Case Study Which Investigates the Role Environmental Toxins Play in Flu Infection

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II. Student Learning Objectives

Students will:

- Be able to distinguish between main types of leukocytes & the role each plays during infection.
- Complete cell differential counts & analyze the data.
- Understand the basic idea & process behind flow cytometry.
- Understand the role environmental toxins play in flu infection.
- Research historical and present-day examples of dioxin pollution in the environment and the resulting impact on humans.
- Perform a Public Service Announcement to present their findings to the rest of the class.

III. State Standards

The following New York State Curriculum Standards are addressed in this unit:

PERFORMANCE INDICATOR 1.2 Hone ideas through reasoning, library research, and discussion with others.

PERFORMANCE INDICATOR 5.2 Explain disease as a failure of homeostasis.

STANDARD 5.2a Homeostasis in an organism is constantly threatened. Failure to respond effectively can result in disease or death.
STANDARD 5.2c The immune system protects against antigens associated with pathogenic organisms or foreign substances and some cancer cells.
STANDARD 5.2d Some white blood cells engulf invaders. Others produce antibodies that attack them or mark them for killing. Some specialized white blood cells will remain, able to fight off subsequent invaders of the same kind.
STANDARD 5.2h Disease may also be caused by inheritance, toxic substances, poor nutrition, organ malfunction, and some personal behavior. Some effects show up right away; others may not show up for many years.
STANDARD 5.2j Biological research generates knowledge used to design ways of diagnosing, preventing, treating, controlling, or curing diseases of plants and animals.

IV. Time Requirements

Total time for the unit is approximately 5 class periods that are each 45 minutes in length, not including time students would need outside of class to complete the Public Service Announcement (PSA). Reviewing the Patient Profiles and completing the Candy Cell Differential Counts would take 45 minutes. Completing the flow cytometry website walk-through and analyzing actual data would take an additional 45 minutes. A facilitated brainstorm and brief lecture about the impact that environmental dioxin pollutants have on the immune system would take 45 minutes. Student research about historical and present-day dioxin pollutants in the environment and the subsequent human impact would take 45 minutes. Student presentations of their PSA would take approximately 45 minutes, depending on the number of total students in the class and the number of groups participating.

V. Materials and Equipment

- Manipulative with 3 different variants
  (e.g. candy in 3 different colors; 3 bags of jellybeans would suffice for a class of 20 students)
- Internet access

VI. Science Background

TCDD became well known as a contaminant of Agent Orange, an herbicide used in the Vietnam War; and later, dioxins were found in the Love Canal, New York. Dioxins are produced in small concentrations when organic material is burned in the presence of chlorine, whether the chlorine is present as chloride ions or as organochlorine compounds, so they are widely produced in many contexts. According to the most recent US EPA data, the major sources of dioxins are:

* Coal fired utilities
* Municipal and medical waste incinerators
* Metal smelting
* Diesel trucks
* Land application of sewage sludge
* Burning treated wood
* Trash burn barrels

*These sources together account for nearly 80% of dioxin emissions

Exposure to high levels of dioxins in humans causes a severe form of persistent acne, known as *chloracne*. High levels of exposures to dioxins have been shown by epidemiological studies to lead to an increased risk of tumors at all sites. Other effects in humans may include: developmental abnormalities in the enamel of children's teeth, central and peripheral nervous system pathology, thyroid disorders, damage to the immune systems, endometriosis, and diabetes.

Most importantly for our purposes, exposure to TCDD enhances inflammatory responses, and is consistently characterized by an increase in the number of neutrophils at the site of antigen challenge.

Exposure to TCDD alters the immunoregulatory balance in the lung so that upon infection, adaptive response are suppressed and inflammatory responses are enhanced.

Which leukocytes are the main players in this saga?

Neutrophils aid in the resolution of infection, yet excessive neutrophil recruitment generally has adverse effects. This is affectionately and informally referred to as the *Goldilocks Principle*, where “too much of a good thing is a bad thing.” The immune system needs the number of neutrophils to be “just right.”

Neutrophils are made by hematopoietic cells in the bone marrow and are then recruited in “waves” to the lung during the innate immune response to flu infection in order to fight the infection. When the 1st wave of neutrophils reaches the lungs, they release their cytotoxic molecules and then turn on a cell-death pathway and begin apoptosis because their job is done. As soon as the 1st wave begins apoptosis, the 2nd wave of neutrophils is recruited from the bone marrow and begins to arrive in the lungs.

