



## Wisconsin Science Olympiad

### Disease Detectives Event

March 2007

\_\_\_\_\_  
*High School Name*

\_\_\_\_\_  
*City*

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## The Eosinophilia-Myalgia Syndrome (EMS)

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This case study is based on an investigation conducted in 1989-90 by the Minnesota Department of Health. It was developed in 2007 by Edward Belongia, MD, Laura Coleman, PhD, RD, and Stacy Holzbauer, DVM, MPH. Drs. Belongia and Coleman are epidemiologists at the Marshfield Clinic Research Foundation, and Dr. Holzbauer is a CDC Epidemic Intelligence Service Officer assigned to the Minnesota Department of Health.

Additional details regarding this investigation are available in the following publication:

Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, Dunnette SL, Pirie PL, MacDonald KL, Osterholm MT. An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *New England Journal of Medicine* 1990; 323:357-65.

*Note: This case study should contain 14 pages with 27 questions. Please check to make sure your copy does not have any missing pages.*

**PART I: An Unexpected Call** It is Thursday, November 9. You are a new epidemiologist at the Minnesota Department of Health, working with a renowned team of public health professionals. Tomorrow is a state holiday, and you are looking forward to a relaxing 3-day weekend.

Just before leaving for the day, you receive a call from a colleague at the Centers for Disease Control and Prevention (CDC) in Atlanta. He tells you that New Mexico is investigating 30 cases of an apparent new disease that causes extreme eosinophilia with severe muscle pain (myalgia) or weakness in affected people. Eosinophilia is an abnormal increase in a type of white blood cell (the eosinophil) that is often associated with allergies or certain parasitic infections. Some patients had muscle biopsies that showed unusual inflammation around the muscle fibers (perimyositis). Their doctors reported that many of the patients were taking a dietary supplement called L-tryptophan (LT). A few of the patients had low-grade fever, but many did not. Several patients also reported joint pain (arthralgias), rash, cough, or swelling of the extremities.

There is a patient with a similar illness in the hospital at the Mayo Clinic in Rochester, Minnesota. Your supervisor asks you to drive down to Rochester and find out more about the case. This patient has pneumonia along with eosinophilia, and she has been taking L-tryptophan for insomnia.

**L-TRYPTOPHAN (LT).** An essential amino acid that is found in protein-rich foods. In the 1980s, it was sold in pharmacies, groceries, and health food stores as a dietary supplement (tablets or capsules). Unlike prescription medications, it was not regulated by the Food and Drug Administration (FDA). Scientific and popular literature suggested that LT might be useful for insomnia, depression, and premenstrual syndrome.

1. Which of the following is **NOT** an appropriate course(s) of action at this point? (2 points)
  - a. Alert the news media to see if the number of cases goes down after people stop taking L-tryptophan.
  - b. Establish a case definition for the new disease.
  - c. Buy bottles of L-tryptophan from local stores and begin animal tests to see if they cause eosinophilia.
  - d. Begin a case-control study of risk factors for this apparent new disease.
  - e. Both A and C.
  
2. Which symptoms should be included in your case definition for this disease? (2 points)
  - a. Eosinophilia
  - b. Fever
  - c. Myalgia
  - d. Headache
  - e. Both A and C
  - f. All of the above

## PART II: The Investigation Begins

On Friday (a state holiday), you discuss these findings with your supervisor and plot a course of action. A quick call to a local pathologist reveals that there will be a conference in town for pathologists starting tomorrow (Saturday). A discussion with a Twin Cities rheumatologist indicates that he has seen 2 or 3 people with a similar illness over the past several months. The characteristics of the illness do not fit any known disease of the muscles or immune system. He thought that many patients with this illness would get a muscle biopsy if they were evaluated by a rheumatologist. You decide that a study must be quickly completed to further evaluate this problem, and an investigation team is assembled. You decide to do a case-control study of risk factors for this illness, which you are calling eosinophilia-myalgia syndrome, or EMS.

After consulting with investigators at the CDC in Atlanta, you agree on the following case definition for EMS: eosinophil count  $>1000$  cells/mm<sup>3</sup>, myalgia or muscle weakness severe enough to affect daily activities, and a muscle biopsy (if done) showing perimyositis (inflammation around the muscle fibers). A muscle biopsy is not required to meet the case definition, but the findings must be consistent with EMS if it was done. Patients with a known cause of extreme eosinophilia (such as trichinosis) were not classified as having EMS.

