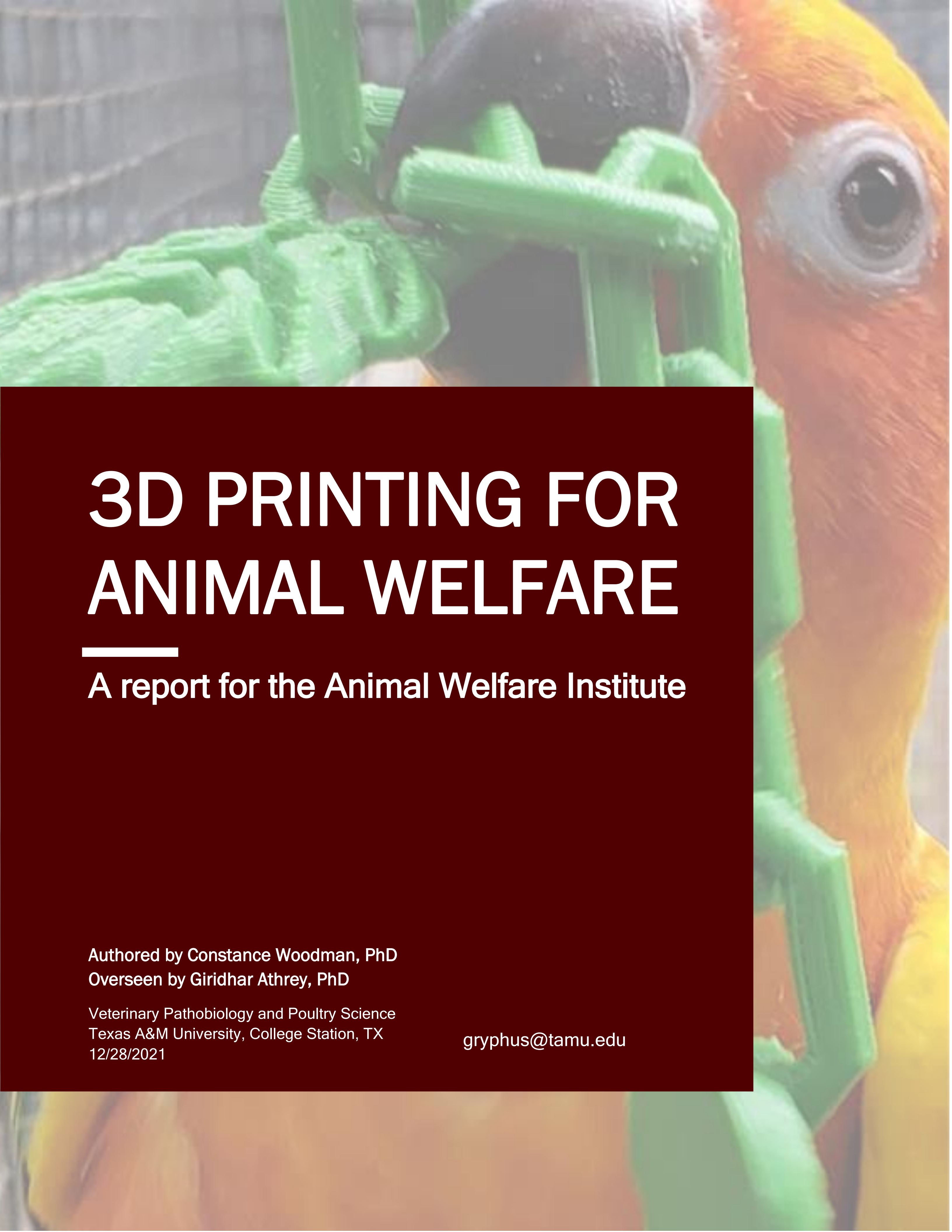

3D PRINTING FOR ANIMAL WELFARE

A report for the Animal Welfare Institute

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Forward to This Report

Why was this report written?

This report was written to help orient animal welfare stakeholders toward 3D printing as an animal welfare solution. When this project was proposed, the general reading and websites about 3D printing offered confusing and often incorrect information related to issues that affect animals, such as plastic toxicity and ease of cleaning. There was not a centralized resource that would discuss issues for laboratory animal welfare. There are a mix of scientific citations as well as clickable links in this report to help quickly orient readers to accurate general reading material, which can be followed with further reading in specialized scientific publications.

The goal of this report is to use a scientific approach to understand the technology's benefits and hazards and how 3D printing is and is not aligned with laboratory needs. The information shared in this report is from laboratory tests and information noted from about 150 communications with 3D printing companies, government regulators, chemical scientists, hobbyists, and plastic manufacturers. By focusing on using printers safely indoors, heavy metals in prints, endocrine disruption caused by prints, sanitizing methods special to prints, and physical hazards of incorrect printing related to animals, this report provides a technically simple way to start conversations about 3D prints in laboratory situations.

The Animal Welfare Institute funded chemical testing to help understand the safety of 3D printing. This report utilized ATS Laboratories, in Marietta, GA, USA. ATS is a major laboratory for safety testing, including human toys. As young humans chew, lick, and salivate on their toys, toy tests reflect potential chemical exposure through oral routes. I felt toy safety tests developed for human children could be informative about the safety of 3D printed objects for animals, who also chew and lick the objects in their surroundings.

Figure 1. Bulk printing of cage furniture and enrichment. Left, bow perches that leave an open central flyway area for birds, maximizing exercise. Center, spinner perches for acrobatic play, with two perching zones for social play. Right, beads and rattles for auditory any rhythm play with manipulation. Upper right, in a box, custom clips for the cage wire used at the facility.

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Justification

Animals Need Affordable Custom Enrichment

Laboratory animals often have specific needs for enrichment, especially non-domesticated animals. The products available for exotic pets and zoo animals, such as parrot or primate toys can often be unsafe such as cotton string impaction or entanglement risks. Or toys may be difficult to clean, such as leather strips that become moldy after being washed. Laboratory-specific enrichment items are often more rugged, easier to mount in animal housing, and can be sanitized using stringent established protocols.

However, laboratory-specific exotic animal products can carry a very high price tag for items such as puzzle feeders, swings with interactive toy stations, etc. Various toys, puzzles, huts, and perches come and go from the market. Laboratory and zoo animal enrichment providers are often familiar with “stocking up” when a useful toy or feeder is available, as they know availability will not be guaranteed in the future.

Custom manufactured devices, such as attachments to animal housings for protected-contact treat feeding and blood draws, tablet holders, or data logger enclosures can be extremely expensive. These devices can increase welfare and provide new ways to minimize stress and increase environmental enrichment but are often cannot be funded under existing enrichment budgets.

Printing objects on-demand changes the costs of enrichment equipment. Instead of paying retail costs per item, the cost is the overhead for printing such as material, electrical, an optional annual printer maintenance plan, and staff time to run the printer. For high-cost specialty items, the printing overhead can be much less than the costs of buying a set of manufactured items. Research has shown that both low-cost and the highest quality industrial printers can all have clinical utility (Chen, Dang & Dang, 2021), so access to the benefits of 3D printing are available at many budget levels.

There are libraries of pre-designed items, such as Thingiverse.com by the MakerBot company, and the NIH 3D exchange at 3dprint.nih.gov. These items can be resized and mixed together with free software such as the Autodesk company’s TinkerCad and Meshmixer, which are free to use. TinkerCAD is geared toward children, a bit fun to use, and easily learned. TinkerCAD allows for combining and changing models without a lot of trouble. Small parts such as adapters and connectors can be replicated in TinkerCad, extending the lifetime of existing enrichment equipment by providing replacement parts that are not otherwise available.

Complex custom items need to be designed specifically for 3D printing. However, the high cost of injection molding setups and other types of manufacture are removed.

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Caregiver Burnout and Secondary Traumatic Stress

When the enrichment process is difficult, due to a lack of supplies, or an inability to provide enough enrichment to meet individual animals' needs, harm can come to the animals' caregiver. Enrichment is especially complex for non-domesticated, long-lived social animals, where there are fewer enrichment supply companies.

For the caretaker, observing a heavily stressed animal can cause compassion fatigue, which is sometimes called "secondary traumatic stress" (Deering, 1996; Figley, 1995; Sabin-Farrell & Turpin, 2003). Compassion fatigue is an existing concept in human caregiving literature that has been more recently brought into animal caregiving (Rank, Zaparanick, & Gentry, 2009). A normal human response to repeatedly observing stress and trauma is the internalization of trauma by the caregiver, which produces issues including occupational and interpersonal problems (Sabin-Farrell & Turpin, 2003). Witnessing animals' stress-related behaviors, such as stereotypy, can harm a caregiver. Often, animal stress is due to a lack of "Biologically relevant" enrichment (Newberry 1995). Biologically relevant enrichment is the addition of care or stimulation, such as toys, conspecifics, and training routines, that improve the biological function of an animal. A wall mural or classical music in the environment may not be biologically relevant, while paired housing for a social animal and foraging opportunities are more likely to be biologically relevant.

Time is a costly and scarce resource for animal caregivers. The lack of time and the pressure to do more than can be done cause the second form of harm to the caregiver, called burnout. Burnout is the result of "prolonged work" and is emotional exhaustion caused by job strain, lack of sense of accomplishment or achievement, and erosion of idealism (Maslach, 1982; Sabin-Farrell & Turpin, 2003). For example, a lack of staff time means that animals do not receive what the daily keepers want very much to offer in zoo setups (Hoy, Murray, & Tribe, 2010). In the home, a lack of time to care for a pet is one of the reasons people do not own pets (Burns, 2012). One of the biggest time commitments for an animal caregiver can be training to reduce stress. For example, Grandin's medical husbandry training for a voluntary antelope blood draw took 118 days (Grandin, 2000). Such time investment is unrealistic for most caregivers.

