

Acknowledgments

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The Plastic Waste Problem

30.7 million tonnes of plastic waste was collected in 2007 by municipalities in the United States alone (United States Environmental Protection Agency, 2008, p. 6). There are several ways of managing plastic waste including recycling, incinerating, and discarding in the landfill. Recycling allows the plastic to be reused in a new form, but only certain types of plastic are recyclable. Incineration is an option which allows plastics to be burned and used as an energy source; however, this method releases toxic particles into the air (Siddiqui, 2009). Finally, putting plastic waste in landfills provides a quick and temporary solution — but in the long term more landfill sites will be required, as plastics can take hundreds of years to degrade (Staniford, n.d.). Thus, the environmental effect of plastics is evident, and the need for better methods of dealing with or reducing plastic waste is constantly increasing.

The science laboratory is a place where large amounts of plastic waste can quickly accumulate. To maintain sterile conditions, laboratory researchers often use disposable plastic equipment that is discarded after a single use. One such piece of equipment is the pipette tip (Figure 1). Micropipettes, or "pipettes" for short, are a category of instruments used to measure small volumes of liquid samples, and are preferred over traditional glass pipettes for performing repetitive tasks that require a high degree of accuracy (Boyer, 2006, p. 22). A plastic tip is fit onto the end of the pipette and disposed of after use. To prevent cross-contamination, these



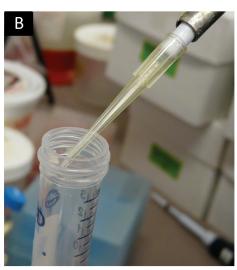


Figure 1: Pipette and Pipette Tip
Pipettes are handheld liquid
measuring devices that use
disposable plastic tips. A) Full view
of a pipette in use, transferring
liquid into a tube. B) Close up of
the plastic tip (in yellow).

tips are not reused, thus generating a large amount of waste (Khadra, Richards, & Robinson, 2000). Furthermore, the disposable tips are packaged and stored in plastic boxes (Figure 2), which are also often discarded after a single use (Staniford, n.d.). Although steps have been taken to address this increasing waste problem, such as using recyclable plastics for the boxes, there is still much that needs to be done for dealing with the waste generated specifically by the plastic tips.

In some labs across the globe, pipette tips are washed and reused to cut down on costs as well as waste (Coloma & Harris, 2004). At Simon Fraser University (SFU), the department of Molecular Biology and Biochemistry (MBB) is a typical example where research labs regularly use pipette tips. Here, just like in many other adequately-funded research facilities, pipette tip washing is not considered as an option due to the possible risk of contamination, as well as the increased amount of work that could be required. However in the teaching labs, where students learn how to conduct experiments using pipettes and pipette tips, there is the potential to test out a possible washing system – since these labs are techniques-based and not results-based, the risk of cross-contamination is not a serious issue. Furthermore, in reference to a system proposed by researchers in Austria, washing should not be time consuming (Khadra et al., 2000). Thus, the SFU MBB teaching labs should consider washing and then reusing disposable plastic pipette tips, to decrease the amount of waste generated, which will also lower equipment costs for the department.



Pipette tips are stored in plastic boxes that have a built-in rack to hold the tips. Once the box is emptied, it is usually discarded. (VWR, 2009)

Figure 2: Pipette Tip Boxes

Pipette Tip Waste

As a fourth year Bachelor of Science Honors student studying MBB at SFU, I have had adequate experience as both a student in teaching labs and as a researcher in an academic lab to notice the alarming amount of plastic waste that is generated — and the waste accumulated from pipette tips surprises me the most. About two years ago, I got to use a pipette for the first time in an MBB lab course. It was such a convenient tool to use that my classmates and I would prefer to use the pipette to measure out all of our samples, even in situations where a graduated cylinder could be used. Graduated cylinders are used to measure millilitre amounts of liquids, and are meant to be washed and reused. On the other hand, pipettes are used mainly for smaller microlitre volumes and have tips that are meant to be discarded. Thus, unintentionally, we were choosing to create more waste because it was more convenient. Furthermore, because we were still getting acquainted with this equipment, often times if we didn't securely fit the tip onto the pipette, it would either fall off or not create the vacuum needed to uptake liquid, causing us to discard the tip and try a new one. So in teaching labs, students can make mistakes in pipette use, which results in unnecessary tip waste.

