Are Three Sheets Enough?

Using Toilet Paper To Teach Science & Mathematics

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The *National Science Education Standards* (National Research Council, 1996) define content areas, skill sets, and processes that provide a foundation on which successful strategies for learning may be developed. The standards identify content areas to develop creative lesson plans that relate theory and practice. Yet, science education should be more about "doing" science, in addition to reading and writing about science (DebBurman, 2002). The approach taken in this paper integrates scientific principles, mathematics, history, and social perspectives to "doing science." This paper describes a scalable (individual through collaborative learning groups), inquiry-based model using an everyday commonplace object to stimulate student curiosity through didactic and hands-on investigation. Demonstrating the object and asking questions (What is this used for? Who invented it? How is it made? Are all types alike? What are its uses? Is there a need to use it? What impact does it have on the environment?) can set the stage for researching history using technology and instrumentation, hypothesis testing, mathematical data analysis, and communication. This model is demonstrated using toilet paper (TP), as our object.

Infectious diarrheal disease accounted for 1.8 million deaths worldwide in 2002 (World Health Organization, 2004). In the United States, acute infectious diarrhea occurs at an annual rate of 99 million episodes in adults, and between 21 and 37 million episodes in children under age five (Lee & Surawicz, 2001). Ingestion of infectious agents in food and water is a primary route of transmission. Unfortunately, one of the most important factors contributing to microbial food contamination is the lack of proper personal hygiene after using the toilet (Farber & Todd, 2000). In fact, the most commonly reported food preparation practice that contributed to foodborne disease, each year from 1983 through 1992, was poor personal hygiene of the food handler (Collins, 1997). For all the education and training that is directed at disease prevention through proper sanitation and handwashing, it seems that there is a dearth of information on the role of toilet paper (TP) use as a barrier to the transmission of enteric organisms (Hughes, 1988).

The main lesson asks the students to survey various TP products, determine product specifications (size, shape, thickness, weight, color, additives, etc.) and make hypotheses relating TP characteristics to tensile strength, absorbancy, and role as a barrier to infectious agents. The latter is presented herein, integrating National Science Standards on scientific inquiry, behavior and adaptation, technological design, personal health, risks and benefits, and the history of science, for example.

Background

TP is reported to have been invented in by the Chinese in 1391 when the Emperor ordered the Bureau of Imperial Supplies to produce 2-foot by 3-foot paper sheets at the rate of 720,000 per year (Toilet Paper World Corp., 2003). It was not until 1857, though, when Joseph Gayetty of the United States packaged the first "therapeutic paper" made of paper and aloe, that personal hygiene entered the industrial marketplace. In 1880, the British Perforated Paper Company produced individual sheets of paper for wiping after using the toilet (World Toilet Organization, 2002). The introduction of rolled and perforated TP is attributed to the Albany Perforated Wrapping Paper Company and the Scott Paper Company between 1877 and 1890 (Virtual Toilet Paper Museum, 1999).

Today, there are over 5,000 different companies producing toilet paper worldwide. The standard sheet of toilet paper is 4.5 x 4.5 inches of 13# thickness, weighing

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approximately 0.22 grams (Toilet Paper World Corp., 2003; Tollefsrud, 2001). Some sheets may be cut smaller than 4.5 inches to vary by as much as 15% less (Toilet Paper World Corp., 2003). Manufacturer specific TP composition appears to be proprietary. However, TP in the United States is generically composed of cellulose (softened wood pulp or cotton) that is washed, bleached, squeezed flat, and rolled. TP composition balances tensile strength with the ability to dissolve in water. TP may be layered, fluffed by air drying, and/or chemically modified to control absorbancy, increase softness, and increase strength. Interestingly, one TP layer (ply) is made of one sheet of 13# thickness paper, while two-ply is made of two sheets of 10# thickness paper (Toilet Paper World Corp., 2003). Various formulations appear to have generated four distinct marketing categories of TP based on "quality;" economy, regular, premium, and super premium (Toilet Paper World Corp., 2003). However, quantitative measurements of absorbency, tensile strength, and final composition are not listed on product pages and there does not appear to be a publicly available index (or rubric) defining each category.

