Accessible version can be found here:
https://www.cdc.gov/nceh/hsb/elearning/toi/Mod4/

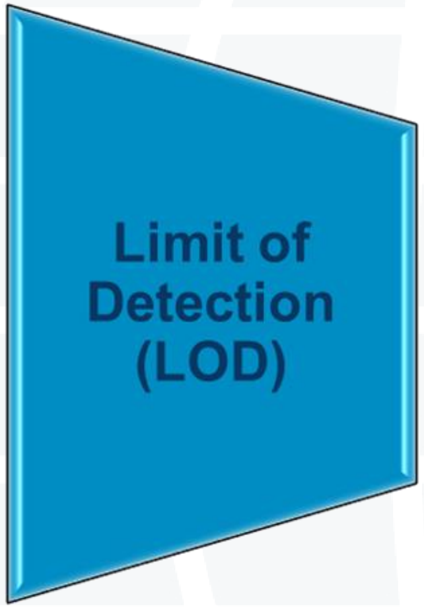
Toxicological Outbreak Investigation Course

Module Four: Analyzing and Interpreting Laboratory Results

Module 4 Objectives

- Describe Limit of Detection (LOD) and two constant values for substituting for values less than LOD
- Identify two common examples of adjusting laboratory results
- Identify and analyze log-normal distribution data
- Describe information needed to interpret laboratory results, specifically toxic threshold and comparison values

Limit of Detection



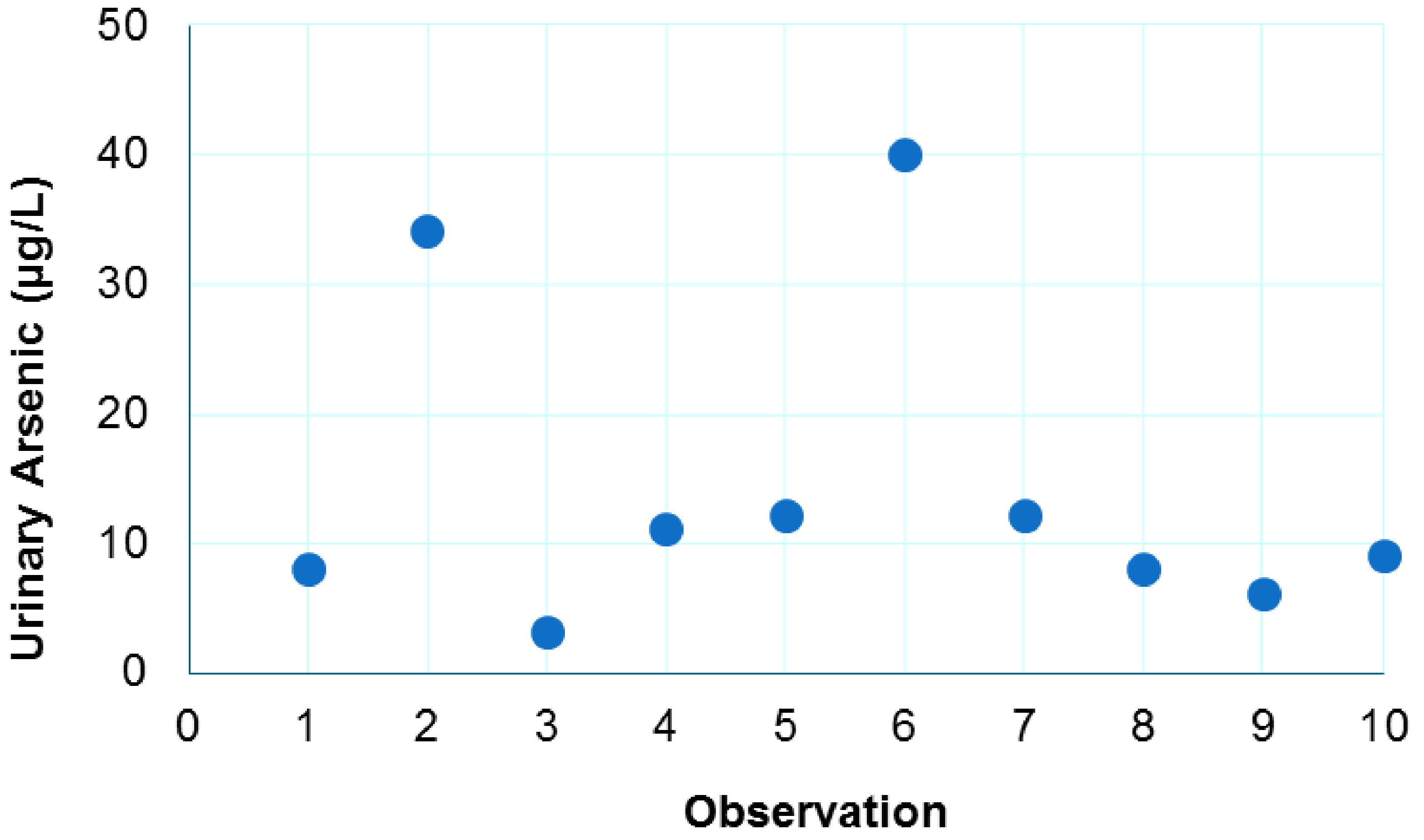
- Most laboratory tests can only detect a toxic agent if there is a certain measureable amount present
- This level is called the limit of detection (LOD)

Limit of Detection (cont.)

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	3
4	11
5	12
6	40
7	12
8	8
9	6
10	9

For example, consider urinary arsenic values collected from patients involved in an acute outbreak investigation.

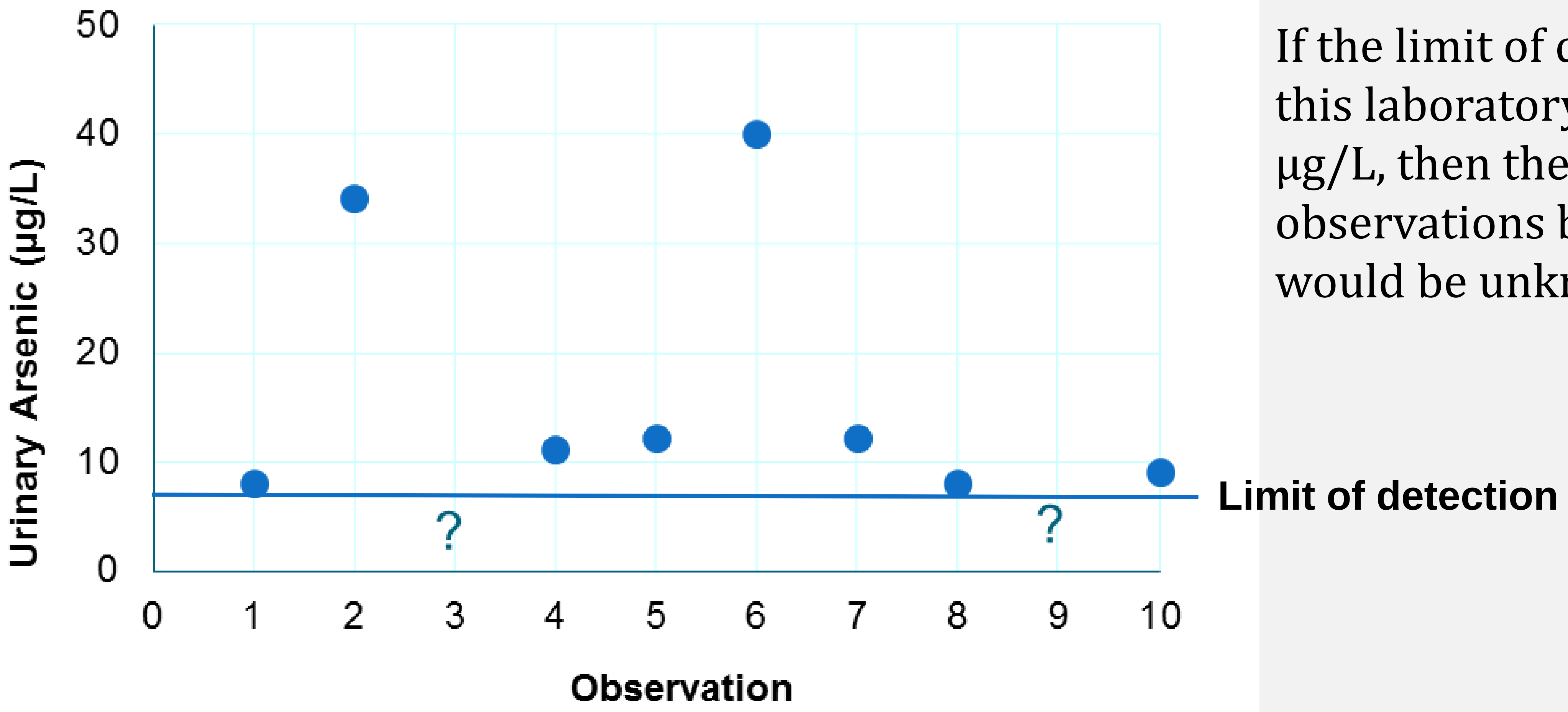
Limit of Detection (cont.)



Plotting them on a graph looks like this.

Each person is an observation along the x-axis, and their corresponding urinary arsenic concentration is on the y-axis.

Limit of Detection (cont.)



If the limit of detection for this laboratory test is 7 µg/L, then the two observations below 7 µg/L would be unknown.

Limit of Detection (cont.)

Observation #	Arsenic Concentration (μg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Observations that are below the limit of detection are usually reported by the lab as <LOD, or Not Detected.

This means the true value is somewhere between 0 and the LOD.

Substituting for Values <LOD

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Can you calculate the median of these values?