3. Which of the following are **NOT** acceptable method(s) to identify cases for the case-control study? (4 points)
- Ask physicians to identify patients they have recently seen who met the case definition and took L-tryptophan;
  - Place an ad in a newspaper asking people to call the health department if they have had muscle pain or weakness in the past month;
  - Ask rheumatologists and other physicians to identify patients they saw in the past 3 months who met the case definition for EMS;
  - Screen blood count results at clinical laboratories to identify all patients with eosinophil count  $>1000$ , and then review their medical records to find those who had myalgia or muscle weakness and no known cause of eosinophilia.
  - Both A and B

4. What is the primary hypothesis in this case control study? (4 points)

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### **PART III: The November Surprise**

It is Sunday, November 12. You are analyzing the results of the case-control study that was conducted over the weekend. Cases were identified over the weekend by contacting rheumatologists and pathologists to identify patients recently diagnosed with eosinophilia and either severe muscle pain or muscle weakness. Potential cases were excluded from the study if they had a known cause of eosinophilia such as trichinosis (a parasitic infection). Controls are identified by random digit dialing, and each case-control pair was matched on age group, gender, and telephone prefix. Both case patients and controls completed a telephone interview regarding diet, illnesses, consumption of undercooked fish or meat, prescription medications, and use of vitamins and dietary supplements before onset of EMS symptoms in the case patient (or similar time period for the control). Based on interviews with twelve cases and twelve controls, you find that all cases consumed LT compared with none of the controls. The p-value for this association is 0.0008. There was no association between EMS and consumption of specific vitamins, other dietary supplements, or prescription medications.

**5.** What is the purpose of matching cases and controls on age group and gender? (4 points)

- a. Matching helps prevent confounding due to age and gender;
- b. The precision of the outcome will be increased because these matching variables are strongly related to L-tryptophan use but not the disease;
- c. Matching will allow you to determine if the risk of EMS varies by age and gender;
- d. All of the above.

**6.** Why did the case and control interviews include questions about consumption of dietary supplements and prescription medications? (4 points)

- a. Because many supplements and prescription medications are known to cause EMS;
- b. To determine whether other supplements or prescription medications may be confounding factors;
- c. Because this information is helpful to identify a dose-response relationship;
- d. All of the above.

**7.** Create a 2 X 2 table showing consumption of LT among cases and controls. Label the rows and columns and show the data in each cell. (4 points)

|  |  |
|--|--|
|  |  |
|  |  |

**8.** What is the odds ratio for L-tryptophan consumption in cases vs. controls? (6 points)

- a.  $0.0008 \times 12 = 0.096$
- b. 12
- c. 0
- d. Undefined

**9.** Do these results prove that L-tryptophan causes EMS? In other words, is this a causal association? Answer YES or NO and explain your answer in 2-3 sentences. (4 points)

YES

NO

Explain: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**10.** Based on information available so far, which of the following are reasonable hypotheses? (4 points)

- a. EMS is triggered by L-tryptophan itself in susceptible individuals;
- b. EMS is triggered by contaminants in L-tryptophan;
- c. The symptoms of EMS cause patients to consume L-tryptophan for relief;
- d. EMS is unrelated to L-tryptophan use.
- e. Both A and B

## PART IV: Showa Leather Epidemiology

You fax a summary of your study methods and results to the FDA and the CDC. After consultation with the Director of the Minnesota Department of Health, you decide to hold a press conference later that day. Newspaper and television stories provide broad coverage about the new illness and they warn the public to avoid taking LT until more is known. The press release asks people to contact the State Health Department if they have consumed LT in the recent past. The next morning, the phone lines in your department are flooded with calls from a worried public. You call in extra staff from field offices to help out. On November 17, the FDA issues a nationwide recall of all L-tryptophan products.

The case-control study was the easy part. Now it's time for some serious shoe leather epidemiology. The next stage of the investigation has 4 main objectives:

- Establish surveillance for EMS cases in Minnesota.
- Assess the prevalence of L-tryptophan consumption in the general population.
- Determine if EMS is associated with L-tryptophan from a specific distributor or manufacturer.
- Test implicated LT tablets for chemical contaminants and other potential disease-causing agents.

