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Toxicological Outbreak Investigation Course

Module Three: Toxicological Laboratory Principles





Module 3 Objectives

- Explain importance of early sample collection
- List laboratory testing considerations
- Identify information needed in sample collection protocols
- Discuss collecting comparison samples in outbreak scenario

Early Sample Collection

- During a toxicological outbreak investigation, collecting environmental samples and biological specimens for laboratory analysis needs to be done as soon as possible
- Some toxic agents may be broken down in the environment or eliminated from the body in minutes to hours after exposure
- The sooner that an environmental sample or biological specimen is collected, the greater the chances that the sample will still contain the toxic agent

Laboratory Testing Considerations

Is laboratory testing needed?

- **Biological testing** may not be needed if environmental samples are found to contain the hypothesized toxic agent,
 - **AND** that toxic agent is clinically compatible with the illness,
 - **AND** the epidemiologic data show an association between illness and exposure.

Laboratory Testing Considerations (cont.)

Is laboratory testing needed? (cont.)

- **Environmental testing** may not be needed if biological specimens are found to contain the hypothesized toxic agent,
 - **AND** that toxic agent is clinically compatible with the illness,
 - **AND** the epidemiologic data show an association between illness and exposure.

Laboratory Testing Considerations (cont.)

Can the etiology be narrowed to a small number of likely toxic agents?

- Thousands of toxic agents can cause illness.
 - Each agent requires a specific, validated method for detection and confirmation.
 - Each sample type, whether biological or environmental (such as urine, blood, or water) requires a different validated method.
- Investigators need a specific idea of what to test for before they start testing.

There is no one single test that can screen for all possible toxic agents at one time.

Laboratory Testing Considerations (cont.)

Is there an accredited laboratory with validated methods that can test for the suspected toxic agents?

- Not all laboratory capabilities are the same.
 - Some laboratories are better than others.
 - Consider how much precision and accuracy is needed when exploring laboratory options.
- Where is the nearest laboratory that can perform the testing?
 - It might be in another country.
 - Are they willing and able to perform the test?
 - What are the export/import requirements for shipping?

There are many toxic agents for which no laboratory test exists.

Laboratory Testing Considerations (cont.)

Is there identifying information to help you interpret the biological specimen or environmental sample?

- This is where the sample log for both the environmental sample and biological specimen is important
- At a minimum, you need to know:
 - Who and where the sample came from?
 - What is it?
 - How was it collected?
 - When was it collected?
 - Was there a chain of custody form completed?

Laboratory Testing Considerations (cont.)

Will you be able to interpret the results?

- Finding a toxic agent in the environmental sample and/or the biological specimen does not always mean that it caused the outbreak.
- Data on what are considered “normal” background levels in the population and the levels that could cause acute illness are helpful for interpretation.
- Were control samples collected, or are there known mean/average values in the population available for comparison?



Outbreak Scenario

- You have determined that the toxidrome fits with a potential carbofuran exposure



What are the next steps to confirm the contamination and exposure?

Develop a Sample Collection Protocol

- Identify and work with a laboratory that will analyze the sample to develop a collection protocol
- Ask the laboratory:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Sample Collection Protocol

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Some analyte(s) can only be detected in specific mediums.

Depending on the amount of time that has passed, the toxic agent itself or only its metabolites might be more likely to be detected in a specific medium.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

When a specific hypothesis has not yet been developed, it is usually best to collect both urine and whole blood.

If a lot of time has elapsed since exposure, there might be a better chance of finding the toxic agent or its metabolite in urine.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Environmental sampling is most useful when you are able to narrow your hypothesis to a small number of potential exposures.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

The more tests you do, the more sample you'll have to collect.

Having extra sample will allow the laboratory to perform repeat testing or validation.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

The laboratory should provide a protocol that specifies when and how to collect the sample.

For example...

