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Alpine Fizz and Male Infertility: A Mock Trial

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Contents

Lesson Plan

TITLE: Alpine Fizz and Male Infertility: A Mock Trial

SUBJECT AREA: Science (biology or chemistry), social studies, health education

OBJECTIVES: At the end of this module, the students should be able to:

- Be critical in their assessment of claims regarding the risks and benefits of health-related products and interventions
- Understand what criteria are used when judging whether an observed association is a causal one
- Finish the sentence: "We know something causes something else when . . ."

TIME FRAME: Three to five 45-minute lessons

PREREQUISITE KNOWLEDGE: None, but it may be helpful to have completed some of the other modules on cohort studies, case-control studies and randomized controlled trials.

MATERIALS NEEDED: Appendices (included at the end of this module)

LINKS TO EDUCATION STANDARDS:

National Science Education:

Unifying Concepts and Processes:

- Evidence, models and explanation
- Change, constancy and measurement

Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Science in Personal and Social Perspectives

- Personal and community health
- Natural and human-induced hazards

History and Nature of Science

- Science as a human endeavor
- Nature of scientific knowledge
- Historical perspectives

National Health Education Standards:

- Students will demonstrate the ability to access valid health information and health-promoting products and services.
- Students will analyze the influence of culture, media, technology and other factors on health.

National Social Studies Standards: To be determined.

Related Internet Sites

Active.com Web site. Available at: http://www.active.com/mtnbiking

Ardmoreite.com Web site. Available at: http://www.ardmoreite.com/stories/032600/liv_abby.shtml

DrWeil.com Web site. Available at: http://www.drweil.com/app/cda/drw_cda.html-command=TodayQA-questionId=3709

Rate It All Web site. Available at: http://www.rateitall.com/item.asp?i=94F1F927-827A-4051-B0D6-20D5E4FC709B

What You Need to Know About Web site. Available at: http://urbanlegends.about.com/library/weekly/aa102799.htm

Background for Teachers

Rationale

This module consists of a mock trial resulting from a fictional class action suit brought by 60 infertile couples in whom the male was a heavy drinker of Alpine Fizz soft drink. The plaintiffs claim that the dye in Alpine Fizz (called Yellow Dye #5, or tartrazine) causes low sperm count, resulting in male infertility. Appendix 1 presents a brief summary of tartrazine's chemical properties and Appendix 2, a brief discussion of male infertility.

In this module, the students will be given a roster of witnesses, with a description of the testimony that each witness is prepared to give. The examination and cross-examination of the witnesses are expected to elicit, from the students, arguments for and against the possibility of a causal association between Alpine Fizz and infertility.

Students will be assigned to one or more roles in the mock trial. It is expected that the teacher will tailor this module according to the size of the class and the time available.

Background on Causality

When a factor is observed to be associated with a disease or other health-related outcome, the challenge is to determine whether the factor is a cause of the disease. An observed association may be due to chance, it may be due to confounding by another factor or it may have arisen because of flaws in study design or execution. Only after these possible explanations have been considered and excluded is it possible to infer that the association may be causal.

For example, it has been found that men who shave less frequently than daily have a higher rate of stroke than men who shave daily. What is not known, however, is whether this is a causal association or whether it is due to confounding. Perhaps the men who shave less frequently have other characteristics (e.g., obesity, smoking) that are related to stroke risk, and it is the overrepresentation of these characteristics among the men who shave less frequently that makes them appear to have a higher stroke risk.

Another challenge when considering the possibility of a causal association is the issue of temporality. In order for a factor to be a cause of a particular health outcome, it must have preceded the onset of that health outcome. Many times, however, the onset of a health outcome is difficult to pinpoint, and the putative risk factor may actually have occurred after the disease began. Other times, information from cross-sectional studies is inappropriately used to infer causality. For example, one may find that persons with chronic lung disease are less likely to be smokers

than persons without the disease. Does this mean that smoking protects from chronic lung disease? No, it may simply reflect the fact that persons who develop chronic lung disease are more likely to quit smoking after their disease is diagnosed. To provide evidence of causality in such a case, one would have to look at whether smoking preceded the onset of disease. Only cohort studies and case-control studies that appropriately account for the time order of the exposure and the disease can definitively address this issue.

In 1964 the U.S. Surgeon General released a report entitled *Smoking and Health*, in which it was concluded that "smoking is causally related to lung cancer." This was a landmark event, marking the first time that the concept of causality was applied to a major public health problem. The criteria used in the report were similar to those proposed by Sir Austin Bradford Hill in 1965. Although Bradford Hill's criteria are the best known, many authors have adapted his list or created their own. One version of the criteria is shown in Appendix 3. Note that these criteria were not developed to be used as a checklist for evaluating whether a particular association could be interpreted as causal. Rather, they were meant to help by making explicit the considerations that are important when inferring causality. For each criterion (except temporality), it is possible to find at least one example of a causal association that does not meet the criterion.

Procedure

1. Assign each student to one or more roles in the mock trial, as shown in Table 1. (Because this is a class action suit, students do not need to play the roles of the plaintiffs, only the plaintiffs' legal team.)

Table 1. Roles to Be Assigned in the Mock Trial

Role	**Comments**
Judge	May be the teacher or one of the students Decides whether objections are relevant and whether evidence is admissible, gives instructions to the jury
Defendants	Representatives of King Kola, manufacturer of Alpine Fizz
Defense legal team	At least two students (may be more, depending on class size)
Plaintiffs	Representatives of the 60 men who claim to suffer from infertility related to drinking Alpine Fizz
Plaintiffs' legal team	At least two students (may be more, depending on class size)
Jury	Variable number of students, depending on class size
Court reporter	Takes notes on proceedings
Witnesses	Teacher assigns students to portray witnesses. A student may portray more than one witness, depending on class size.