Cytospin slides consist of actual stained cells from the tissue of an organism. (In the case of flu infection, the cells come from the lungs, which would not be possible to obtain when working with

Pulse

(adapted from www.webmd.com)

Results

Your pulse is the rate at which your heart beats. Your pulse is usually called your heart rate, which is the number of times your heart beats each minute (bpm).

Normal resting heart rate

The chart below shows the normal range of a resting heart rate (pulse rate after resting 10 minutes) in beats per minute, according to age. Many things can cause changes in your normal heart rate, including your age, activity level, and the time of day.

Resting heart rate

Age or fitness level	Beats per minute (bpm)
Babies to age 1:	100–160
Children ages 1 to 10:	

Blood Pressure

(adapted from www.webmd.com)

What Is "Normal" Blood Pressure?

There are several categories of blood pressure, including:

1. **Normal:** Less than 120/80
2. **Prehypertension:** 120-139/80-89
3. **Stage 1 high blood pressure:** 140-159/90-99
4. **Stage 2 high blood pressure:** 160 and above/100 and above

What Causes High Blood Pressure?

The exact causes of high blood pressure are not known. Several factors and conditions may play a role in its development, including:

- Smoking
- Being overweight or obese
- Lack of physical activity
- Too much salt in the diet
- Too much alcohol consumption (more than 1 to 2 drinks per day)

1.

Chédiak-Higashi Syndrome

(adapted from www.emedicine.medscape.com)

Background

Chédiak-Higashi syndrome (CHS) was described by Beguez Cesar in 1943, Steinbrinck in 1948, Chédiak in 1952, and Higashi in 1954. Chédiak-Higashi syndrome is a rare childhood autosomal recessive disorder that affects multiple systems of the body. Patients with CHS exhibit hypopigmentation of the skin, eyes, and hair; prolonged bleeding times; easy bruisability; recurrent infections; abnormal natural killer cell function; and peripheral neuropathy. Morbidity results from patients succumbing to frequent bacterial infections or to an accelerated-phase lymphoproliferation into the major organs of the body. Most patients who do not undergo bone marrow transplantation die of a lymphoproliferative syndrome, although some patients with CHS have a relatively milder clinical course of the disease.

Pathophysiology

CHS is an autosomal recessive immunodeficiency disorder characterized by abnormal intracellular protein transport. The CHS gene was characterized in 1996 as the *LY*

X. Candy Cell Differential Count Directions

1) Use candy! (M&Ms, Skittles, Runts with different shapes, jellybeans- the mottled ones can look like neutrophils)

2) Lymphocytes should be the darkest color. (Representative of the fact that they have almost no cytoplasm visible.)

3) Lay the candy in a small snack-size ziploc snack bag so that the candy covers the bottom of the bag completely (each bag can represent a “microscope slide”)

*Relative # of pieces of candy for each type

	Patient A	Patient B

XII. Flu Infection & Environment Research Directions

1) Facilitated student brainstorm: Why would one person get sicker with the flu than others?
**Hopefully someone comes up with the environment; if not, may need to lead them in that direction.*

2) Tie in the mouse as a research model. Although we can't get lung

XIV. References

Campbell, Neil A. and Jane B. Reece (2008). *Biology AP Edition*. 8th Ed. Pearson.

Gibbs, Lois M. (1995) *Dying From Dioxin*. South End Press.

Institute of Medicine of the National Academies. (2003) *Dioxins and Dioxin-like Compounds in the Food Supply: Strategies to Decrease Exposure*. The National Academies Press.

Murphy, K.M., Travers, P., and M. Walport. (2007) *Janeway's Immunology*. 7th Ed.

Teske, S. *et al., Activation of the aryl hydrocarbon receptor increases pulmonary neutrophilia and diminishes host resistance to influenza A virus.* Am J Phusiol Lung cell Mol Physiol. 2005. **289:** p. 111-124.

http://www.unsolvedmysteries.oregonstate.edu/flow_01

www.toxicology.org

www.niehs.nih.gov

www.cdc.gov

www.mayoclinic.com

www.webmd.com

www.emedicine.medscape.com

III. Patient Profiles

Patient A

Patient History:
Mitchell D. is a 25 year old African-American male. He works full time for a marketing firm in Birmingham, Alabama. He exercises at the gym 2-3 times per week, and plays in a slow-pitch softball league during the summer. He has been a smoker since age 15, and currently smokes approximately a pack of cigarettes per day. He had his gall bladder removed in 2005.