3D printing can reduce both secondary traumatic stress and burnout. If the 3D printed objects solve difficult welfare needs through on-demand specialty enrichment, then there will be less animal stress for caregivers to internalize. If low-cost, on-demand enrichment means being able to acquire less time-intensive options, then burnout is reduced. For example, if staff usually packs treats and paper shreds into paper towel tubes, then a set of fill-from-the-back mounted puzzle foragers could help. In a fill-from-the-back forager, a foraging item mix is poured in from a bucket without ever opening the cage. Re-fill is walking and pouring as opposed to getting a team together to stuff tubes, and then opening animal enclosures to place or hang enrichment in the enclosure.

3D Printing Glossary

Basics for Understanding Animal Welfare Printing

This glossary provides general knowledge about 3D printing necessary to understand the rest of the report. This glossary was developed from talking to participants in a 3D printing-themed Veterinary Innovation Summit workshop held by the author. As a note, this report uses plastic acronyms, such as “PLA” which stands for Polylactic acid. Because 3D printing is driven by hobbyists, many sales and information websites use only the acronym of the plastic-type, not its chemical name. This can create confusion going between scientists and 3D printing options. To align this report with the way products are sold and information appears in online discussions, acronyms are used.

ABS - A strong plastic that has a lower melting point compared to other FDM/FFF filaments. Depending on the masterbatch, softens enough to be extruded around 230 C. The material is hygroscopic, meaning it absorbs moisture from the air. After too much exposure to humidity, the filament will print more poorly, forming strings, but can be stored unprotected on a shelf for a few weeks in most climates. When heated, ABS filament gives off fumes that can cause adverse reactions in human beings, such as headaches. Printing systems designed for ABS filament will include a closed chamber with an air filter, designed to filter out irritating molecules. Test with any high-temperature dishwashing process for durability after several wash cycles, in the Author’s experience, some batch types can weaken with extended water contact but as it starts out as a strong, durable material, weakening can often have a negligible effect.

Annealing – Putting a completed print in an oven and running a specific heating and cooling cycle. Annealing is done to improve the strength, surface appearance, and water tightness of prints. Developing one’s own annealing process for a given filament + printer combination takes tweaking an existing method through repetition and testing until the method works at your site. If done incorrectly, annealing can cause “off-gassing” where plastic gives off fumes, which can be dangerous. Annealing is usually optional but is a required step but is required for unusual specialist filaments, such as PPS.

Bed adhesive – A chemical to help the first layer of filament stick to the printer bed. Glues can range from non-toxic, such as Elmer’s brand gluestick to a variety of chemicals that should probably not be in direct contact with an item that will have animal contact.

FFF/FDM - A type of 3D printing. Fused filament fabrication (FFF), also known under the trademarked term fused deposition modeling (FDM) owned by the Stratasys company. A general term for printing using a roll of filament that is heated, extruded, and the extrusion laid down in layers onto the print bed to build up an object. Most often refers to printing in plastic

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though specialty approaches to printing concrete, chocolate, wax, clay, and glass have also been called FFF. FFF is widely utilized by hobbyists and professionals. There are variations on FFF, such as the use of pellets instead of filament, which allow for faster print speeds of larger objects but these are not yet mainstream. FFF is different than forms of printing, of which there are several, such as sintering powder into a solid (selective laser sintering, SLS), spraying and hardening liquid droplets (polyjet), or solidifying resins with ultraviolet light (Stereolithography printing, SLA).

High-temperature filament – Filaments that have useful mechanical and chemical properties but require being printed with expensive specialty printers with heated chambers and certain supplies, such as bed glue. Not necessary for making useful 3D prints. Filaments include PEEK, CFPEEK, ULTEM brand filaments, PSU, PPS, CFPA6, PEI, PPSU, and others. As the VisionMiner.com Frequently Asked Questions page states, “Is High Temperature FDM easy? No.” The difficulty can be made worthwhile by properties of the material, such as 3XDtech.com’s advertisement that “PPS is completely insoluble in any known solvent under 200°C”.

Masterbatch - The filament made from the base material (i.e., PLA, ABS, PETG, PVB) with additives to turn the base plastic into a printer filament. Additives determine the properties of the final filament. Additives can include elastomers to make the material flexible, preservatives to keep the material shelf-stable, colorants, and more exotic additives to allow for special effects. It is important to remember that the base material may be non-toxic but the masterbatch may have additives that are dangerous for animals or could be unexpected.

A “PLA” filament would seem like a good choice for animals as PLA is used for food containers and in small amounts, breaks down into harmless lactic acid if ingested. However, PLA is the “base” material, the filament contains more than PLA. For example, the Polymaker Company provides detailed information about their filaments upon request. In the information provided to the author, the Polymaker company used BHT (butylated hydroxytoluene) as a preservative. One would not expect BHT in pure PLA plastic. Despite being advertised for youth and hobby projects many filaments’ dust is probably unsafe to breathe, chew on, have extended skin contact, or ingest due to additions to the masterbatch. Surprising additives include glass beads, carbon fiber, light-reflective fibers, wood pulp, and toxic pigments.

Natural or virgin filament - Masterbatch without any colorants. Useful for applications where the total number of additives is minimized.

PEEK – A high-temperature filament that does well through multiple autoclave cycles, one of the most popular high-temperature filaments. However, it is difficult to print, requires higher-end printers, and is not commonly utilized, so the total available knowledge about using the material for 3D printing is low. Part of a class of “high temperature” filaments.

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PETG - A strong, waterproof plastic that is useful for outdoor and underwater applications. Before printing, the roll of filament is greatly hygroscopic, which means it will absorb moisture from the air, then print improperly. If not used soon after opening, PETG filament rolls are stored in a “dry box” sold by the supplier to prevent exposure to humidity. Can be oven-dried to reduce moisture content prior to printing if improperly stored. More difficult to print with as it requires higher printing temperatures and a heated bed, meaning the 3D printer is more expensive. Depending on the masterbatch, PETG softens enough to be extruded at 240 C. Test with any high-temperature dishwashing process for durability after several wash cycles. **At time of writing, can be purchased as food-grade certified from the Filaments.ca company. This material will off-gas toxic fumes if accidentally autoclaved.**

PLA - Polylactic acid. A plastic made from a carbohydrate source, so sometimes called “corn plastic” in reference to being made from processed maize. Pure PLA breaks down into lactic acid and can be safely composted in an industrial composting facility where high temperatures can fully break it down. An extremely common and easy-to-use base material for 3D printing. Depending on the masterbatch, softens enough to be extruded at 210 C. Expected to melt in sanitizing dishwasher cycles, typically balloons out in a high-temperature dishwasher as air trapped inside the model is heated. **At the time of writing, can be purchased as food-grade certified from the Filaments.ca company.**

Printer bed – Also called a base plate. A sheet of material under the printhead. Various bed types include BuildTak brand, stainless steel, glass, carbon fiber sheets, or PEI (often Kapton brand tape) sheets. Some people cover their build bed each print with a layer of masking tape to avoid wear and tear on the bed material. Adding a disposable “blue tape” also increases filament adhesion without adding a bed adhesive and creates a pull-away layer to allow for easier printed part removal from the bed. A flexible built plate, such as a metal plate with a BuildTak sheet allows easy “popping” of prints from the print bed. A solid, perfectly smooth bed, such as glass, can make object removal very difficult. Solid bed removal can require metal scrapers, a glue stick bed adhesive layer and soaking, or occasionally breaking the glass in the process of removing the object. A “raft” can make removing a flat-bottomed object from a non-flexible bed feasible.

PVB - A plastic with similar properties to PLA, that is soluble in isopropyl alcohol. This allows the plastic to be surface smoothed in an alcohol vapor or spray chamber, creating a less porous surface with a commonly used and relatively safe chemical. Depending on the masterbatch, softens enough to be extruded at 220 C. The Polymaker company produces an inexpensive vapor chamber called the “Polysher” to go with their Polysmooth line of PVB filaments. In the author’s experience, it can take weeks to months for the Polysmooth PVB surface to become completely hard after surface smoothing. Items to be treated should be made in bulk, well ahead of time. Once fully re-hardened the items are quite durable around small animals.

Raft – Loose layers of material connecting the object to the print bed, created in a slicer software. The use of a raft can protect fine details from being torn away during bed removal, or deformed by being printed directly onto the bed, squishing the first layer or two. The use of a raft also prevents direct contact of the 3D printed object with bed adhesive. When using bed adhesives that have an unknown safety profile, a raft will prevent the object from absorbing and transferring the glue's chemicals to an animal. Rafts also allow for flat-bottom objects to be more easily removed from a solid print bed.

