had you put the entire Decision Unit in the jar
  - enough mass
  - enough increments
  - sample correctness
- If we cannot collect the entire Decision Unit, we will have sampling error (the ability for the sample to truly represent the Decision Unit)

Sampling

- About objectives, not about
  - the perfect sampling tool
  - the perfect sampling technique
  - the universal sampling plan
- Estimating parameters
  - average
  - percentage
  - detect/non-detect
- Making decisions
  - no universal/perfect statistics



Flow Chart for *Defensible Decisions*



Contact Information

Charles Ramsey
EnviroStat, Inc.
970-689-5700
PO Box 339
Vail, AZ 85641
chuck@envirostat.org
www.envirostat.org