Current Vitals:

Body Temperature: 104^0 F

Blood Pressure: 80/60

O_2 Saturation: 86%

Pulse: 106 beats per minute

Symptoms:
Mitchell presents with a sore throat, headache, *myalgia* (non-specific muscle aches in his arms, legs, and back), a nonproductive cough, and *rhinorrhea*. Additionally, he has a feeling of pressure behind his eyes and sensitivity to light, as well as a burning sensation in his chest. He has not had much of an appetite, but when he has eaten it resulted in indigestion.

Patient B

Patient History:
Paige L. is a 27 year old Caucasian female. She is a high school Spanish teacher in Austin, Texas. She runs regularly, having completed two marathons in the past three years. She has adhered to a vegan diet for the past five years. Her only regular medication is an oral contraceptive. Paige traveled to Argentina 2 months ago.

Current Vitals:

Body Temperature: 100^0 F

Blood Pressure: 118/76

O_2 Saturation: 98%

Patient C

Patient History:
Jen H. is an 18 year old female of Latino descent. She is a Biology major at the University of Rochester. She rides her bike to school every day, weather-permitting. She has Chediak-Higashi syndrome. She currently takes *Colchicine* as an anti-inflammatory agent to decrease leukocyte motility and phagocytosis in inflammatory responses that result from her syndrome.

Current Vitals:

Body Temperature: 102^0 F

Blood Pressure: 125/82

O_2 Saturation: 92%

Pulse: 95 beats per minute

Symptoms

IV. Candy Cell Differential Counts

Objective:
Students will learn how to perform a differential count and use the results to help diagnose patients' illnesses.

Background:
A nasal wash was performed on Patients A, B and C. Cells from each patient's nasal wash were put on a microscope slide and sent to the lab for analysis. Cells were stained with a dye called *hematoxylin and eosins*. Note: Each group in class will have a different "slide" that is representative of one field-of-view from that particular patient's blood smear slide.

White blood cells (leukocytes) can be divided into *granulocytes* and *agranulocytes*, and then further divided into 5 subgroups. For our purposes, we will focus on the types of leukocytes below:

Macrophages
Visible cytoplasm; highly variable shapes.

"Macrophage" Color: _____

Lymphocytes
Small, round cells. Almost no cytoplasm visible.

"Lymphocyte" Color: _____

Neutrophils
Polymorphonuclear (Nucleus has crazy, multilobular shape.)

"Neutrophil" Color: _____

Procedure:

- Obtain the "slides" for Patients A, B, & C from your instructor.
- Fill in the corresponding color for each "leukocyte" on the proper line above.
- Count the number of each "leukocyte" type for Patients A, B, & C, and record your results below in Table 1.
- Use your data from Table 1 in order to calculate the percentage of each type of leukocyte for Patients A, B, & C in Table 2.
- Compile data from Table 2 for the entire class and record the results in Table 3.

Table 1: Number of Leukocytes

	Patient A	**Patient B**	**Patient C**
# Macrophages			
# Neutrophils			

Lawrence Lab Data (Actual Cytospin picture for "Patient B**")**

Lawrence Lab Data (Actual Cytospin picture for "Patient C**")**

VI. A Guided Tour of Dioxins in the Environment and an Overview of Flow Cytometry

Adapted from Oregon State University's
Unsolved Mysteries of Human Health: How Scientists Study Toxic Chemicals and Health

Dioxins & Flow Cytometry

Directions:
-Go to the site: http://www.unsolvedmysteries.oregonstate.edu/flow_01
(Courtesy of Nancy Kerkvliet & the Environmental Health Sciences Center at Oregon State University)
-Begin on the page entitled *Going With the Flow***.** Read through each pg)4(a,23nt)-MC /P <</MCID 0 >>BDC 1

11. Are there still major sources of dioxins in the United States? Why or why not?

12. What are examples of activities that continue to contribute to the release of low levels of dioxins into the environment?