SLA - Stereolithography printing. The print is created by shining light at the bottom of a tray of liquid resin and the solidified resin is lifted out of the tray. A very popular hobbyist and professional type of 3D printing that is widely adopted. This method of printing was not utilized for this report as there are (1) a more limited number of materials, (2) many prints are delicate, (3) there are serious toxicity concerns for any uncured resin in the print, (4) some resins break down and become yellowed and sticky months or years after printing, making the longevity of parts an issue. There are biocompatible or exceptionally strong resins available but the cost is currently higher than FFF plastics. **Due to the risk of giving animals under-cured and thus toxic SLA, this kind of printing is not utilized in this report.**

Slicer software – A computer program that generates printer instructions, called GCODE, from a 3D model. Popular slicing software titles include Cura, Simplify3D, and ideaMaker. Slicers determine layer height, speed of the print, rate of extrusion of the filament, and other details. The slicer software adds features to help the print that are not found in the STL model, such as support material to hold up parts of the printed piece so there is no drooping during the print. Or a skirt connecting the part to the print bed to help small parts adhere to the print bed, rafts to separate the printed piece from the build plate for easier removal, and wipe towers that help clean the printhead between colors.

STL - A filetype that contains information about the triangles that make up a 3D model, and to a limited extent, some versions of STL record the color of those triangles. The more triangles in a model, the larger the file size. A good 3D model for printing does not contain smaller details than the printer can produce. Many microscopic details are just a waste of computer memory and may make the final surface bumpy. It is possible to create an overly complex model (such as one >100 megabytes) that takes a very long time to download, process and causes errors in the printing process. There are new formats that have special features that are better suited for 3D printing, such as 3MF, X3G, and OBJ. However, STL is the most popular file type for consumer printing. STL can be referred to in several ways, often referred to as "Standard Triangle Language", or "Standard Tessellation Language" and Occasionally, "STereoLithography" according to the Library of Congress https://www.loc.gov/preservation/digital/formats/fdd/fdd000504.shtml.

UFP – Ultrafine Particles, those below a micron in diameter. Fine dust produced in FFF/FDM printing can interact with organisms' lungs, skin, and eyes, causing irritation or toxicity. Can be reduced by enclosed printers with true HEPA filters, for example, MakerBot company's

Method filter system or the Raise3D company's activated charcoal HEPA filtration system. Keeping the printer in a room with ventilation reduces the concentration of UFP personnel contact. Wiping down the inside of the printer and rinsing finished prints can carry away UFP that has settled upon surfaces. According to the US EPA, Ultrafine particles that go deeply into the lungs can enter the bloodstream (https://www.epa.gov/pmcourse/particle-pollution-exposure).

VOC - Volatile organic compounds. These are carbon compounds that decrease air quality and have been shown to be produced by the filament heating and extrusion process (Zhang et al, 2017; Zhang et al, 2018). A well-known example is formaldehyde. These chemicals can be concentrated in indoor air. They are defined as air pollutants by the US Environmental Protection Agency (https://www.epa.gov/air-emissions-inventories/what-definition-voc.)

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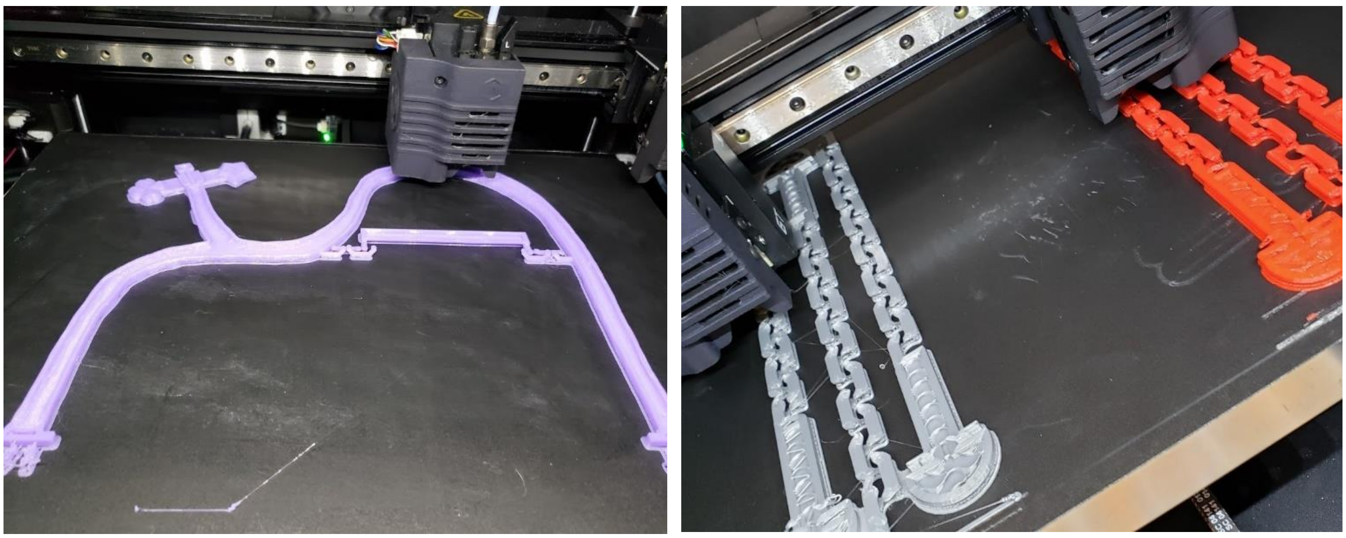
Author’s Use Case Examples

As this author works with birds, these examples are from bird projects before support from AWI. There are a variety of facilities using 3D printing for animal welfare and these use cases are only a few of many possibilities.

Figure 2.

Print-in-place designs to create complex activity areas. Print-in-place designs require little to no assembly but possess multiple parts. Left, a halfway printed activity station bow perch with a built in spinner manipulation toy and a built in swing on in the upper right. Right, bulk printing chain and perch combinations to hang inside the bow perch activity stations.



Specialty mounts for perching. Left, a pop-on natural branch holder to allow for natural chewing and bark stripping behavior. Right, a bird utilizing a natural branch perch, note evidence of healthful gnawing behavior.

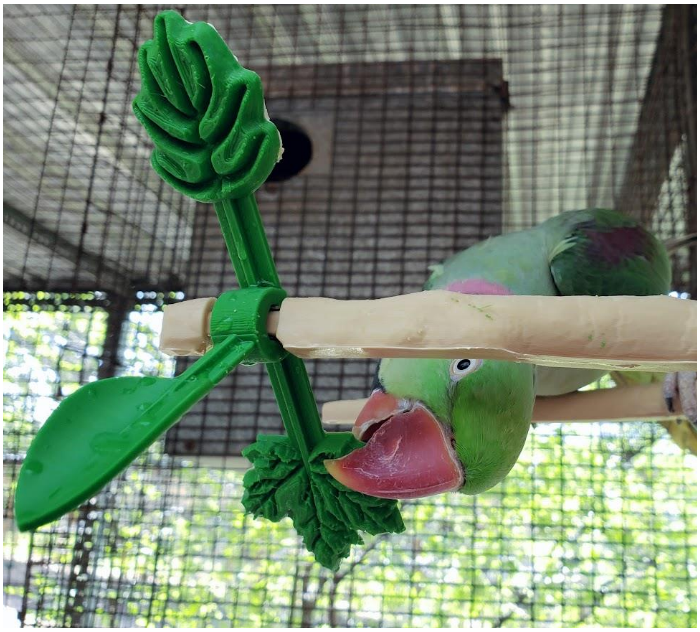
Figure 3.

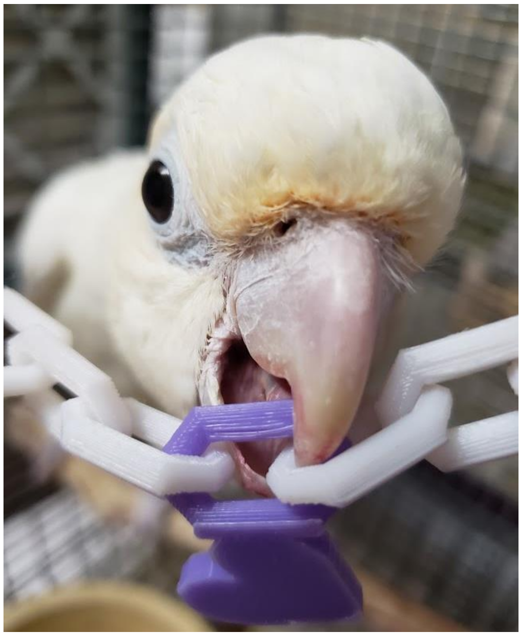
Custom data logger housings. These egg-shaped data logger shells allowed for data collection during nesting season without being intrusive to the animals.

Tablet holders. Customized to different types of enrichment and research. Left, a battery holder, feeder devices, and tablet holder mount for automated enrichment. Right, and external, adjustable holder for recording cage activity and displaying stimulation.

Figure 4.

Hanging interactive manipulatable toys. Left, a “spinner” toy on a Y-shaped perch allowing for acrobatic play. Right, colored charms on a chain allow for manipulation and texture play. Both were printed in place, requiring no assembly.





Cage furniture items. Left, funnel to deliver treats and novelty items without opening the door or contacting the bird. Right, custom end caps to more easily hang a textured PVC pipe swing.