2. In the pretrial period, the students are given the roster of witnesses with the description of the testimony that each witness is prepared to give. The roster and descriptions are summarized in the table in Appendix 4, which should be distributed to all students.

3. The teacher distributes the criteria for causation shown in Appendix 3 and reviews them with the students.

4. The legal teams peruse the roster of witnesses and decide which witnesses they will call. This process should be done with the participation of all the students because it represents one of the most important learning activities of this module. In making the decision about whether to call a witness, the students should discuss whether the witness's evidence will support the plaintiffs or the defendants. Not all potential witnesses have to be chosen. The teacher may set a limit on the number of witnesses each legal team calls.

5. Once the students have decided which witnesses will be called by each legal team, they should plan the cross-examination of each of the witnesses by the other side. Throughout the process of selecting witnesses, preparing testimony and preparing cross-examination, the teacher should verify that the students understand why the evidence is supportive of one side or the other and what principles are demonstrated (see Table 2), using the criteria for causation as a guide.

6. The teacher may assign one or more students to be the court reporter. Alternatively, the teacher may ask the students who assume the roles of witnesses to write a summary of the testimony they would give if called as a witness, and may ask the students who are members of the legal teams to write a summary of their examination and cross-examination.

Procedures continue on page 17.

Table 2. Witness Roster (Teacher's Version)*

	Witness for Which Side?	Cross-Examination Arguments	Principles Demonstrated
1. Plaintiffs Rumors have been circulating for several years relating to the dye called FD&C Yellow No. 5, scientifically known as Tartrazine but commonly called Yellow Dye #5 in Alpine Fizz to low sperm counts in men who consume the chartreuse-colored drink. Several chat rooms post messages that put forward the belief that young men's fertility, sexual attractiveness or sexual potency could be adversely affected by drinking Alpine Fizz. For the purposes of this exercise, let us imagine that a group of heavy Alpine Fizz drinkers met in one of these chat rooms and discovered that they were all having trouble with fertility. All were married, all had been trying unsuccessfully to get their wives pregnant for more than one year, and all drank at least three cans of Alpine Fizz every day. They launched a Web site and urged people with the same problem to communicate with them. After about a year they had assembled about 60 couples with infertility problems that they attributed to the men's drinking of Alpine Fizz. They hired an impressive legal team and filed a class action suit.	Plaintiffs	The fact that some men drink Alpine Fizz and become infertile does not necessarily mean that there is a causal association. Millions of people drink Alpine Fizz and millions of people are infertile, and it is to be expected that some people who drink Alpine Fizz will also be infertile. Even if it is not a coincidence, the association may be the result of confounding by a third factor (e.g., engaging in extreme sports, use of recreational drugs). For example, men who use recreational drugs (some of which can, it is believed, lower sperm counts) may also be more likely to drink Alpine Fizz, creating an apparent (but not causal) association between Alpine Fizz and low sperm counts.	• Chance • Confounding
2. Defendant The manufacturer of Alpine Fizz, King Kola, claims that there is absolutely no scientific	Defendant	There are at least 60 men who became infertile after drinking large quantities of Alpine Fizz, and there	• Anecdote • No comparison group

continue

	Witness for Which Side?	Cross-Examination Arguments	Principles Demonstrated
evidence that tartrazine, caffeine, or any other substance found in Alpine Fizz is related to infertility. All of the soft drink's ingredients are approved by the Food and Drug Administration (FDA), the federal agency that regulates such matters. The manufacturer claims that the fact that some men drink Alpine Fizz and are infertile is simply coincidence and that there is no causal association between the two.		are many more who have visited the Web site. In some cases men who stopped drinking Alpine Fizz almost immediately regained their fertility.	
3. Harry G. Walker Mr. Walker is an 80-year-old man who drank Alpine Fizz all his life and continues to consume at least two bottles a day. He has fathered 12 children and is prepared to testify that his soft drink consumption has had no effect on his fertility.	Defendant	The fact that one man has consumed large quantities of the drink and has maintained a high level of fertility does not negate the possibility that Alpine Fizz causes infertility in most men. Because Mr. Walker is 80 years old and Alpine Fizz did not become widely available until the 1960s, most or all of his Alpine Fizz consumption must have occurred after his children were born.	• Anecdote • Exceptions do not invalidate the rule • Temporality
4. Mary Stewart-Swanson Ms. Stewart-Swanson owns Mary's Deli in Springfield, Connecticut. After a few months of drinking large quantities of Alpine Fizz (more than four cans per day) she began having numerous symptoms, including rash, diarrhea and shortness of breath. She is prepared to testify that after she stopped drinking Alpine Fizz, all her symptoms disappeared.	Plaintiffs	The fact that one woman has consumed large quantities of the drink and has experienced symptoms such as these does not prove that the drink was the cause of the symptoms. These symptoms are quite common, and it may be coincidence that the symptoms appeared after the woman starting drinking large amounts of Alpine Fizz and disappeared after she	• Anecdote • Analogy • Chance