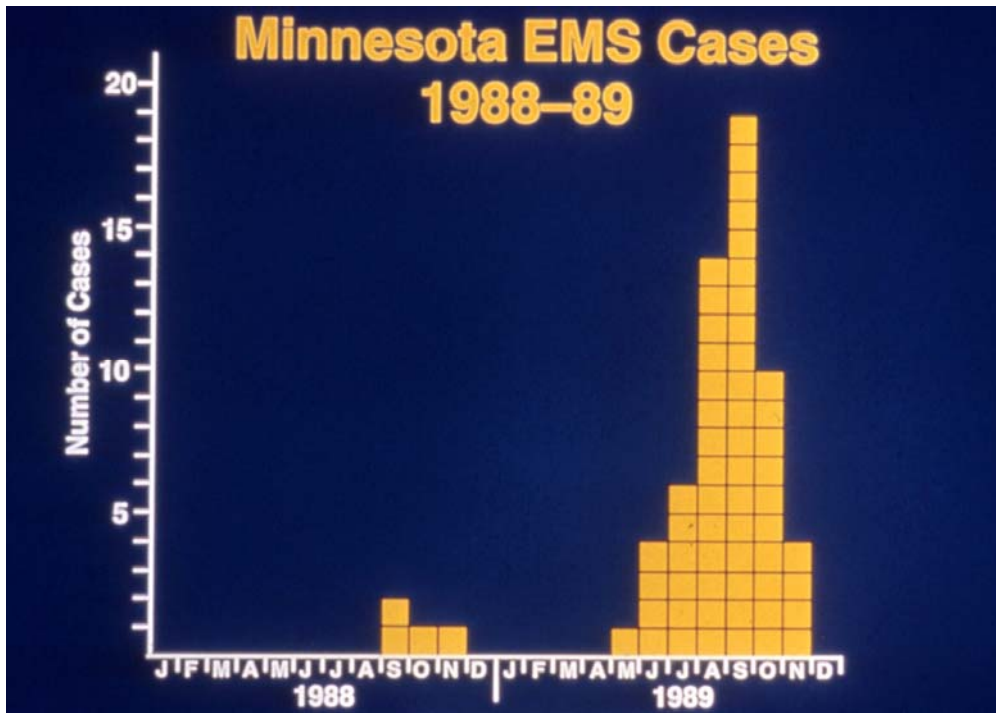
11. Which of the following is **NOT** an appropriate method to determine if EMS is associated with all L-tryptophan products, or only with L-tryptophan from a particular manufacturer or distributor? (4 points)

- a. Conduct a study by giving different brands of L-tryptophan to healthy people (after obtaining informed consent) and see who develops eosinophilia;
- b. Expose rodents to implicated and non-implicated lots of L-tryptophan to determine which ones develop pathologic abnormalities that resemble EMS;
- c. Conduct laboratory studies to find out if implicated and non-implicated lots of L-tryptophan are harmful to human muscle cells or fibroblasts (connective tissue cells);
- d. Conduct a case-control study of EMS patients and asymptomatic L-tryptophan users;
- e. All of the above

### Surveillance:

Soon after the press conference, you initiate surveillance by sending a letter to all licensed chiropractors, primary care physicians, and rheumatologists to request reporting of patients with unexplained eosinophilia and myalgia. The news media reports that people who took LT and have muscle pain or weakness should contact their doctor and the Minnesota Department of Health. Your staff contacts each patient meeting the case definition and completes a structured interview to determine the date of illness onset, history of LT use, and symptoms.

Here is the epidemic curve you constructed to show the date of onset for the 62 EMS cases in Minnesota:



The following table shows descriptive statistics for the EMS cases in Minnesota.

|  | Number of Cases |
|--|-----------------|
| Female   | 55              |
| Resident of Minneapolis-St. Paul   | 56              |
| Hospitalized for EMS   | 18              |
| Taking L-tryptophan when illness began or within 4 months prior to onset | 62              |

The median age of case patients was 45 years, with a range of 4 to 77 years.

12. When did the outbreak begin in Minnesota (provide month and year)? (4 points)

\_\_\_\_\_

**13.** Which of the following are possible explanations for the observation that the majority of EMS case patients are female? (4 points)

- a. L-tryptophan use is more common in women than in men;
- b. L-tryptophan has different physiologic effects in men and women;
- c. Women often take higher doses of L-tryptophan than men;
- d. Women are more susceptible to develop EMS compared to men;
- e. All of the above.

**14.** Which of the following are appropriate strategies to find out if L-tryptophan use is more common in women compared to men? (4 points)

- a. Conduct a random digit dialing telephone survey to assess the prevalence of L-tryptophan use in men and women;
- b. Conduct a focus group that includes both women and men who use L-tryptophan;
- c. Request marketing and advertising data from US companies that sell L-tryptophan;
- d. Conduct a case-control study of female EMS cases vs. male controls.
- e. A and D

You decide to conduct a random digit dialing telephone survey of households in the Minneapolis-St. Paul area to find out if L-tryptophan use is different in men and women, and whether it has changed over time. At each participating household, a structured interview is completed to assess tryptophan use by all household members over the past 10 years.