However, aside from this accumulation created by inexperienced users, there are also many protocols in the lab which require numerous amounts of tips to be used. An example of such a protocol is crystal plating. The lab where I did my undergraduate honors thesis specializes in determining structures of proteins by using a method called crystallography. This technique uses X-ray beams that are directed towards a protein crystal, and the resulting diffraction pattern is studied to determine the structure (Branden & Tooze, 1999, p. 374). However, forming the protein crystal in the first place is a challenge on its own. In order to get a crystal, drops of purified protein are placed in wells surrounded by a buffer that will hopefully provide the correct conditions to form the crystal. Since this method is based on trial and error, multiple conditions are set up using 24-well or 96-well plates. This process of setting up the drops in the wells, known as crystal plating, requires a new pipette tip to be used for each of the buffers that is added. In the hanging drop method of plating, one pipette tip can be used for all of the

protein drops, as the tips do not come in contact with the buffer (Branden & Tooze, 1999, pp. 375-376). But if the sitting drop method of crystal plating is used, then each protein drop must also require a new pipette tip as the protein is mixed in with some of the buffer – in other words, to prepare a 96-well sitting drop plate, at least 192 tips will be used (96 for the different buffers, and 96 for the protein drops). From this example, we can see how much pipette tip waste can add up in just an hour's work.

Current Solutions Available

The amount of plastic waste that is being generated in the lab is apparent not only to researchers, but to equipment manufacturers as well. Fortunately, in the last two decades, steps have been taken to help minimize the waste generated by pipette tips and the plastic boxes they are stored in.

Refill Systems

In the 1990's, multiple patents were created for the designs of pipette tip refill systems (Kelly & Rainin, 1997; Lemieux, 1997). Today, improved versions of these systems are available for researchers to minimize their plastic waste. These systems allow the plastic boxes that contain pipette tips to be reused, by refilling the racks that are inside (Figure 3) (VWR, 2009). For the laboratory research industry as a whole, this is a great innovation as pipette tip boxes are often discarded once emptied, and new ready-to-use boxes are purchased (Staniford, n.d.). But for smaller research labs such as those at SFU, this system isn't necessary as there are





Figure 3: Pipette Tip Refilling Systems

Companies, such as VWR, offer refilling systems to reuse plastic pipette tip boxes. This figure illustrates a simple two-step system which allows all 96 tips to be refilled at once. This provides convenience in comparison to manually racking one tip at a time. (VWR, 2009)

already some environmentally friendly practices in use. For example, in the teaching labs and most research labs at SFU, pipette tips are bought in bulk, and manually racked into the empty boxes. Then the full boxes are sterilized via autoclaving, which is a method that "use[s] moist heat (steam) under pressure to destroy microorganisms" (SFU Environmental Health & Safety (EHS), 2008, p. 61). After autoclaving, the tips are ready for use. Since manual refilling is still being done at SFU, the tip refilling systems do not offer much of a reduction in the accumulation of plastic waste – instead, they might increase the amount since the systems themselves have additional plastic components, which later become of no use.

Recycle Logo

Another recent step taken by manufacturers is introducing a recycling logo on the tip boxes (VWR, 2009). Majority of tips and boxes today are made of polypropylene (PP) which comes under the category of #5 plastics (Figure 4) (Khadra et al., 2000; Thermo Fisher Scientific Inc., 2009 p. 48). The plastic waste can accumulate in numerous amounts, and some labs go "through enough plastic pipette tip boxes a month to fill a small back-yard pool" (Grimm, 2007a). Incorporation of a recycle logo now allows proper management of the plastic waste, which would otherwise be incinerated or sent to the landfill. However, even though most municipalities in Canada and the United States recycle #5 plastics, #5 laboratory plastics are not as widely accepted due to the risk of possible contamination (Ragan, 2007). This has driven some members of the research community to create their own recycling programs, such as the

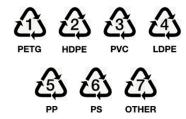


Figure 4: Universal Plastic Recycling Logos

Plastic products that can be recycled are labelled with logos such as these. Most pipette tips and boxes are formed of polypropylene (PP) which is a #5 plastic, and the corresponding #5 logo can now be seen on the tip boxes.

(Thermo Fisher Scientific Inc., 2009)

facility at the National Cancer Institute at Frederick (NCI-Frederick) in the United States. In its first few years, from 2003 to 2006, the NCI-Frederick pipette tip box recycling program recycled 52,189 pounds of plastic from more than 300 research labs on site (Figure 5). Furthermore, this is a "reduction of 45 metric tons of Carbon Dioxide" greenhouse gas emissions (Ragan, 2007). From the success of this one program, the impact of recycling pipette tip boxes is evident, and thus the option of recycling the boxes should be available for all facilities. But one point to note here is that for every one of these pipette tip boxes, 96 tips were originally racked in it (VWR, 2009). And this leads to the concern of what to do with the tips.