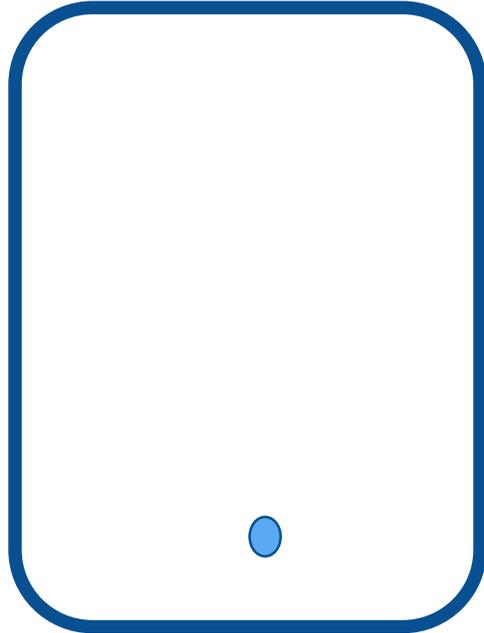
- If you are collecting urine, does it need to be a first morning void?
- If you are collecting a soil sample, does it need to be taken from the top layer?

When collecting biological specimens or environmental samples, personal protection should be worn.

Heterogeneity in Environmental Samples

- When collecting an environmental sample, consider how it is distributed in the air, water, soil, etc.
- For example, consider a bag of corn/maize flour that contains a toxic agent

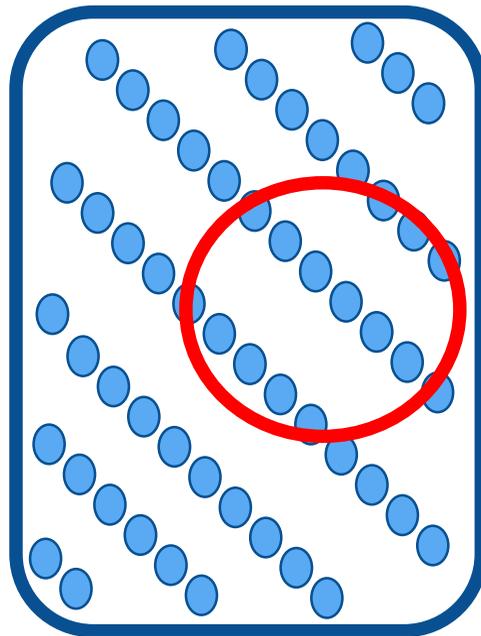
Heterogeneity in Environmental Samples



The rectangle on the left represents the bag of flour.

The blue circle represents a toxic agent.

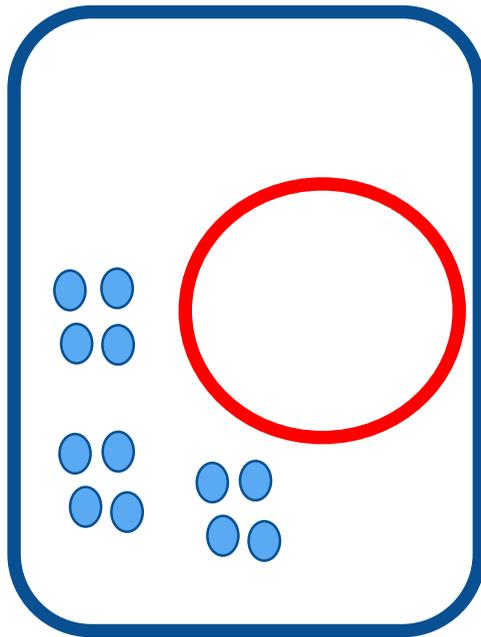
Homogeneity in Environmental Samples



A single sample might be sufficient for collection

If a toxic agent is distributed evenly, this is a **homogenous** distribution.

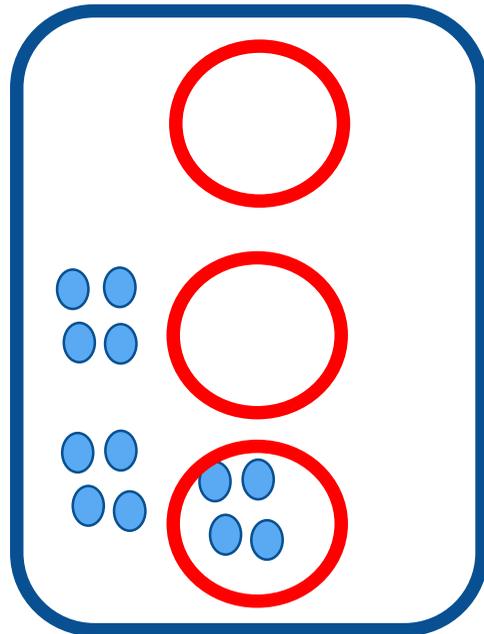
Heterogeneity in Environmental Samples



A single sample might miss the toxic agent

If a toxic agent is NOT distributed evenly, this is a **heterogenous** distribution.