You also decide to conduct a case-control study among LT users to determine if EMS is associated with all LT or only certain brands or manufacturers of LT. You want to find out if case-patients were more likely to consume LT from a particular company or manufacturer relative to control LT users. Since you are mainly interested in the cause of the EMS epidemic, you decide to exclude anyone who became ill before the epidemic started.

**15.** Which of the following groups would **NOT** be appropriate to serve as controls in this case-control study? (4 points)

- a. L-tryptophan users identified through the random digit dialing telephone survey;
- b. L-tryptophan users who are asymptomatic and called the Minnesota Department of Health after the public warning was issued;
- c. People who purchased L-tryptophan from a specific department store chain (identified through credit card records);
- d. All of the above groups are NOT appropriate as controls.

Testing LT Tablets or Powder for Impurities:

Your supervisor mentions that an immunologist at the Mayo Clinic is one of the world's leading authorities on eosinophilic diseases. You call Dr. G. and learn that he and his postdoctoral fellow (Arthur) have already started some preliminary tests of LT provided by patients at the Mayo Clinic. They did not find any evidence of bacterial or viral pathogens, and all the pills were at least 99% pure LT. You head down to the Mayo Clinic to discuss your investigation with them, and they enthusiastically agree to collaborate and test LT lots from case patients and controls. They suggest a laboratory procedure called high performance liquid chromatography (HPLC) to look for chemical impurities in the LT products. They quickly learn that LT from different manufacturers has a characteristic HPLC pattern, or 'fingerprint' due to differences in the manufacturing processes and resulting trace impurities.

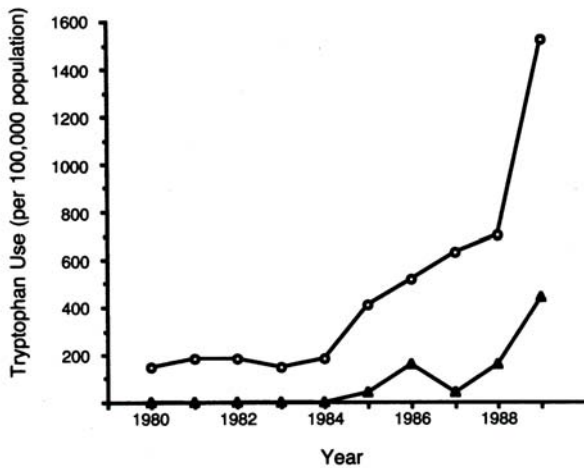
Fortunately, you have a colleague down the hall (Craig) who has experience with product tracebacks. He also knows a lot of baseball trivia. Craig makes contacts with the major LT distributors and retail chains, and he learns about the complex network of LT manufacturing and distribution. All LT in the world is made by six companies in Japan. The manufacturer with the largest market share is called Showa Denko K. K. People in the health food industry tell you that Showa Denko has about 70% market share of the world's tryptophan market.

LT powder is imported into the US by several different wholesalers who turn it into tablets. It then moves to distributors and eventually to stores. Distributors purchase LT from a variety of sources, and the manufacturer cannot be determined based on the retail brand alone. However, every retail bottle of LT has a lot number which can be traced back through the distribution network to determine the manufacturer and production date. As LT bottles are turned in by case patients and controls, Craig begins the slow process of tracing the retail lot number back to find out which company produced it.

## PART VI: Crunching the Numbers - Investigation Results

### Household Survey of LT Use:

Your random digit dialing telephone survey reached 2162 eligible households in the Minneapolis-St. Paul metropolitan area, and 93% agreed to participate. Overall, 101 of 5188 household members had consumed LT at some time during in the past 10 years. The prevalence of LT use by year is shown in the following graph.



Prevalence of L-tryptophan use in Minneapolis-St. Paul residents since 1980. Circles show use in women and triangles show use in men.

Using these data and available Minnesota census data, you estimate that 7400 women used LT products in the Minneapolis-St. Paul area during the year before the outbreak (1988), and 16,400 women used LT products in 1989. In 1988, 4 cases of EMS occurred among women living in Minneapolis-St. Paul. In 1989, this increased to 44 cases.

