Accessible version can be found here:
https://www.cdc.gov/nceh/hsb/elearning/toi/Mod7/

Toxicological Outbreak Investigation Course

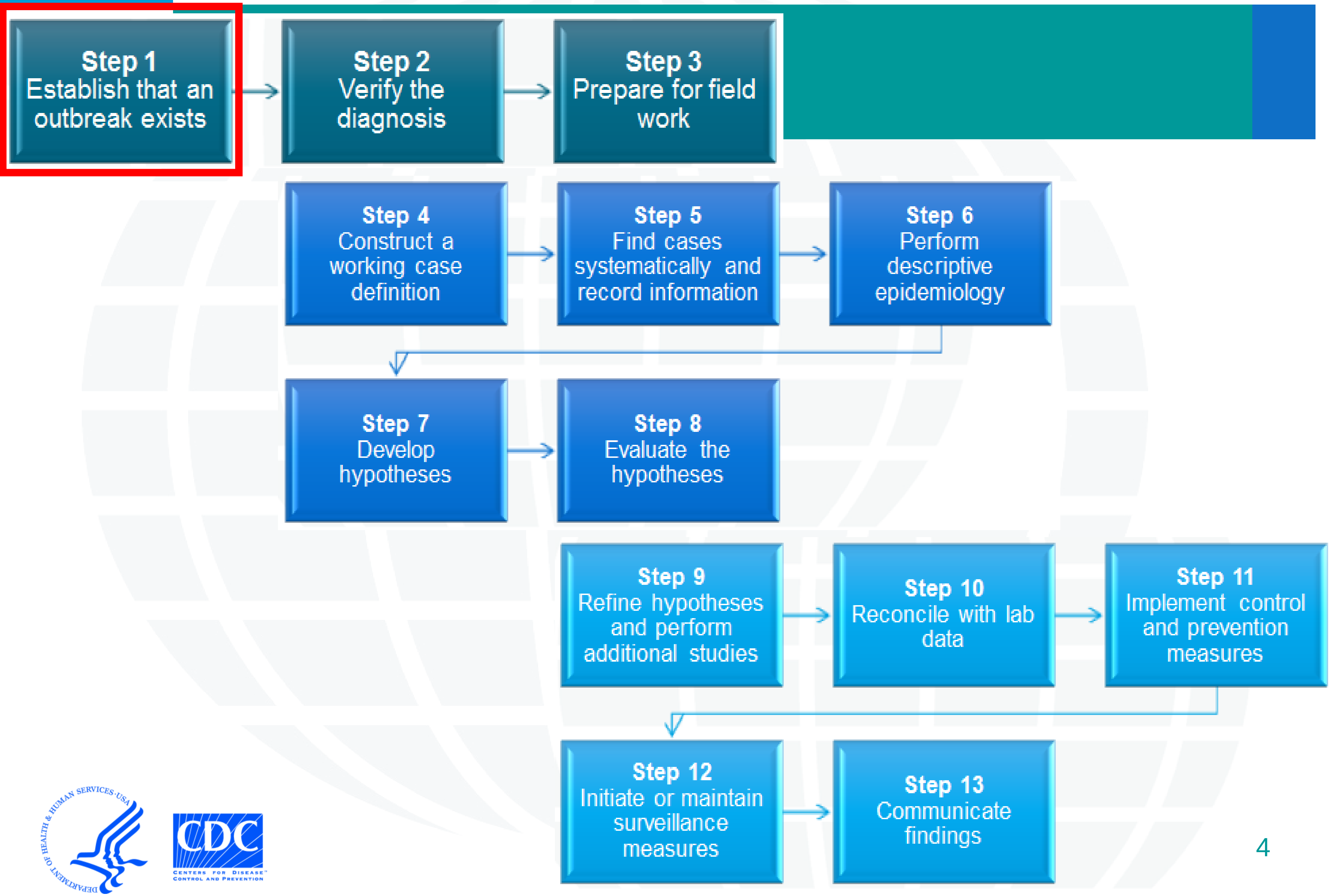
Module Seven (International):
Case Study

Module 6 Objectives

- Apply the steps of an outbreak investigation to a toxicological outbreak case study
- Interpret results of biologic and environmental samples
- Describe the purpose of relevant forms from the Toxicological Investigation Tool Kit

Introduction

- This case study is based on an investigation conducted in Bangladesh in 2009
- Some details have been modified



Step 1: Establish that an outbreak exists

The Call

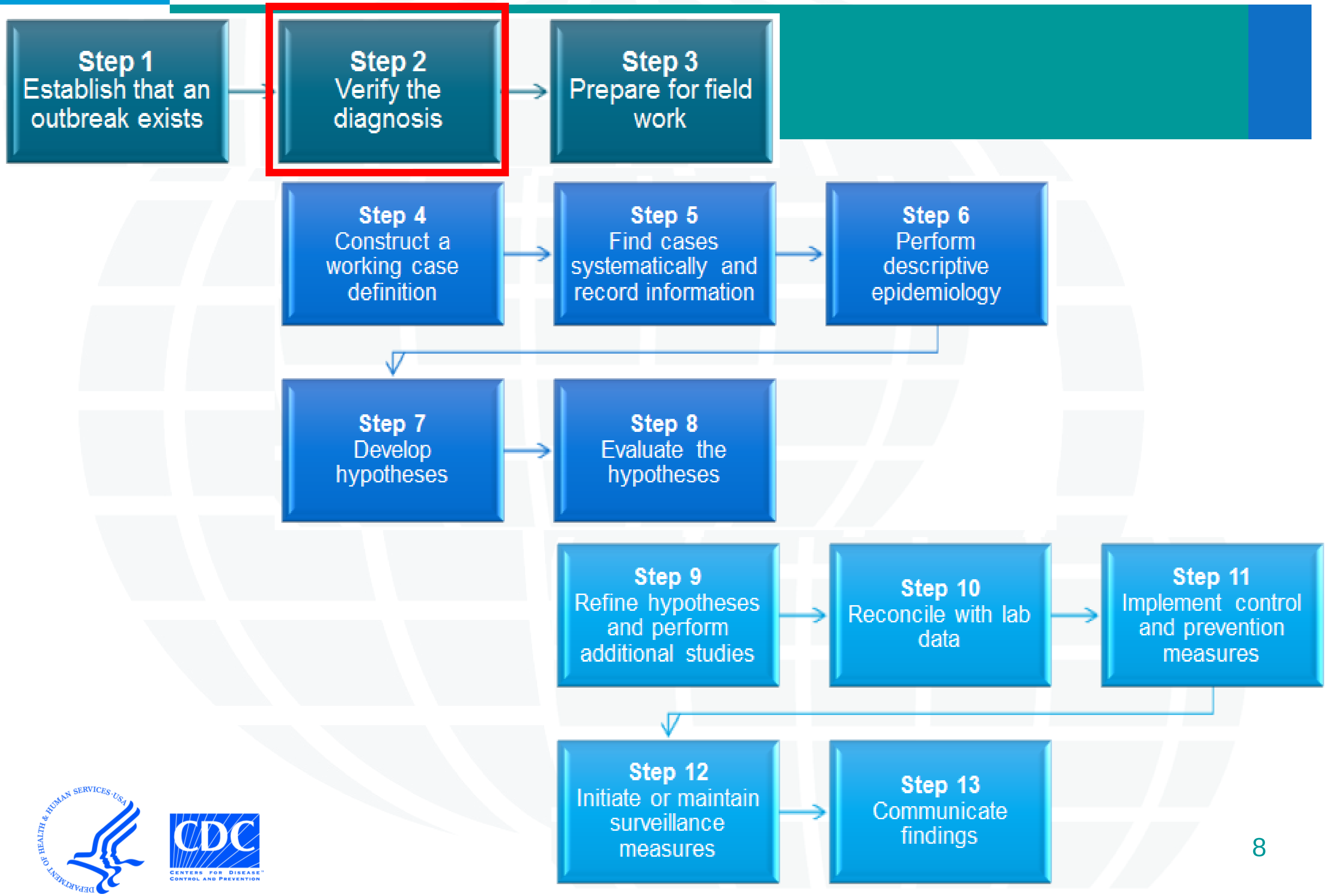
- In April, a doctor at a rural hospital contacts the Ministry of Health
- That month, he had seen 11 children in the hospital with sudden illness; 3 had died
  - Symptoms included difficulty breathing, frothy oral discharge, and loss of consciousness
  - The children were aged 7 months to 10 years
  - The children all resided in a rural farming village

Background Information

- The Ministry of Health speaks with the local doctors and learns more information
  - Several calves and puppies in the region had become suddenly ill and died around the same time that the children had become ill
  - A similar group of illnesses occurred at this time last year
  - Last year, laboratory tests were negative for influenza, Japanese encephalitis, and Nipah virus

Background Information (cont.)

- The Ministry of Health decides that this is an outbreak
  - They decide to investigate to find the cause of the outbreak and address public concerns
- **Your team gets called into action**



Step 2: Verify the diagnosis

Toxicological Outbreak

The Ministry of Health realizes this might be a toxicological outbreak, because it meets these criteria:

- Prior tests for an infectious disease were negative
- Fever has not been mentioned as a prevailing symptom
- Illness seemed to have a rapid onset
- So far, all reported cases had similar symptoms
- There were concurrent animal illnesses reported

What is the most important thing to do when learning an outbreak might have been caused by a toxic agent?

Toxicological Outbreak (cont.)

The Ministry of Health realizes this might be a toxicological outbreak, because it meets these criteria:

- Prior tests for an infectious disease were negative
- Fever has not been mentioned as a prevailing symptom
- Illness seemed to have a rapid onset
- So far, all reported cases had similar symptoms
- There were concurrent animal illnesses reported

What is the most important thing to do when learning an outbreak might have been caused by a toxic agent?

- Consult with a toxicologist.
- Gather biological samples as early as possible.