In contrast to the numerous scientific and lay publications on the impact of fecal contamination of worker hands and the role of hand washing on disease transmission, the students may find no study that directly compares TP quality with prevention of bacterial transmission. Hypotheses may suggest that TP does not prevent bacterial transmission, that TP thickness (ply), layers, or additives may prevent bacterial contamination of hands, or that marketing categories reflect absorbancy and thus transmission of bacteria. The experimental setup is relatively low tech, inexpensive and, importantly, easy for students to use. We present our evaluation of eight commercially-available TP products, considering quality (assigned by the manufacturer) and thickness (1 vs. 2 ply), for their ability to restrict *E. coli* transmission to an agar surface.

Materials & Methods

Bacterial Preparation, Growth Conditions & Safety

Escherichia coli K99 (#31616) was obtained from the American Type Culture Collection (Manassas, VA). Overnight cultures were grown at 35˚C in nutrient broth (Becton Dickson Co., Sparks, MD), and diluted with sterile water to a density similar to skim milk (ca. 1 x 10^8 colony forming units/mL if using plate counts or 0.095 OD_{600} if using a spectrophotometer) just prior to use. Nutrient agar (Becton Dickson Co., Sparks, MD) was prepared in petri plates (VWR/SP, Pittsburgh, PA) and refrigerated until used. (Nutrient broth, agar, and water may be sterilized by heating to boiling in a microwave three successive times. Agar plates should be poured once the flask is not too hot to hold, but before the agar solidifies in the flask.) Nutrient agar plates were warmed to room temperature prior to each experiment.

E. coli is potentially infectious material and should not be handled without proper training. Use of *E. coli* should be in a well labeled area indicating that a potentially infectious material is present and that good laboratory work practices (also known as universal precautions) are used. These include having protective eyewear and clothing, and disinfectant (10% chlorine bleach in water) readily available. Eating, drinking, use of cosmetics, gum, and tobacco products are strictly prohibited when working with bacteria. Hands should be washed thoroughly in the event of any exposure to bacteria and after completion of the manipulation, culture, and counting of bacteria. Work areas should be cleaned with disinfectant when work is completed. All contaminated materials should be decontaminated by chemical (soaked in 10% bleach for at least 30 minutes) or physical (incineration or autoclaving) methods prior to disposal. Additionally, local college, university, or public health microbiologists may offer additional advice on disposal.

Table 1.
Comparison of TP products evaluated for restricting *E. coli* transmission.

Category	Ply	Sheet Size (in x in)
Economy	1	4.5 x 3.9
Regular A	1	4.5 x 4.0
Regular B	1	4.5 x 4.0
Premium (rippled)	1	4.5 x 4.0
Premium (+ Aloe) A	1	4.5 x 4.0
Premium (+ Aloe) B	1	4.5 x 4.0
Super* Premium A	2	4.5 x 4.0
Super* Premium B	2	4.5 x 4.0

* The word "super" appears to mean 2-ply.

Toilet Paper

Eight brands of commercially available toilet paper (TP) were purchased off the shelf. Quality classification (assigned by manufacturers) was used to identify the original TP brand (Table 1). TP packages were opened with thoroughly-washed hands so as to prevent accidental bacterial contamination. (Alternatively, bacteria-free gloves may be used, if available.) Sections of six perforated sheets were removed from each roll and used individually or combined to create up to five layers of TP. TP (or layers) were held across the open petri dishes of nutrient agar by ring stands and clips, so as to keep the paper from touching the agar surface.

Bacterial Transfer & Enumeration

Using good laboratory practice (see above), a one mL suspension of *E. coli* was dripped in one center spot onto the TP (suspended one inch above the agar surface) using a plastic, one mL pipet (VWR/SP, Pittsburgh, PA). The wet TP remained over the agar for 30 seconds to permit the liquid to flow onto the agar surface. The control plates were made by the direct delivery of *E. coli* to the agar surface, from the same height as the experimental groups. Triplicate culture dishes of control and experimental groups were subsequently incubated overnight at 35˚C. A paper grid was drawn to measure the amount of bacterial surface growth on agar. Concentric circles (Figure 1) were plotted to account for 10%, 25%, 50%, 75%, and 100% of the petri plate surface using the formula, area = Πr^2. A numerical value of 1, 2, 3, 4, or 5 was then assigned to each incubated plate so as to represent 1-10, 11-25, 26-50, 51-75, and 76-100% bacterial confluence, respectively. The value "0" was used to represent the lack of bacterial growth.