**16.** What was the EMS attack rate among female LT users in Minneapolis-St. Paul during 1988? *Show your work.* (6 points)

- 54 cases per 100,000 female L-tryptophan users
- 595 cases per 100,000 female L-tryptophan users
- Cannot be calculated because the investigation did not begin until 1989
- 4 cases per 100,000 female L-tryptophan users

**17.** What was the EMS attack rate among female LT users in Minneapolis-St. Paul during 1989? *Show your work.* (6 points)

- 54 cases per 100,000 female L-tryptophan users
- 268 cases per 100,000 female L-tryptophan users
- 44 cases per 100,000 female L-tryptophan users
- 5 cases per 100,000 female L-tryptophan users

**18.** True or false: The attack rate is the cumulative incidence of disease in a specified time period. (2 points)

- True  
 False

**19.** What was the rate ratio for EMS occurrence in female L-tryptophan users in 1989 relative to 1988? *Show your work.* (6 points)

- a. 3.9  
b. 0.2  
c. 5.0  
d. 11.0

**20.** Do these results suggest that the EMS epidemic of 1989 was entirely due to the dramatic increase in the number of L-tryptophan users that year? (2 points)

- Yes  
 No

Case-Control Study of L-Tryptophan Users:

The case-control study used 2 different control groups. Thirty-three people in the first control group (called *self-referred* controls) were selected from asymptomatic LT users who called the Minnesota Department of Health after the press conference on November 12. All the self-referred controls agreed to provide blood samples, and their eosinophil counts were normal. The second control group (*random* controls) was identified through the random digit dialing telephone survey. There were 24 random control subjects who used LT after May 1989 and had no symptoms. The median amount of LT consumed per month was 40.5 grams for EMS patients, 6.0 grams for random controls, and 15.0 grams for self-referred controls.

**21.** Which of the following are potential limitation(s) of using these asymptomatic L-tryptophan users as one control group? (4 points)

- a. There is a potential for bias;  
b. They may not be representative of all L-tryptophan users;  
c. They may have undiagnosed EMS;  
d. Both A and B  
e. None of the above

The following table shows the L-tryptophan manufacturer for products consumed by EMS patients and controls.

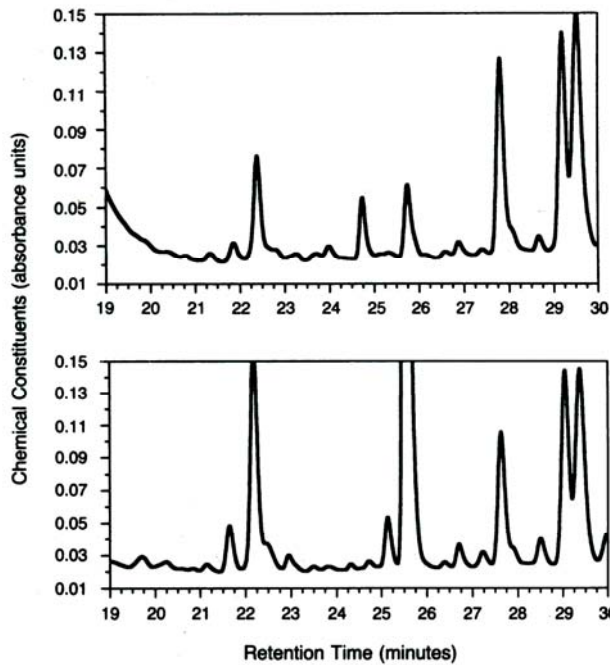
|                            | Tryptophan Manufacturer |                    |         | TOTAL |
|----------------------------|-------------------------|--------------------|---------|-------|
|                            | Showa Denko<br>K.K.     | Other<br>Companies | Unknown |       |
| Case patients              | 29                      | 1                  | 22      | 52    |
| Random controls            | 5                       | 4                  | 15      | 24    |
| Self-referred<br>controls  | 16                      | 10                 | 7       | 33    |
| Combined<br>control groups | 21                      | 14                 | 22      | 57    |

**22.** What is the odds ratio for consumption of Showa Denko L-tryptophan in case patients compared to the combined control group? Do not include cases or controls with 'unknown' L-tryptophan manufacturer. (4 points)

- a. 18.1
- b. 19.3
- c. 23.2
- d. 7.7

HPLC Analysis of LT Retail Lots:

HPLC analysis of Showa Denko tryptophan demonstrated 33 peaks, each representing a trace chemical impurity other than LT (shown on next page). Comparison of individual peaks in case and control retail lots demonstrated only 1 peak that was significantly associated with case lots. You decide to call this "Peak E" because it was always your favorite letter or perhaps because it reminds you of eosinophilia. Peak E was present in 9 of 12 case lots and 3 of 11 control lots.