Team Consults with Local Toxicology Experts

- The team knows it is important to collect biologic samples as soon as possible
- They contact the district hospital and learn that blood samples have been collected from 7 cases and are now being stored in a freezer
- They ask the district hospital to complete a specimen log to record descriptive information along with the specimens

Sample Log

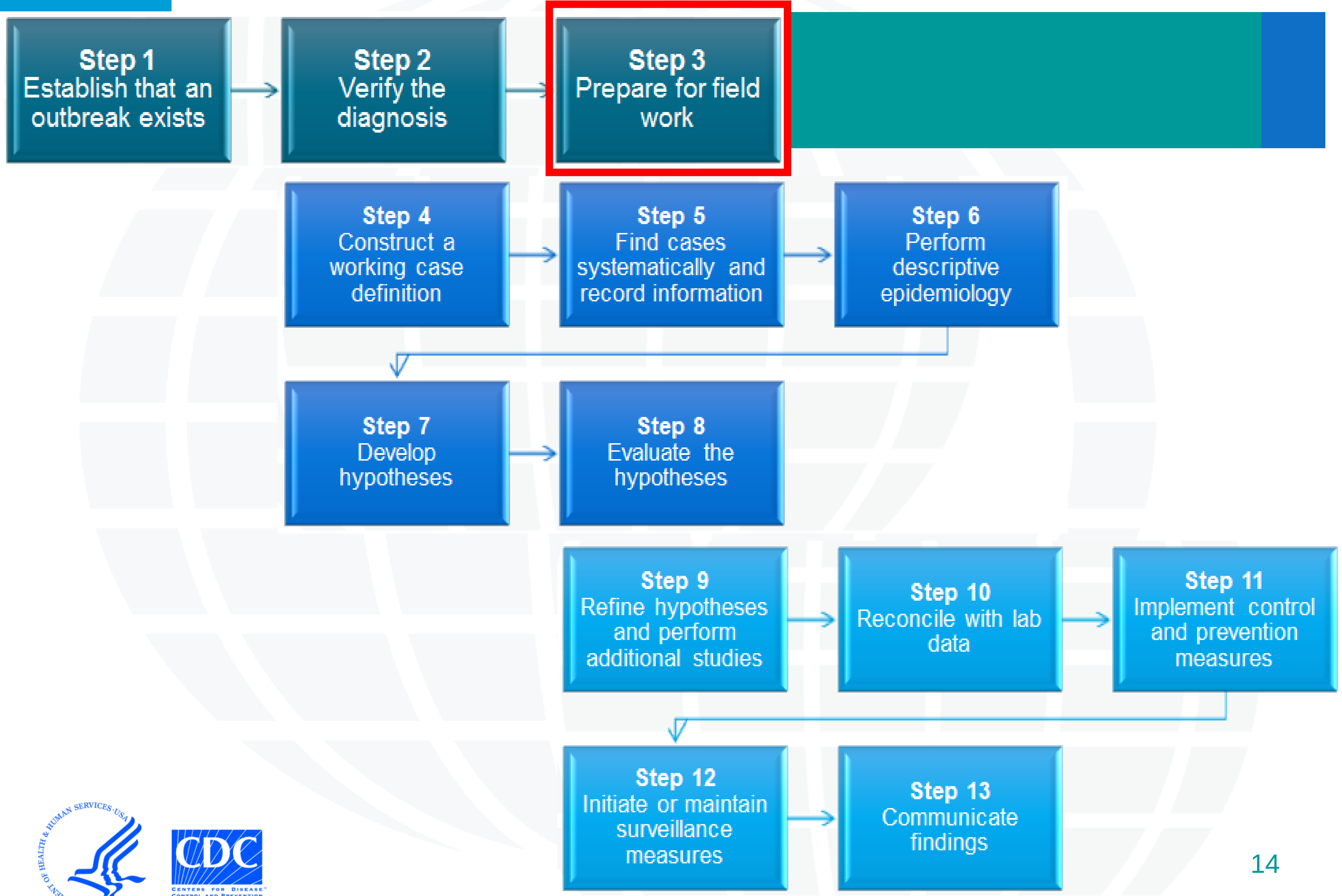
What information do you want to collect on the specimen log?

Sample Log (cont.)

What information do you want to collect on the sample log?

- Identifying information (e.g., person's name)
- Collection date and time
- Sample type (e.g., blood, urine)
- Location where the sample was collected
- Descriptive data (e.g., age, sex)

Tool Kit: Sample Log



Step 3: Prepare for field work

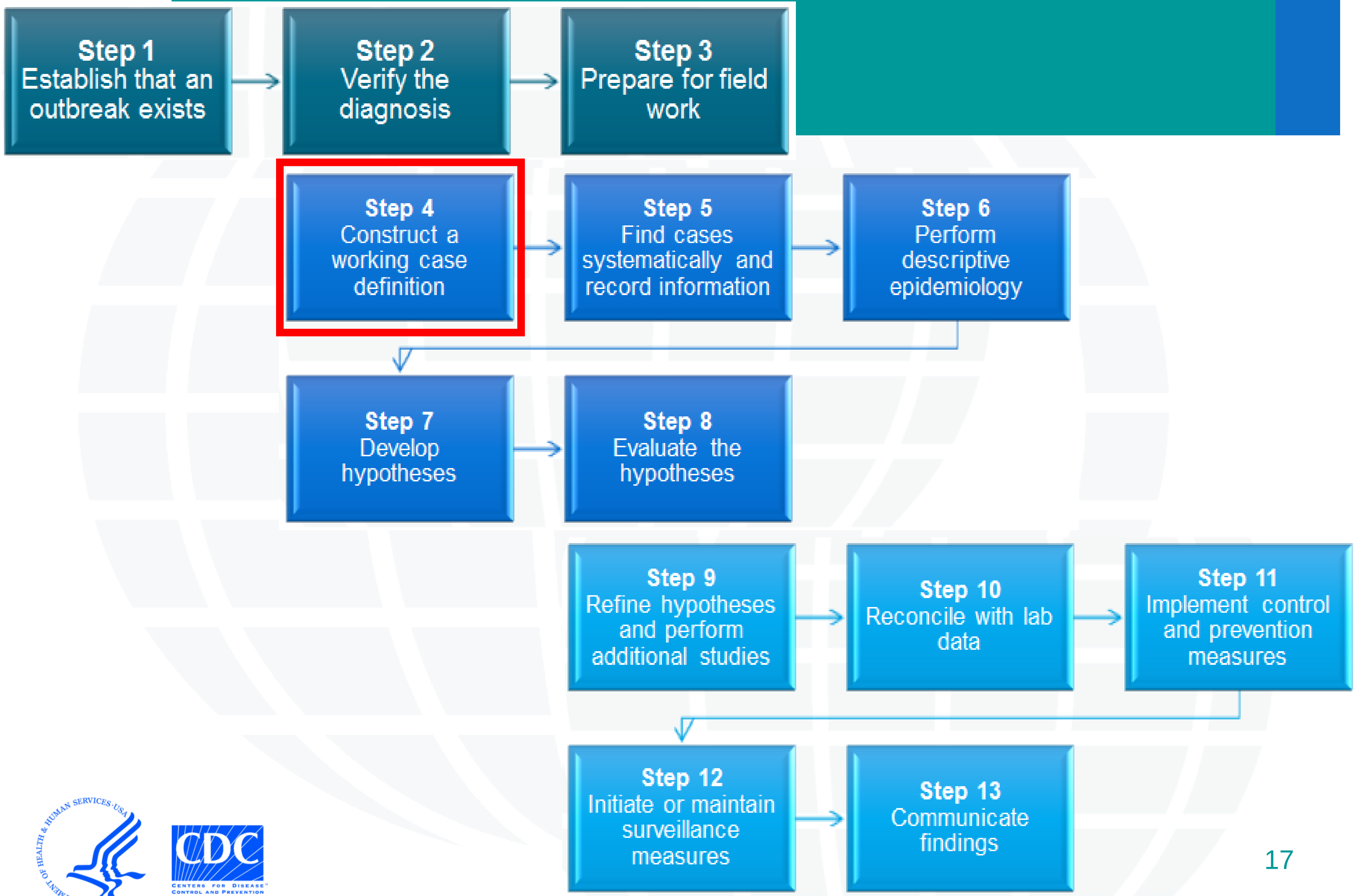
Investigation Objectives

- The team begins to plan for the field investigation
- They develop four objectives:
  - Determine the extent of the outbreak
  - Describe the illness
  - Identify the etiology
  - Identify the route of exposure

Assemble the Team

They also assemble a team:

- Toxicologist
- Laboratorian
- Epidemiologist
- Anthropologist



Step 4: Construct a working case definition

Additional Symptoms

- The team speaks with the clinician who saw the patients and reported the most common symptoms:
  - Difficulty breathing
  - Excessive sweating
  - Frothy oral discharge
  - Loss of consciousness
  - Convulsions/fits
  - Urinary incontinence
  - Vomiting
  - Weakness in arms or legs

Case Definition

Develop a working case definition.

A good case definition includes:			
WHAT Clinical signs and symptoms	WHO Person characteristics	WHERE Place characteristics	WHEN Time characteristics

Hint: Consider what, who, where, when

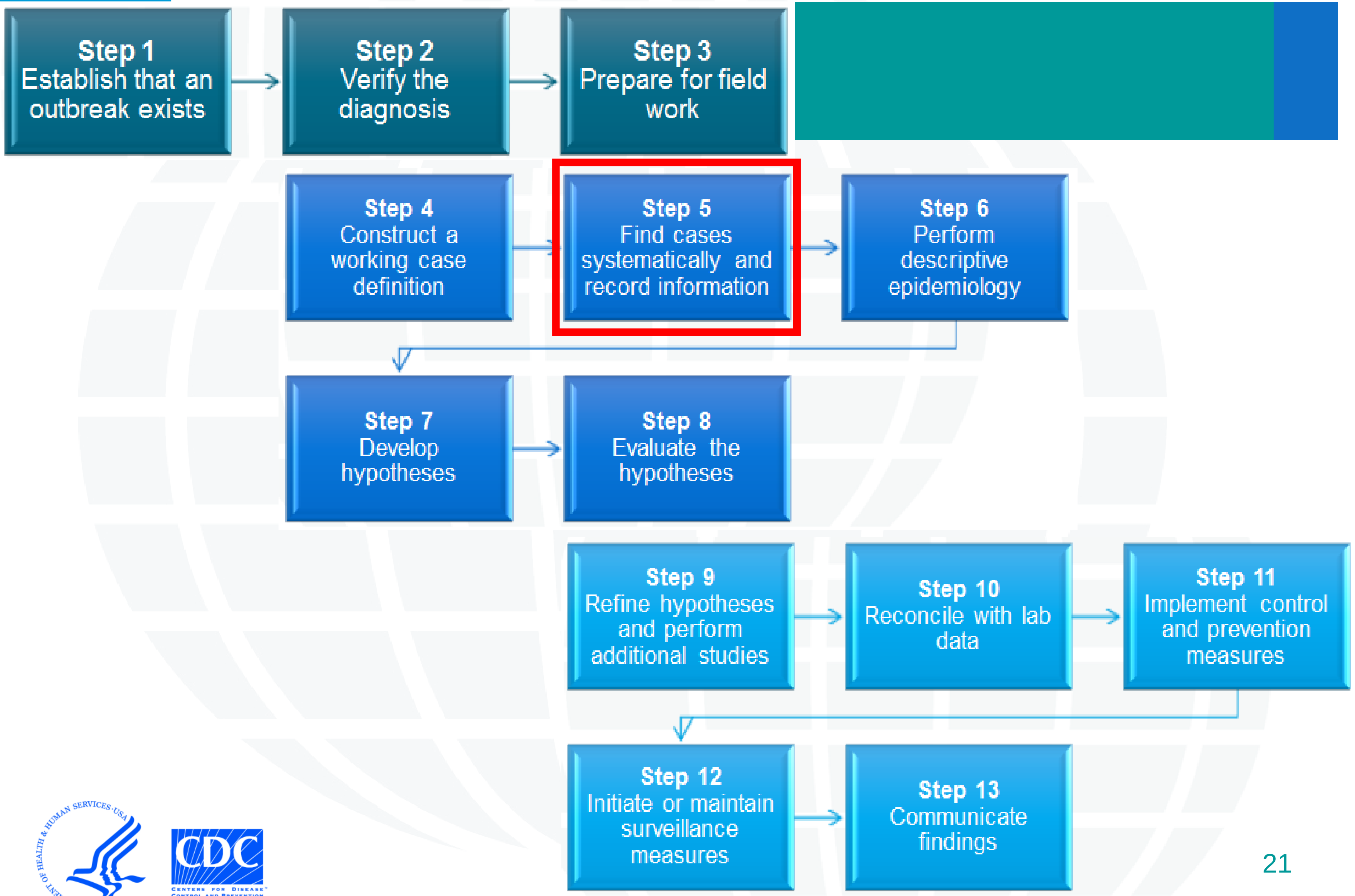
Case Definition Developed by Team

- Any two of the following symptoms occurring on the same day:
  - Difficulty breathing
  - Excessive sweating
  - Frothy oral discharge
  - Loss of consciousness
  - Convulsions/fits
  - Urinary incontinence
  - Vomiting
  - Weakness in arms or legs

- Among persons aged 10 years or less
- Residing in Village A
- Occurring on or after March 1

The team decides to require the presence of at least two of the symptoms, to be more specific and reduce the risk of categorizing other sick people as cases.