continue

		stopped drinking Alpine Fizz. In any case it is known that tartrazine can cause allergic reactions in some people. This fact is not relevant to the question of whether tartrazine causes male infertility, as the biological mechanism leading to allergy is quite different from that leading to infertility.	
5. Gregory Marchildon Mr. Marchildon is a Madison, Wisconsin, man who claims he is sure that Alpine Fizz lowers sperm count. He is prepared to testify that he always drinks Alpine Fizz before intercourse and none of his sexual partners has ever become pregnant.	Plaintiffs	The fact that one man regularly consumes the drink before intercourse and has never impregnated anyone does not prove that Alpine Fizz causes infertility.	• Anecdote • Exceptions do not invalidate the rule
6. Michelle Cabano Ms. Cabano is an executive at Bornstein, Burrard, Benson and Cabano, a well-known New York City advertising agency. She developed the advertising campaign linking Alpine Fizz to extreme sports. Some of these sports (e.g., dirt biking, motocross, mountain biking) are reputed to cause low sperm count in men. She is prepared to testify that it may be that men who engage in these sports are more likely than others to drink Alpine Fizz and that it is the sports activity, not the Alpine Fizz, that is the cause of the infertility.	Defendant	The link between extreme sports and infertility is tenuous and based on little scientific evidence.	• Confounding
7. Laszlo Nagy Mr. Nagy is the Chief Executive Officer of ABC Chemicals, a New Jersey company that manufactures dyes (including Yellow Dye #5), artificial flavorings and other chemicals for the food	Defendant	The fact that there has never been a report of Alpine Fizz–related infertility does not mean that such a condition does not exist.	• Absence of evidence does not mean absence of association

continue

	Witness for Which Side?	Cross-Examination Arguments	Principles Demonstrated
industry. He is prepared to testify that more than two million pounds of tartrazine are used every year in medicines, beverages, desserts, processed vegetables and cosmetics. Because a 1986 FDA advisory committee concluded that Yellow Dye #5 can cause allergic skin reactions (such as hives and rashes), manufacturers who use tartrazine must list it on their labels. However, Mr. Nagy will testify that there has never been any evidence that the dye is related to male infertility.			
8. Patrick L. Rodriguez Mr. Rodriguez is a representative of the American Caffeine Foundation. He is prepared to testify that although Alpine Fizz contains very high levels of caffeine (higher than either Cola Rite or King Kola), there is evidence to suggest that caffeine actually increases the motility and effectiveness of sperm cells.	Defendant	This testimony is irrelevant because the plaintiffs believe it is the dye, not the caffeine, that is the culprit.	• Irrelevant information
9. Janet S. Fang Ms. Fang is Vice President for Public Affairs for the Soft Drink Manufacturers Association of America. She is prepared to testify that Alpine Fizz has been consumed by millions of people since the drink was first introduced in the 1950s and yet King Kola has never received a consumer inquiry or complaint about the effects of Alpine Fizz on male fertility or potency. Her testimony will be based on the premise that if there were such effects they would have become apparent by now.	Defendant	The fact that there has never been a report of Alpine Fizz–related infertility does not mean that such a condition does not exist.	• Absence of evidence does not mean absence of association

continue

10. Vanessa Johnson, Ph.D. Dr. Johnson is an organic chemist who specializes in dyes. She will testify that the chemical structure of tartrazine (commonly known as Yellow Dye #5) is similar to that of other dyes, some of which are known to cause infertility or carcinogenesis. She will also discuss some of the other health effects (e.g., allergy) of Yellow Dye #5.	Plaintiffs	The fact that other dyes cause infertility does not mean Yellow Dye #5 does. Even though Yellow Dye #5 may cause other conditions, it does not mean it also causes infertility.	• Analogy
11. Pragati Desai, Ph.D. Dr. Desai is a toxicologist who studies the carcinogenic effects of artificial dyes on animals. She has also studied the effects that other chemicals have on infertility.	Plaintiffs	The fact that some dyes have been found to cause cancer or infertility in animals does not prove that Yellow Dye #5 causes infertility in men.	• Analogy • Difficulty in extrapolating results of animal studies to humans
12. Roger Davis, M.D., Ph.D. Dr. Davis is a physician who is an expert on fertility. He is prepared to testify that he has never encountered a case of Alpine Fizz–related infertility in his practice.	Defendant	The fact that Dr. Davis has never observed a relation between Alpine Fizz and infertility in his practice does not mean that such an association does not exist. He may simply never have asked his patients about Alpine Fizz consumption.	• Absence of evidence does not mean absence of association
13. Greg Markowicz, DrPH Dr. Markowicz is an epidemiologist who has studied men who donate sperm at a sperm bank. In his study Dr. Markowicz asked sperm donors to complete a questionnaire, and one of the questions asked whether they drank Alpine Fizz. His findings indicate that men who drank one to two 20-oz. bottles of Alpine Fizz per day had a significantly lower sperm count than men who drank Alpine Fizz less frequently. Men who drank three or more bottles per day had an even lower sperm count.	Plaintiffs	The fact that those who drank a lot of Alpine Fizz had lower sperm count may be due to some other characteristic (a confounding variable) that is associated with both Alpine Fizz and sperm count. Also, there is no information about timing. It may be that men began drinking Alpine Fizz after they began to have lower sperm count. If so, Alpine Fizz cannot be causally related to low sperm count.	• Confounding • Temporality • Biological gradient