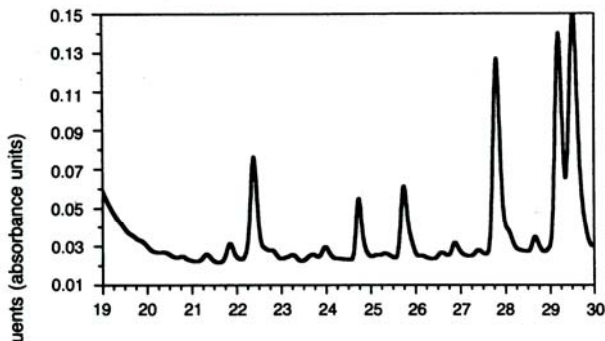
Case Lot

Control Lot

HPLC of Tryptophan Manufactured by Showa Denko, K.K.:

Absorbance was measured at 216 nm. Trace chemical constituents with retention times between 19 and 30 minutes are shown. Different trace constituents have different retention times. The upper chromatogram shows the results for a lot that was consumed by case patients with EMS. The lower chromatogram shows results for a lot that was present in LT consumed only by controls. Peak E (in the upper chromatogram) represents a chemical constituent that was present significantly more often in the retail lots consumed by case patients relative to those lots consumed by controls. Differences in the heights of peaks are not important, and minor variations in the baseline absorbance are not important.

**23.** In the HPLC graphs, each impurity has a unique retention time, represented by a peak. Identify peak E in the HPLC plot of LT associated with EMS cases (graph below). Draw an arrow to point to peak E. (2 points)



Case Lot

**24.** Based on this information, can you conclude that peak E causes EMS? (2 points)

- Yes
- No

You may recall that one patient consumed LT that was manufactured by a company other than Showa Denko K.K. That patient consumed one lifetime bottle of LT, and some tablets were still remaining in her bottle. HPLC analysis of those tablets demonstrated a 'fingerprint' that was characteristic of LT manufactured by Showa Denko, K.K., and peak E was present.

**25.** Based on this information, which of the following are plausible explanation(s) for the fact that 1 patient with EMS consumed L-tryptophan that was traced to another manufacturer? (4 points)

- a. There was an error in the product traceback;
- b. The other manufacturer purchased L-tryptophan powder from Showa Denko and then sold it as their own product;
- c. EMS was triggered by something other than L-tryptophan in this patient;
- d. The L-tryptophan bottle that was provided by this patient was not the one that triggered her illness;
- e. Both A and B

**26.** Which of the following factors are important to consider when making a determination if particular lots of Showa Denko L-tryptophan caused EMS. (4 points)

- a. Strength of association between Showa Denko LT and EMS;
- b. Consistency of findings in other populations and studies;
- c. Ingestion of Showa Denko L-tryptophan occurred before EMS symptoms developed;
- d. People who developed EMS consumed higher doses of Showa Denko LT compared to those who remained well (dose-response effect).
- e. All of the above

Now that the investigation is winding down, Craig decides to test your knowledge of baseball trivia. He poses this question:

**27.** Which of the following is NOT one of Yogi Berra's famous quotes? (**NOT SCORED**)

- a. "If you don't know where you're going, you'll end up someplace else."
- b. "You can observe a lot by watching."
- c. "The future ain't what it used to be."
- d. "Ninety percent of this game is mental. And the other half is physical."
- e. Yogi said all of the above.

***YOU ARE FINISHED! PLEASE TURN IN THE COMPLETED CASE STUDY.***

## Epilogue

The epidemiologic investigation found that changes in the manufacturing process at Showa Denko led to the introduction of a chemical that triggered EMS. The implicated LT was produced after they switched to a new genetically modified strain of bacteria and simultaneously reduced the amount of carbon for purification. Since the product was not regulated, the company did not have to demonstrate the safety of these changes prior to implementation. The chemical structure of peak E was rapidly determined. It was an unstable dimer of LT, and it did not cause illness in laboratory animals. It was ultimately discarded as a possible cause of EMS. The case lots of LT had several other impurities that were absent in the control lots, but the ultimate cause of EMS has still not been determined.

National surveillance for EMS identified over 1500 cases, including 27 deaths. The number of new cases fell dramatically after LT was taken off the market in November 1989. Unfortunately, many patients with EMS have continued to have symptoms for years afterward. Although LT remains off the market, other dietary supplements are consumed by the public without regulation or oversight by the FDA. Since we still do not know what caused EMS, we do not know if the same impurity could be generated in a different product and create a similar outbreak in the future.

1. Which of the following is **NOT** an appropriate course(s) of action at this point? (2 points)

- f. Alert the news media to see if the number of cases goes down after people stop taking L-tryptophan.
- g. Establish a case definition for the new disease.
- h. Buy bottles of L-tryptophan from local stores and begin animal tests to see if they cause eosinophilia.
- i. Begin a case-control study of risk factors for this apparent new disease.