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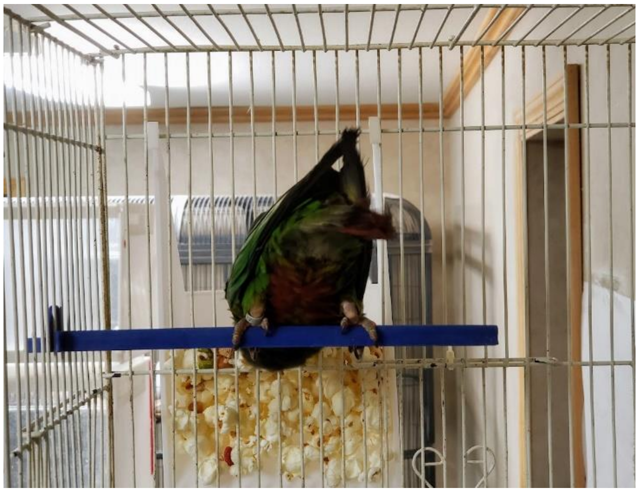
Figure 5.

Custom equipment to solve care problems. Left, a device to correct a severe underbite in a nestling age cockatoo. Right, a silver-colored bucket caddy to allow staff to organize feed out more effectively during outdoor colony maintenance, using dishwasher-safe food containers to improve hygiene.

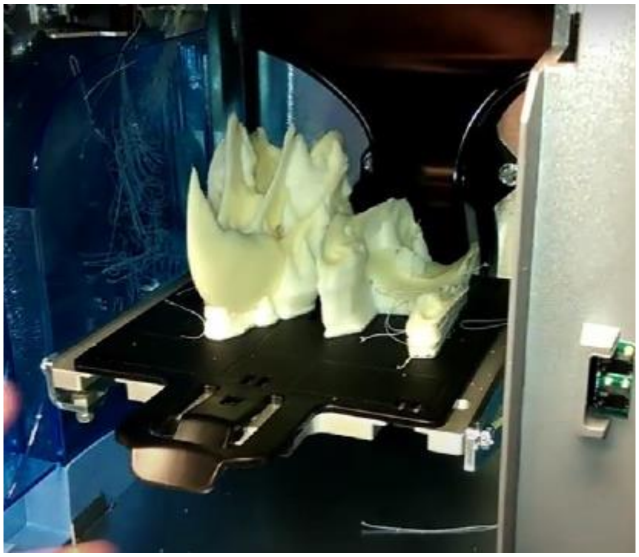
Manipulatable loose toys for carrying. Left, a translucent rattle with a brightly colored bead printed inside. Right, algae wafer foraging puzzle for the office fish, who is an honorary bird. The fish must fight gravity to access food.

Figure 6.

Foraging puzzles. Plastic hampers to turn powder-coated cage wire into foraging puzzles. Printed in strong PETG and virgin ABS plastics for durability. Left, note the blue 3D printed PLA perch to create a foraging station.



Training models for staff workshops. Left, ABS parrot skull embedded in wash-away support inside Stratasys Mojo Printer, used for training safe restraint. Right, hollow 2mm thick false shell, ABS plastic, holding up metal weight to illustrate the plastic's strength.



General Safety When 3D printing

Safety concerns for Humans

During printing, printers can release ultrafine particles and volatile organic compounds, called VOCs, into the air. The first controlled studies in this area are recent, such as Zhang et al (2017) and Zhang et al (2018) for excellent introductory materials about the types and health risks of emissions. A general audience review of these studies can be found at the technology news site Gizmodo, https://gizmodo.com/new-study-details-all-the-toxic-shit-spewed-out-by-3d-p-1830379464. A more chemistry-oriented review of volatiles and electron microscopy of ultra-fine particles (UFPs) can be found at Chemical & Engineering News https://cen.acs.org/articles/96/i13/3-D-printer-emissions-raise.html .

MakerBot, a 3D printing company that helped develop initial air quality studies, has a video seminar (which also helps sell their air filter system) available at https://www.makerbot.com/stories/engineering/pro-webinar-how-to-3d-print-in-a-safe-environment/ .

Undesirable emissions are related to the temperature of the nozzle and the filament being utilized. A rule of thumb is the hotter the nozzle, the more emissions. Research is ongoing. For example, while both ABS and PC filament emissions should be avoided, in lab tests PC gave off more particles than ABS (Farcas et al, 2019).

The additives that change a base plastic, such as ABS, into a masterbatch of filament, such as MatterHackers ABS+, can change without notice. It is not always known what gasses or particles will come out of the nozzle during printing. Additives include preservatives, elastomers, and other chemicals. For example, carbon nanotube additives to a filament were associated with an increase in the most hazardous VOCs (Potter, Al-Abed, Lay & Lomnicki, 2019).

While occasional exposure is likely not problematic, a worker who has a printer in their office is going to be more at risk of chronic exposure to 3D print emissions than someone who only comes into a printer room to pick up finished objects. Online discussion forums, such as Reddit's 3DPrinting forum, https://www.reddit.com/r/3Dprinting/, note headaches in response to ABS plastic printing.

When setting up a print space, The National Institute for Occupational Safety and Health (NIOSH) has a useful safety poster to hang near the printer to help people think about 3D printing safety https://www.cdc.gov/niosh/docs/2020-115/default.html.

Primary Safety Concerns for Animals

When making custom items, a caregiver can provide new types of enrichment complete with new types of hazards. Talking through ideas with other enrichment specialists can help identify dangers. Animal safety and 3D prints relate to mechanical and chemical hazards.

Figure 7. Left, chewing on a soft PLA plastic has resulted in likely ingestion of fine particles worn away from the chain links during chewing play. Right, a molded set of plastic chain links from a harder plastic. Despite equal time in an animal enclosure, much less plastic has been chewed away during manipulative play due to hardness. The injection-molded yellow chain was subsequently left outdoors for a year to show grime build-up over a long period. Compare the yellow chain to the PLA chain in Figure 12.

Mechanical hazards include entanglement with thin, flexible filament strings, choking on parts that can be broken off and swallowed, inhalation of dust from chewing or scratching a print, getting a toe or head entrapped within a ring or hollow shape, intestinal blockage from ingestion, toxicity from ingestion, and blunt trauma from swinging or falling items. Sources of mechanical danger can come from poor 3D printing practices. The selection of soft, brittle plastic for a heavy chewing animal will lead to ingestion, or the object breaking down into swallowable, small pieces. A strong plastic that is printed into a thin, object will be broken apart as though it were soft plastic because durability requires multiple printed layers of material.

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Figure 8. Incorrect printer settings can cause layers to loosely adhere. Here, the height between layers was too great and the layers could be peeled apart, creating an entanglement hazard and intestinal impaction hazard. Left, An extreme close-up of loosely adhered layers, with visible separation of the layers. Center, the short pieces of extrusion peeling away could perforate the gut create an intestinal impaction. Right, the long, unadhered extrusions peel away from the object creating an entanglement hazard that could snare an animal.

Chemical hazards include irritation caused by chemicals that irritate the surface of the eyes, skin, and gut, as well as physiological changes or toxicity caused by chemicals entering body tissues. For example, non-toxic bed glue on the surface of a print might cause temporary chemical irritation to the eyes but have no long-term health effects. A plastic that contains heavy metals, BPA, and other endocrine disruptors could cause issues in growth and reproduction. In the human-built environment, there are “everywhere chemicals” that animals’ bodies are always exposed to due to our habits and the materials we use to construct the built environment. I have known a lab where daily use of a cleaning agent was associated with low fertility of mice, similar to the findings Melin et al (2014). A plastic that contains a toxic colorant or additive could cause poisoning and damage to the liver, kidneys, brain, and other organ systems, if the animal is chewing on, licking, or drinking out of the plastic.

Chemical risk is harder to manage than mechanical risk as we cannot visualize the potential risk by looking at a product. A soft plastic that has general safe ingredients to chew on in its virgin masterbatch recipe sheet might have a toxic dye for coloring when colored green, for example. This is why the author prefers using the Filaments.ca company’s certified Food Safe colored PLAs and PETG products for toys that will be chewed or have food contact, to reduce the risk of dye toxicity. For aquatic applications (see Figure 5), I soak 3D prints in a slightly acidic, warm water bath for a few days before use with aquatic animals. That way, if there is any initial heavy leeching from the plastic into the water, the worst of the leeching will occur in the bath prior to use in an aquarium or pond. I tend to use uncolored, virgin filament for aquatic applications.

As a reference point for discussing vertebrate exposure to chemicals, this author looks to the United States or European Union regulations for items that have contact with the mouth and mucus membranes or are likely to be ingested. Useful categories include toys with modeling clay and baby pacifiers regulation. In the United States, the Consumer Product Safety Commission is an excellent starting place to understand minimizing chemical exposure, such as https://www.cpsc.gov/Business--Manufacturing/Business-Education/Business-Guidance/Pacifiers-Business-Guidance. While these safety guidelines apply to human biology and may not be relevant for other species, we can start with human/primate safety to talk about this new technology.

The PolyMaker manufacturer provides extensive datasheets about their products upon request. Other groups may provide partial or not batch information. Manufacturers can treat their ingredients as trade secrets, which is a legitimate practice in a high competition industry. A popular company, Fiberology, would not explain what they use to create metallic effects in their “silk” effect filaments and thus, I was unable to evaluate that material’s safety for use with animals.