They decide to go back to March 1st, in case it was occurring sooner but was just missed.



Step 5: Find cases systematically and record information

Find Cases Systematically and Record Information

The team heads to the village to determine the extent of the outbreak

How would you search for additional cases?

Find Cases Systematically and Record Information (cont.)

- The team searches for additional cases by:
  - Visiting nearby hospitals
  - Contacting public health officials in neighboring districts
  - Asking village leaders and family members for reports of similar illness
- No additional cases are found; it appears the outbreak is not ongoing.

Line List Excerpt

The team constructs a line list to record information about the cases. An excerpt of this line list is shown below:

Age	Sex	Start Date	Cold Skin?	Excessive Sweating?	Outcome	Urine Collected?
3	M	April 2	Yes	Yes	Died	Yes
4	F	April 7	Yes	Yes	Survived	Yes
8	M	April 8	No	Yes	Survived	No
8	F	April 10	Yes	Yes	Survived	Yes
3	M	April 13	Yes	Yes	Survived	Yes

What is missing in this line list?

Line List Excerpt (cont.)

The team constructs a line list to record information about the cases. An excerpt of this line list is shown below:

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4	F	April 7	Yes	Yes	Survived	Yes
8	M	April 8	No	Yes	Survived	No
8	F	April 10	Yes	Yes	Survived	Yes
3	M	April 13	Yes	Yes	Survived	Yes

What is missing in this line list?

Required
Name/ID
Date of Urine Collection

Optional
Address
Time of presentation

Tool Kit: Sample Line List

Hypothesis-Generating Interviews

- The team conducts hypothesis-generating interviews with the physician who saw the cases to learn more about the timeline of illness
- The physician recalls three cases in detail, as shown on the following slides

Patient #1

- A female aged 2 years was brought to the Emergency Room
- According to her parents:
  - She was quite healthy
  - After breakfast, she suddenly developed weakness in her right leg while she was playing with other children; this forced her to lie down on the ground
  - Frothy discharge started coming through her mouth and nose, and she became unconscious; she also had convulsions and severe trouble breathing

Patient #1 died on her way to hospital.

Patient #2

- A child aged 3 years was brought to the Emergency Room presenting with severe trouble breathing and frothy discharge through mouth and nose
- The child was semiconscious and gradually became unconscious
  - Pupils were pinpoint and not reacting to light
  - Whole body was sweaty, cold, and clammy
  - Heart rate and breathing were fast; pulse 179 beats/min, respiratory rate 62 breaths/min, and lungs full of fluid
- The patient was provided oxygen and intravenous fluids, and received atropine, antibiotics, and steroids

Patient #2 recovered completely.

Patient #3

- A child about 8 years of age was brought to the Emergency Room at 8.30 am
- The patient presented with severe trouble breathing, frothy discharge through the mouth, and loss of control of bowels
  - Pupils were pinpoint (very small)
- The patient was provided oxygen and intravenous fluids, and received atropine, antibiotics, and steroids

Patient #3 recovered completely.

Toxidrome

The team reviews a list of classic toxidromes to see if one of these matches with the clinical picture

Do any of the toxidromes on the following slides match the clinical picture?

Tool Kit: Toxidromes Chart

Toxidrome Excerpt: Page 1 of 3

Toxidrome	Signs and Symptoms	Potential Toxic Agent
Opioid	Lethargy, miosis, respiratory depression. Can progress to coma, pulmonary edema, hypotension, bradycardia	• Opium/snuff • heroin • Prescription medications: • Codeine • Hydro/oxycodone • Hydro/oxymorphone • Fentanyl • Desomorphine aka krokodil
Anticholinergic	Cutaneous flushing, hyperthermia, dry skin, mydriasis, dry mucous membranes, disorientation, hallucination, seizures, tachycardia, hypertension, urinary retention	• Belladona alkaloids • Jimson Weed/Datura • Brugmansia • Diphenhydramine

Toxidrome Excerpt: Page 2 of 3

Toxidrome	Usual Signs and Symptoms	Potential Toxic Agents
Hallucinogen	Disorientation, hallucination, panic	• Peyote • Psilocybin mushrooms • LSD • PCP • Lysergic acid containing plants: morning glory, Hawaiian woodrose
Sympathomimetic	Tachycardia, hypertension, hyperthermia, diaphoresis, mydriasis, hyperreflexia, anxiety, seizures	• Ma Huang (ephedrine) • Amphetamines • Cocaine • Khat and other cathinones • Bath salts

Toxidrome Excerpt: Page 3 of 3

Toxidrome	Signs and Symptoms	Potential Toxic Agent
Cholinergic crisis	Salivation, diarrhea, lacrimation, bronchorrhea, diaphoresis, urination Miosis, fasciculations, weakness, bradycardia or tachycardia, hypotension or hypertension, altered mental status, seizures	• Nicotine • Organophosphate insecticides • Carbamate insecticides • Medicinal carbamates (e.g., physostigmine)

Toxidrome

- The team decides that the illness most closely resembles a **cholinergic crisis**
- Based on this, and because the outbreak occurred in a farming region, they narrow their focus to organophosphate and carbamate insecticides

Hypothesis-Generating Interviews

The team interviews local farmers and family members to figure out what insecticides are in the area

What are two questions you might ask farmers?

Hypothesis-Generating Interviews (cont.)

Tool Kit: Qualitative Epidemiological Questions

The team interviews local farmers and family members to figure out what insecticides are in the area

What are questions you might ask farmers?

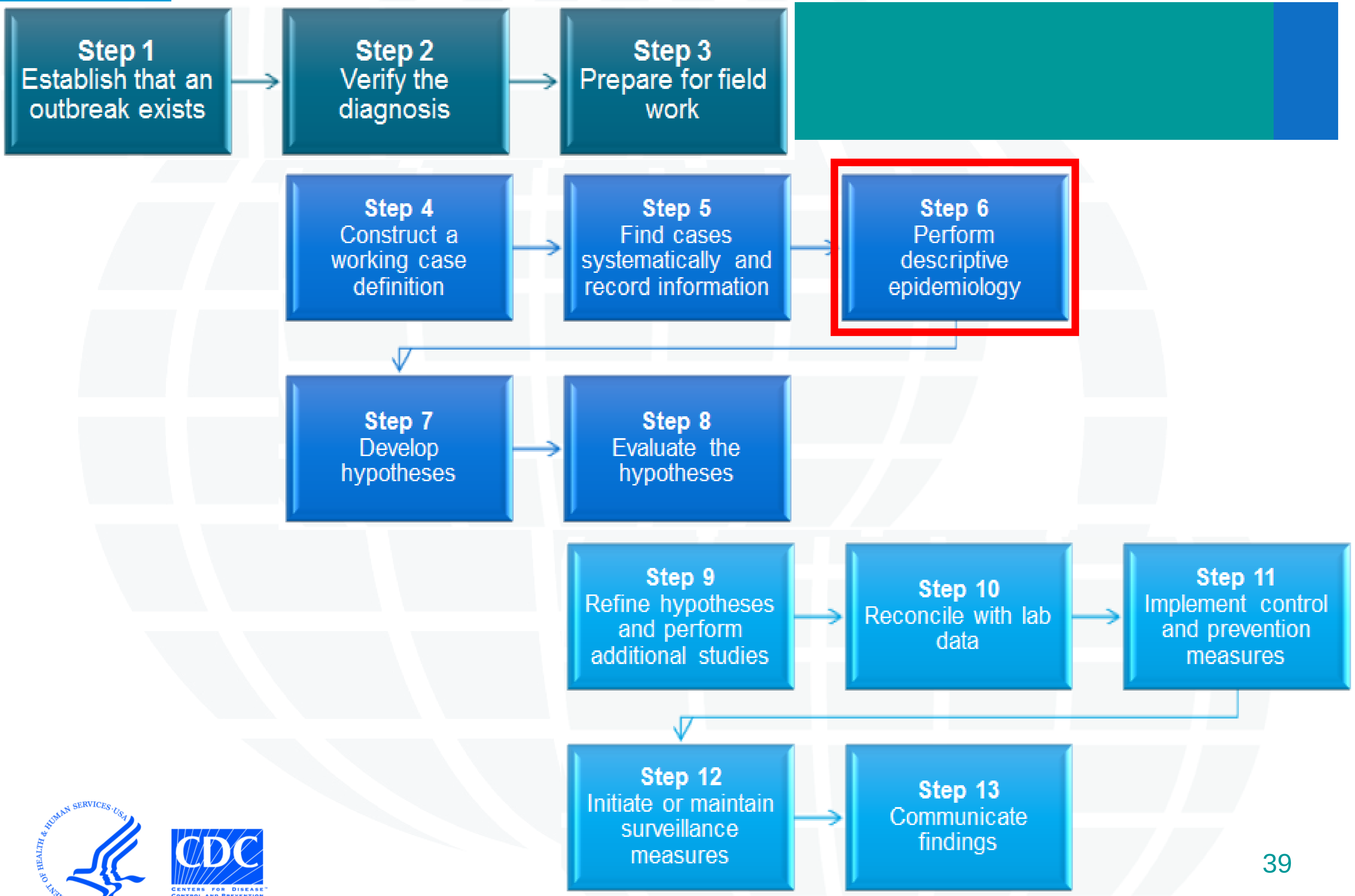
- What types of insecticides are used in farming?
- When is the last time those insecticides were applied?
- Where were the insecticides purchased?
- Where are the insecticides stored?

Hypothesis-Generating Interviews (cont.)

- Several farmers report spraying chemicals on their fields to help keep the pests away
- Two insecticides that are reported by multiple farmers include carbofuran and diazinon

Hypothesis-Generating Interviews (cont.)

Chemical	Purpose	Application
Carbofuran	• Rice paddy • Home vegetable gardens	• Purchased in pellet form • Mixed in a large bowl with urea and thrown by hand into wet paddy fields
Diazinon	• Rice paddy • Home vegetable gardens	• Mixed with urea and spread on paddy field and by hand • Mixed with top soil in vegetable gardens • Also mixed with water and sprayed on rice paddy fields



Step 6: Perform descriptive epidemiology

Step 6
Perform descriptive epidemiology

Descriptive Epidemiology

- Summarize the data you collected during the case finding and hypothesis-generating interviews by…
  - Person
  - Place
  - Time

Step 6
Perform descriptive epidemiology

Scenario: Attack Rates

	Cases (n=50)	Total number of children (n=500)	Attack rate
Age			
≤1 year	5	100	
3 year	22	100	
4 years	18	100	
8 years	3	100	
10 years	2	100	
Sex			
Male	45	250	
Female	5	250	
Village			
A	2	125	
B	38	125	
C	8	125	
D	2	125	

Summarizing by person…

Investigators refer to the line list they created and calculate attack rates…

How do you calculate an attack rate?

Step 6
Perform descriptive epidemiology

Scenario: Attack Rates (cont.)