**j. Both A and C.**

2. Which symptoms should be included in your case definition for this disease? (2 points)

- g. Eosinophilia
- h. Fever
- i. Myalgia
- j. Headache

**k. Both A and C**

l. All of the above

3. Which of the following are **NOT** acceptable method(s) to identify cases for the case-control study? (4 points)

- f. Ask physicians to identify patients they have recently seen who met the case definition and took L-tryptophan;
- g. Place an ad in a newspaper asking people to call the health department if they have had muscle pain or weakness in the past month;
- h. Ask rheumatologists and other physicians to identify patients they saw in the past 3 months who met the case definition for EMS;
- i. Screen blood count results at clinical laboratories to identify all patients with eosinophil count >1000, and then review their medical records to find those who had myalgia or muscle weakness and no known cause of eosinophilia.

**j. Both A and B**

4. What is the primary hypothesis in this case control study? (4 points)

L-tryptophan causes (or is associated with) EMS

5. What is the purpose of matching cases and controls on age group and gender? (4 points)

- a. Matching helps prevent confounding due to age and gender;
- b. The precision of the outcome will be increased because these matching variables are strongly related to L-tryptophan use but not the disease;
- c. Matching will allow you to determine if the risk of EMS varies by age and gender;
- d. All of the above.

6. Why did the case and control interviews include questions about consumption of dietary supplements and prescription medications? (4 points)

- a. Because many supplements and prescription medications are known to cause EMS;
- b. To determine whether other supplements or prescription medications may be confounding factors;
- c. Because this information is helpful to identify a dose-response relationship;
- d. All of the above.

7. Create a 2 X 2 table showing consumption of LT among cases and controls. Label the rows and columns and show the data in each cell. (4 points)

|      | Cases | Controls |
|------|-------|----------|
| + LT | 12    | 0        |
| - LT | 0     | 12       |

8. What is the odds ratio for L-tryptophan consumption in cases vs. controls? (6 points)  $([12 \times 12] \div [0 \times 0])$

- a.  $0.0008 \times 12 = 0.096$
- b. 12
- c. 0

d. Undefined

9. Do these results prove that L-tryptophan causes EMS? In other words, is this a causal association? Answer YES or NO and explain your answer in 2-3 sentences. (4 points) (2 points for correct answer to T/F + 2 points for correct explanation)

YES

NO

Explain: The results do not prove that LT causes EMS because association ≠ causation

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10. Based on information available so far, which of the following are reasonable hypotheses? (4 points)

- a. EMS is triggered by L-tryptophan itself in susceptible individuals;
- b. EMS is triggered by contaminants in L-tryptophan;
- c. The symptoms of EMS cause patients to consume L-tryptophan for relief;
- d. EMS is unrelated to L-tryptophan use.

e. Both A and B

11. Which of the following is **NOT** an appropriate method to determine if EMS is associated with all L-tryptophan products, or only with L-tryptophan from a particular manufacturer or distributor? (4 points)

- a. Conduct a study by giving different brands of L-tryptophan to healthy people (after obtaining informed consent) and see who develops eosinophilia;
- b. Expose rodents to implicated and non-implicated lots of L-tryptophan to determine which ones develop pathologic abnormalities that resemble EMS;
- c. Conduct laboratory studies to find out if implicated and non-implicated lots of L-tryptophan are harmful to human muscle cells or fibroblasts (connective tissue cells);
- d. Conduct a case-control study of EMS patients and asymptomatic L-tryptophan users;
- e. All of the above

12. When did the outbreak begin in Minnesota (provide month and year)? (4 points)

May 1989

13. Which of the following are possible explanations for the observation that the majority of EMS case patients are female? (4 points)

- a. L-tryptophan use is more common in women than in men;
- b. L-tryptophan has different physiologic effects in men and women;
- c. Women often take higher doses of L-tryptophan than men;
- d. Women are more susceptible to develop EMS compared to men;

e. All of the above.

14. Which of the following would be a reasonable strategy to find out if L-tryptophan use is more common in women compared to men? (4 points)

- a. Conduct a random digit dialing telephone survey to assess the prevalence of L-tryptophan use in men and women;
- b. Conduct a focus group that includes both women and men who use L-tryptophan;
- c. Request marketing and advertising data from US companies that sell L-tryptophan;
- d. A and D

15. Which of the following groups would **NOT** be appropriate to serve as controls in this case-control study? (4 points)

- a. L-tryptophan users identified through the random digit dialing telephone survey;
- b. L-tryptophan users who are asymptomatic and called the Minnesota Department of Health after the public warning was issued;
- c. People who purchased L-tryptophan from a specific department store chain (identified through credit card records);
- d. All of the above groups are NOT appropriate as controls.