Introduction to Optional Surface Finishing

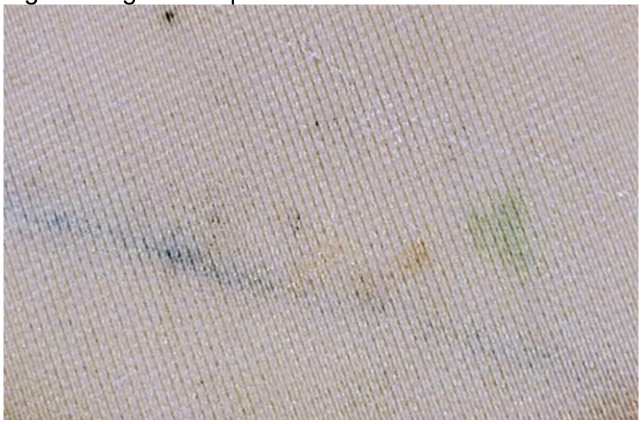
3D Prints and Surface "Finishing"

Finishing is the process of changing the surface of the print. This process includes removing excess material such as a skirt that holds the print to the print bed, the raft that raises the print from the print bed, or support material that keeps the prints from drooping during printing.

One step of finishing is treating the print's surface. There are multiple types of surface finishing. The most basic surface finishing is sanding. Sanding a print is very time intensive and generates plastic dust that reduces air quality and may irritate eyes and skin. Sanding is generally inappropriate for an animal facility.

Why finish surfaces?

Figure 9. This is a 5x zoom photo of a PLA print made for this report. The print was used as the cap of a water reservoir for several months. The black line is a colony of mold growling along a flaw inside the print. The green and orange patches are colonies of algae living in the spaces between extrusions.



A smooth surface is easy to clean, allowing for the removal of grime and pathogens by soaking and wiping. The textured surface of an FDM/FFF print traps feces, urine, and food residues. This can be partially remedied with a scrub brush and soaking but total cleaning requires additional and effort. In prints that are not watertight, organic material and water will infiltrate deep into the print, creating a habitat for microbes and fungi. A finished surface is more resistant to water infiltration, reducing grime to a more manageable surface problem.

The 3D printed object is not like vitrified glass, meaning fused solid in heat any without gaps. The plastic has many small pores and cracks in addition to gaps between layers. 3D printed objects are generally going to catch and retain some amount of organic material and moisture compared to glass, ceramic, and metal objects.

The Polymaker company explains that even a solid appearing object "[. . .] might have micro-cracks and holes which are invisible to the naked eye even though the prints look smooth. Bacteria are prone to grow in these micro-cracks and holes and thus we do not recommend using the printed parts in food contact applications." https://polymaker.com/polyterra-pla-info/.

Types of surface finishes

Another kind of finishing is annealing. In annealing, the printed object is placed in a calibrated oven for a limited amount of time, melting the layers together more tightly and changing the surface. Annealing takes a lot of experimenting until the process is done right. A good annealing workflow is extremely useful but can be difficult to exactly replicate from facility to facility. If annealing is performed incorrectly, some plastics can produce toxic fumes or secondary species of unexpected and unwanted chemicals when overheated.

Flame polishing is another type of finishing. In flame polishing, an object has the flame of a hand torch passed over the surface, briefly and intensely heating the surface to smooth it. This is a hit-or-miss process and often can scorch or ruin a print.

Tumbler polishing involves placing the print in a rolling canister a vibrating bed, filled with polishing media. This may take two steps, with a coarse and then a fine media. The size and shape of the tumbling media is based on the smallest details of the print. The tumbling media must be able to get inside small details of the print in order to polish them. Tumbling can take more than a day, sometimes two days, depending on the process selected. I suggest Kramer Industries, https://www.kramerindustriesonline.com/, for setting up a tumbling workflow. Large vibrating tables are expensive, as is media. The tumbling media can last a long time after purchase and Kramer was willing to explain the whole process, in detail, over the phone.

Like tumbler smoothing, bead blasting involves media contacting the surface of the print. Bead blasting, similar to sandblaster, uses plastic beads to even out the surface. Sand blasting can create a pitted, rough surface effect, while bead blasting may produce a more smooth matte finish.

Figure 10.

Surface smoothing of PVB prints. Left, a normally printed surface. Right, a similar print that has been smoothed by 90% isopropyl alcohol mist for half an hour.

Original, left. Tumble polished, right. Used with permission, from www.kramerindustriesonline.com/tumbling-3d-printed-plastic-parts

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The least time-intensive form of surface finishing is chemical smoothing. The 3D print is exposed to a bath or vapor of a solvent, which causes the surface to flow. The print is removed from the solvent and the solvent is allowed to off-gas, leaving behind the plastic object. As an example, here is a beginner's guide to vapor smoothing from the All3DP educational website https://all3dp.com/2/abs-smoothing-a-beginners-guide-to-abs-vapor-smoothing/.

The safety issues with associated chemical surface finishing are exposure to chemicals, potential fire hazards from creating concentrations of flammable vapor, retained solvent within the object, accidental creation of secondary species during chemical smoothing, and giving an object to an animal before the solvent has completely off-gassed. If the object remains in contact with the smoothing chemical for too long, the object may deform or completely melt into a solution!

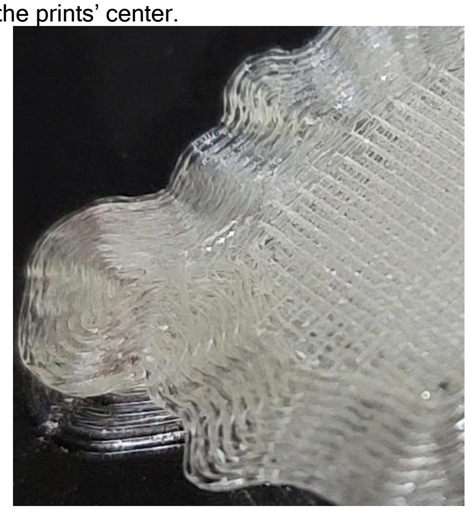
A solvent used in this report, DipSmooth-100 by Reliance Specialty Products, had a concerning number of warnings on the material safety data sheet that came with the solvent. I utilized it in an outdoor area with high airflow, goggles, and thick chemical-resistant gloves. The Dip-Smooth also ate its way deep into the prints if left in the bath for more than a few seconds, deeply saturating the print. However, the product was extremely effective and fast, allowing for rapid chemical smoothing. Adding a specific solvent into a laboratory 3D printing process should involve consulting with a chemist to identify if there are risks to a certain masterbatch and solvent combination, making sure the chemist understands that a masterbatch can include surprising ingredients. It may be necessary to analyze samples to be sure a solvent fully leaves the filament, without leaving behind unwanted chemicals.

Off-gassing can take a few minutes or several months depending on the combination of plastic, solvent, and the duration of contact. Checking the 3D printed object's hardness by pressing the tip of a tool into the object and wafting the air around the object to detect an odor of solvent are ways to check if a chemical smoothing process is complete. This author noted that PolyMaker Polysmooth material did not fully regain its hardness, using a fingernail depression test, for several months after smoothing a batch of beads for a hanging perch play station at half an hour using 90% isopropyl alcohol. As parrots are strong chewers, this meant the toys were not available to the animals for more than three months. For non-chewing situations, the Polysmooth prints were suitably hardened within one week of smoothing.

Improving the surface quality through printing

3D prints can be watertight or even airtight, meaning the object provides a barrier to water and air. A true watertight print prevents the microbial colonies seen in the above photo. In industrial 3D printing, watertight plumbing parts are routinely printed and tested. The industrial approach would likely be out of the price range for most animal facilities. In the 3D printing hobby, watertight and airtight parts have been made without specialty setups. I corresponded to Mikey77, author of a watertight print tutorial on the Instructables website https://www.instructables.com/3D-Printing-Make-Water-Tight-and-Air-Tight-Contain/ during the creation of semi-watertight prints.

Figure 11. This is a print in progress. The multiple wall layers of this print are visible on the outside edge, while the basket-like infill pattern, which is not watertight, is visible in the prints' center.



The slicer software that turns a 3D model into instructions for the printer has changeable settings. To develop a watertight printing method for a specific printer model, it is best to contact the printer company and ask if they can e-mail a profile of watertight settings for your slicing software.

The general approach to creating a watertight print is to increase the extrusion rate of the "wall" filament to make thicker lines of plastic with fewer gaps between them. Additionally, several "wall" layers on the outside of the print, laid parallel to each other, help create a water barrier.

Slightly increasing the temperature of the hotend of the printer can make the filament more flowy and sticky, thus more able to seal gaps. Too much flow and too much heat will clog the nozzle, as the material creeps backward up the nozzle due to backpressure and heat. This "heat creep" will cause a clog inside the nozzle during printing, or as soon as the printhead cools. For this report, I increased temperature by 10 degrees Celsius, and increased the flowrate by 10% and adjusted those settings for each material until print quality was reliable and did not form bubbles on the surface when submerged in water for a few minutes. I removed the filament immediately after printing to prevent a clog from solidifying in the printhead.

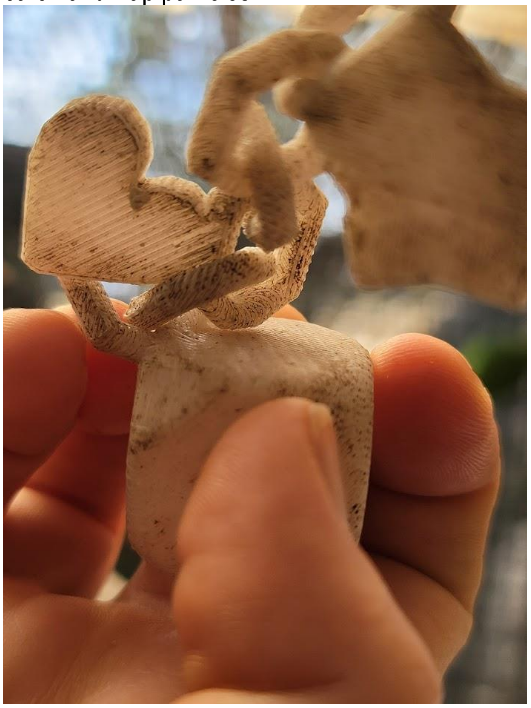
Another technique that may help with watertightness is called ironing. Ironing involves going over layers twice with the print head and extruding a small amount of material to fill gaps while the second pass of the printhead smooths the layer. Ironing each layer will double the print time and thus is not a good solution for producing items on demand. Not all slicing software has an iron setting, and some iron settings will only iron the top layers.