Substituting for Values <LOD (cont.)

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Can you calculate the median of these values?

Yes, the median is 11.5 µg/L

Substituting for Values <LOD (cont.)

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Can you calculate the average of these values?

Substituting for Values <LOD (cont.)

Observation #	Arsenic Concentration (μg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Can you calculate the average of these values?

No. A value of "<LOD" cannot be summed or averaged.

Substituting for Values <LOD (cont.)

- To calculate a mean or perform statistical tests, investigators need to assign a numeric value to levels that are <LOD; these values should not be ignored
- The choice of substitution will affect any subsequent descriptive or statistical analyses on the data
- There are two constant values that are commonly substituted for values that are <LOD:
  - $LOD/2$
  - $LOD/\sqrt{2}$

Substituting for Values <LOD (cont.)

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

In this example, where the LOD = 7 µg/L:

- $\frac{LOD}{2} = \frac{7}{2} = 3.5\mu g/L$
- $\frac{LOD}{\sqrt{2}} = \frac{7}{\sqrt{2}} = 4.9\mu g/L$

Substitution Differences

	Original Data	Ignoring values <LOD
	8	8
	34	34
	<LOD	
	11	11
	12	12
	40	40
	12	12
	8	8
	<LOD	
	9	9
Mean	N/A	17

Substitution Differences (cont.)

	Original Data	Ignoring values <LOD	Substituting 0
	8	8	8
	34	34	34
	<LOD		0
	11	11	11
	12	12	12
	40	40	40
	12	12	12
	8	8	8
	<LOD		0
	9	9	9
Mean	N/A	17	13

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Substitution Differences (cont.)

	Original Data	Ignoring values <LOD	Substituting 0	Substituting $\frac{LOD}{2}$
	8	8	8	8
	34	34	34	34
	<LOD		**0**	**3.5**
	11	11	11	11
	12	12	12	12
	40	40	40	40
	12	12	12	12
	8	8	8	8
	<LOD		**0**	**3.5**
	9	9	9	9
Mean	N/A	17	13	14

Substitution Differences (cont.)

	Original Data	Ignoring values <LOD	Substituting 0	Substituting $\frac{LOD}{2}$	Substituting $\frac{LOD}{\sqrt{2}}$
	8	8	8	8	8
	34	34	34	34	34
	<LOD		**0**	**3.5**	**4.9**
	11	11	11	11	11
	12	12	12	12	12
	40	40	40	40	40
	12	12	12	12	12
	8	8	8	8	8
	<LOD		**0**	**3.5**	**4.9**
	9	9	9	9	9
Mean	N/A	17	13	14	14

Advanced Methods

- There are other ways to account for values that are <LOD that may be more advanced than substitution.
- One method is to use survival analysis procedures that are adjusted to account for left censoring (as opposed to the right censoring that is more commonly experienced).
- Note: you may need to consult with a statistician or other scientist familiar with such methods.

For further reading:

- Helsel, D.R. (2005). Nondetects and data analysis: Statistics for censored environmental data. New York: John Wiley & Sons
- Helsel, D.R. (2012). Statistics for Censored Environmental Data Using Minitab and R. New York: John Wiley & Sons

Making Adjustments to Lab Results

Making Adjustments to Lab Results

- It may be necessary to adjust toxicological laboratory results to account for how concentrated or diluted the specimen is.
- Two common examples:
  - Adjusting for creatinine in urine specimens
  - Adjusting for proteins or lipids in serum specimens

Example: Urine Concentration Adjustment

- Urine dilution can vary…
  - Between people
  - Within a single person during the day
- Factors that affect this dilution include water consumption and general health status
- The dilution can affect the concentration of toxic agents in a urine specimen

Note: Picture on the right shows three urine specimens taken from one person with during different times of the day. Note how the color varies from concentrated to dilute.

Example: Urine Concentration Adjustment

- Levels of toxic agents in urine may decrease if a person drinks a lot of water; and conversely may go up if the person is dehydrated
- Adjusting for urine creatinine can help account for differences in urine dilution
- Common formula:

Analyte concentration (µg/g creatinine) =

$$\frac{\textit{Concentration of toxic agent}\ (\mu g/L)}{\textit{Creatinine concentration}\ (mg/dL)}$$

Note: The analyte and creatinine levels are analyzed from the same urine specimen.

Urine creatinine excretion is constant across and within individuals, such that changes in the ratio will reflect changes in biomarker excretion.

Example: Urine Concentration Adjustment

Analyte concentration (µg/g creatinine) =

$$\frac{\boldsymbol{Concentration\ of\ toxic\ agent}\ (\mu \boldsymbol{g/L})}{\boldsymbol{Creatinine\ concentration}\ (\boldsymbol{mg/dL})}$$

Observation #	Toxic agent in urine (µg/L)	Creatinine level (g/L)	Adjusted measurement (µg/g creatinine)
1	7	1.1	6.4
2	34	0.7	49
3	26	1.3	20

Example: Adjusting for Proteins or Lipids in Serum Specimens

- Similarly, a serum level of an analyte can be divided by a lipid measurement in the same specimen in order to obtain a "lipid adjusted" result
- When an adjustment is made, results are expressed as the analyte units/gram of lipid (in case of lipid adjustment)

If someone has high amounts of lipids in their serum, serum levels may be higher for a substance that binds to lipids (lipophyllic)

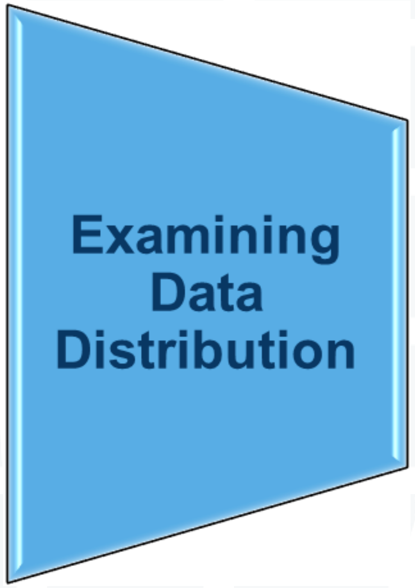
Examples of toxicants hat are lipophilic and require adjustment for the amount of lipid in the specimen:

- Polychlorinated biphenyls (PCBs)
- Organochlorine pesticides

Making Adjustments to Laboratory Results

- Adjustment is a way to standardize lab results
- Adjustments are not always needed -- for example, for toxic agents that do not bind to lipids in the blood, you would not adjust for lipid concentration.
- Consult the laboratorian or a toxicologist for guidance on if and how adjustments should be made for your specimens

Examining Data Distribution



Typical Infectious Outbreak Data

- **Infectious** outbreak investigations often result in dichotomous (e.g. present or absent) laboratory data.

Observation #	Salmonella	Shigella
1	Present	Absent
2	Present	Absent
3	Absent	Absent
4	Present	Absent
5	Absent	Absent

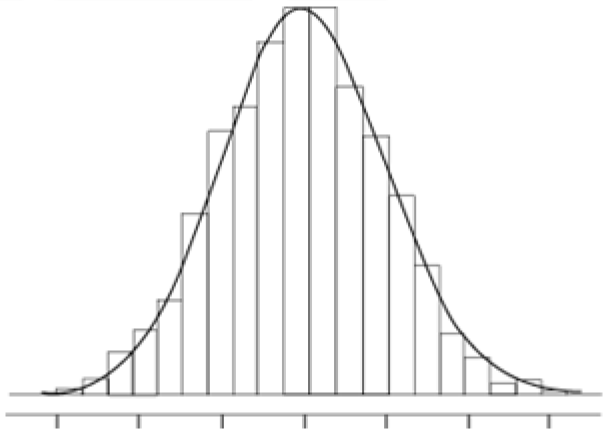
Typical Toxicological Outbreak Data

- **Toxicological** outbreak investigations often result in continuous laboratory data
- Before analyzing continuous variables, their distribution must be checked for normality