continue

	Witness for Which Side?	Cross-Examination Arguments	Principles Demonstrated
14. Gillian McDermott, Ph.D. Dr. McDermott is an epidemiologist who has done a study on patients at a fertility clinic. She found that the men in her study who drank one or more 12-oz. cans of Alpine Fizz per day had an average sperm count similar to that of men who drank Alpine Fizz less frequently.	Defendant	Because this study was done among patients at a fertility clinic, the men may have had a lower sperm count on average than the general population. Therefore, all the men in this study, regardless of their Alpine Fizz drinking habits, may have had low sperm count, which would make it harder to detect a difference between the Alpine Fizz drinkers and nondrinkers, even if such an association existed.	• Selection bias
15. Luz Rivera, Ph.D. Dr. Rivera is a clinical psychologist who will testify that in her experience, men who become depressed as a result of infertility may turn to stimulants (such as Alpine Fizz) to compensate for their feelings of depression.	Defendant	There is no scientific evidence to confirm her statements.	• Temporality
16. John Thompson Mr. Thompson is an activist who set up a Web site that collects the stories of men who drank Alpine Fizz and became infertile as a result.	Plaintiffs	The Web site asks only for men who drank Alpine Fizz and became infertile. It does not ask for men who drank Alpine Fizz and did not become infertile.	• Anecdote • Selection bias • No comparison group
17. Harry Humphrey Mr. Humphrey is an herbalist who believes that all synthetic chemicals, including Yellow Dye #5, are hazardous to health.	Plaintiffs	This is irrelevant.	• Irrelevant information • Personal opinion

continue

18. Franz Lichter, M.D. Dr. Lichter, a Swiss radiologist, has done a study comparing male mountain bikers with men who are not mountain bikers. He is prepared to testify that mountain bikers have a lower sperm count and less motile sperm than men who are not mountain bikers, and that mountain bikers are more likely to have abnormalities in their scrotum. He believes that the observed infertility among the plaintiffs may be due to their participation in extreme sports (which is more common among men who drink Alpine Fizz) rather than to the drink itself.	Defendant	Having low sperm count or low sperm motility does not make it impossible to conceive. Also, there is no evidence that Alpine Fizz drinkers are in fact more likely to engage in extreme sports.	• Confounding
19. Georgeanne Parker, Ph.D. Dr. Parker is an epidemiologist who has compiled statistics on average sperm counts among U.S. males during every year between 1971 and 2001. She also has statistics on the average annual per capita consumption of Alpine Fizz in the United States during the same period. Her statistics clearly demonstrate that sperm counts declined and Alpine Fizz consumption increased over the period in question.	Plaintiffs	Mean sperm count and mean per capita consumption of Alpine Fizz are aggregate statistics, based on an entire population. This type of data cannot be used to infer relationships on the individual level. (It is called **ecologic fallacy** when aggregate data are used to infer relationships on the individual level.) The fact that Alpine Fizz consumption increased in the U.S. population does not mean that it increased among men who were at risk of becoming infertile. Besides, there are many factors other than Alpine Fizz consumption that changed over the time period and that may be the cause of the lower	• Confounding • Temporality • Ecologic fallacy

continue

	Witness for Which Side?	Cross-Examination Arguments	Principles Demonstrated
		sperm counts. Also, it is impossible with these data to ascertain that Alpine Fizz consumption preceded lowering of the sperm count.	
20. Anatoly Pritovich, Ph.D. Dr. Pritovich is a reproductive biologist. He will testify that the sperm count is a measure of the total number of sperm present in a man's semen, usually recorded in millions of sperm per milliliter of semen. Sperm count varies widely among human males. A normal count is regarded as being greater than 20 million/ml. A man is considered at risk of being infertile if his sperm count is less than 20 million/ml of semen. However, some men with low sperm counts are fertile, whereas others with higher counts prove to be infertile. Dr. Pritovich will testify that a measure of sperm count is not a completely reliable method of assessing fertility.	Defendant	Although some men with low sperm count are fertile and some men with high sperm count are infertile, it is clear that a man with low sperm count is more likely to be infertile than a man with higher sperm count.	• Outcome definition and measurement • Difficulty in detecting effects when background variability is high

*The teacher should caution students that all of the individuals and some of the information described in this roster are fictional. They were created for the purposes of this module, and any resemblance to real people or situations is purely coincidental.

Procedures (continued) from page 8.

7. Before proceedings begin, the judge sets the stage by explaining the importance of assessing causality in a complex world. The judge explains that the fundamental question the jury must answer in this case is whether drinking Alpine Fizz *causes* male infertility. The judge instructs the jury to disregard any preconceptions they might have about the issue at hand. The fact that many consider the association between Alpine Fizz and infertility to be an urban legend does not exclude the possibility that there is in fact a causal association. (However, the teacher should warn students that Alpine Fizz should not be used as a method of contraception.)

8. The mock trial takes place, with each side having the opportunity to call its witnesses. Each witness called may be cross-examined by the other side.

9. The jury deliberates, reaches a verdict, and announces its verdict.

10. The verdict is discussed by all students in the class. (The teacher may use the worksheet in Appendix 5 to structure this discussion.) The teacher asks the class to assess how likely it is that the correct verdict has been reached. Do the students believe, on the basis of the evidence, that Alpine Fizz causes male infertility? If the losing side were to appeal the verdict, what further evidence would it like to have? The teacher could, at this stage, introduce the criteria for causation listed in Appendix 3.

11. The teacher points out that the example used in this mock trial is hypothetical. Court cases follow legal rules for determining causality that are appropriate for the goals of the litigation but are not necessarily consistent with the principles of the scientific method. However, epidemiologists, health care providers and policymakers, as well as students, are regularly faced with the challenge of deciding whether a particular association is a causal one. The teacher may at this point review the types of studies that are often used by epidemiologists when they are exploring the possibility of an association between an exposure and a disease. Appendix 6 gives a very brief overview of epidemiologic study designs and the potential of each to shed light on causal associations.