Sanitizing 3D Prints in the Laboratory

A cleaning standard for many facilities is autoclaving. Autoclaving is where 3D printed objects tend to be weak. Filaments may melt, produce dangerous chemicals, or become physically weakened by the autoclaving process. Sanitizing baths for low-temperature filaments, or high-temperature dishwashers for higher melting point filaments, may generally be better options than autoclaving.

The Stratasys 3D printing company worked with the University of Texas El Paso to characterize the effects on 3D prints of autoclaving, flash autoclaving, ethylene oxide gas, hydrogen peroxide gas plasma, and gamma radiation (Perez et al, 2012). The materials used were five ABS-derivative and four specialty filaments, PC, PC-ISO, PPSF, and Ultem 9085. The study examined how much bacteria was left behind by different sanitizing methods, as well as the damage to the 3D prints caused by the methods. Autoclaving left behind some bacteria, while all other methods, including flash autoclaving, were 100% effective at sterilizing the sample object. In the study, autoclaving and flash autoclaving were the most likely to damage prints, while gas, plasma, and gamma radiation created no visible damage. The study recommended using PC-ISO, PPSF, or ULTEM 9085, if autoclaving is desired.

Figure 12. A 3D print with an untreated surface, showing grime build-up. This chain toy was hung near an outdoor animal area for one year to show the problems of 3D printed textures, whereas the injected-molded chain in figure 7, which was hung for the same amount of time, does not catch and trap particles.



Minimizing water and chemicals absorbed

METHODOLOGY

A 3D model with a nubbly surface texture was selected. The shape was selected for increased surface area to maximize the opportunity for water absorption. Prints had six wall layers, six bottom layers, six top layers, and a semi-hollow infill. Prints of four types of materials were created, (1) Filaments.ca food-safe certified PLA, (2) Polymaker virgin PVB, (3) ZYLtech ABS, and (4) Filaments.ca food-safe certified PETG.

ZYLtech ABS was utilized as the author read that higher-end ABS filament with strengthening additives were less easily chemically smoothed. ZYLtech is a budget filament and did not have special additives for extra strength or chemical resistance. Other filaments were selected based on their reputation of high quality. For each material, regular and watertight settings were developed per filament type using a series of test prints. Once printer settings were established, a quantity of 10 objects was printed per factor, per filament type. Factors were (1) default print settings, (2) Watertight approximate settings, (3) chemically smoothed surface of a default print, (4) chemically smoothed surface of a watertight print.

Figure 11. Samples, center, in labeled bags with individual labels, next to a milligram scale. Left, a drying basket for suspending samples to dry after weighing.

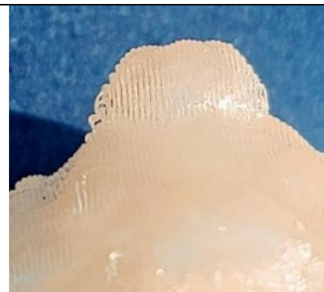
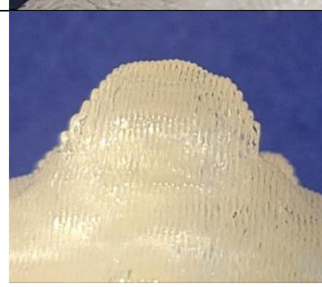
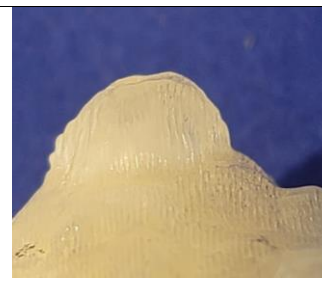
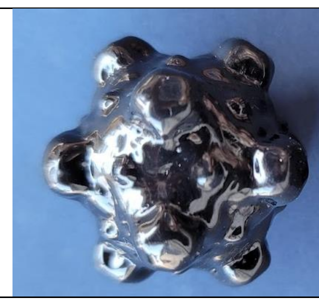
Chemical smoothing utilized one of three solvents, appropriate to each material type, per Table 1.

Prints were weighed on a warmed up US Solid brand milligram scale. Before and after each batch of weighing, the scale was checked against a calibration weight to assure milligram-resolution accuracy.

The prints were then cleaned and sanitized as though in a laboratory situation. As high-temperature dishwashing is not appropriate for some lower temperature 3D print materials, a chemical soak in sodium hypochlorite, Clorox brand household bleach, was selected instead. The sanitizing soak was based on Emory University's publicly available sanitizing SOP for high-level disinfection http://www.iacuc.emory.edu/documents/313_Sanitation%20of%20Research%20Equipment%20Used%20with%20Animals.pdf.

In batches, each print type was rapidly agitated with a scrub brush for one minute in 45.6C tap water to simulate hand washing under hot water to remove surface debris. Scrubbing was immediately followed by 12 minutes soaking in disinfectant. The samples were sunk into the disinfectant by covering them with an inverted plastic bowl. The solution was 10% Clorox household bleach, diluted in 21.1C room temperature reverse osmosis water. During the soak, the samples were shaken briefly to remove air bubbles from the surface. After the soak, each batch was removed and placed on a tray. One at a time, the samples were blotted and rolled on a dry towel to remove all visible surface moisture, and immediately weighed.

Table 1. Chemical smoothing for surface treatments.

Filament type	Solvent method	Before treatment	After treatment	Off-gas period
Filaments.ca FoodSafe PLA (uncolored)	DipSmooth-100 by Reliance Specialty Products, 2-second dip and swirl.			36 hours
Filaments.ca FoodSafe PETG (uncolored)	DipSmooth-100, by Reliance Specialty Products, 2-second dip and swirl in a strainer.			36 hours
PolySmooth PVB (uncolored)	90% Isopropyl alcohol fog, Polysmooth brand Polysher chamber, for 20 minutes.			1 week
ZYLTech ABS (black)	100% Acetone vapor in foil pan for 1 hour.			36 hours

RESULTS

Prior to washing, the variability between prints was examined to understand similarity across prints when creating identical items for the laboratory. Before chemical smoothing treatment, print batches varied from 1% to 3% from the average weight for that batch, even though all prints were from the same printer model, following the same instruction set, utilizing the same material. After chemical treatment, print batches varied from 1% to 15% from the average weight of the batch.

After washing and weighing, outliers were detected by graphing the factor groups for visual clustering. Only one visual outlier occurred. Out of 160 formal samples and 10 early test samples, only one had such a poor print quality that it had a leak in its wall and absorbed water into the internal infill. This outlier was a chemically smoothed PETG print. Likely, it had a flaw which the DipSmooth treatment opened up into a pore on its surface, melting a tunnel into the loosely filled inside of the part. This outlier gained 0.5 gm of weight during the test and was removed from the analysis. Of parts that completed printing successfully, this outlier represents a 0.59% part failure rate during cleaning.

T-tests for unequal variance were run to compare the weight change of the default prints against each treatment type, such as PLA normal versus PLA watertight. For each comparison, treated groups were all significantly different from normal, default print settings ($p<0.001$). Two of the treatment groups showed similar average change, so additional t-tests were conducted to understand if the small differences were significant changes in water absorption. Smoothed and smoothed watertight PLA's weight changes were not statistically different from each other ($p>0.1$). PETG's the smoothed and watertight samples were somewhat significantly dissimilar ($p<0.01$).

Table 2. Treatment per 3D Print and Average Change in Weight Immediately After Washing

Type	Treatment	Avg change (gm)	Type	Treatment	Avg change (gm)
ABS	Smoothed	-0.023	PLA	Sm. & Watertight	0.001
ABS	Sm. & Watertight	-0.014	PLA	Smoothed	0.001
ABS	Watertight	0.011	PLA	Default	0.092
ABS	Default	0.027	PLA	Watertight	0.294
PETG	Smoothed	0.001	PVB	Sm. & Watertight	0.002
PETG	Sm. & Watertight	0.003	PVB	Watertight	0.034
PETG	Watertight	0.028	PVB	Smoothed	0.137
PETG	Default	0.094	PVB	Default	0.302

The ABS plastic samples that were chemically smoothed all lost weight after washing and soaking. The ABS weight losses were in excess of the scale's variability when weighing the same object repeatedly, suggesting a genuine but small loss of mass during washing, despite the opportunity for water absorption.

DISCUSSION

Generally, a watertight or chemically smoothed surface absorbed less water during washing and sanitizing. A watertight and smoothed print may be "overkill" as those treatments yielded similar results to smoothing alone. Smoothed prints may outperform watertight prints for absorption but require a chemical bath or vapor set up and off-gassing time. A watertight print may be less effective than a chemically smoothed print but it is a much simpler process. Less water infiltration is good for animals. Less water absorbed means less moisture and organic material for microbes. Less absorption of cleaning solutions means less buildup of cleaning chemicals as solutions evaporate. A chemically smoothed print is much easier to surface clean with wipe downs where visible grime is unacceptable and may increase the risk of pathogens.