Heterogeneity in Environmental Samples

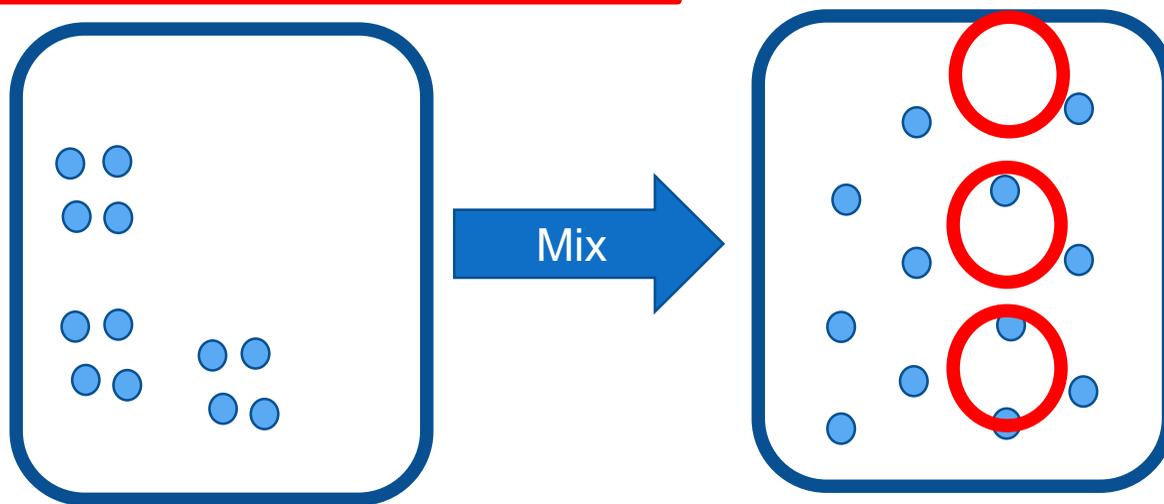


You might collect multiple smaller samples from different locations in the bag

A heterogenous distribution requires a different sampling strategy.

Heterogeneity in Environmental Samples

Or, you might mix it to more evenly distribute the toxic agent prior to collection



Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Some toxic agents can react with specific materials; therefore, the type of container or collection material needs to be considered.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Many samples need processing after collection.

For example...

- A blood specimen may need to be centrifuged to separate the serum.
- A water sample may need an acid preservative added.

Personal protection should be worn during sample processing.

Sample Label

- All samples need a label (such as an ID sticker or write the information on a small piece of paper and tape securely to the sample)
- Information to include on label:
 - Who/what the sample was drawn from
 - ID or barcode
 - Time sample was collected





Activity: Sample Log

- A log sheet or sample log should be completed
- A sample log is systematic way to record the descriptive information about each sample



What type of information is usually collected on a sample log?



Activity: Sample Log (cont.)



What type of information is usually collected on a sample log?

Who

- ID#
- Name

What

- Type of medium
- Any pertinent details on the sample

When

- Collection date and time

Where

- Collection location



Sample Log

Institution name:

Institution address:

Institution phone:

| Sample ID | Collection date | Collection time | Person collecting the sample (Name) | Location where collected (GIS coordinates if possible) | Type of sample (urine, blood, water, food, etc.) | Amount of sample (units) | Collected from (Name) | Collected from (ID) | Observations (visible debris, tampering, etc) |
|-----------|-----------------|-----------------|-------------------------------------|--|--|--------------------------|-----------------------|---------------------|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

A modifiable Sample Log is available in the Tool Kit.

Sample Label and Log: Common Problems

- If samples are missing a label or a log, then investigators might be...
 - Unable to determine who/what the samples came from (for example, did they come from cases or controls?)
 - Unable to determine what the sample is (particularly if it is an environmental sample)
 - Unable to determine how many half lives may have passed between exposure and sample collection

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where samples should be shipped?