	Cases (n=50)	Total number of children (n=500)	Attack rate
Age			
≤1 year	5	100	5%
3 year	22	100	22%
4 years	18	100	18%
8 years	3	100	3%
10 years	2	100	2%
Sex			
Male	45	250	18%
Female	5	250	2%
Village			
A	2	125	2%
B	38	125	30%
C	8	125	6%
D	2	125	2%

How do you calculate an attack rate?

Attack rate:

$$\frac{\#\ \boldsymbol{cases}\ with\ exposure}{\#\ \boldsymbol{children}\ with\ exposure}$$

Step 6
Perform descriptive epidemiology

Scenario: Attack Rates (cont.)

	Cases (n=50)	Total number of children (n=500)	Attack rate
Age			
≤1 year	5	100	5%
3 year	22	100	22%
4 years	18	100	18%
8 years	3	100	3%
10 years	2	100	2%
Sex			
Male	45	250	18%
Female	5	250	2%
Village			
A	2	125	2%
B	38	125	30%
C	8	125	6%
D	2	125	2%

The attack rate was highest among…

- 3 and 4 year old children
- Males
- Children who lived in village B

Based on these findings, you might do further informal data collection to determine what these groups had in common.

Symptom Frequency

The team describes symptom frequency:

Symptoms	n	%
Cold skin	10	91
Excessive sweating	9	82
Frothy discharge	9	82
Weakness in arms or legs	8	73
Loss of consciousness	7	64
Difficulty breathing	5	45
Fatigue	5	45
Convulsions	5	45
Fever	3	27
Vomiting	3	27
Confusion	3	27

Summarizing by person…

Observations

- The team performs observations in the village to determine possible ways that children could have been exposed to pesticides
- They note that the village consists of a cluster of houses surrounded by rice paddy and vegetable fields

Summarizing by place…

Observations (cont.)

- The team follows a small number of children around for the morning and observe what types of activities they perform
- They choose the morning hours because that is when most cases became ill

Village Map

They draw a map of the village so they can better visualize where cases resided

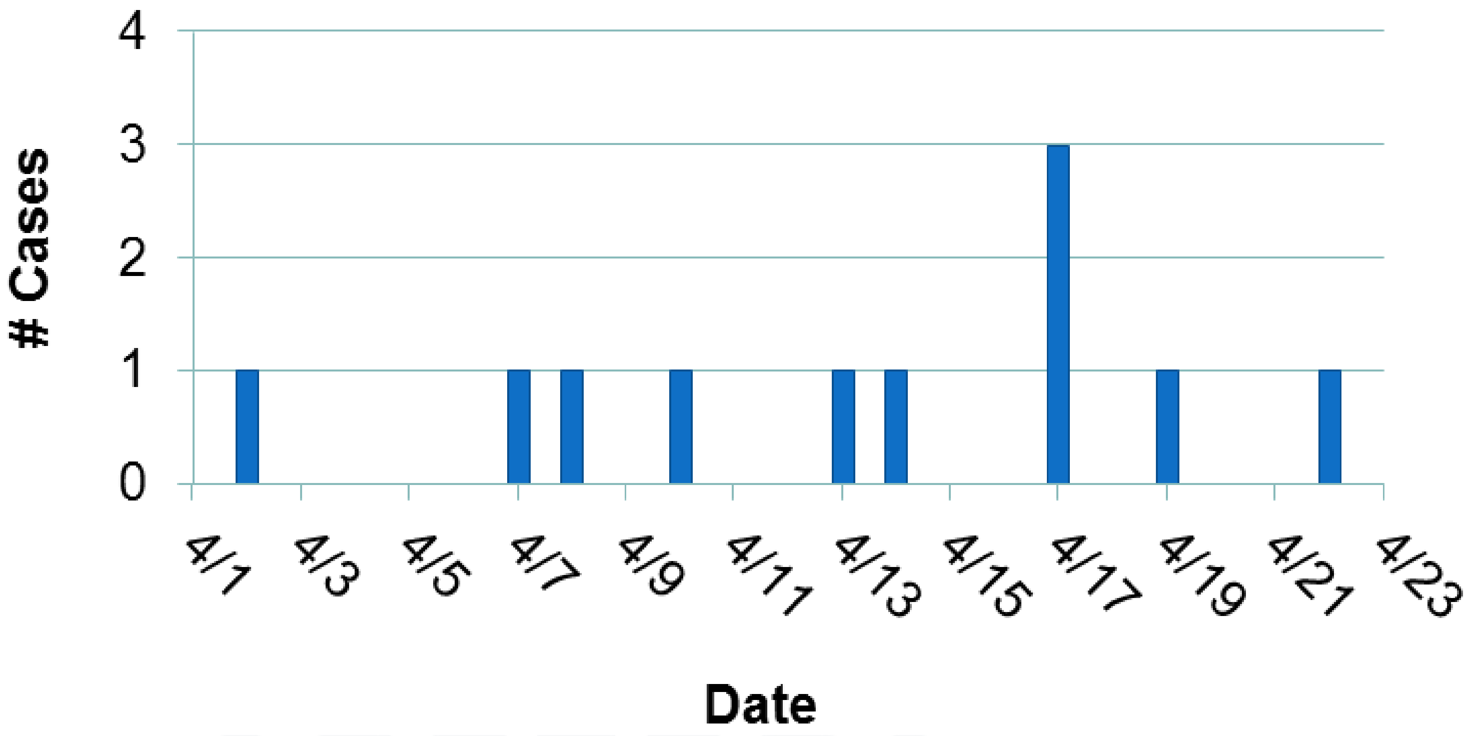
Observational Summaries

- Children spend most of their time playing outside, in and around a mud pile
- Children play unobserved; family members do not pay much attention to what they do
- Children sometimes eat unripe mangoes that fall on the ground overnight; the mangoes are not washed before eating

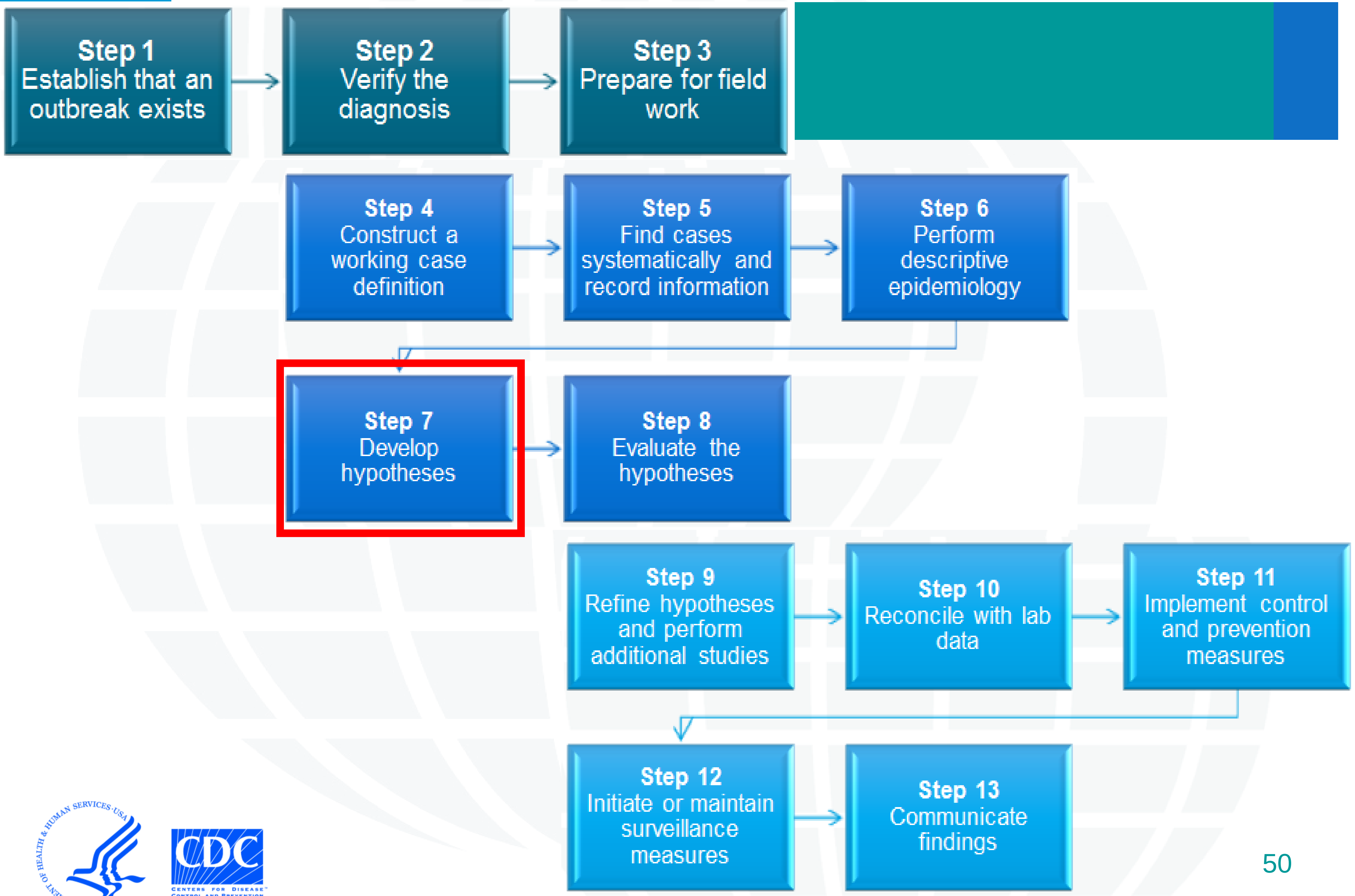
Summarizing by place…

Descriptive Epidemiology

The team constructs an epi curve:



Summarizing by time…

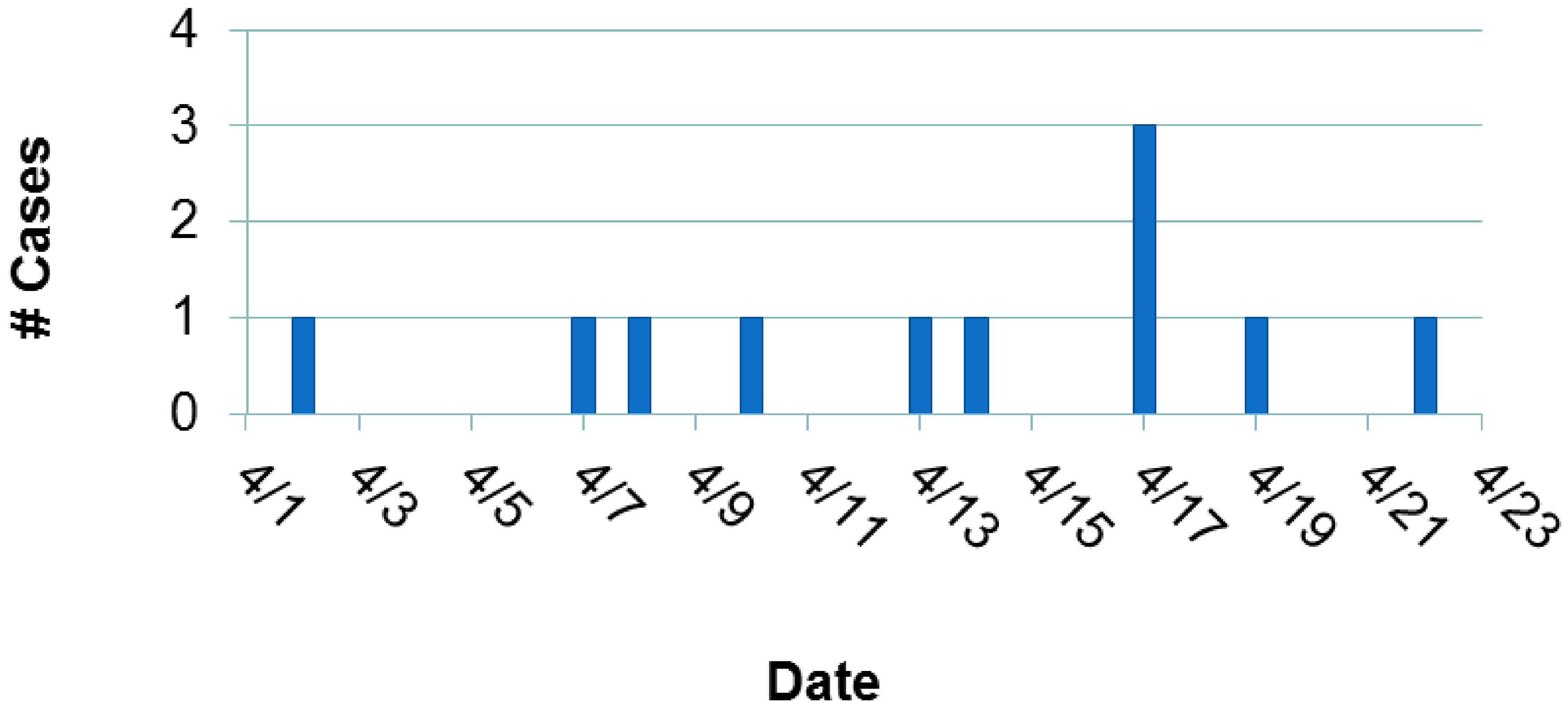


Step 7: Develop hypotheses

Hypothesis - Etiologic Agent

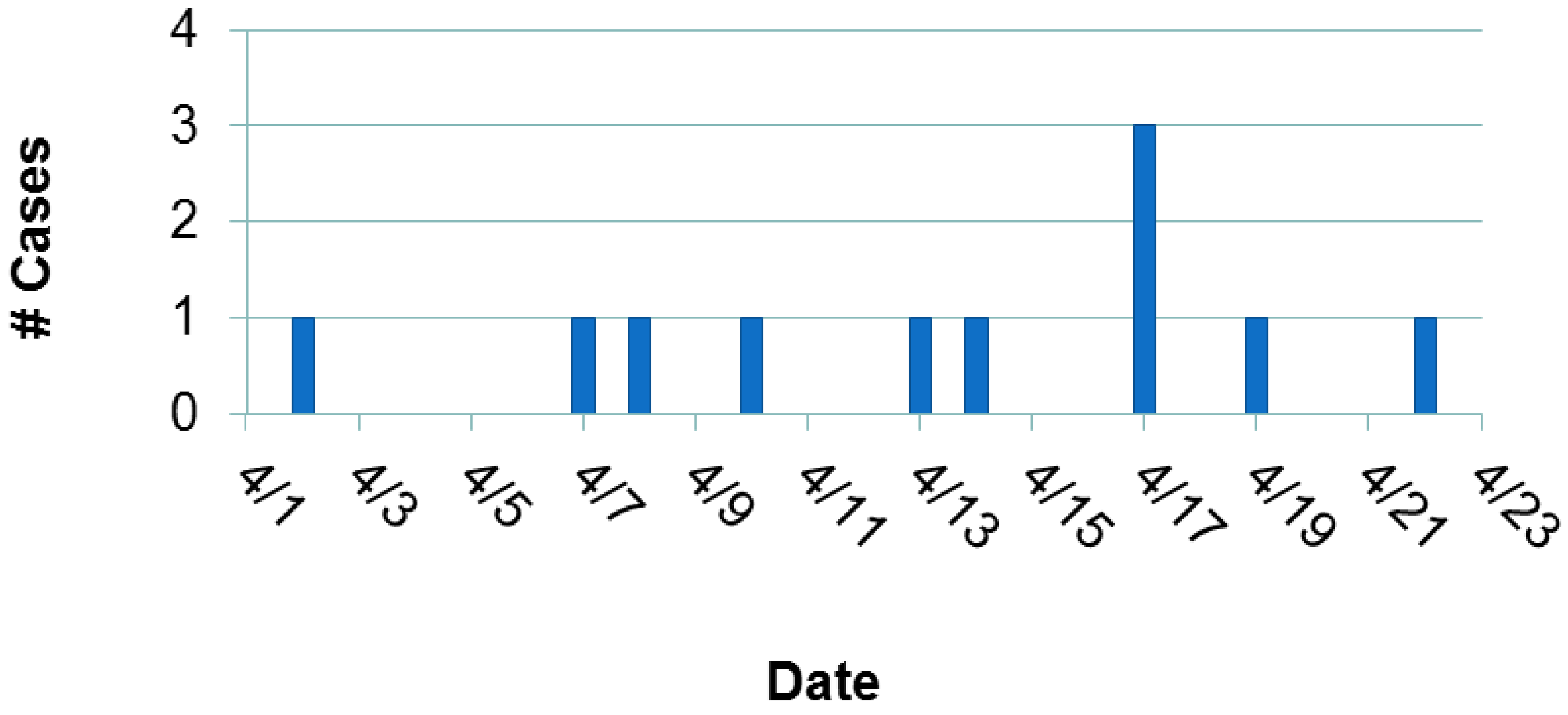
- The team previously developed a hypothesis that the etiologic agent is carbofuran or diazinon insecticide
- They would like to develop a hypothesis for what exposure caused the outbreak

Develop Hypothesis



What is one hypothesis you might draw from the epi curve?

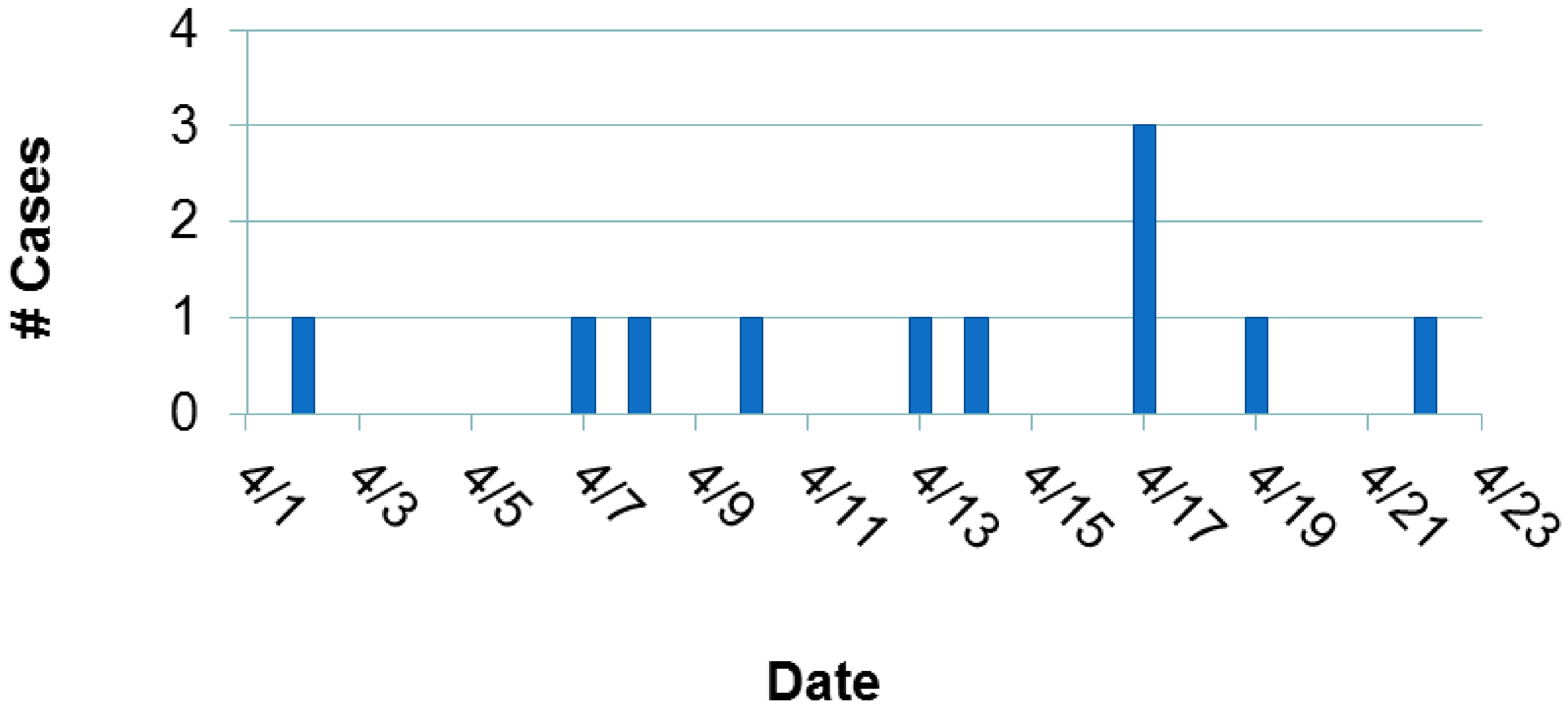
Develop Hypothesis (cont.)



What is one hypothesis you might draw from the epi curve?

- Based on the epi curve, it does not appear that all children were exposed during a single event
- If that had been the case, we would have expected the cases to have occurred within a shorter range of time

Develop Hypothesis (cont.)