The loss of mass in the smoothed ABS plastic is not explained. The ABS weighings were mixed in with some samples from other types, in mixed batches. The other sample types did not follow the weight loss pattern seen in ABS. Combined with satisfactory pre and post-batch calibration checks on the scale, this indicates actual weight loss in the vapor-smoothed ABS despite being wet. One option is that the ABS had absorbed moisture from the air and that moisture was driven off during chemical smoothing. Perhaps some element of the masterbatch volatilized and was lost to the air during smoothing? Alternatively, it may be that the ingredients in the masterbatch for this particular ABS were not as stable as expected and treated material was subsequently shed or scraped away during washing. While negative weight loss occurred sporadically in other sample groups, the majority of other negative results were a -0.001 difference, which are probable scale errors at the limit of the scale’s accuracy. Mass loss associated with cleaning of vapor-smoothed 3D printed ABS plastic should be tested across multiple brands to see if this situation is unique or to be expected, and if the cause is harmless or of concern.

It is not known if the lost plastic mass could end up in an animal’s gut or mucus membranes, despite the prints appearing to have hardened after smoothing. The Polysmooth prints were still slightly soft after off-gassing for a longer period but did not lose mass during washing.

The PLA absorbed more water when printed at watertight settings. While at first, this seems counter-intuitive, it may be because the increased amount of extruded material created tiny, drooping loops on the underside of the part. In Table 1, row 1, the before photo of the PLA shows droopy layers not seen on the other prints. It may be important, with such a low melting point filament, to tightly “dial in” the temperature so the layers are as well-adhered as possible while still being maximally sticky and melted. Increasing temperature and flow in the PLA as much as I did may have been overkill, creating external drooping layers areas that captured water despite a thick solid internal wall to block water from reaching the print’s infill. In the future, I would use closely fitting support material to prop up the underside of a PLA print, or only watertight print PLA that did not have overhangs to the shape, to avoid drooping. When the surface loops were chemically smoothed, the weight gain associated with watertight PLA ceased to exist.

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Metal Deposition by Printers

Presence of Heavy Metals in 3D Prints

A common refrain on 3D printing advice forums is that the metal in the nozzles and the printhead could contaminate a 3D printed object during printing so it is not known when items are “food safe”. The contact with metal occurs when the filament is squeezed and pushed by a gear system, which may or may not be metal, into a metal tube or “throat”, which then leads into a metal extrusion nozzle. This experiment was carried out to understand if printers added meaningful amounts of heavy metals to 3D printed objects. The experiment included a duplication two printers from the brand and duplication of filament material from two rolls of the same brand to start examining variance within brands.

METHODOLOGY

To test this potential issue, a commonly used test shape used in 3D printing, a toy boat called a “benchy”, was printed on six different printers across three 3D printer lab facilities. The “benchies” averaged 12gms each. Each print ran for approximately an hour and a half. All test prints were made from one of two rolls of filament, Esun brand PLA, in white. Two rolls were needed as different diameters of filament are utilized by different printer models. Esun is popular for printing labs and was readily accepted for use by each facility. Each printer had been already heavily used for production work, representing what to expect from a working printer making many objects.

The models of printer that utilized 1.75mm filament were the Prusa MK3S with stock parts, Dremmel 3D20 with stock parts, Raise 3D E2 with a Raise3D brand premium hardened nozzle, and an Anycubic Chiron with stock parts. The model of printers that utilized the 2.85mm filament spool were the Ultimaker 3 with stock parts, and Ultimaker S5 with stock parts.

The five printed “benchies” and a control sample of both unprinted filaments, left over after all the prints, were analyzed for total metals by ATS laboratories, https://atslab.com/, a group that provides toy safety testing for manufacturers and has specialty knowledge in testing plastic toys for contaminates. ATS is Consumer Product Safety Commission (CPSC)-Accepted Testing Laboratory. This means that under Section 14(a)(3)(E) of the United States Consumer Product Safety Act, ATS is included in up-to-date list of entities that have been accredited to assess conformity with children's product safety rules.

The samples were placed in plastic food storage bags, labeled, and mailed to ATS Labs. ATS was agreed to use pressurized air to blow any environmental dust from the surfaces of the prints before testing.

RESULTS

Figure 12. Full heavy metals report from ATS (summarized with ASTM standards below).

Applied Technical Services

CHEMICAL TEST REPORT

Ref. 351210-1 **Date** March 30, 2021 **Page** 1 **of** 2

Customer: Texas A&M University, 400 Raymond Stotzer Pkwy, #4467, College Station, TX 77843

Attention: Constance Woodman

Purchase Order #: N/A Part /Name: See Below

Material Designation: Various

Special Requirement: Samples prepared using acid hot plate and / or microwave digestion techniques.

Lab Comment: ICP atomic emission techniques utilized to analyze for heavy metals per ASTM E1479-16.

Test Results

Composition: (parts per million, mg/kg)

Identification	ATS #	Total As	Total Ba	Total Cd	Total Cr	Total Hg	Total Ni	Total Pb	Total Sb	Total Se	Total Zn
DREMMEL	1	<5	<5	<5	<5	<10	<5	<5	<5	<5	<5
RAISE3D	2	8	<5	<5	<5	<10	<5	<5	9	<5	<5
UM3	3	7	7	<5	<5	<10	<5	<5	11	<5	<5
UM5	4	<5	6	<5	<5	<10	<5	<5	9	<5	<5

As – Arsenic; Ba – Barium; Cd – Cadmium; Cr – Chromium; Hg – Mercury; Ni – Nickle; Pb – Lead; Sb – Antimony; Se – Selenium; Zn – Zinc

ACCREDITED
CHEMICAL TESTING CERT #1886.02

Prepared by: Digitally signed by Kyle Banyas Date: 2021.03.30 09:05:11 -04'00' K. Banyas
Chemist

Approved by: Justin Burmeister Digitally signed by Justin Burmeister Date: 2021.03.30 10:12:37 -04'00' J. Burmeister
Manager

ATS300, 05/2010

Applied Technical Services

CHEMICAL TEST REPORT

Ref. 351210-1 **Date** March 30, 2021 **Page** 2 **of** 2

Customer: Texas A&M University, 400 Raymond Stotzer Pkwy, #4467, College Station, TX 77843

Attention: Constance Woodman

Purchase Order #: N/A Part /Name: See Below

Material Designation: Various

Special Requirement: Samples prepared using acid hot plate and / or microwave digestion techniques.

Lab Comment: ICP atomic emission techniques utilized to analyze for heavy metals per ASTM E1479-16.

Test Results

Composition: (parts per million, mg/kg)

Identification	ATS #	Total As	Total Ba	Total Cd	Total Cr	Total Hg	Total Ni	Total Pb	Total Sb	Total Se	Total Zn
PRUSA	5	7	<5	<5	<5	<10	<5	<5	8	<5	<5
CHIRON	6	<5	<5	<5	<5	<10	<5	<5	<5	<5	<5
CONTROLTHIN	7	<5	<5	<5	<5	<10	<5	<5	8	<5	<5
CONTROLTHICK	8	<5	6	<5	<5	<10	<5	<5	7	<5	<5

As – Arsenic; Ba – Barium; Cd – Cadmium; Cr – Chromium; Hg – Mercury; Ni – Nickle; Pb – Lead; Sb – Antimony; Se – Selenium; Zn – Zinc

ACCREDITED
CHEMICAL TESTING CERT #1886.02

Prepared by: Digitally signed by Kyle Banyas Date: 2021.03.30 09:05:21 -04'00' K. Banyas
Chemist

Approved by: Justin Burmeister Digitally signed by Justin Burmeister Date: 2021.03.30 10:12:49 -04'00' J. Burmeister
Manager

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ATS300, 05/2010

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Table 3. Results Summary of ATS Heavy Metal Testing for “Benchies”

Sample	eSUN PLA white spool	Detected metals ppm
Filament only	1.75 mm	8 lead
Prusa MK3S	1.75 mm	7 arsenic, 8 lead
Anycubic Chiron	1.75 mm	None
Dremmel 3D20	1.75 mm	None
Raise3D E2	1.75 mm	8 arsenic, 9 lead
Filament only	2.85 mm	6 barium, 7 lead
Ultimaker 3	2.85 mm	7 arsenic, 7 barium, 11 lead
Ultimaker 5	2.85 mm	6 barium, 9 lead

Table 4. From ASTM Standard ASTM F963-17: Maximum Soluble Migrate Element in ppm (mg/kg) for Modeling Clays Included as Part of a Toy*

Antimony	Arsenic	Barium	Cadmium	Chromium	Lead	Mercury	Selenium
60	25	250	50	25	90	25	500

*This ASTM moideling clay toy standard is more stringent in its heavy metal limits than the general toy standard. As other animal species may be more sensitive to heavy metals, the more conservative of the two limits tables were selected for comparison.

DISCUSSION

Both eSUN spools had acceptable heavy metal concentrations when compared to the ASTM standard for human toys that include modeling clay. The toy with modeling clay standard is more stringent than the general toy standard as modeling clay is more easily ingested than solid toys.