12. It is important to complete this module by giving the students a realistic example of the difficult process of assessing whether there is a causal association between an exposure and a disease. The association between smoking and lung cancer is such an example. We recommend that the students be asked to read the section (pages 54–69) on tobacco and lung cancer in the book *Investigating Disease Patterns: The Science of Epidemiology,* by P. D. Stolley and T. Lasky (New York: Scientific American Library; 1998).

13. Ask the students to finish the sentence: "We know something causes something else when . . ."

Assessment

1. Ask students the following question:

   It is now universally accepted that cigarette smoking is causally related to lung cancer. Review pages 54 to 69 in the book *Investigating Disease Patterns: The Science of Epidemiology,* by P. D. Stolley and T. Lasky (New York: Scientific American Library; 1998), which describes how that causal association was established.

   Now imagine that you were given an unlimited amount of funding to put together a team of scientists including, among others, biologists, chemists, and epidemiologists. The objective of the team is to determine if there is an association between Alpine Fizz and decreased sperm count and, if there is, whether it is causal. How would you go about doing this? Answer this question using what you learned from reading the passage from Stolley and Lasky's book, as well as what you have learned in your science classes. Provide an outline of the different types of studies that you would use and explain your choices. Specify which study you would conduct first and give a logical sequence of the studies that would follow. If you use human participants in your studies, what are some ethical issues that you must consider when designing these studies?

2. Ask students the following question:

   Look closely at the criteria for causation. For each criterion, think about whether this is a necessary condition for causation. For example, is it necessary for a causal association to display a biological gradient? For each criterion, see whether you can think of a situation in which an association could be causal even though it does *not* meet the criterion.

3. Ask students the following question:

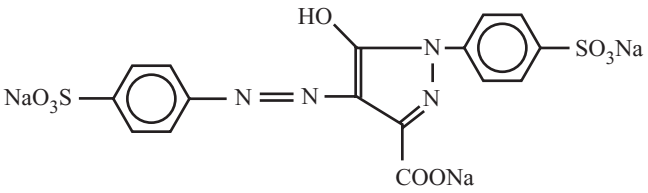
   The Internet has made it easier for all types of urban legends to spread. Have you heard or read about asbestos in tampons or antiperspirants causing breast cancer? A description of these rumors and urban legends can be found at Web sites such as Hoaxbusters, available at http://hoaxbuster.ciac.org. What are the potential social and health consequences of such urban legends? What is an appropriate reaction when hearing or reading about a health risk or a health benefit? How can you decide if what you have heard or read is in fact an urban legend?

4. Give students an article from the popular press (e.g., *Time* or *Newsweek*) about the evidence that a particular exposure (e.g., cell phone use) may cause a particular outcome (e.g., brain cancer). Identify statements in the article that describe evidence for causation. Ask students to alter the statements in a way that would make the evidence more persuasive and in a way that would make the evidence less persuasive.

Appendix 1: Chemical Properties of Tartrazine (Yellow Dye #5)

Common name	Tartrazine
Suggested name	Tartrazine
Other names	
C.I. number	19140
C.I. name	Acid yellow 23
Class	Azo
Ionisation	Acid
Solubility in water	Soluble
Solubility in ethanol	Sparing
Absorption maximum	425
Colour	Yellow
Empirical formula	$C_{16}H_9N_4O_9S_2Na_3$
Formula weight	534.385

Structural formula:



Tartrazine is often used saturated in cellosolve (2-ethoxy ethanol) to differentiate other acid dyes while counterstaining the tissues yellow. Lendrum's phloxine tartrazine method for Paneth cell granules is a good example. It is rarely used for other purposes.

References:

Conn's Biological Stains
R. D. Lillie.
Williams & Wilkins, Baltimore, MD, U.S.A.

The Merck Index, 12 ed.
Susan Budavari, Ed.
Merck & Co., NJ, USA.

Aldrich chemical catalogue
Aldrich, Milwaukee, WI, USA.

Source: StainsFile Web site. Available at: http://members.pgonline.com/~bryand/StainsFile/dyes/19140.htm

Appendix 2: Male Infertility

What is infertility?

Infertility is defined by the American Society for Reproductive Medicine (ASRM) as a disease of the reproductive system that impairs the body's ability perform the basic function of reproduction. Although conceiving a child may seem to be simple and natural, the physiological process is quite complicated and depends on the proper function of many factors, including the following, as listed by the ASRM:

- production of healthy sperm by the man
- production of healthy eggs by the woman
- unblocked fallopian tubes that allow the sperm to reach the egg
- the sperm's ability to fertilize the egg
- the ability of the fertilized egg to become implanted in the uterus
- adequate embryo quality

Who is affected by infertility?

The average chance to conceive for a normally fertile couple having regular, unprotected intercourse is approximately 25 percent during each menstrual cycle. In most couples, conception occurs within about 12 months. However, infertility affects about 10 percent of couples of childbearing age. Infertility is not just a woman's concern. A problem with the male is the sole cause, or a contributing cause, of infertility in about 40 percent of infertile couples. About one-fourth of infertile couples have more than one cause or factor related to their inability to conceive. About 10 to 15 percent of couples have no identifiable cause for their infertility after medical investigation.

What are the risk factors for men regarding infertility?

The following is a list of risk factors related to male infertility (also called male factor or male factor infertility):

- history of prostatitis or genital infection
- testicular trauma or torsion

- history of precocious puberty (puberty occurring at a young age) or delayed puberty (puberty occurring at an older age)
- exposure to toxic substances or hazards on the job, such as lead, cadmium, mercury, ethylene oxide, vinyl chloride, radioactivity, and x-rays
- cigarette or marijuana smoke
- heavy alcohol consumption
- exposure of the genitals to high temperatures
- hernia repair
- undescended testicles
- prescription drugs for ulcers or psoriasis
- DES taken by mother during pregnancy
- mumps after puberty

What causes male factor infertility?