Figure 5: Pipette Tip Box Waste
Bags full of empty pipette tip boxes, such as these, are recycled at the NCI-Federick recycling facility.
(Ragan, 2007)

Solution for Pipette Tips

Despite initiatives being taken for reducing the waste from pipette tip boxes, not much is being done in regards to the pipette tips themselves. In the SFU labs, tip boxes are not accumulating as much as the tips, and an alternative is needed to specifically reduce the tip waste. The two main solutions for this problem are recycling and reusing. Recycling is possible since the tips are composed of the #5 polypropylene plastic. However, the risk involved in letting these plastics (which, unlike the tip boxes, come in direct contact with chemicals) combine with other municipal plastics and be reused to create consumer goods, could make recycling less appealing to the public. Washing and reusing, on the other hand, is a better approach, as it will keep the tips in the same lab, and in contact with the same or similar chemicals. Furthermore, in some places of the world, the idea of reusing tips has already been embraced.

For research labs that can afford to buy new pipette tips, there is no immediate need to reuse them. However, for low funded labs in third world countries, and even in Canada, reusing tips is the only way to continue on with work. For example, to help out his lab, Nataniel Mamani, a researcher at the Universidad Mayor de San Andrés in Bolivia, created a contraption out of a plastic jar and tubing to wash tips (Figure 6) (Coloma & Harris, 2004). Using bleach and soap to clean the tips, Mamani is able to reuse them and reduce costs for his lab. Applying a similar scheme to the labs at SFU can raise several issues such as 1) how can the tips be thoroughly washed to avoid contamination, and 2) what is the labour and fiscal cost of washing the tips? Since laboratory research is very competitive, risking years of work by using a possibly contaminated pipette tip does not seem appealing, and is the main concern of most researchers (Grimm, 2007b). Hence, there is a lack of exploration on this topic.



Figure 6: Nataniel Mamani's Tip Washer
Bolivian researcher Nataniel Mamani
created this simple pipette tip washer
which helps him reduce costs and plastic
waste. (Coloma & Harris, 2004)

Contamination Problem

In 2000, a research paper was published in the *Journal of Immunological Methods* by a team of Austrian scientists, which directly addresses the question of contamination by washing pipette tips. Although the authors suggest a method which allows tips to be "washed and reused at least ten times without introducing detectable variability into the results," at the end of the paper it is also noted that the "commercial viability of this tip washing system is probably limited or non-existent" due to the general reluctance towards the notion of washing tips (Khadra et al., 2000). The protocol developed by these researchers is quite simple, and is summarized in Table 1. The apparatus used is shown in Figure 7.

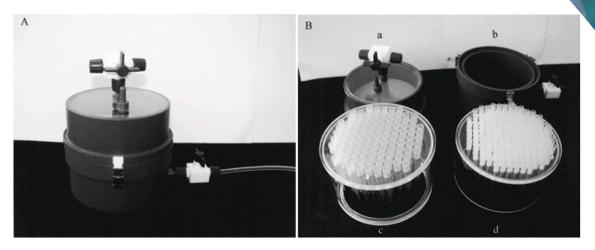


Figure 7: Apparatus used by Khadra et al. (2000)

This figure shows the apparatus used by Khadra et al. in their proposed pipette tip washing system.

A) Fully assembled and closed container. B) The components of the system are shown where a) is the top lid, and b) is the bottom contailiner. This apparatus can be used to clean different sized tips as c) shows the holder for large tips and d) shows the holder for small tips. (Khadra et al., 2000)

Table 1 – Brief Overview of the Pipette Tip Washing System Developed by Khadra et al. (2000)

- 1) tips are placed into the apparatus, and soaked in the Decon 90™ cleaning solution for 45 minutes
- 2) solution is removed, apparatus is refilled with fresh solution, and the tips are soaked for 20 minutes
- 3) solution is removed, apparatus is filled with distilled water, and the tips are soaked for 10 minutes
- 4) water is replaced with fresh distilled water, and the tips are soaked for another 10 minutes
- 5) water is removed, and the tips are dried in a 37°C incubator overnight

Using a similar method in the SFU labs would not be too difficult or time consuming. Also, in step 4, instead of using an incubator, the pipettes could be dried and sterilized more quickly using an autoclave. Because of the size and cost of an autoclave, low funded labs may not have access to one, and thus an incubator may suffice. However at SFU, there are three main autoclaves available, which are located in the Biology, Kinesiology, and MBB departments (SFU EHS, 2008, p. 61). So by slightly revising this protocol, the level of sterilization of the tips increases as autoclaves allow even "infectious laboratory wastes (petri dishes, pipettes, culture tubes, glassware, etc.) [to] be effectively decontaminated" (Public Health Agency of Canada, 2004, p. 79).