Observation #	Arsenic (µg/L)	Lead (µg/dL)
1	8	3
2	34	4
3	10	4
4	11	3
5	12	5

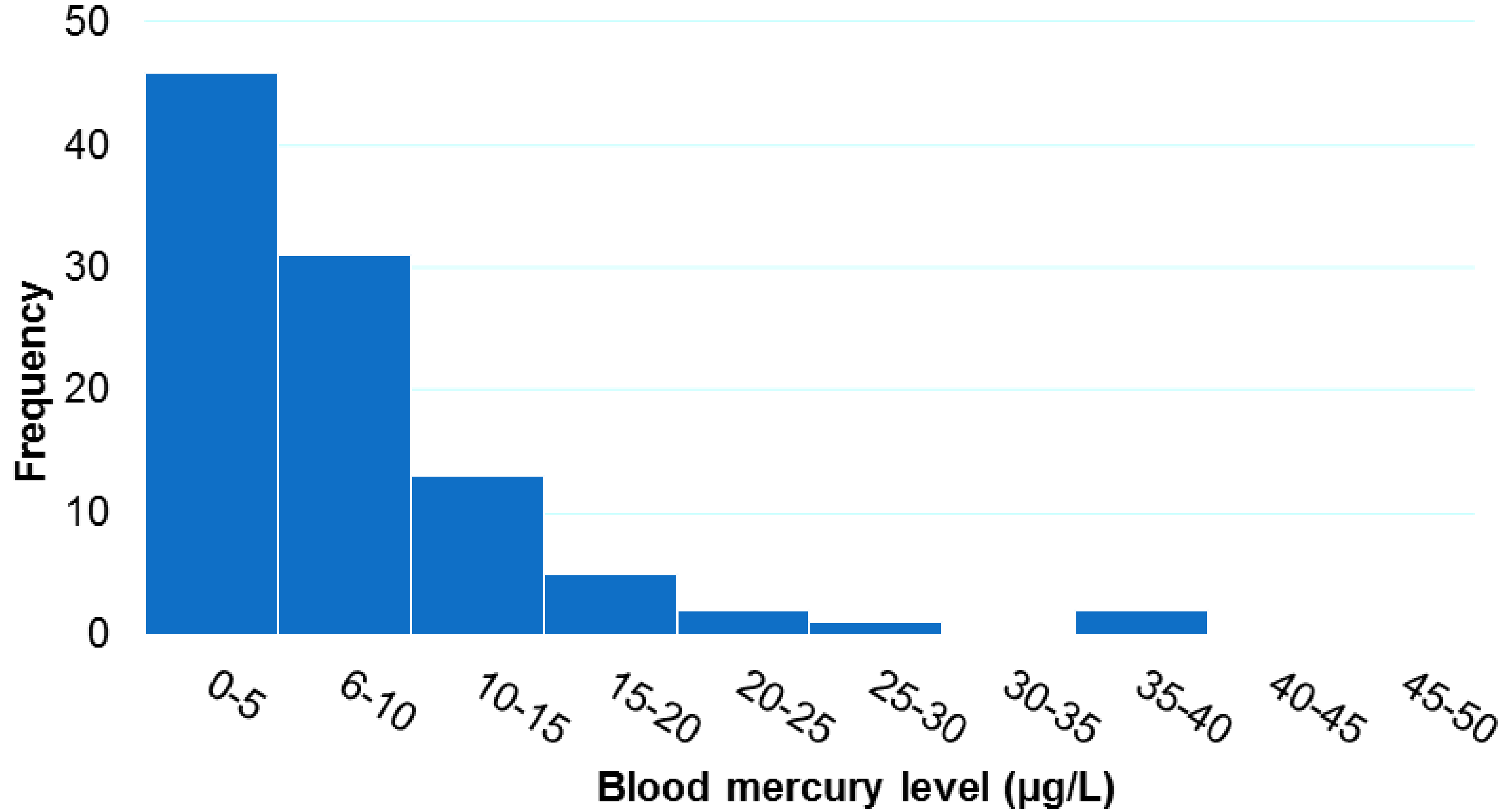
Normal Distribution

- Also called:
  - Bell-shaped curve
  - Parametric distribution
  - Gaussian distribution



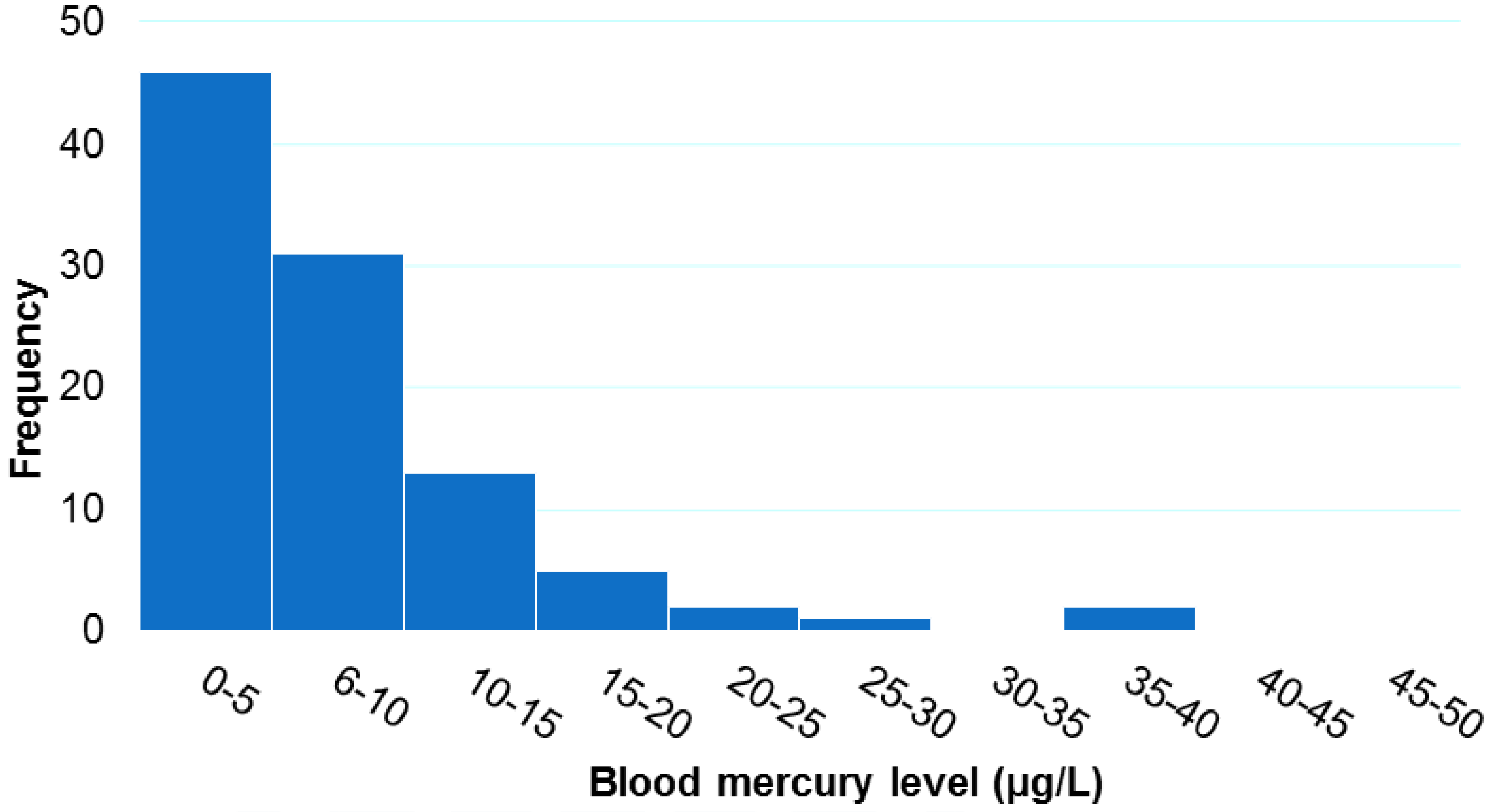
- If data are normally distributed, you can calculate means and compare groups using t-tests

Examining Data Distribution



Are these values normally distributed?

Examining Data Distribution (cont.)

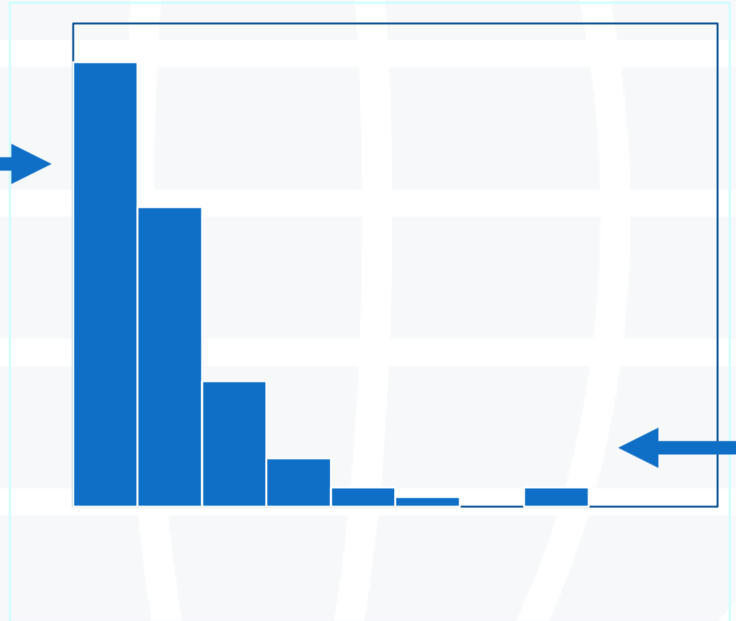


Are these values normally distributed?

No. Most participants have low levels, and a small number have high levels.