The main causes of male infertility can be divided into the following categories:

- Sperm disorders: Problems with the production and maturation of sperm are the most common causes of male infertility. Sperm may be immature, abnormally shaped, or unable to move properly. Or, normal sperm may be produced in abnormally low numbers (oligospermia) or seemingly not at all (azoospermia). This problem may be caused by many different conditions, including the following:
  - Infectious diseases or inflammatory conditions such as the mumps virus
  - Endocrine or hormonal disorders such as Kallmann's syndrome or pituitary problem
  - Immunological disorders in which some men produce antibodies to their own sperm
  - Environmental and lifestyle factors
  - Genetic diseases (most are associated with sperm abnormalities, either directly or indirectly):
    - Cystic fibrosis: An inherited condition that typically involves the lungs and pancreas, but can present also as a cause of infertility with or without mild sinus problems; 6 to 10 percent of men with obstructive azoospermia have congenital bilateral absence of the vas deferens (CBAVD), which means they were born without the vas deferens; of these, 70 percent may have cystic fibrosis or carry a mutation in the cystic fibrosis gene.

- Noonan syndrome: An inherited condition which can occur in either males or females. In males, this syndrome can cause abnormal gonadal (testicular) function.
- Myotonic dystrophy: An inherited condition with progressive multisystem involvement, resulting in infertility (underdeveloped testes and abnormal sperm production), in some cases.
- Hemachromatosis [Hemochromatosis]: An inherited condition affecting iron storage. Eighty percent of men with hemochromatosis have testicular dysfunction.
- Sickle cell disease: An inherited condition affecting the normal production of hemoglobin.
- Sex reversal syndrome: A male who has the sex chromosomes of a genetic female (XX, instead of XY), resulting in azoospermia and other characteristics.
- Androgen receptor gene mutations: An inherited condition in which a man is genetically male (46,XY), but has infertility due to a defect in receptors for testosterone.
- Chromosomal abnormalities: Men with an extra X sex chromosome, known as Klinefelter syndrome, often do not produce sperm or produce very low quantities of sperm.
- Chromosome rearrangements: In some persons, there are the usual number of chromosomes (46) in the nucleus (center) of cells, but rearrangements in the chromosome material, where a piece of a chromosome has exchanged places with another, has taken place; men with either azoospermia or oligospermia have a higher frequency of chromosome rearrangements than is found in the general population.
- Deletions in the Y chromosome: In some persons, there are the usual number of chromosomes (46) in the body cells, but small sections of the Y chromosome are missing or deleted; anywhere from 3 to 30 percent of men with either azoospermia or oligospermia have deletions in the Y chromosome.

  It is important to understand that men who have genetic problems which cause their infertility, such as a deletion in the Y chromosome, can pass this problem to their sons, who would also have infertility, if they elect to use their own sperm in achieving a pregnancy.

- Anatomical abnormalities: Obstructions of the genital tract can cause infertility by partially or totally blocking the flow of seminal fluid. Some of these abnormalities may be of congenital (present at birth) origin or the result of a genetic defect. Others could have occurred due to infection or inflammation of the urogenital tract, surgery that left scar tissue in the genital tract, or the presence of varicose veins in the scrotum (scrotal varicoceles).
- Immotile cilia syndromes: In this condition, the sperm count is normal but the spermatozoa are nonmotile such as in Kartagener's syndrome, an inherited disorder.

- Mitochondrial deletions: Mitochondria are structures in the cell responsible for energy production. There are actually a set of genes in the mitochondria, separate from the normal chromosome set contained in the nucleus. Recently, it has been discovered that these genes, when altered or deleted, can affect a person's health and/or fertility.
- Liver disease, renal disease, or treatment for seizure disorders
- Other factors: Other factors may arise from the defective delivery of sperm into the female genital tract, which could be caused by impotence or premature ejaculation.

How is male factor infertility diagnosed?

In addition to a complete medical history and physical examination, diagnostic testing for male factor infertility may include the following:

- Multiple semen analysis—at least two semen examples are collected on separate days to examine the semen and sperm for various factors, such as semen volume, consistency, and pH, and the sperm count, motility, and morphology (shape).
- Other tests (to determine the cause of sperm abnormalities or diseases of the male reproductive system)

Treatment for male factor infertility:

Specific treatment for male factor infertility will be determined by your physician based on:

- your age, overall health, and medical history
- extent of the disease
- your tolerance for specific medications, procedures, or therapies
- expectations for the course of the disease
- your opinion or preference

There is a range of treatment options currently available for male factor infertility. Treatment may include:

- Assisted reproductive technologies (ART): This type of treatment may include the following:
  - Artificial insemination: Artificial insemination involves the placement of relatively large numbers of healthy sperm either at the entrance of the cervix or into the partner's uterus, bypassing the cervix, to have direct access to the fallopian tubes.