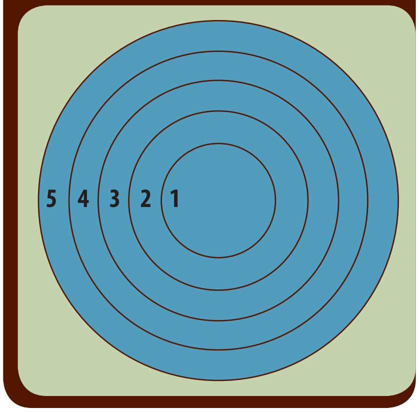
Mathematics & Statistics

Students will need to measure the petri plate diameter, calculate its surface area, and then calculate the diameters of concentric circles needed to represent the various surface area percentages. The students can calculate mean and standard deviation (or standard error) of the mean or use statistical software for determining descriptive statistics and tests for significant differences. Students may also calculate differences between means, as formulas are readily available online as well as in books. Most inexpensive calculators can also perform descriptive statistics and several tests used to compare the means of paired data sets. The project described herein can be shared between science and mathematics classes to further integrate the two disciplines. We report our numerical scores as mean ± standard error of the mean. Our data were evaluated by analysis of variance with significant differences between means identified by the Tukey-Kramer Multiple Comparisons Test using GraphPad InStat® software (alpha levels of 0.05 and 0.01). However, comparison by students' *t*-test (without software) would also demonstrate differences between the data set means.

Results

Collecting, analyzing, and reporting data become central aspects of the experimental component of the exercise. These tasks can be assigned to individuals or to groups. Tables and graphs may be used to summarize data. Photographs of culture results and students at work can be added to the other products generated during the exercise. Students may communicate the results of their project by presenting their research as a written report, in a poster, a table-top presentation demonstrating activities in the project, and/or orally.

Figure 1.
A grid of concentric circles used to measure the amount of bacterial growth on the surface of agar petri plates (not to scale). A numeric value was assigned to each plate based upon the approximate surface area of bacterial growth. A numerical value of 1 represented 1-10% surface growth, 2 represented 11-25% surface growth, 3 represented 26-50% surface growth, 4 represented 51-75% surface growth, and 5 represented 76-100% surface growth. A value of "0" was assigned to plates on which no bacteria grew.



In our example, eight TP products were evaluated for their ability to restrict the transmission of *E. coli* suspended in water. Six of the products were 1-ply thick and two products were 2-ply thick. Control plates had heavy bacterial growth that typically covered greater than 75% of the agar surface, resulting in an area score of 5.0±0 (n=3). Table 2 reports the data obtained from triplicate evaluations of one to five sheets of the 1-ply TP. Four of the six 1-ply products prevented the transmission of *E. coli* when four or five sheets of TP were used (Table 2). Up to five sheets of the Economy or Regular A TP did not prevent the transmission of *E. coli*.

Two super premium TP products (2-ply thick) were also evaluated for their ability to restrict the transmission of *E. coli* suspended in water. Four to five sheets of these 2-ply products were also required to prevent the transmission of *E. coli* (Table 3).

Discussion

Integrating several national science content standards, such as scientific inquiry, behavior and adaptation, technological design, personal health, risks and benefits, and the history of science, may seem ominous. We present a model whereby theory and practice merge by focusing on commonplace objects that many students may find humorous, intriguing, and even "nonscientific." Extensions, like those presented herein, relating

Table 2.
Growth of *E. coli* on agar surface after passage through various layers of 1-ply toilet paper (mean ± SEM) for triplicate samples. Numerical values represent approximate surface area growth as determined by a grid of concentric circles.