How would I most likely be exposed to dioxin?

- How would YOU most likely be exposed to dioxin?

The Immune System and Foreign Invaders

10) What are the main functions of red blood cell (*erythrocytes*), platelets, and white blood cells (*leukocytes*)?

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What can you use to study damage to the immune system?

15) What is *flow cytometry*?

16) What do scientists "measure" about each type of immune system cell?

17) What advantage does using a flow cytometer have over counting cells on a microscope?

But what exactly **does the flow cytometer do?**

18) Summarize the three components of what the flow cytometer can do.

In the Lab

As you learned, one of the jobs of the immune system is to destroy any mutated cells which can develop into tumor cells. To understand how dioxin affects this part of the immune system, mice were

Sample Preparation

21) What is the purpose of the *fluorescent markers?* (Note: Each marker is a specific antibody to a protein that you want to measure that has a fluorochrome attached to it)

22) What is the purpose of the *fluorochromes***?**

23) For this experiment, you will use two types of fluorochromes to bind to two different proteins on the CTLs in order to differentiate them from other cells. One fluorochrome will emit green light and the other yellow light. We will call the two different proteins to which these fluorochromes attach Protein Y and Protein G (for the yellow and green fluorochromes).

**According to this information, how will you distinguish the CTLs?*

Flow Cytometry- How Does It Work?

24) The tissue sample is broken up into single cells and held in a test tube, which is placed into the flow cytometer. The liquid containing the cells is drawn up from the test tube and pumped into the flow chamber. Summarize the following 6 steps below (*move your mouse over the components to learn more*!):

1. Flow Chamber

2. Laser

3. Light Detector

4. Filters

5. Color Detectors

6. Computer

Data Analysis: What does a histogram tell me?

25) What does the *x-axis* represent, and what does this mean?

26) What does the *y-axis* represent, and what does this mean?

27) What will be shown in *each* of the 4 quadrants?

Quadrant 1

Quadrant 2

Quadrant 3

Quadrant 4

28) Draw and label a picture of the *x-axis*, *y-axis*, and *Quadrants 1 – 4*

29) Using the information above, where will the CTLs appear on the histogram?

Data from the Dioxin Experiment

30) What does the histogram of the *nonimmune mouse* tell us?

31) What does the histogram of the *vehicle control mouse* tell us?

32) What does the histogram of the *dioxin treatment mouse* tell us?

Solve It!

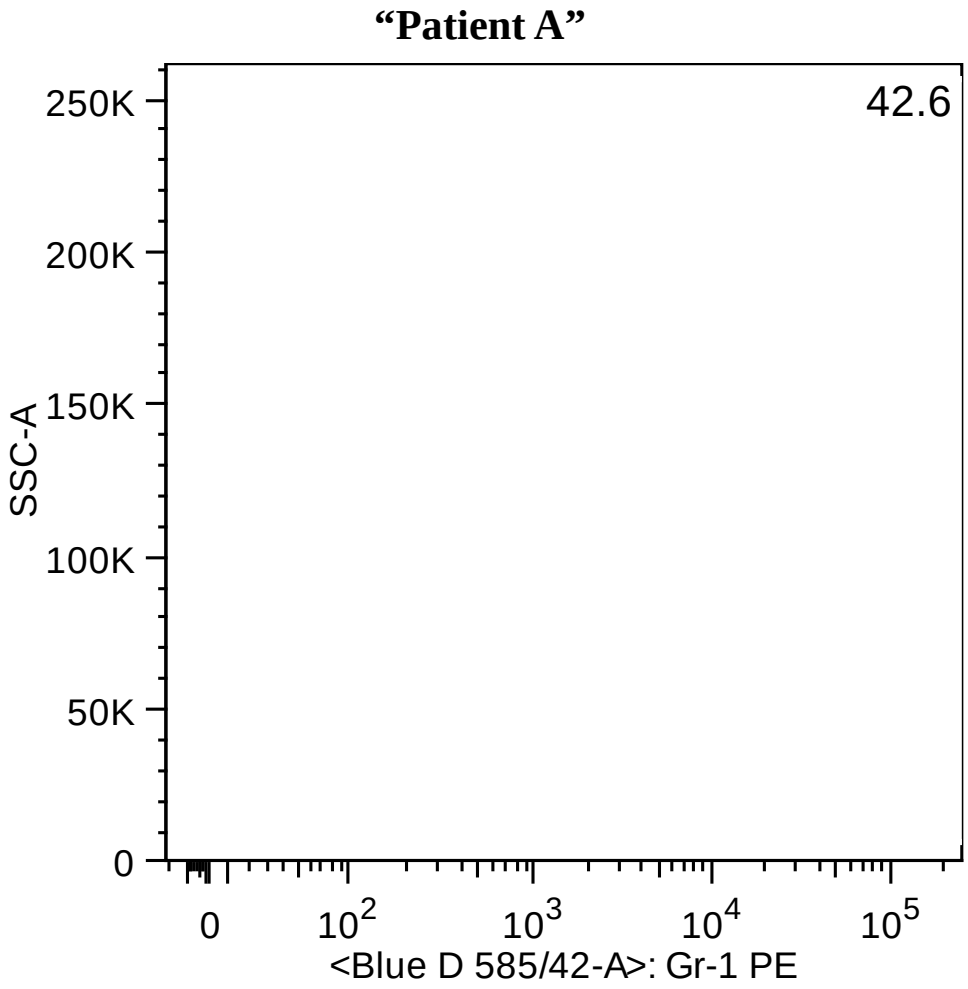
33) Why did the mouse that was exposed to dioxin have low CTL counts? (*Click on the correct answer choice & write the answer here.*)