  - IVF, GIFT, and other techniques: In vitro fertilization (IVF) or gamete intra-fallopian transfer (GIFT) have been used for the treatment of male infertility. As is the case with artificial insemination, IVF and similar techniques offer the opportunity to prepare sperm in vitro, so that oocytes are exposed to an optimal concentration of high-quality, motile sperm.
  - Microsurgical fertilization (microinjection techniques such as intracytoplasmic sperm injection, or ICSI): This treatment is used to facilitate sperm penetration by injection of a single sperm into the oocyte. Fertilization then takes place under the microscope.
- Drug therapy: A small percentage of infertile men have a hormonal disorder that can be treated with hormone therapy. Hormonal imbalances caused by a dysfunction in the mechanism of interaction between the hypothalamus, the pituitary gland, and the testes directly affect the development of sperm (spermatogenesis). Drug therapy may include gonadotrophin therapy, antibiotics, or another medication deemed appropriate.
- Surgery: Surgical therapy in male infertility is designed to overcome anatomical barriers that impede sperm production and maturation or ejaculation. Surgical procedures to remove varicose veins in the scrotum (varicocele) can sometimes serve to improve the quality of sperm.

Source: University of Utah Health Sciences Center Web site. Available at: http://www.med.utah.edu/healthinfo/adult/men/infertil.htm

Appendix 3: Criteria for Causation

Criterion	Comment
Temporality*	Cause must precede effect. This is a necessary condition for causality.
Biological gradient*	Causality is supported when higher levels of exposure lead to more severe disease or higher incidence of disease.
Magnitude of the association*	Strong associations are more likely to be causal than weak associations.
Consistency	If an association is causal, findings should be consistent with other data.
Biological plausibility	If an association is causal, it should be plausible in light of scientific knowledge about the biological mechanisms involved.
Coherence	A causal interpretation should not conflict with what is known of the natural history and biology of the disease.
Experimental evidence	The existence of experimental evidence supports a causal interpretation.
Analogy	Analogy with other known associations strengthens the credibility of a causal association.

***Note:** Criteria with an asterisk can be applied to findings of a single study. Other criteria are applied to a series of studies on a particular topic.

Appendix 4: Roster of Potential Witnesses

Witness	Testimony
Plaintiffs	Rumors have been circulating for several years relating the dye called Yellow Dye #5 (or tartrazine) in Alpine Fizz to low sperm counts in men who consume the chartreuse-colored drink. Several chat rooms post messages that put forward the belief that young men's fertility, sexual attractiveness or sexual potency could be adversely affected by drinking Alpine Fizz. For the purposes of this exercise, let us imagine that a group of heavy Alpine Fizz drinkers met in one of these chat rooms and discovered that they were all having trouble with fertility. All were married, all had been trying unsuccessfully to get their wives pregnant for more than one year, and all drank at least three cans of Alpine Fizz every day. They launched a Web site and urged people with the same problem to communicate with them. After about a year, they had assembled about 60 couples with infertility problems that they attributed to the men's drinking of Alpine Fizz. They hired an impressive legal team and filed a class action suit.
Defendant	The manufacturer of Alpine Fizz, King Kola, claims that there is absolutely no scientific evidence that tartrazine, caffeine or any other substance found in Alpine Fizz is related to infertility. All of the soft drink's ingredients are approved by the Food and Drug Administration (FDA), the federal agency that regulates such matters. The manufacturer claims that the fact that some men drink Alpine Fizz and are infertile is simply coincidence and that there is no causal association between the two.
Harry G. Walker	Mr. Walker is an 80-year-old man who drank Alpine Fizz all his life and continues to consume at least two bottles a day. He has fathered 12 children and is prepared to testify that his soft drink consumption has had no effect on his fertility.
Mary Stewart-Swanson	Ms. Stewart-Swanson owns Mary's Deli in Springfield, Connecticut. After a few months of drinking large quantities of Alpine Fizz (more than four cans per day), she began having numerous symptoms, including rash, diarrhea and shortness of breath. She is prepared to testify that after she stopped drinking Alpine Fizz, all her symptoms disappeared.

continue

Witness	Testimony
Gregory Marchildon	Mr. Marchildon is a Madison, Wisconsin, man who claims he is sure that Alpine Fizz lowers sperm count. He is prepared to testify that he always drinks Alpine Fizz before intercourse, and none of his sexual partners has ever become pregnant.
Michelle Cabano	Ms. Cabano is an executive at Bornstein, Burrard, Benson and Cabano, a well-known New York City advertising agency. She developed the advertising campaign linking Alpine Fizz to extreme sports. Some of these sports (e.g., dirt biking, motocross, mountain biking) are reputed to cause low sperm count in men. She is prepared to testify that it may be that men who engage in these sports are more likely than others to drink Alpine Fizz and that it is the sports activity, not the Alpine Fizz, that is the cause of the infertility.
Laszlo Nagy	Mr. Nagy is the Chief Executive Officer of ABC Chemicals, a New Jersey company that manufactures dyes (including Yellow Dye #5), artificial flavorings and other chemicals for the food industry. He is prepared to testify that more than two million pounds of tartrazine are used every year in medicines, beverages, desserts, processed vegetables and cosmetics. Because a 1986 FDA advisory committee concluded that Yellow Dye #5 can cause allergic skin reactions (such as hives and rashes), manufacturers who use tartrazine must list it on their labels. However, Mr. Nagy will testify that there has never been any evidence that the dye is related to male infertility.
Patrick L. Rodriguez	Mr. Rodriguez is a representative of the American Caffeine Foundation. He is prepared to testify that although Alpine Fizz contains very high levels of caffeine (higher than either Cola Rite or King Kola), there is evidence to suggest that caffeine actually increases the motility and effectiveness of sperm cells.
Janet S. Fang	Ms. Fang is Vice President for Public Affairs for the Soft Drink Manufacturers Association of America. She is prepared to testify that Alpine Fizz has been consumed by millions of people since the drink was first introduced in the 1950s, and yet King Kola has never received a consumer inquiry or complaint about the effects of Alpine Fizz on male fertility or potency. Her testimony will be based on the premise that if there were such effects, they would have become apparent by now.
Vanessa Johnson, Ph.D.	Dr. Johnson is an organic chemist who specializes in dyes. She will testify that the chemical structure of tartrazine (commonly known as Yellow Dye #5) is similar to that of other dyes, some of which are known to cause infertility or carcinogenesis. She will also discuss some of the other health effects (e.g., allergy) of Yellow Dye #5.