	Number of TP Sheets				
TP Type	1	2	3	4	5
Economy	5.0±0	4.7±0.3	4.0±0.6	3.7±0.3	3.0±0.6*
Regular A	5.0±0	4.7±0.3	4.0±0.6	2.7±0.7*	1.7±0.9**
Regular B	4.3±0.3	2.0±1.0	1.7±0.9*	0**	0**
Premium (rippled)	4.7±0.3	2.3±1.2	0.7±0.7**	0**	0**
Premium (+ Aloe) A	4.7±0.3	3.3±0.3*	0.7±0.7**	0**	0**
Premium (+ Aloe) B	5.0±0	2.0±1.2*	0.7±0.7**	0**	0**

* Significant difference among means by Tukey-Kramer Multiple Comparisons Test ($p \leq 0.05$) as compared with bacterial control (5.0±0).

** Significant difference among means by Tukey-Kramer Multiple Comparisons Test ($p \leq 0.01$) as compared with bacterial control (5.0±0).

everyday objects to science may stimulate curiosity and increase student interest in science. For example, investigation revealing the invention of TP in the 1800s begs the question of what was used before. This question opens the door for discussion about personal hygiene, germ theory of disease, and societal change resulting from invention. Questions regarding the composition of TP can lead to a chemistry discussion and paper-making exercises. Furthermore, questions on the real and perceived function of TP can lead to experimentation. Physical and chemical measurements may be taken. TP function may be tested. In these cases, numerical data are generated, analyzed, interpreted, and communicated. Thus the student uses the tools of science to do science. Importantly, as in this case, doing the science fosters appreciation for scientific inquiry; teaches specific skills used by scientists; stimulates reflection on personal hygiene practices; demonstrates causal relationships; reinforces mathematic, reading and communication skills; and leads to an appreciation of science and technology as agents of societal change.

Table 3.

Growth of *E. coli* on agar surface after passage through various layers of 2-ply toilet paper (mean ± SD) for triplicate samples. Numerical values represent approximate surface area growth as determined by a grid of concentric circles.

	Number of TP Sheets				
TP Type	1	2	3	4	5
Super Premium A	4.7±0.3	3.3±0.3	1.3±0.9**	0.3±0.3**	0**
Super Premium B	3.7±0.3	2.0±1.0**	0.7±0.7**	0**	0**

* Significant difference among means by Tukey-Kramer Multiple Comparisons Test ($p \leq 0.05$) as compared with bacterial control (5.0±0).

** Significant difference among means by Tukey-Kramer Multiple Comparisons Test ($p \leq 0.01$) as compared with bacterial control (5.0±0).

In our example, a perception that TP is a barrier to fecal bacteria is readily changed by the data. In fact, the perception that products marketed as "super," "premium," or other superlatives are superior in all aspects can also be refuted by our data. While categories of quality and marketing strategies are designed to sell products, we quickly learn from researching the history of TP and the experiments that 2-ply (super) is no better than 1-ply when protection from fecal bacteria is tested. It should be noted that "library" research is as important as "bench" research when teaching science. The historical information regarding TP presented previously was readily obtained using traditional and online sources. As this type of lesson is readily scalable, we suggest that teachers permit each student to search for background information, participate in the hands-on activities, and communicate results; because learning science is by doing science. Of note is the fact that while significant differences between some experimental groups and the control were identified, any growth of *E. coli* is indicative of potential hand contamination. This presents another opportunity to discuss statistical tests and their use for data analysis.

Importantly, everyday objects can be used to impart context to science and mathematics. Integration of content from other disciplines (history, art, social science, etc.) into traditional science lessons permits students (and teachers) to identify concrete examples of how advances in science and mathematics influence societal change. Conversely, students can be asked to relate societal trends, or needs, to the invention and use of the object. One additional tangent to the lesson presented herein could trace the papermaking process; another, the marketing strategies used to sell the product in a Victorian culture and yet another; the influence of the product on art, music, and drama.

In conclusion, it is suggested that commonplace items can be used to teach science by asking students to do science with them. The use of TP, for example, offers many opportunities to investigate form and function, collect and analyze numerical data, and consider safe hygiene practices.

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