Sometimes, placing a sample in the refrigerator or freezer, or preventing its exposure to light, is required to slow the degradation or metabolism.

The laboratory you are working with will be able to provide you with the correct storage conditions for your samples.

Sample Collection Protocol (cont.)

- Common considerations:
 - What type of sample should be collected?
 - How much sample should be collected?
 - How should it be collected?
 - What type of container should be used?
 - What needs to happen to the sample after collection?
 - What temperature should it be stored at?
 - How long can it be stored?
 - How and where the sample should be shipped?

Some samples will only last a certain amount of time, depending on the storage temperature.

The laboratory you are working with can tell you about storage temperature and duration.

Laboratory Testing Considerations

- Toxicological lab testing can be expensive and resource-intensive
- Results can sometimes take weeks or months to receive
- Many times, the outbreak may be over and cases will have stopped occurring
- Need to determine if laboratory testing is needed

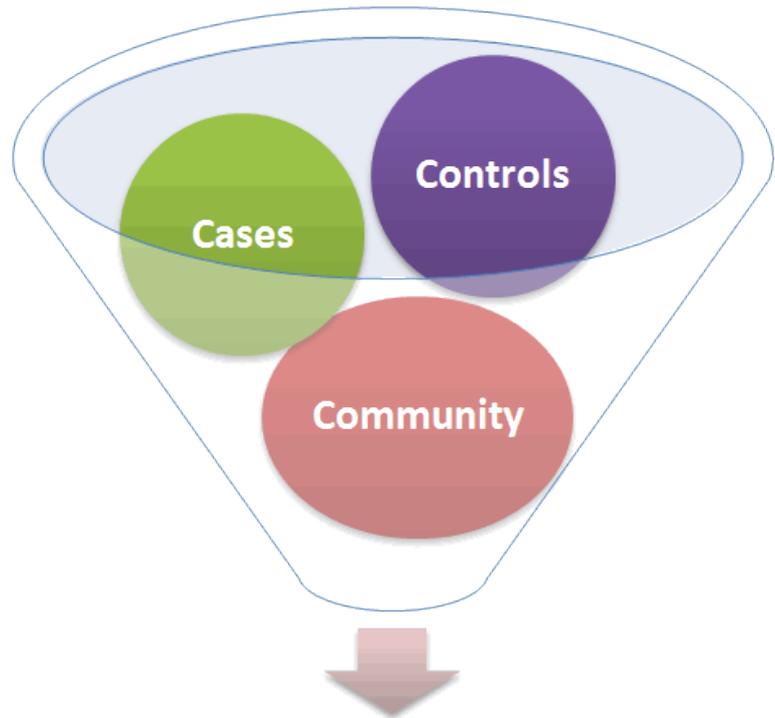


Outbreak Scenario



What next steps must you consider to determine if carbofuran tested among cases is higher than what you would expect in the population or if it is causing the outbreak?

Laboratory Data: Comparison Samples



Comparison Samples

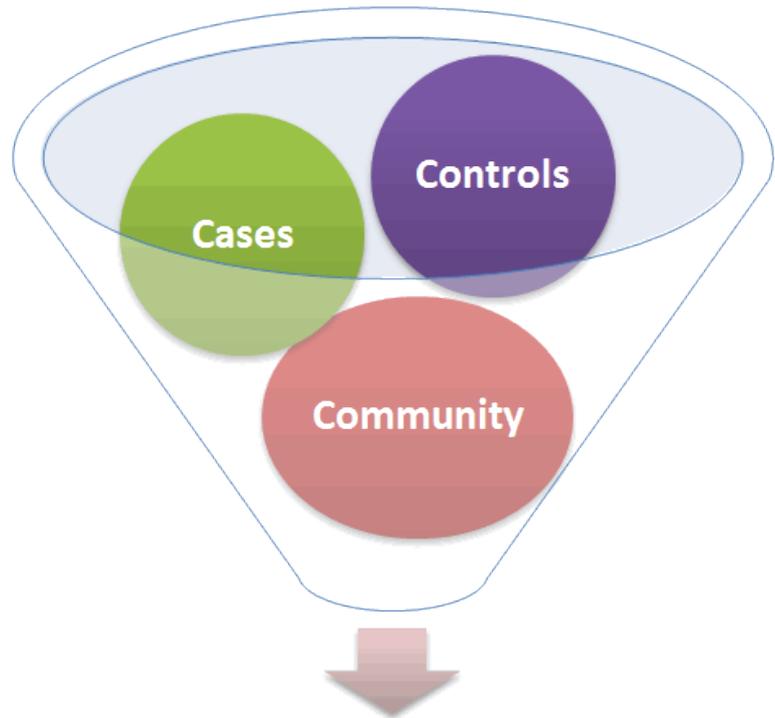
- Collect comparison samples to determine if the levels found in cases are higher than people who did not get sick
- Comparison samples are usually collected from one of more of these three groups:
 - Controls
 - Cases
 - Community