Log-Normal Distribution

Many people with levels that are low or <LOD

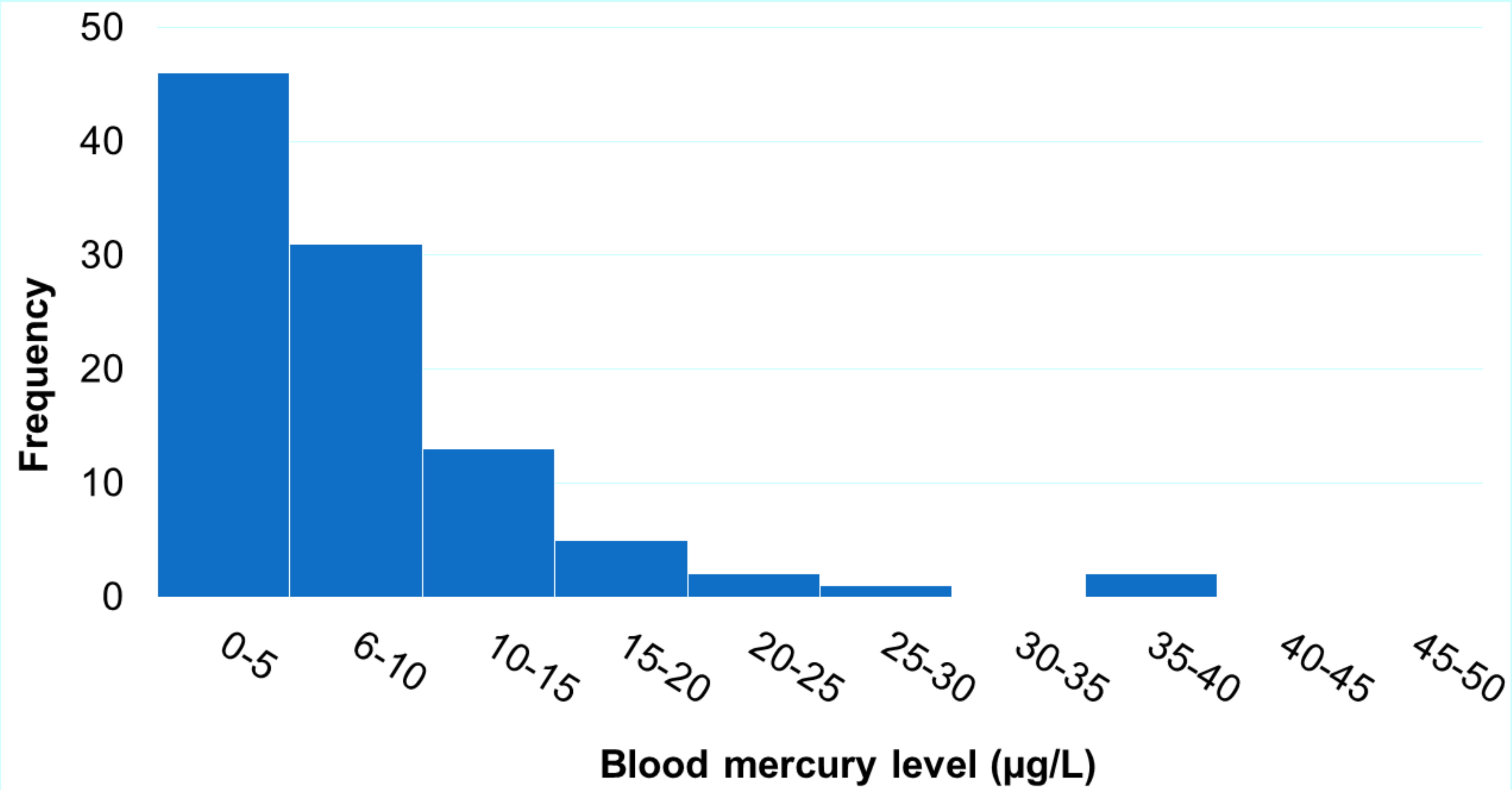


Small number of people with levels that are very high

When toxic agents are measured in people, they often have a **log-normal** distribution.

Log-normal distributions can become normally distributed when you take the natural log of each of the measurements.

Examining Data Distribution



This histogram shows blood mercury levels in 100 cases from an outbreak investigation.

Values that were below the limit of detection received a substitution of $LOD/\sqrt{2}$.

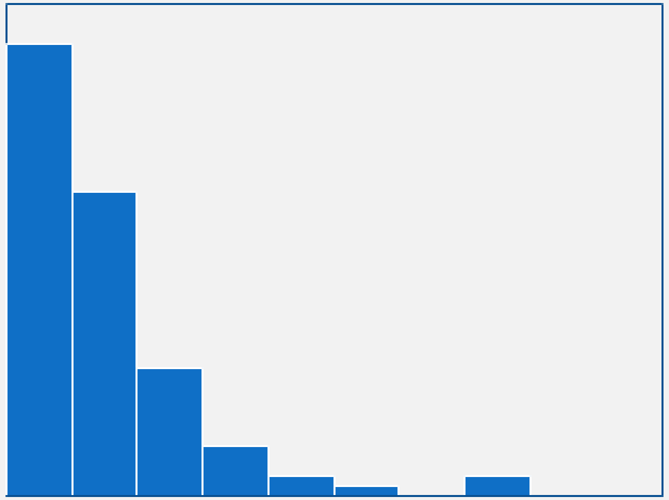
Examining Data Distribution (cont.)

If you calculate a mean on data that are not normally distributed, what is the typical result? (Select one)

a) The mean will be higher than the median

b) The mean will be lower than the median

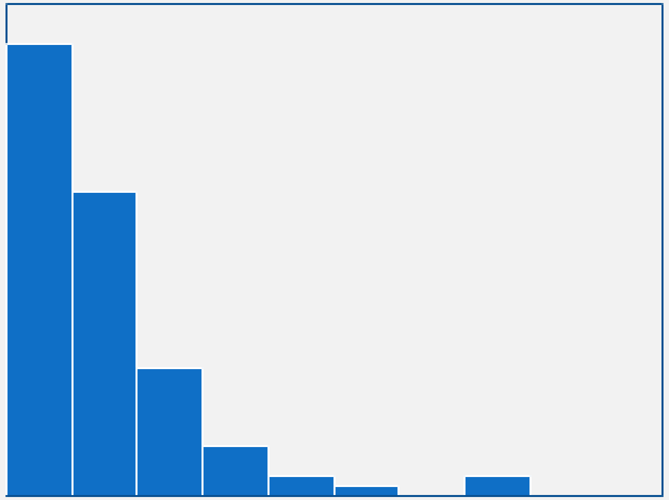
c) The mean will be about the same as the median



Examining Data Distribution (cont.)

If you calculate a mean on data that are not normally distributed, what is the typical result?

a) **The mean will be higher than the median**



Analyzing Log-Normal Data

There are two common ways to analyze log-normal data:

1) Transform the data to make them normally distributed
   - Then, use parametric statistics (for example, the t-test) to compare levels between groups
2) Keep the data as they are, and use nonparametric statistics (for example, the Kruskal-Wallis test) to compare levels between groups

Note: Parametric is defined as assuming the value of a parameter (e.g. levels, data) for the purpose of analysis.

Assumptions of parametric data are:

- random sampling is used to gather the data, and
- the distribution of the levels fall within a possibility of a normal distribution.

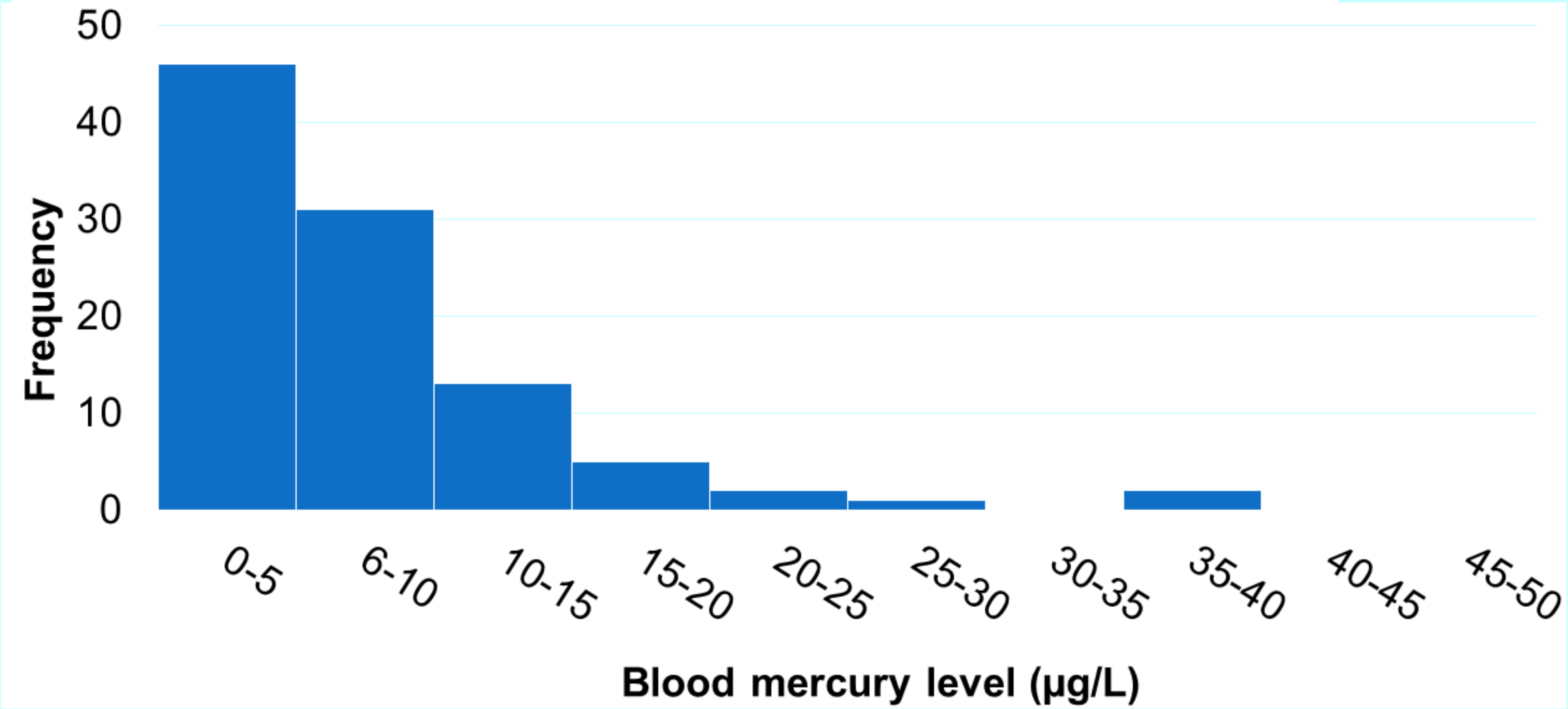
Log Transformation

Log transformation means calculating the natural log of each value.

- For example
  - 0.8 ➡ Ln(0.8) = -0.2
  - 10 ➡ Ln(10) = 2.3
  - 167 ➡ Ln(167) = 5.1
- Log transforming values pulls very high values in, resulting in a distribution that is closer to normal.