Furthermore, instead of washing with only a cleaning agent such as Decon90™ suggested by Khadra et al., bleach (sodium hypochlorite) can also be used, as seen by the technique established by the Bolivian lab (Coloma & Harris, 2004). Scientists working for the Royal Canadian Mounted Police (RCMP) forensic laboratories also use bleach for washing their stainless steel pipette tips, and justify the choice by stating that "bleach (6−10% sodium hypochlorite commercial stock) has been recognized as one of the best chemical disinfectants available. It effectively destroys nearly all disease-producing micro-organisms," and they write that even a 2% bleach solution is sufficient (Frégeau, Lett, Elliott, Yensen, & Fourney, 2008, p. 647). Despite the fact that the RCMP researchers are using this approach on non-disposable stainless steel pipette tips (which are specially designed for their robotic pipetting systems), bleach can also be used on the plastic tips since the chemical is recommended for cleaning other plastic equipment in the lab (Thermo Fisher Scientific Inc., 2009, p. 43).

So in terms of contamination, there seems to be some evidence supporting the notion that pipette tips can be thoroughly cleaned. However, since the number of papers available on this subject is low, it can be difficult to start implementing this system in all of the labs at SFU. But for the teaching labs, especially in the MBB department where pipette tips are regularly used, the washing system can be tried out — since the purpose of these labs is to teach students techniques instead of producing ground-breaking results, the washing system can be tested here without worrying about possible cross-contamination. Also, only the pipette tips that come in contact with water, buffer solutions or other non-hazardous chemicals will be washed — for example, any tip that touches biological samples such as bacterial or tissue cultures should still be disposed of in the biohazardous waste containers. In this manner, no serious risk will be posed to the students as the tips will not have been in contact with hazardous materials. Thus, by washing and reusing tips in the teaching labs, the education and the safety of the students will not be affected and numerous tips can be diverted away from the landfill.

Labour and Fiscal Costs

While washing and reusing pipette tips offers a great opportunity to reduce plastic waste, there is also the concern of the amount of time and labour required to perform this task. In comparison to cleaning glass pipettes, which is done regularly in some of the MBB labs at SFU, the method proposed by Khadra et al. is much faster. Glass pipette washing takes usually a few hours to do, and uses many litres of water — a continuous flow of water at rates from 2L per minute to 12L per minute is used to fill up a tank of pipettes, and the cycle is repeated multiple times to wash and rinse the glass pipettes (Nalge Company, 1988; UCLA Merchant Lab, 2008). For plastic pipette tips, using a cleaning system similar to the one by Khadra et al. would not involve continuous water flow, and excessive water would not be wasted. Also, in regards to concerns of extra electricity costs for using the autoclaves to sterilize the tips, it should be noted that since new tips are also autoclaved, there will not be any extra autoclaving performed. And finally, finding someone to do the work should not be difficult as undergraduate students look for volunteer opportunities to gain experience in the lab.

Any costs that would be necessary, would be for finding materials that could function as the apparatus — but many of these parts can be found in the lab. Since the proposed washing system is based on soaking the tips in a cleaning agent and in water, a simple way of creating this could be by using an empty tip rack from a tip box, and using it to hold the tips. A wide container or bucket could be used to contain the cleaning solutions. Hence, since the operation and labour costs will be minimal, attempting to wash and reuse pipette tips is a fairly plausible solution for reducing plastic waste in the teaching labs.

Washing Pipette Tips is a Beneficial Approach

While maintaining sterilized conditions in laboratory research, numerous pipette tips are used. A bag of 1000 pipette tips costs around \$20, and can be used up quite quickly (VWR, 2009). If using a pipette tip washing system leads to a lab buying one less bag of tips a month, that can become a savings of \$240 a year. Furthermore, if this system were to be applied to just 10 MBB research labs at SFU, the savings increase to over \$2000 a year for the department. However, because of the uncertainty of the system's ability to fully decontaminate the tips, washing pipette tips can first be implemented in only the MBB teaching labs. Here, the students can continue to learn various techniques using pipettes, without worrying about significant effects to their results. As consumption of plastic materials continues to grow worldwide, making a small difference in pipette tip waste management can help start a trend toward researching more environmentally friendly practices in the lab. After all, since reducing plastic waste in the lab helps protect the environment, washing and reusing pipette tips benefits everyone, and not only members of the scientific research community.

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