Why is this research important to me?

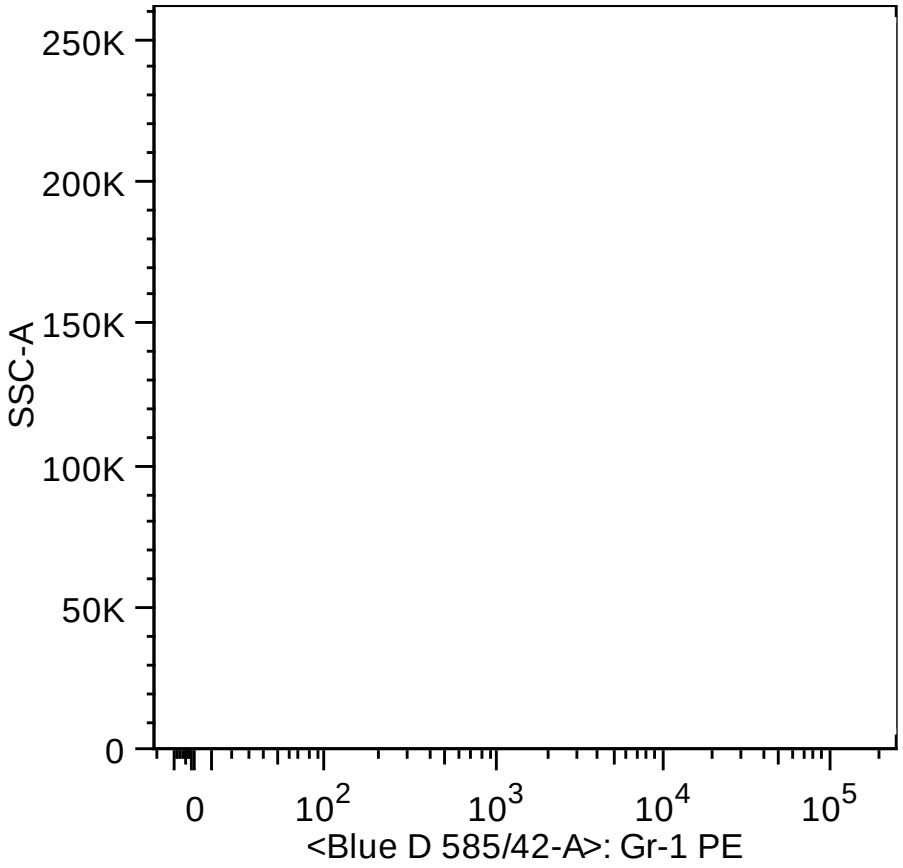
34) Using the yellow box on the page, explain why animal models are important in toxicology research.

35) What are some limitations of animal models?