16. What was the EMS attack rate among female LT users in Minneapolis-St. Paul during 1988? *Show your work.* (6 points)

- a. 54 cases per 100,000 female L-tryptophan users ( $4 \div 7400 = .00054 \times 100,000 = 54 \text{ per } 100,000$ )
- b. 595 cases per 100,000 female L-tryptophan users
- c. Cannot be calculated because the investigation did not begin until 1989
- d. 4 cases per 100,000 female L-tryptophan users

17. What was the EMS attack rate among female LT users in Minneapolis-St. Paul during 1989? *Show your work.* (6 points)

- a. 54 cases per 100,000 female L-tryptophan users
- b. 268 cases per 100,000 female L-tryptophan users ( $44 \div 16,400 = .00268 \times 100,000 = 268 \text{ per } 100,000$ )
- c. 44 cases per 100,000 female L-tryptophan users
- d. 5 cases per 100,000 female L-tryptophan users

18. True or false: The attack rate is the cumulative incidence of disease in a specified time period. (2 points)

- True
- False

19. What was the rate ratio for EMS occurrence in female L-tryptophan users in 1989 relative to 1988? *Show your work.* (6 points)

- a. 3.9
- b. 0.2
- c. 5.0 ( $268 \div 54 = \sim 5.0$ )
- d. 11.0

20. Do these results suggest that the EMS epidemic of 1989 was entirely due to the dramatic increase in the number of L-tryptophan users that year? (2 points)

Yes

No

21. Which of the following are potential limitation(s) of using these asymptomatic L-tryptophan users as one control group? (4 points)

a. There is a potential for bias;

b. They may not be representative of all L-tryptophan users;

c. They may have undiagnosed EMS;

d. Both A and B

e. None of the above

22. What is the odds ratio for consumption of Showa Denko L-tryptophan in case patients compared to the combined control group? Do not include cases or controls with 'unknown' L-tryptophan manufacturer. (4 points)

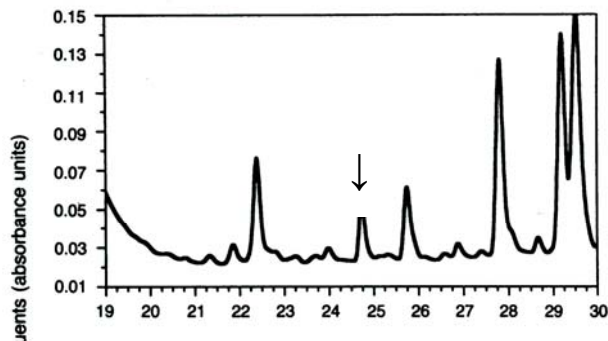
a. 18.1

b. 19.3 ( $[29 \times 14] \div 21 = 19.3$ )

c. 23.2

d. 7.7

23. In the HPLC graphs, each impurity has a unique retention time, represented by a peak. Identify peak E in the HPLC plot of LT associated with EMS cases (graph below). Draw an arrow to point to peak E. (2 points)



**24.** Based on this information, can you conclude that peak E causes EMS? (2 points)

Yes

No

**25.** Based on this information, which of the following are plausible explanation(s) for the fact that 1 patient with EMS consumed L-tryptophan that was traced to another manufacturer? (4 points)

a. There was an error in the product traceback;

b. The other manufacturer purchased L-tryptophan powder from Showa Denko and then sold it as their own product;

c. EMS was triggered by something other than L-tryptophan in this patient;

d. The L-tryptophan bottle that was provided by this patient was not the one that triggered her illness;

e. Both A and B

**26.** Which of the following factors are important to consider when making a determination if particular lots of Showa Denko L-tryptophan caused EMS. (4 points)

a. Strength of association between Showa Denko LT and EMS;

b. Consistency of findings in other populations and studies;

c. Ingestion of Showa Denko L-tryptophan occurred before EMS symptoms developed;

d. People who developed EMS consumed higher doses of Showa Denko LT compared to those who remained well (dose-response effect).

e. All of the above

**27.** Which of the following is NOT one of Yogi Berra's famous quotes? (NOT SCORED)

a. "If you don't know where you're going, you'll end up someplace else."

b. "You can observe a lot by watching."

c. "The future ain't what it used to be."

d. "Ninety percent of this game is mental. And the other half is physical."

e. Yogi said all of the above.