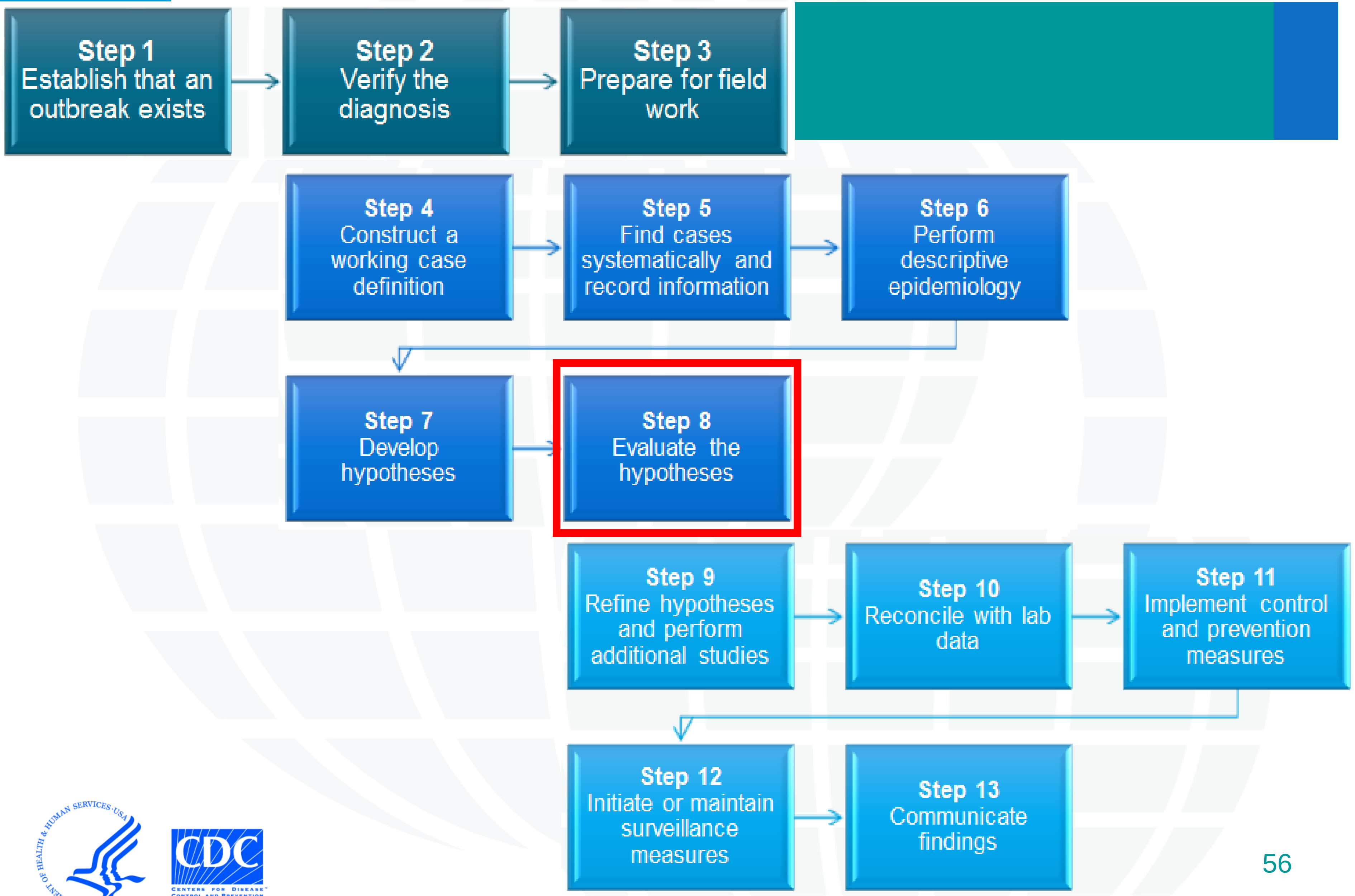


What is one hypothesis you might draw from the epi curve? (cont.)

- Thus, they hypothesize the risk must have been present in the environment over a period of time

Develop Hypothesis (cont.)

- The team hypothesizes that there was not a single exposure that caused the illness, but rather an exposure that occurred multiple times
- Based on their observational summaries, they decide to target their investigation:
  - To exposure to mud, or
  - To exposure to eating food that has dropped to the ground



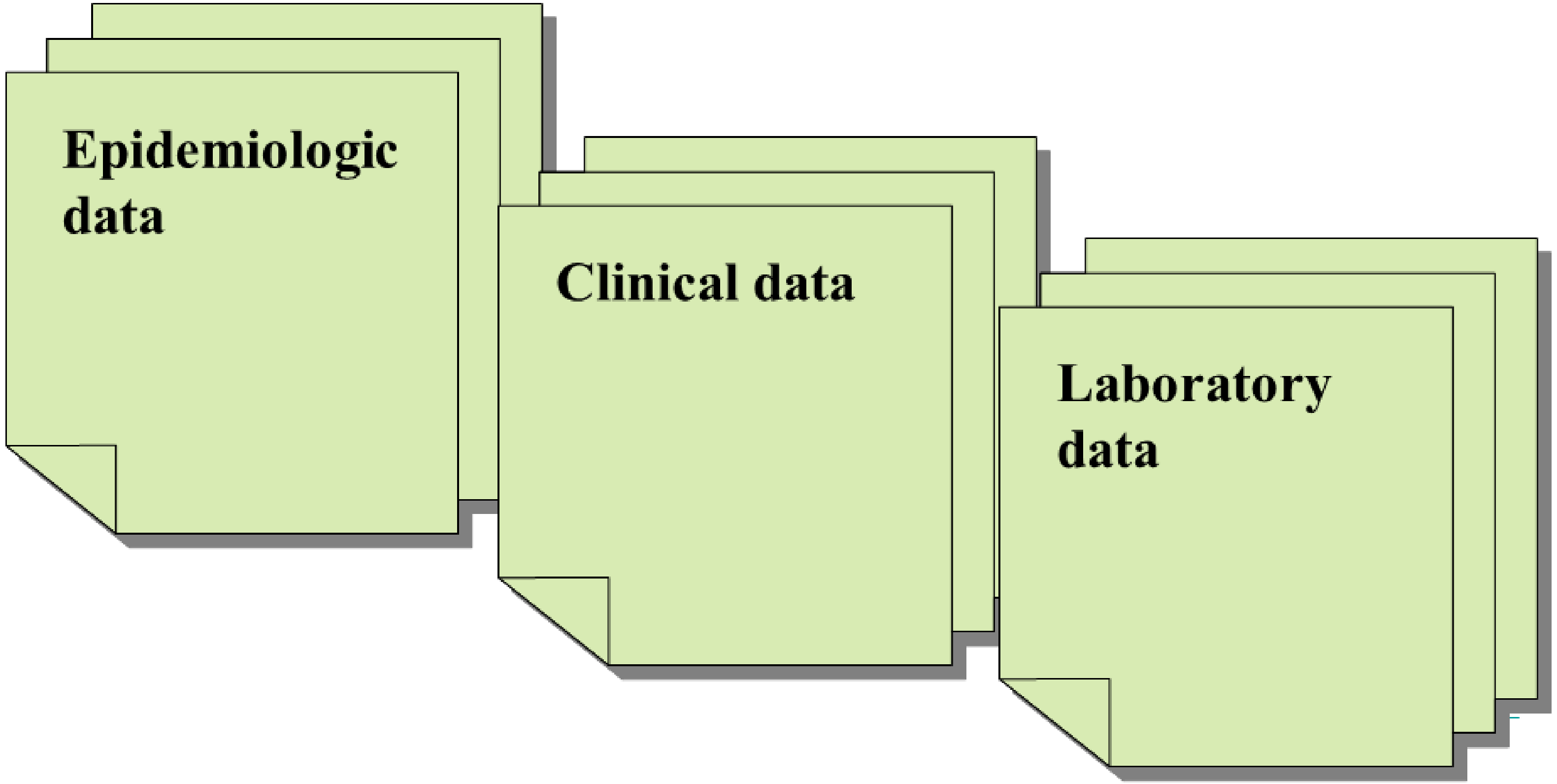
Once you have developed hypotheses, the next step is to conduct a study to evaluate the hypotheses.

Step 8: Evaluate the hypotheses

Step 8
Evaluate the hypotheses

Types of Data Collected

A typical investigation involves collecting:



Case-Control Study

The team conducts a case-control study

What would you consider when selecting controls?

Epidemiologic data

Case-Control Study (cont.)

The team conducts a case-control study

What would you consider when selecting controls?

- Controls should be as similar as possible to cases
- Because all the cases are children, it might be better to select controls who are children

Case-Control Study (cont.)

- The team aims to try and enroll 3 controls for every 1 case
  - They decide to match based on age
- They need to develop an epidemiologic questionnaire to collect data on potential exposures

Tool Kit: Sample Questionnaire

Questionnaire Modification

Design a question to ask study participants about exposure to mud

Questionnaire Modification (cont.)

Design a question to ask study participants about exposure to mud

Things to consider:

- In addition to asking about what the cases had been exposed to, they should attempt to quantify how much exposure occurred
- They should focus these questions on the time period right before the illness occurred

Questionnaire Modification (cont.)

The team decides to ask these questions about mud:

Did your child play in mud on [date]?

If yes, how many times did your child play in mud on [date]?

Biologic Sample Collection

- Most organophosphate and carbamate insecticides have short half-lives, ranging from hours to days
- The team decides to collect blood samples from controls, in order to serve as a comparison group

Environmental Sample Collection

- The investigators decide to collect mud samples.
- They decide not to collect any samples of food on the ground, because …

How should they choose where to collect mud samples from? What are some factors they should consider?

Environmental Sample Collection (cont.)

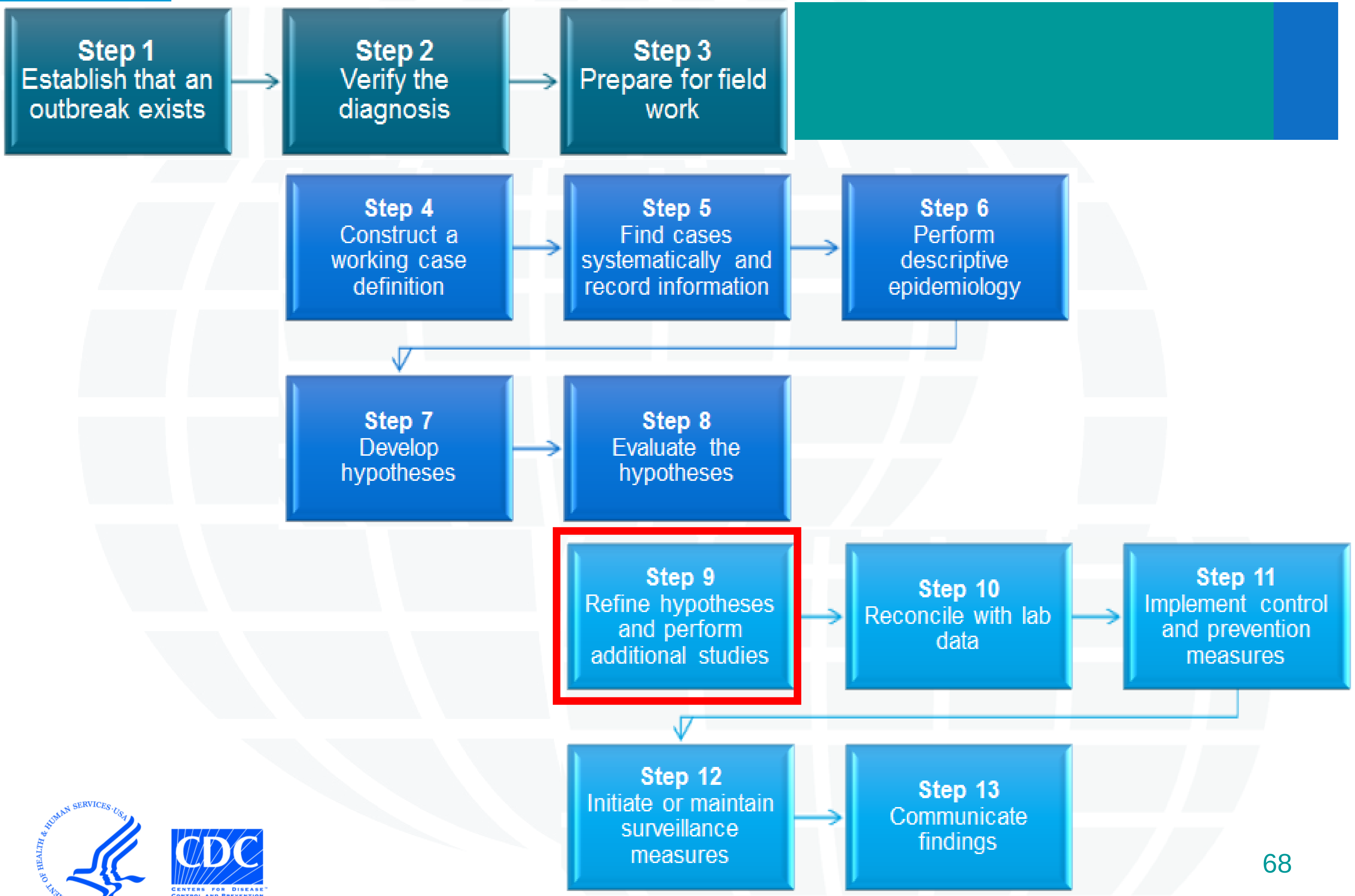
How should they choose where to collect mud samples from? What are some factors they should consider?