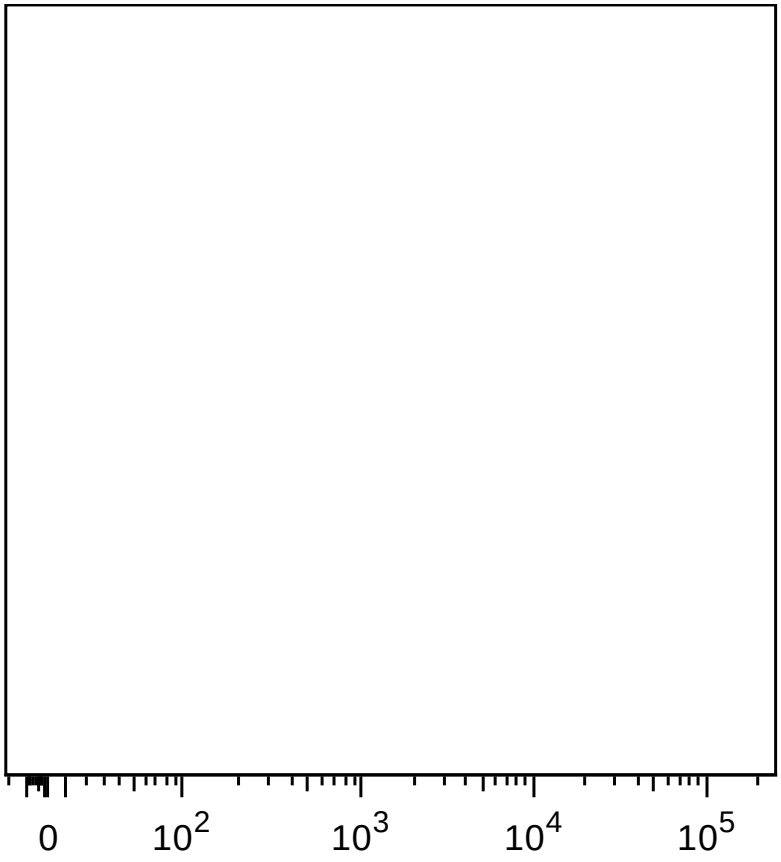
VII. Lawrence Lab Flow Cytometry Data



“Patient B”


250K
200K
150K
100K
50K
0
SSC-A
0
10^2
10^3
10^4
10^5
<Blue D 585/42-A>: Gr-1 PE

“Patient C”



VIII. Websites for Dioxin Research

World Health Organization (WHO)

http://www.who.int/en/

-Search “dioxins & their effects on human health”

U.S. Food & Drug Administration

http://www.fda.gov/

-Search “Questions & Answers about Dioxins”

United States Environmental Protection Agency

http://www.epa.gov/

-Search “Dioxin Exposure Initiative”

-Search “Electronic Waste and eCycling”

-Search “PCBs”

-Search “Love Canal”

-Search “Times Beach”

United States Department of Veteran’s Affairs

http://www.publichealth.va.gov

-Search “Agent Orange”

IX. Culminating Task: Public Service Announcement

Task

You and your group members must sketch and complete a 60 second Public Service Announcement (PSA) which will summarize the following key points:

I. Explanation of how pollutants in the environment affect the immune system's ability to fight flu infection.

-Focus on the innate immune system

II. One *historical* example of dioxin pollution in the environment

-What caused this type of dioxin pollution?

-What was the resulting effect on people and the environment?

III. One *current* example of dioxin pollution in the environment

-What is the resulting effect on people and the environment?

-What can your audience do to prevent this pollution from happening?

Tips for developing a PSA

*These guidelines are drawn from the Ad Council PSA kit.

Make sure your campaign idea deals with a significant public problem for which a solution can be offered. Do not develop a campaign which arouses public concern but offers no solution.

Media messages are fleeting. One compelling central message, clearly presented with a simple call for action, is the most effective.

Make sure your PSA can answer the following questions:

Target Audience:

- Who is the intended audience?
- Are there any barriers to understanding the message? (Adjust your level of "science speak" to fit your target audience).

Message:

- What is the proposed message?
- What do I want the person who is watching this to understand? (Look to the *Task* above).

Action Step:

- What is the call to action? What do I want the person to do?
- What can an individual do in the home or community?
- How will the action solve the problem?

Significance of Issue to the Public:

- What is the proposed issue? Describe the problem and why it is important to the public.
- Are there any statistics involved which might be useful?