Conducting epidemiological studies can help you find the answer. A critical part of an epidemiological investigation is collecting comparison samples.

Module 5 will discuss two types of epidemiological study designs to consider in toxicological outbreak investigations: case-control study and cohort study.

Controls who are a part of your case-control study

Laboratory Data: Comparison Samples



Comparison Samples

- Comparison samples are usually collected from one or more of these three groups:
 - Controls
 - Cases
 - Community

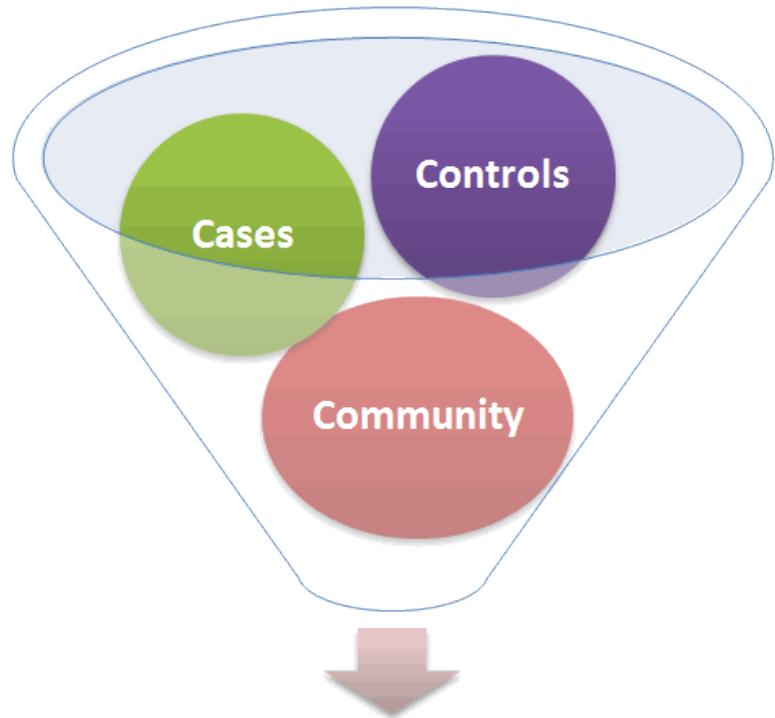
Biological specimens

- Follow-up specimens from cases.
- Useful if the suspected toxic agent has a short half-life and specimens were collected during the illness, and some time later.

Environmental samples

- If you suspect a contaminated source of flour, and if a case has multiple types of flour, then you might grab a sample from each bag.

Laboratory Data: Comparison Samples



Comparison Samples

- Comparison samples are usually collected from one or more of these three groups:
 - Controls
 - Cases
 - Community

Biological specimens
Specimens previously collected from the community. For example, if there are urine or blood specimens stored at a local clinic.

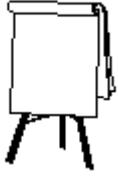
Environmental samples
Food samples from the local market.

Summary

- It is important to collect biological and environmental samples as early as possible
- Identify a lab that will conduct laboratory testing and work with the lab to develop the sample collection protocol

Summary (cont.)

- Collecting samples from a comparison population will assist with determining whether the toxic agent is the source of the outbreak
- Laboratory testing can be expensive and resource-intensive



Module Conclusion



What questions do you have about the information presented in this module?