- Collect two types of samples:
  1) Samples that seem to be associated with illness (for example, the mangoes that cases ate prior to becoming ill, or the mud that children were playing with before they became ill)
  2) Samples that do not seem to be associated with illness (for example, samples from a different part of the village)

Environmental Sample Collection (cont.)

How should they choose where to collect mud samples from? What are some factors they should consider? (cont.)

- Collect samples based on what cases would have been exposed to
  - For example, if children were playing on a mud pile, then it would make more sense to collect samples from the surface of the pile as opposed to digging in and collecting mud from the inside



Step 9: Refine hypotheses and perform additional studies

Refine Hypotheses and Perform Additional Studies as Needed

- The team collected epidemiologic data from 11 cases and 29 controls
- Because they performed a case-control study, they calculate odds ratios for the exposures

Analytic Epidemiology (2x2 Table)

Play in mud?	Case	Control	Total	% Ill
Yes	9	9	18	50%
No	2	20	22	9%
Total	11	29	40	
% Exposed	82%	31%		

How would you compare the strength of association between exposure and case status?

Standard Two-by-Two Table

	Case	Control
Exposed	a	b
Unexposed	c	d

Odds ratio = ad / bc

If there is no association between exposure and disease, then odds ratio will equal 1.0.

The higher the risk ratio or odds ratio, the stronger the association between exposure and disease.

Analytic Epidemiology (2x2 Table)

Play in mud?	Case	Control	Total	% Ill
Yes	9	9	18	**50%**
No	2	20	22	**9%**
Total	11	29	40	
% Exposed	**82%**	**31%**		

Can we conclude that playing in mud caused the outbreak?

Calculate Odds Ratio (OR)

$$OR = \frac{9 * 20}{2 * 9}$$

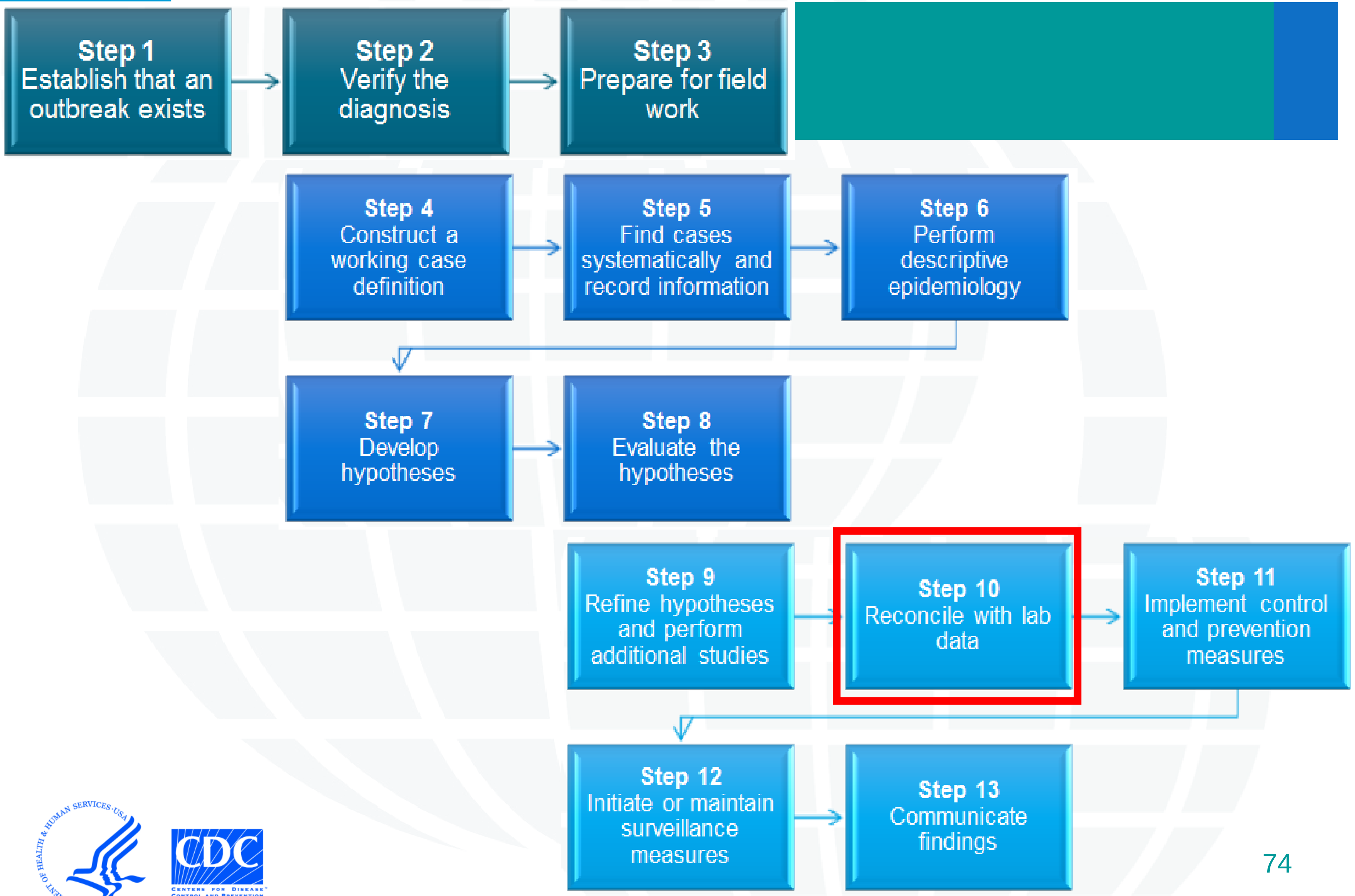
$$OR = \frac{180}{18}$$

$$OR = 10$$

Analytic Epidemiology

Can we conclude that playing in mud caused the outbreak?

- The odds ratio of 10 suggests a strong association between playing in mud and becoming a case
- However, it is possible there could be a confounding factor
- It would be strongest if we can detect high levels of insecticide in the mud where children were playing



Step 10: Reconcile with laboratory data

Reconcile with Laboratory and Environmental Data

The team examines the results of the biologic samples

	Cases (n=7)	Controls (n=13)
Carbofuran		
% Detected	29%	0%
Range	9.5 to 1061	<LOD
Diazinon		
% Detected	0%	0%
Range	<LOD	<LOD

Serum Results

- Carbofuran has a very short half-life in the body
- The team decides to stratify based on how soon the blood sample was collected after presentation to the hospital

Serum Results (cont.)

	<2 hours (n=2)	>10 hours (n=5)
Carbofuran		
% Detected	100%	0%
Range	9.5 to 1061	<LOD

The 2 cases who had samples collected within two hours of presentation to the hospital had detectable levels of carbofuran.

The 7 cases who had samples collected more than 10 hours after presentation to the hospital did not have detectable levels of carbofuran.

Serum Results (cont.)

- These data suggest that carbofuran may be the etiologic agent that caused the illness, because levels are higher in cases than controls

Serum Results (cont.)

- These data suggest that carbofuran may be the etiologic agent that caused the illness, because levels are higher in cases than non-cases
- There is not much known about how much carbofuran is required to be ingested before it will cause illness

Serum Results (cont.)

- These data suggest that carbofuran may be the etiologic agent that caused the illness, because levels are higher in cases than non-cases
- There is not much known about how much carbofuran is required to be ingested before it will cause illness
- However, from the little data that does exist, it appears that the levels in the biologic samples from the two cases were high enough to cause the toxic syndrome that was seen

Environmental Sample Results

- The investigators also receive data on the environmental samples that had been collected
- The table on the next slide shows carbofuran and diazinon levels in various environmental samples

Levels of Carbofuran and Diazinon in Environmental Samples

Type of Sample	Carbofuran (µg/kg)	Diazinon (µg/kg)
Mango #1	<LOD	<LOD
Mango #2	<LOD	<LOD
Mango #3	<LOD	<LOD
Mango #4	<LOD	<LOD
Soil #1	68	<LOD
Soil #2	0.8	<LOD
Soil #3	417	<LOD
Soil #4	0.3	<LOD

Team Consults with Toxicologists for Interpretation of Environmental Data

- None of the environmental sample tests results seem to be high enough to have made the children sick
- The highest level detected was 417 µg/kg
  - This would be safe even if a child had consumed a couple of teaspoons of this amount every day

Levels of Carbofuran in Environmental Samples

What are some of the reasons why the levels were not that high in the environmental samples?

Levels of Carbofuran in Environmental Samples (cont.)

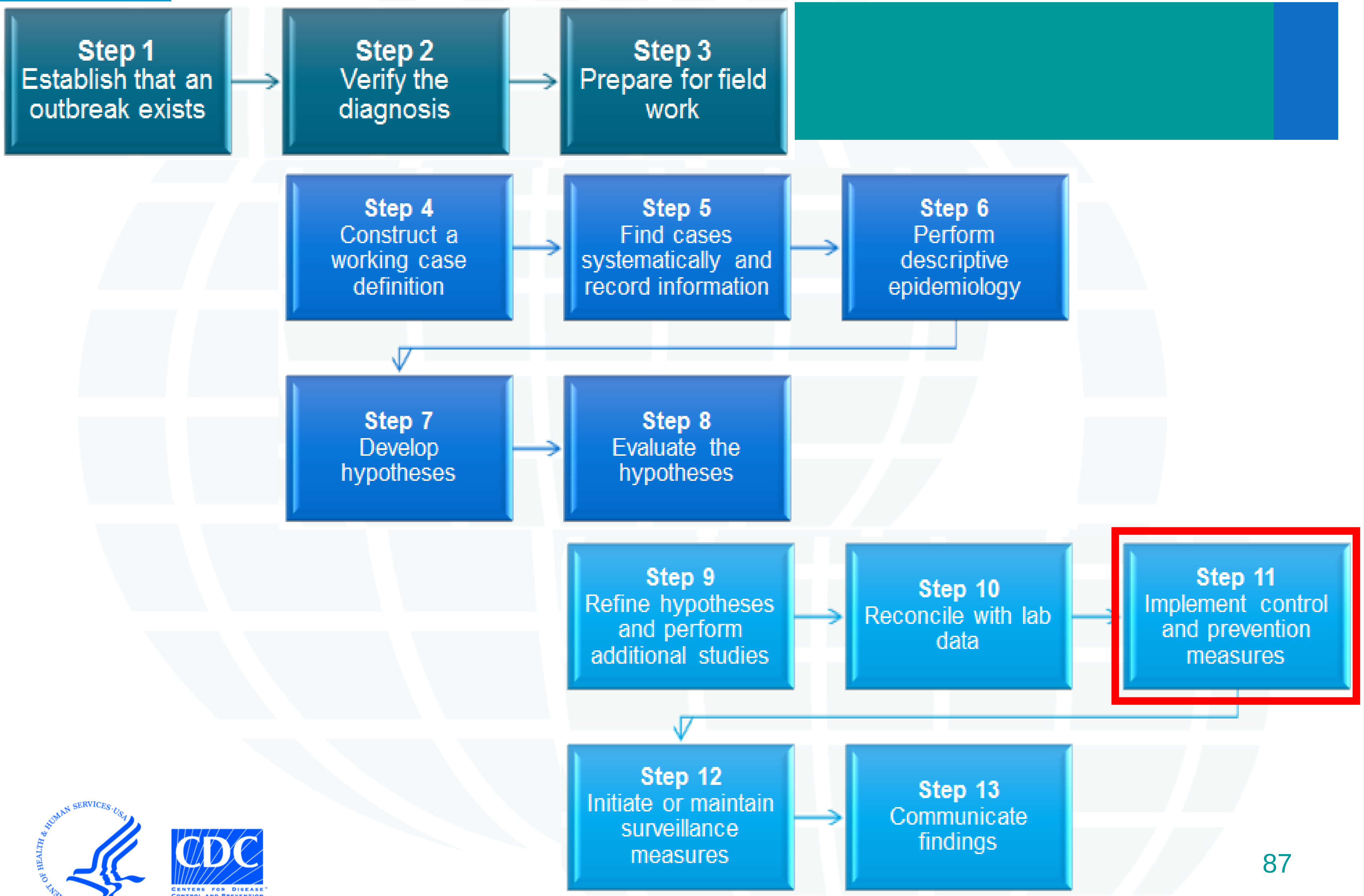
What are some of the reasons why the levels were not that high in the environmental samples?