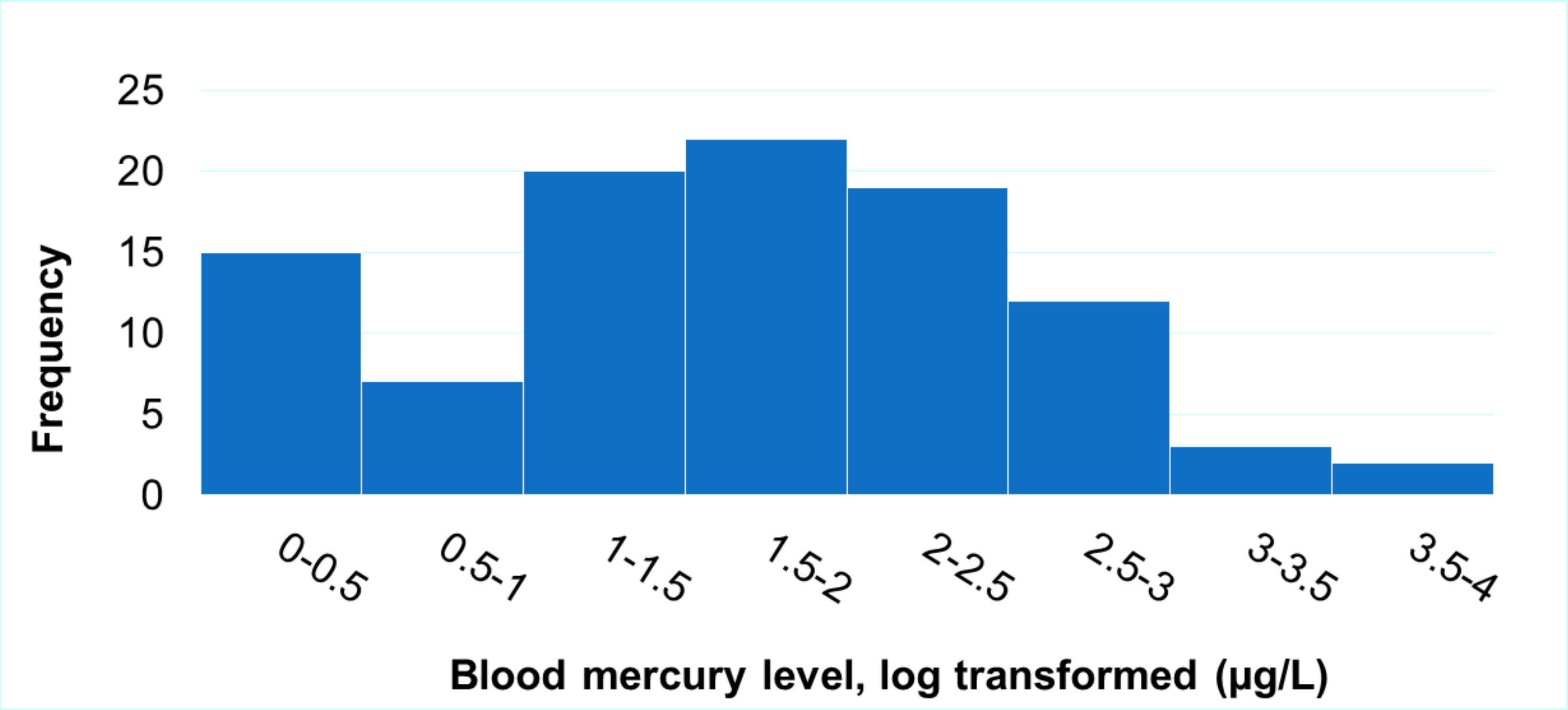
Log Transformation (cont.)

Original data:



Log Transformation (cont.)

Log-transformed data:



Geometric Mean

- Log-transforming toxicological laboratory data usually results in data that are approximately normally distributed.
    - This allows you to calculate a geometric mean
- Geometric mean = Average of log transformed data
- Arithmetic mean = Average of non-transformed data

Geometric Mean (cont.)

Steps to calculate a **geometric mean** on data with a log-normal distribution:

1) Perform substitution for values that are <LOD
2) Perform adjustments (such as accounting for creatinine or lipids, if necessary)
3) Log-transform the data
4) Calculate the mean of the log-transformed data
5) Exponentiate the mean

Calculate Geometric Mean

Observation #	Arsenic Concentration (µg/L)
1	8
2	34
3	<LOD
4	11
5	12
6	40
7	12
8	8
9	<LOD
10	9

Calculate the geometric mean of the data.

Calculate Geometric Mean (cont.)

Case ID #	Level of …(µg/L)	Step 1: Log-transform
1	8	ln(8) = 2.1
2	34	ln(34) = 3.5
3	4.9	ln(4.9) = 1.6
4	11	ln(11) = 2.4
5	12	ln(12) = 2.5
6	40	ln(40) = 3.7
7	12	ln(12) = 2.5
8	8	ln(8) = 2.1
9	4.9	ln(4.9) = 1.6
10	9	ln(9) = 2.2

Step 2: Calculate the mean of the log-transformed data

2.1 + 3.5 + 1.6 + 2.4 + 2.5 + 3.7 + 2.5 + 2.1 + 1.6 + 2.2 = 24

$$\frac{24}{10} = 2.4$$

Step 3: Exponentiate the mean

exp(2.4) = 11

Geometric mean = 11

Calculate the geometric mean of the data.

NOTE: <LOD values were substituted with values equal to the

$$\frac{LOD}{\sqrt{2}} = \frac{7}{\sqrt{2}} = 4.9\mu g/L$$

Examining Data Distribution

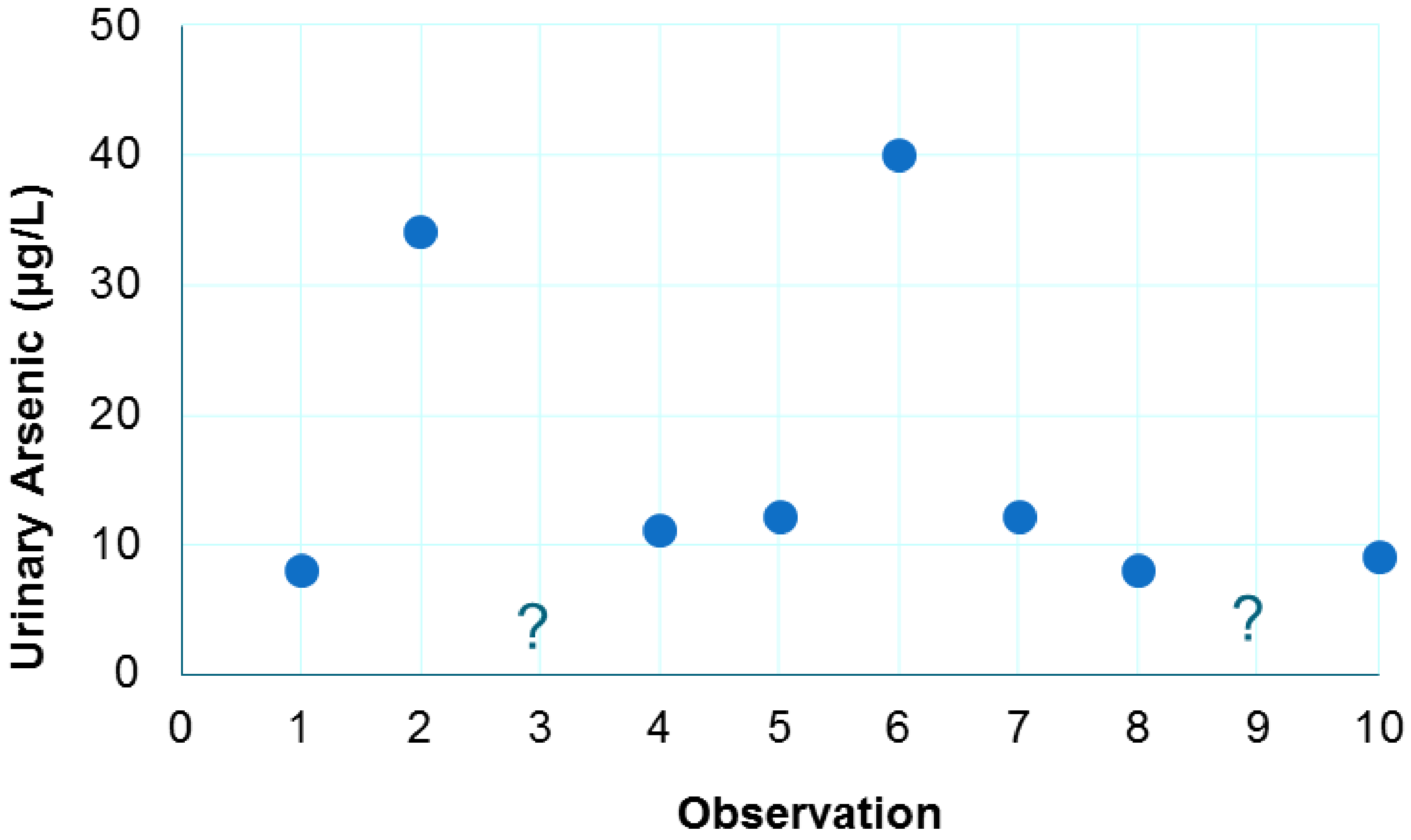
- The geometric mean (11 µg/L) is closer to the median (11.5 µg/L) than the arithmetic mean (14 µg/L) calculated earlier
- This pattern in biologic data is common – when data are log-normal, the arithmetic mean is skewed higher
- For log-normal data, the geometric mean is a better indicator of the midpoint of the data compared to the arithmetic mean