Witness	Testimony
Pragati Desai, Ph.D.	Dr. Desai is a toxicologist who studies the carcinogenic effects of artificial dyes on animals. She has also studied the effects that other chemicals have on infertility.
Roger Davis, M.D., Ph.D.	Dr. Davis is a physician who is an expert on fertility. He is prepared to testify that he has never encountered a case of Alpine Fizz–related infertility in his practice.
Greg Markowicz, DrPH	Dr. Markowicz is an epidemiologist who has studied men who donate sperm at a sperm bank. In his study, Dr. Markowicz asked sperm donors to complete a questionnaire, and one of the questions asked whether they drank Alpine Fizz. His findings indicate that men who drank one to two 20-oz. bottles of Alpine Fizz per day had a significantly lower sperm count than men who drank Alpine Fizz less frequently. Men who drank three or more bottles per day had an even lower sperm count.
Gillian McDermott, Ph.D.	Dr. McDermott is an epidemiologist who has done a study on patients at a fertility clinic. She found that the men in her study who drank one or more 12-oz. cans of Alpine Fizz per day had an average sperm count similar to that of men who drank Alpine Fizz less frequently.
Luz Rivera, Ph.D.	Dr. Rivera is a clinical psychologist who will testify that in her experience men who become depressed as a result of infertility may turn to stimulants (such as Alpine Fizz) to compensate for their feelings of depression.
John Thompson	Mr. Thompson is an activist who set up a Web site that collects the stories of men who drank Alpine Fizz and became infertile as a result.
Harry Humphrey	Mr. Humphrey is an herbalist who believes that all synthetic chemicals, including Yellow Dye #5, are hazardous to health.
Franz Lichter, M.D.	Dr. Lichter, a Swiss radiologist, has done a study comparing male mountain bikers with men who are not mountain bikers. He is prepared to testify that mountain bikers have a lower sperm count and less motile sperm than men who are not mountain bikers, and that mountain bikers are more likely to have abnormalities in their scrotum. He believes that the observed infertility among the plaintiffs may be due to their participation in extreme sports (which is more common among men who drink Alpine Fizz) rather than to the drink itself.

continue

Witness	Testimony
Georgeanne Parker, Ph.D.	Dr. Parker is an epidemiologist who has compiled statistics on average sperm counts among U.S. males during every year between 1971 and 2001. She also has statistics on the average annual per capita consumption of Alpine Fizz in the United States during the same period. Her statistics clearly demonstrate that sperm counts declined and Alpine Fizz consumption increased over the period in question.
Anatoly Pritovich, Ph.D.	Dr. Pritovich is a reproductive biologist. He will testify that the sperm count is a measure of the total number of sperm present in a man's semen, usually recorded in millions of sperm per milliliter of semen. Sperm count varies widely among human males. A normal count is regarded as being greater than 20 million/ml. A man is considered at risk of being infertile if his sperm count is less than 20 million/ml of semen. However, some men with low sperm counts are fertile, whereas others with higher counts prove to be infertile. Dr. Pritovich will testify that a measure of sperm count is not a completely reliable method of assessing fertility.

Note: All of the individuals and some of the information described in this roster are fictional. They were created for the purposes of this module and any resemblance to real people or situations is purely coincidental.

Appendix 5: Posttrial Worksheet

1. How likely do you think it is that the correct verdict has been reached? Do you believe, on the basis of the evidence, that Alpine Fizz causes male infertility?

2. If the losing side were to appeal the verdict, what further evidence would it like to have?

Appendix 6: Epidemiologic Study Designs

	Description	Potential to Infer Causality
Randomized Controlled Trials	• Participants with a particular disease or condition are assigned by randomization to a group that receives the experimental treatment or to a group that does not. • Treated and untreated participants are followed over time to determine whether they experience an outcome (e.g., recovery).	• Very strong potential to infer causality. • Randomization reduces potential for selection bias. • Randomization tends to make the groups comparable with respect to known and unknown confounding variables.
Cohort Studies	• Exposed and unexposed participants without disease are followed over time to determine how many acquire the disease.	• Strong potential to infer causality. • Confounding may be a problem because factors that determine whether or not a person is exposed may also be related to the outcome.
Case-Control Studies	• Odds of exposure among persons with the disease (cases) is compared with odds of exposure among persons without the disease (controls).	• Moderate potential to infer causality. • Confounding may be a problem because factors that determine whether or not a person is a case may also be related to the exposure. • Selection bias and recall bias are particular problems.
Cross-Sectional Studies	• The status of individuals with respect to one or more characteristics is assessed at one point in time.	• Rarely possible to infer causality. • Cannot determine whether exposure preceded disease.
Ecologic Studies	• The units of analysis are populations or groups of people, rather than individuals. • Each individual in the population is characterized by the average for the population.	• Rarely possible to infer causality. • An association observed between variables on an aggregate level does not necessarily represent the association that exists at an individual level.
Case Series	• All participants have the disease (or the exposure).	• Not possible to infer causality. • No comparison group.