- Toxic agents are not always distributed evenly throughout media; we do not know if these values are representative of what children were exposed to
- The carbofuran could have broken down or washed away before these samples were collected

Conclusions

Given this information, the investigators conclude:

- The etiologic agent was carbofuran, due to…
  - Higher levels in cases compared to controls
  - Levels were higher when collected immediately after illness
- The source of exposure is unknown
- It is possible that there was not a single exposure source, but rather children may have been exposed in different ways, even in the same village



Step 11: Implement control and prevention measures

Implement Control and Prevention Measures

- Cases had stopped occurring prior to the team's arrival, and no further cases occurred during their investigation
- It appeared that whatever the source of carbofuran was in the environment, it had likely gone away
  - Perhaps a recent rainstorm had washed away pesticide residue from the fields
- Thus, there was not an urgent need to implement control and prevention measures

Control and Prevention Measures

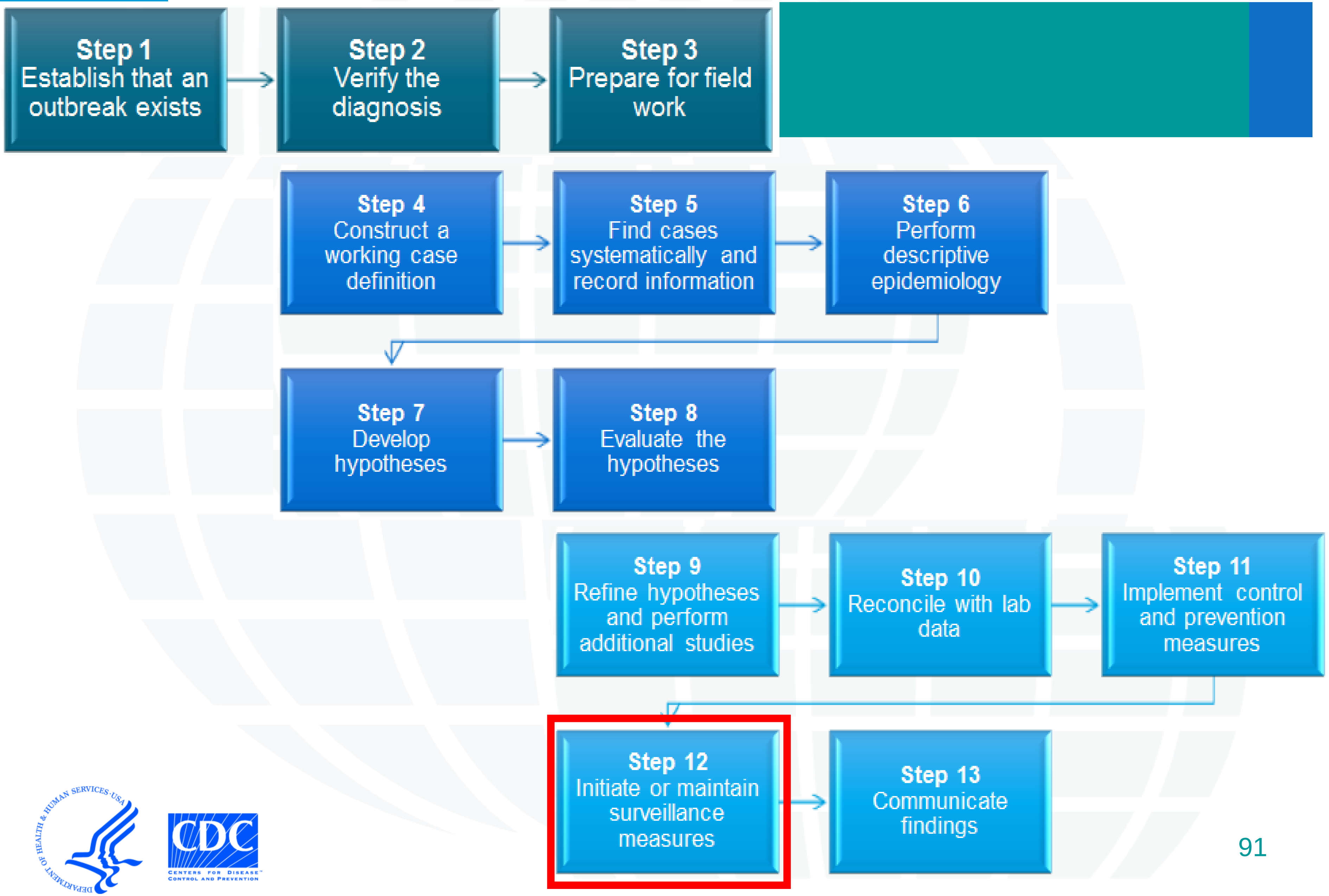
Farmers are not likely to stop spraying potentially toxic chemicals on their fields, because not using these chemicals could mean that pests could destroy crops and families would not have enough food to eat

What are possible control and prevention measures that could be considered?

Control and Prevention Measures (cont.)

What are possible control and prevention measures that could be considered?

- Wash fruits and vegetables before eating them
- Follow instructions for proper use of pesticides; do not apply too much, and wear proper protective equipment
- When a pesticide is applied, keep children away from the area for at least a couple of days

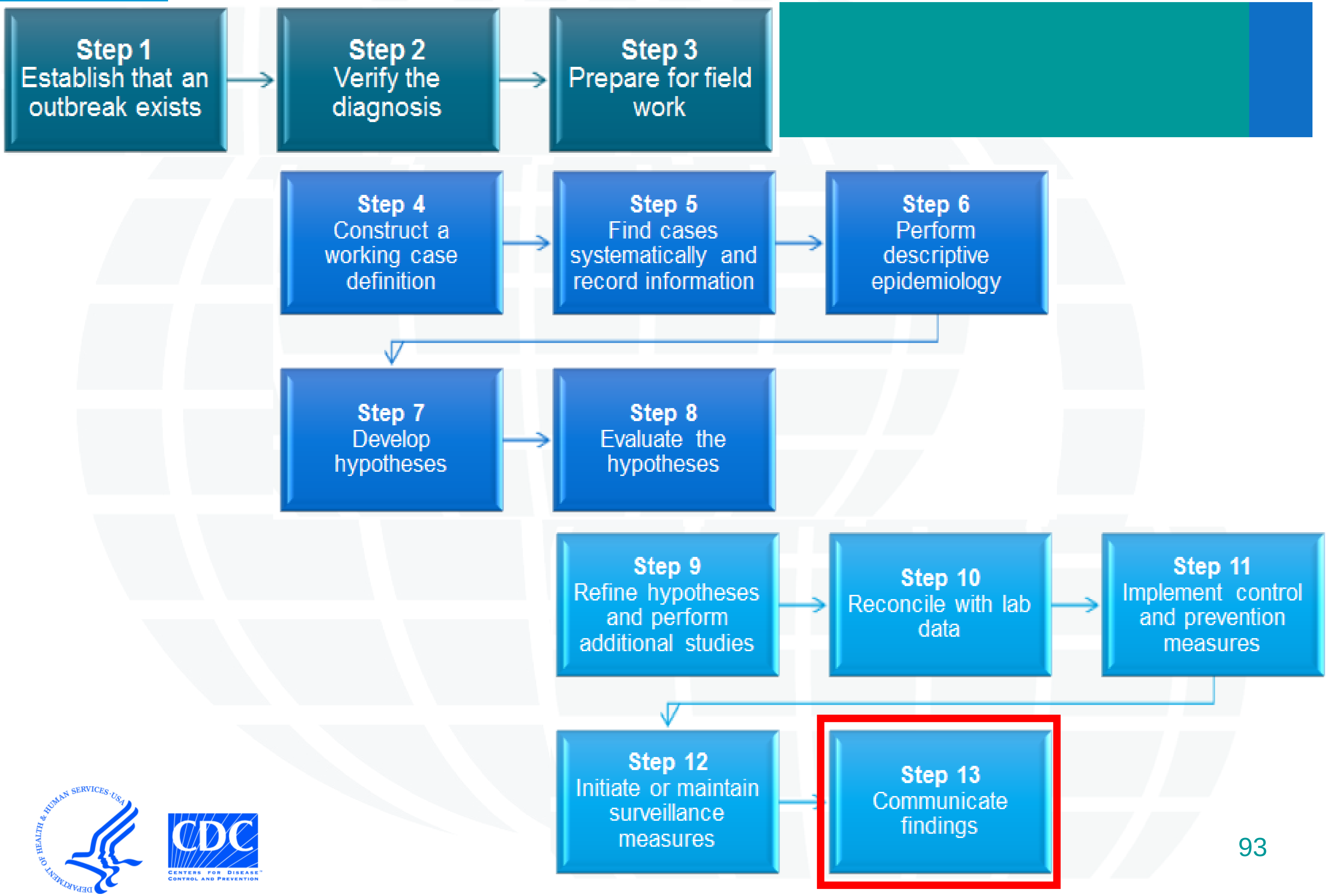


Step 12: Initiate or maintain surveillance measures

Surveillance

The team meets with local public health leaders to discuss whether they should initiate surveillance

What would be the pros and cons of initiating surveillance?



Step 13: Communicate findings

Communicate Findings

- The team communicates their findings to the local community
- They know the community will want to know why carbofuran was not detected in all cases

Develop some talking points to explain why carbofuran was not detected in all cases

Communicate Findings (cont.)

Develop some talking points to explain why carbofuran was not detected in all cases

- Like all toxic agents, pesticides are metabolized and eventually leave the body
- Not all cases had biologic samples collected immediately upon presentation to the hospital

Communicate Findings (cont.)

Develop some talking points to explain why carbofuran was not detected in all cases (cont.)

- Maybe the particular pesticide that caused illness was extremely toxic, and only a very small amount was needed to make a person sick
- Maybe this level is below the threshold at which the laboratory can detect the pesticide in a sample (below the limit of detection, <LOD)

Module Conclusion

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

Thank you for your participation!