Interpreting Laboratory Results

Interpreting Laboratory Results

- Biologic specimens and environmental samples usually contain many toxic agents
- Detecting a toxic agent in a biological or environmental sample does not mean the toxic agent caused the outbreak; it might be a normal background level
- We need to interpret the levels to determine whether they are high enough to have likely caused the outbreak

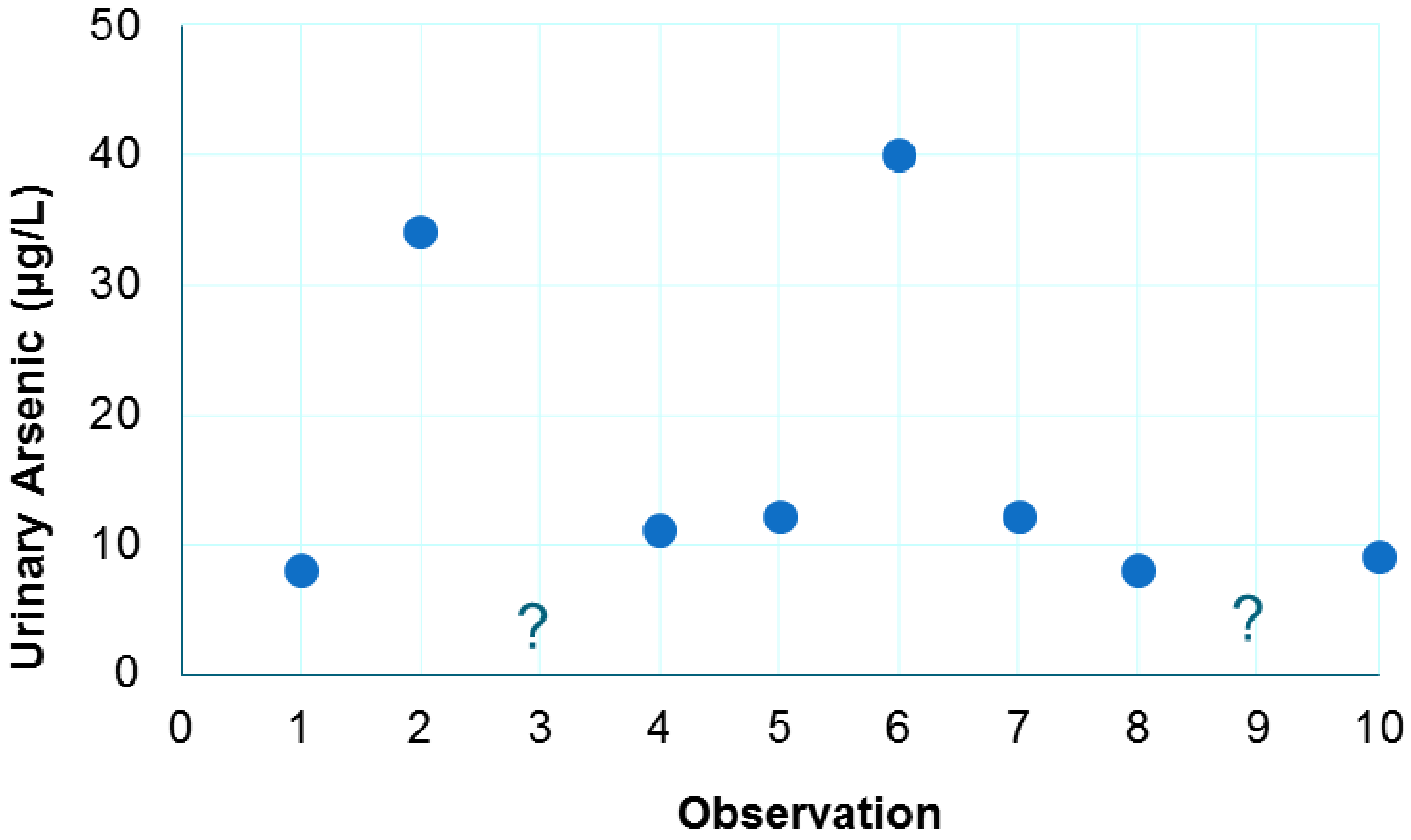
Interpreting Laboratory Results (cont.)



Consider this hypothetical scenario from earlier. Could these urinary arsenic levels have caused illness?

What information do we need to know?

Interpreting Laboratory Results (cont.)

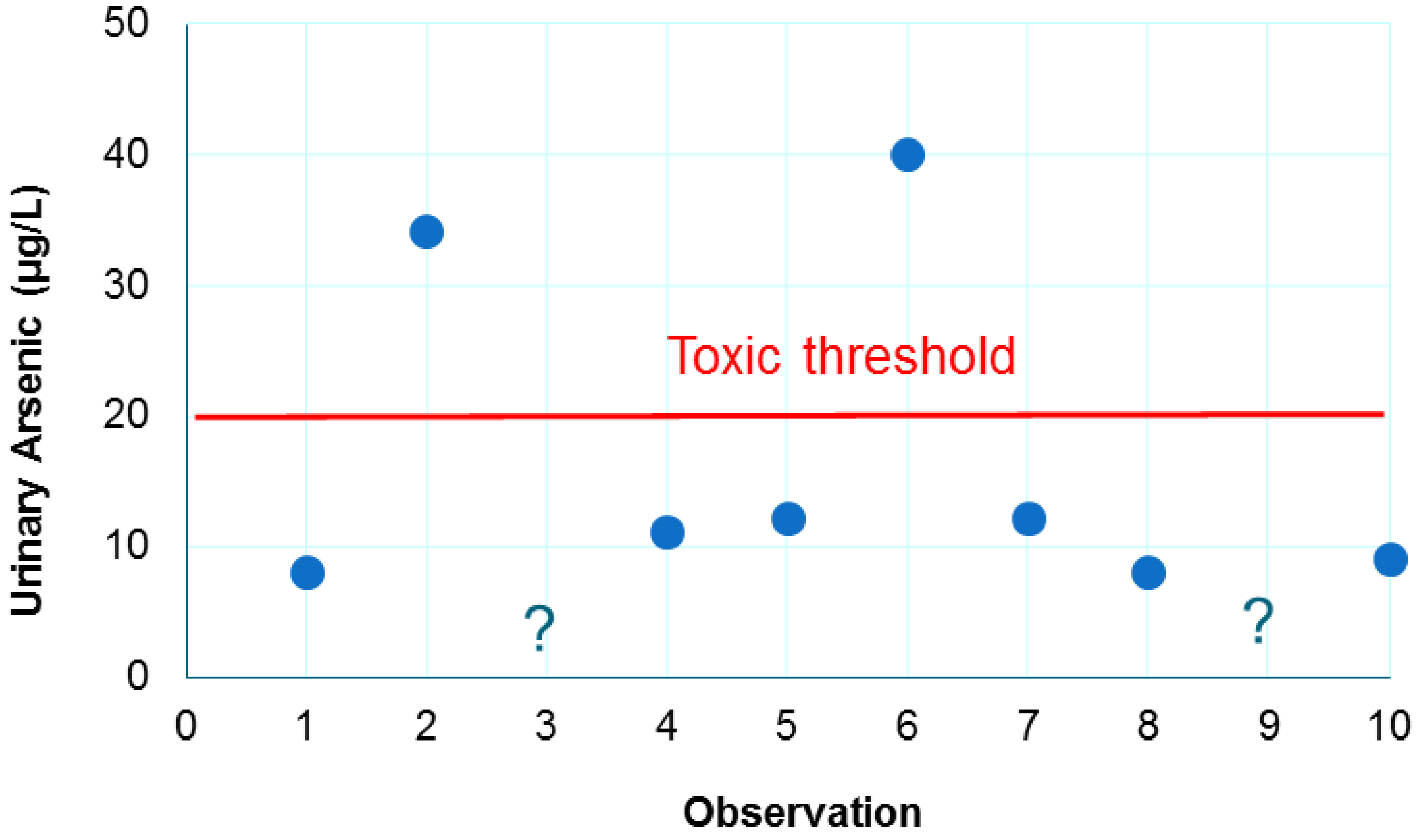


What information do we need to know?

We need to know:

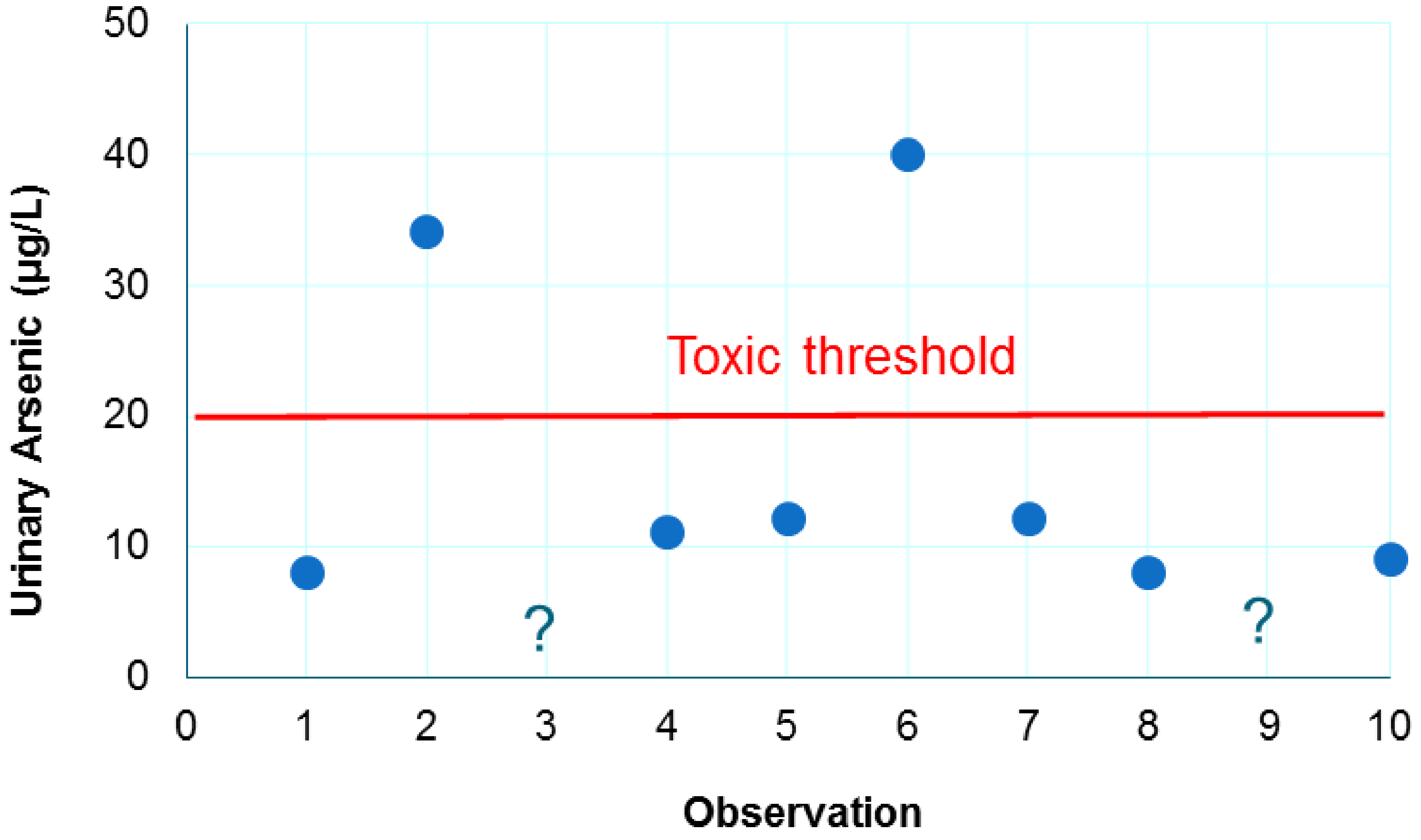
- What is the toxic threshold?
- When were the specimens collected? Do these levels represent the level they were exposed to?
- Did these individuals share a common exposure?

Toxic Threshold



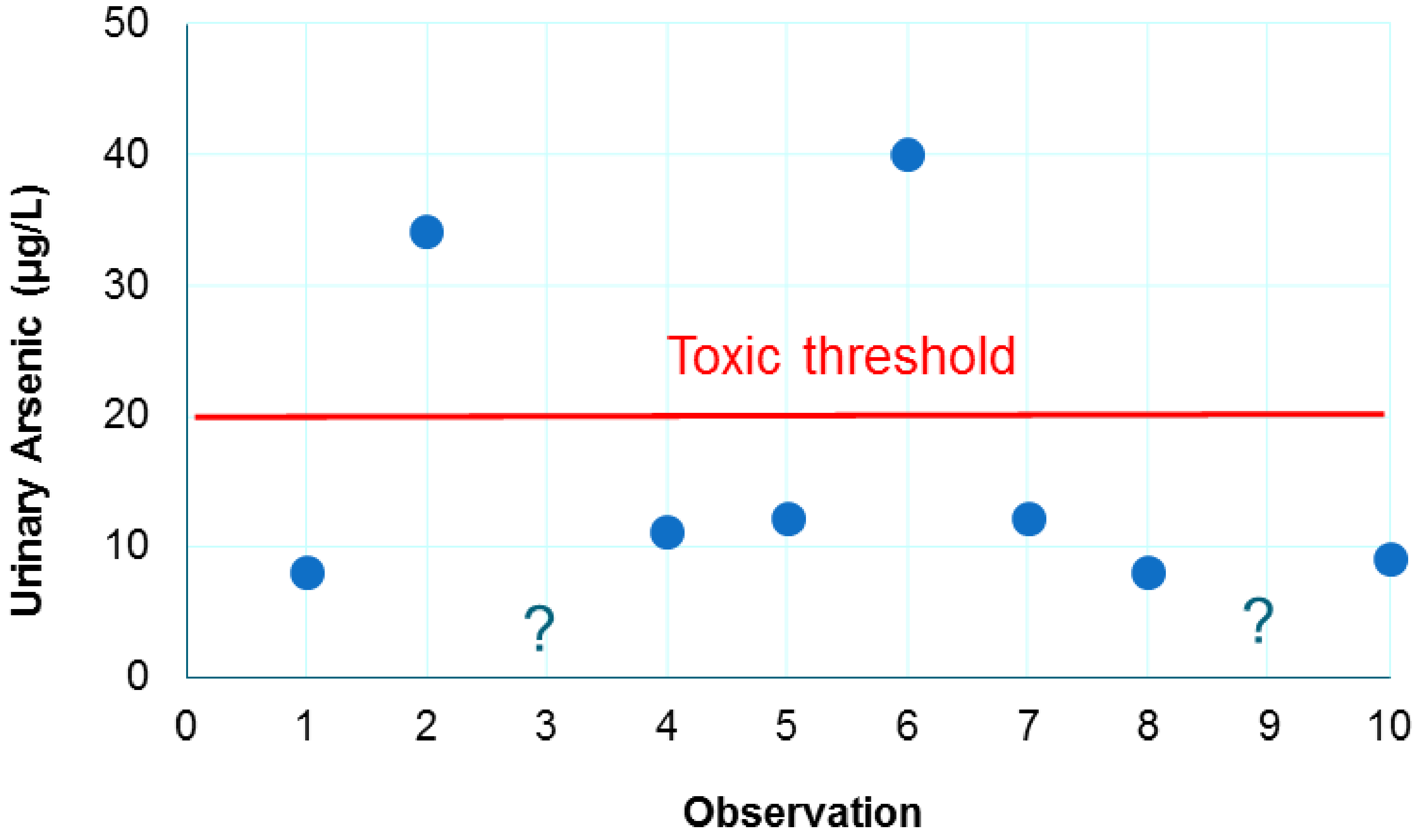
If the toxic threshold is 20 ug/L, then you might conclude that arsenic exposure could be causing sickness in individuals #2 and #6.

Toxic Threshold (cont.)



If the toxic threshold is 20 µg/L, is it possible that the toxic agent made the other individuals sick?

Toxic Threshold (cont.)



If the toxic threshold is 20 µg/L, is it possible that the toxic agent made the other individuals sick?

Yes.

- It is possible the toxic agent was more metabolized (later sample collection).
- The individuals may have been more susceptible.

Comparison Values

- For most toxic agents, we do not know the toxic threshold
- Other comparison values are needed
  - Comparison samples collected from your investigation
  - Published values from previous outbreaks or research studies

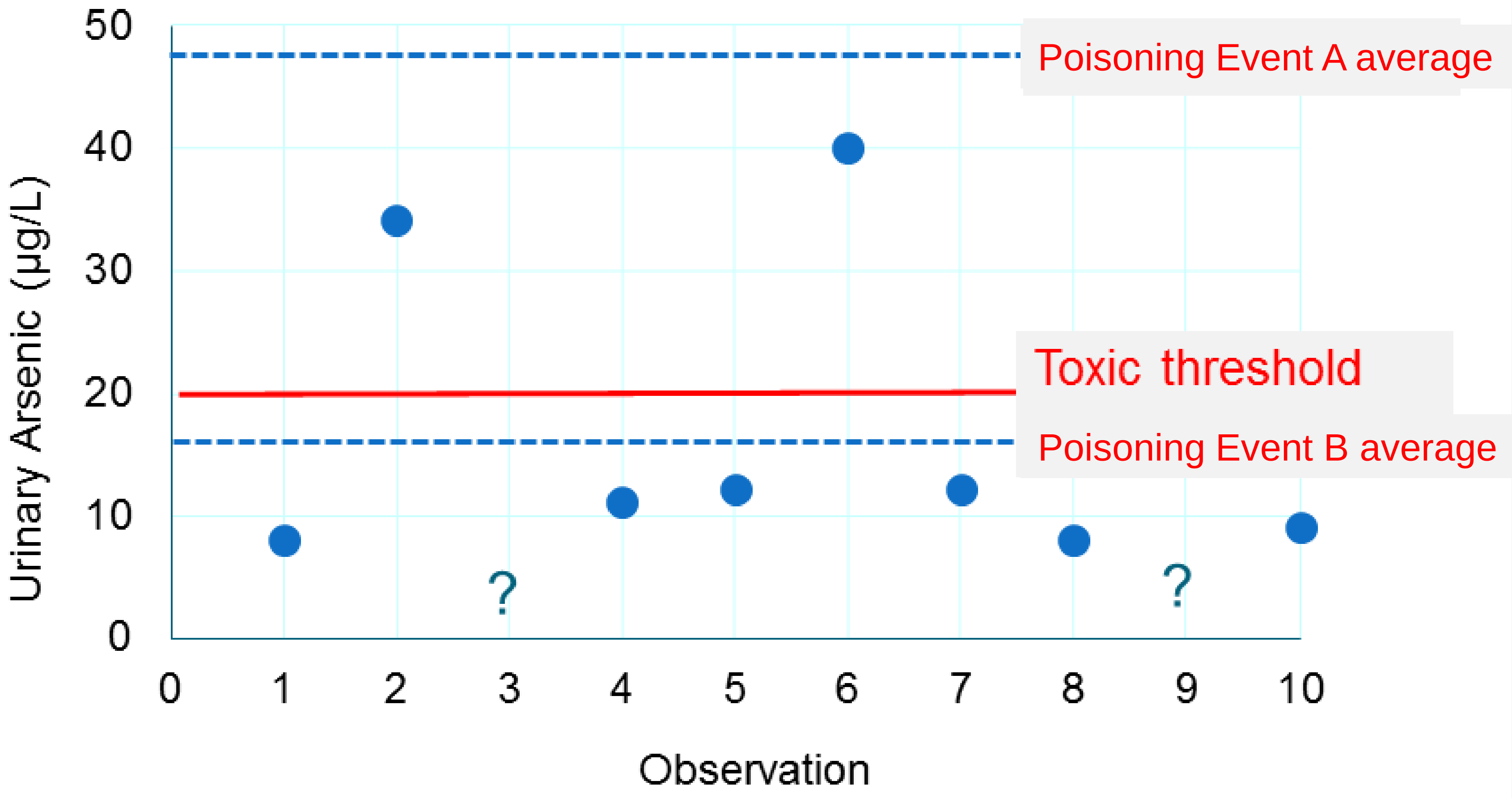
We have good data for interpreting toxic levels for:

- Carbon Monoxide
- Cyanide
- Toxic Alcohols (ethylene glycol, isopropanol, methanol)
- Lead

Do not have good data for interpreting toxic levels for:

- Organophosphates
- Pyrethroids
- Hydrofluoric acid

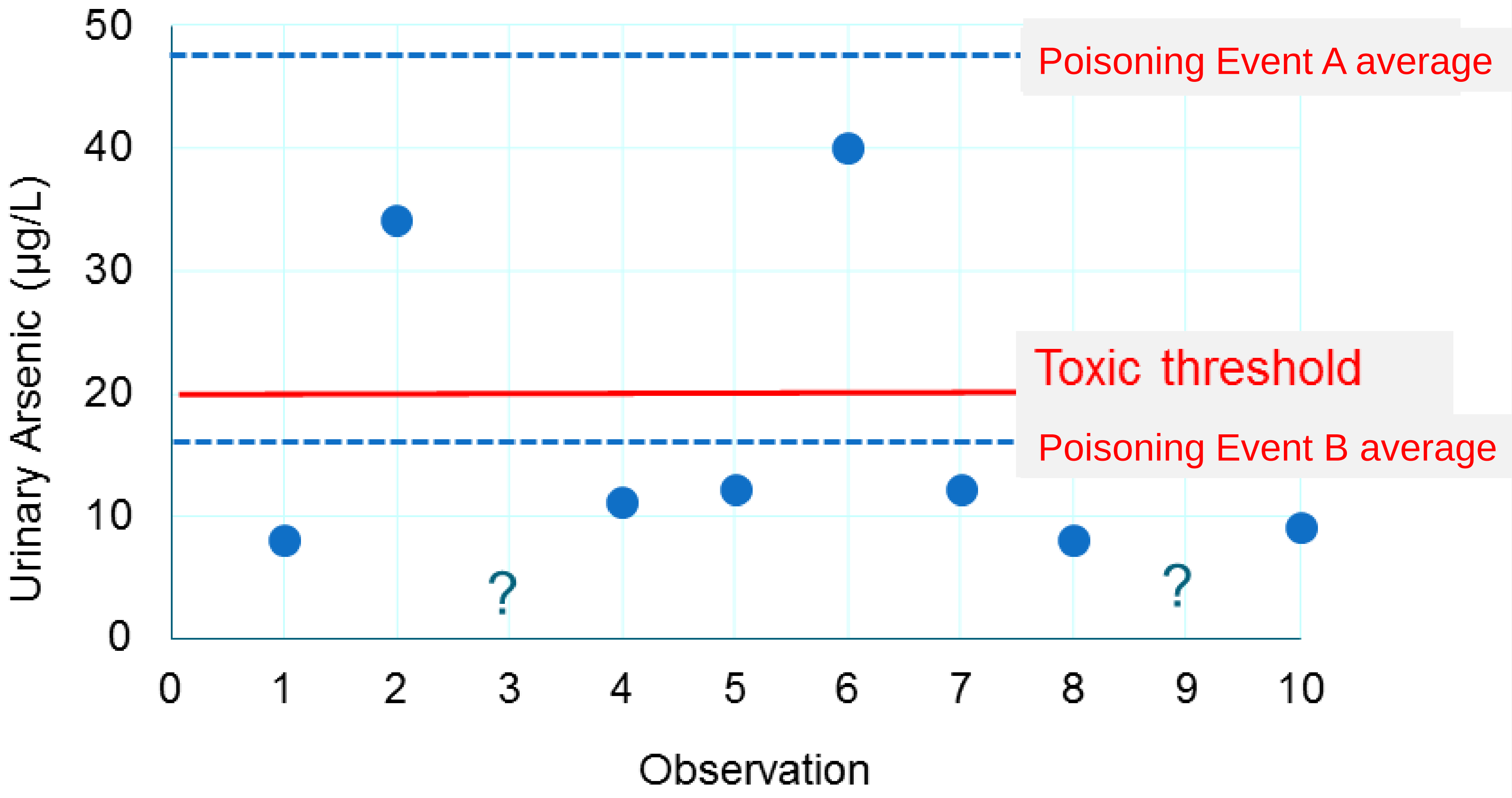
Inconclusive Results



If comparison values range from 18 to 48 µg/L, then your results are inconclusive.

What are some reasons for inconclusive results?

Inconclusive Results (cont.)



What are some reasons for inconclusive results?

- Individual variation in metabolism and susceptibility
- Long time lapse between exposure and sample collection
- Arsenic may not be the exposure of concern

Interpreting Laboratory Results: Considerations

- The hardest part of communicating toxicological outbreak findings is correctly interpreting and communicating the laboratory data
- There are some considerations to keep in mind when developing talking points to communicate the results of your biological specimen and environmental sample analysis

Interpreting Laboratory Results: Considerations (cont.)

Reasons for not finding a particular toxic agent:

- The agent might not have caused the outbreak

Interpreting Laboratory Results: Considerations (cont.)

Reasons for not finding a particular toxic agent:

- The agent might not have caused the outbreak
- The toxic agent may have already been metabolized or broken down before the sample was collected

Interpreting Laboratory Results: Considerations (cont.)

Reasons for not finding a particular toxic agent:

- The agent might not have caused the outbreak
- The toxic agent may have already been metabolized or broken down before the sample was collected
- To be detected, the toxic agent must be present at levels that are above the method's LOD
  - A toxic agent could be <LOD, yet still high enough to cause illness
- It may have had a heterogeneous distribution and perhaps was missed

Interpreting Laboratory Results: Considerations (cont.)

- Just because a particular toxic agent was identified, does not mean it caused the outbreak
- People are continuously exposed to toxic agents
- Typical baseline exposures might vary from region to region and between different groups of people

Summary Review

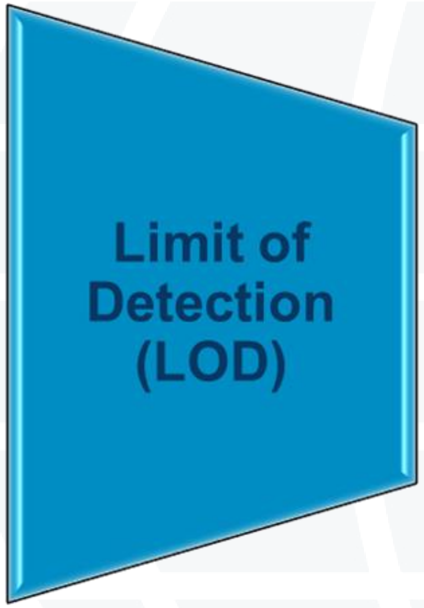
- Limit of Detection (LOD)
- Making Adjustments to Lab Results
- Examining Data Distribution
- Interpreting Laboratory Results

Summary Review (cont.)

Limit of Detection (LOD)

- What is Limit of Detection (LOD)?
- What are the two constant values that are commonly substituted for values that are <LOD?

Summary Review (cont.)



- Observations that are below the limit of detection are usually reported by the lab as <LOD, or Not Detected
- Two constant values that are commonly substituted for values that are <LOD:
  - LOD/2
  - LOD/√**2**

Summary Review (cont.)

Making Adjustments to Lab Results

- **What are the two common examples of adjusting toxicological laboratory results?**

Summary Review (cont.)

Making Adjustments to Lab Results

- Two common examples of adjusting toxicological laboratory results:
  - Adjusting for creatinine in urine specimens to account for a specimen's concentration or diluteness.
  - Adjusting for proteins or lipids in serum specimens to account for chemical binding to these entities.

Summary Review (cont.)

Examining Data Distribution

Toxicological outbreak investigations often result in continuous laboratory data

- **Before analyzing continuous variables, their distribution must be checked for ___________ ?**
- **When toxic agents are measured in people, they often have a _____________ distribution**

Summary Review (cont.)

Examining Data Distribution

- Toxicological outbreak investigations often result in continuous laboratory data
- Before analyzing continuous variables, their distribution must be checked for normality
- When toxic agents are measured in people, they often have a **log-normal** distribution

Summary Review (cont.)

Interpreting Laboratory Results

For most toxic agents, we do not know the toxic threshold

- **What other comparison values are needed?**

Summary Review (cont.)

Interpreting Laboratory Results

- What is the **toxic threshold**?
- For most toxic agents, we do not know the toxic threshold - other **comparison values** are needed
  - Comparison samples collected from your investigation
  - Published values from previous outbreaks or research studies

Module Conclusion

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