During printing, tiny amounts of metals appeared to have been introduced into some samples. The small amounts could be contamination from the printer’s metal parts or dust in the printing facility, incorporated during melting and extrusion, or pre-existing contamination of that local portion of the filament, occurring during production of the filament. The lack of any lead in the Dremmel and Anycubic printers, when lead was found in both control samples, could be due to a lack of metal in that section of the filament, or an issue with the test’s accuracy. Loss of metal due to vaporizing of metal into fumes is unlikely as the temperature to extrude PLA filament is below the melting point of lead.

If human safety limits are acceptable for animals, then 3D printed objects from these model printers are within the most stringent ASTM limit for heavy metals in toys. Such objects are likely safe for primates, birds, rats, and other laboratory animals when it comes to metal content.

Endocrine disruptors in 3D printed filament

The Potential Chemicals in 3D Prints

In reviewing the kinds of health issues caused by exposure to plastics, disruption to the endocrine by BPA and Phthalates system was a concern (Warner & Flaws, 2018). These chemicals have been found in 3D printing filaments during 3D printer research (Gu, Wensing, Uhde, Satlhammer, 2019). For laboratory or pharmaceutical work, reducing the presence of these chemicals reduces potential confounding factors that could affect animal physiology. As much as possible, we apply the ethic of reducing the total number of animals needed in research. Reducing chemical contaminants can reduce the need for experimental replication due to confounding factors.

BPA, bisphenol A, is a chemical found in some plastics that mimics estrogen. There is ample research on the unwanted effects of BPA in rats and monkeys (i.e., Larsen, 2015). Other chemicals can replace BPA in manufacture, such as bisphenol-S (BPS) or bisphenol-F (BPF), which may cause similar health issues to BPA (Rochester & Bolden, 2015). Due to the ability for manufacturers to substitute BPA with similar chemicals, knowing if plastic is BPA-free is not a complete answer about this group of estrogen mimics. Knowing if BPA is not present is a useful starting point as it is historically common in manufacture.

A second group of chemicals of concern are phthalates. The health consequences of these chemicals are less well understood, as health problems can appear at very low doses rather than high doses, and the effects can be subtle. In animal studies, phthalates have had effects on animals' cardio-vascular systems (Mariana & Cairrao, 2020) and their reproductive systems (Habert, Livera, & Rouiller-Fabre, 2014; Weaver, et al. 2020). Phthalates can have antiandrogenic effects, meaning they block the messaging chemicals related to testosterone and affect sex characteristics and reproductive development.

Regulation and laws to limit BPA in humans include the banning of BPA from human infant cups and bottles, as well as the banning of BPA from adult and child drinking containers. The state of Washington has used a detection of 1ppm as a yes/no threshold to determine if a product contains BPA, using an LC/MS/MS test. Specifically, liquid chromatography-tandem mass spectrometry, following Environmental Protection Agency Method 1694. This same test was carried out by ATS laboratories on 3D filament samples.

In personal communication for this project, a polymer chemist indicated that they did not expect phthalates to be changed when run through the lower temperatures printheads common to tested 3D print materials, so measurements of masterbatch filament should represent the phthalates found in printed results and visa versa.

METHODOLOGY

Samples of 3D filament material were placed in plastic bags, weighed to make sure they exceeded ATS minimum requirements, and mailed to ATS Laboratories. ATS was requested to blop away any dust or surface particles with compressed air before testing. Tests for BPA and phthalates were selected based on recommended methods from the United States Consumer Protection Safety Commission (CPSC).

There are eight phthalates regulated in US law and identified for testing by CPSC for children's product testing. They are di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), diisononyl phthalate (DINP), diisobutyl phthalate (DIBP), di-n-pentyl phthalate (DPENP), di-n-hexyl phthalate (DHEXP), and dicyclohexyl phthalate (DCHP). Various literature support being cautious with these chemicals. For example, potent antiandrogens that can affect female animal development include DEHP, BBP, and DINP (Barakat & Ko, 2018).

Phthalates samples were prepared by ATS according to CPSC-CH-C1001-09.3 sample preparation method. The phthalates were quantified using gas chromatography-mass spectrometry (GC-MS). This test is used for the detection of phthalates in children's toys.

Samples of 3D filament were selected based on company reputations of high quality and reliable manufacture, across different filament types. Filaments.ca has worked to meet US food safety regulations and their food-safe certified virgin PLA and virgin PETG were tested. Matterhackers has an excellent reputation in the US 3D printing community, so their white ABS+ was tested. PolyMaker has a global reputation and provides excellent documentation about its masterbatch ingredients to consumers, their virgin PVB was tested.

Funded by the Animal Welfare Institute https://awionline.org/

RESULTS

Figure 13. Full report from ATS BPA, left, and phthalate testing, right. The product names have been added to sample ID codes for clarity.

APPLIED TECHNICAL SERVICES

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CHEMICAL TEST REPORT

Ref. 351210-3 **Date** March 24, 2021 **Page** 1 **of** 1

Attention: Constance Woodman

Materials Specification: N/A

Customer: Texas A&M University
400 Raymond Stotzer Pkwy, #4467
College Station, TX 77843

Test Methods: ATS 367 Rev. 1 – Analysis of Bisphenol A (BPA) by LC/MS/MS

P.O.# CC

Sample: See below

Test Results

Sample	Results: Bisphenol A parts per trillion (ng/kg)	Results: Bisphenol A parts per billion (µg/kg)
Filaments.ca Foodsafe PLA ALPHA.PLA	N.D. < 100 ppt	N.D. < 100 ppt
Filaments.ca Foodsafe PETG BETA.PETG	80500	80.5
MatterHackers ABS+ GAMMA.ABS	N.D. < 100 ppt	N.D. < 100 ppt
PolyMaker Polysmooth PVB DELTA.PVB	N.D. < 100 ppt	N.D. < 100 ppt

The samples were analyzed for Bisphenol A with a detection limit of 100 parts per trillion (ppt) ng/kg.
N.D. < 100 ppt – None Detected, less than 100 parts per trillion

ACCREDITED
CHEMICAL TESTING CERT #1888.02

Prepared by: William Carson, Senior Chemist

Approved by: J. Burmeister, Manager
Digitally signed by Justin Burmeister Date: 2021.03.24 12:44:59 -04'00'

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ATS302, 01/2010

Applied Technical Services

CHEMICAL TEST REPORT

Ref. 351210-2 **Date** March 30, 2021 **Page** 1 **of** 1

Customer: Texas A&M University, 400 Raymond Stotzer Pkwy, #4467, College Station, TX 77843

Attention: Constance Woodman

Purchase Order #: N/A Part #/Name: See Below

Material Designation: Polymers

Special Requirement: N/A

Lab Comment: GC/MS was used to identify and quantify the phthalate compounds according to CPSC-CH-C1001-09.4.

Test Results

Composition: (parts per million, mg/kg)

Identification		DPENP§	DHEXP§‡ (DnHP)	DCHP§	DIBP§	DEHP§‡	DnBP§‡	BBP§‡	DINP§‡	DIDP‡	DNOP
PETG	9	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
PLA	10	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
ABS	11	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
PVB	12	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.

*N.D. – None Detected <50 ppm: Di-N-Pentyl Phthalate (DPENP), Di-N-Hexyl Phthalate (DHEXP), Dicyclohexyl Phthalate (DCHP), Diisobutyl Phthalate (DIBP), Di-Ethyl Hexyl Phthalate (DEHP), Di-N-Butyl Phthalate (DnBP), Butyl Benzyl Phthalate (BBP), Di-Isononyl Phthalate (DINP), Di Isodecyl Phthalate (DIDP) and Di-n-Octyl Phthalate (DnOP)

§CPSIA Subsection 108(a) requirement for phthalates
‡CA Prop 65 requirements for phthalates

Prepared by: S. Tan, Senior Chemist
Digitally signed by Sze Tan Date: 2021.03.30 10:04:22 -04'00'

Approved by: Justin Burmeister J. Burmeister, Manager
Digitally signed by Justin Burmeister Date: 2021.03.30 10:12:08 -04'00'

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ATS300, 05/2010

The finding of 80.5 ppb in the Filaments.ca PETG would pass the Washington State total detection threshold of 1 ppm, as it converts to 0.0805 ppm. Even though BPA is detected in this sample, it was detected in very small amounts that can probably be considered negligible. BPA was not present in the other three samples.

The eight phthalates identified as those of highest concern by the United States and requiring testing by CPSC were not present in any of the four samples.

DISCUSSION

While these were small total n-values, there were no concerning concentrations of endocrine-disrupting chemicals in the four plastics types across three brands. These results are different than the common expectation that the plastics will have significant problematic chemical concentrations. Such expectations are often discussed on online forums, where most discourse about 3D printing occurs.

CONCLUSIONS

Based on the results of low to no endocrine-disrupting chemicals found, as well as low to no heavy metals found, I would be comfortable creating hutches, exercise devices, climbing structures, etc., from 3D prints that would be chewed on by animals and used around growing infant animals, especially if food-safe certified or virgin materials are used to reduce the risk of colorant toxicity.

In these results, heavy metals and endocrine disruptors were not present at concerning levels when they were detected. For short-term liquid contact, common materials used in 3D prints are probably fine. However, due to the texture of the prints, effort should be taken to avoid untreated, stagnant water contacting the part. Chlorinated water use, flushing clean water over parts before use, watertight printing, or event internal chemical smoothing of liquid contact parts are suggested to keep microbial colonies reduced inside the 3D printed parts. For brief liquid contacts, such as a pipe that carried water to a basin for drinking or bathing, this author would flush those systems for 30 seconds before use to clear any leached chemical and remove microbes or biofilms, then use the water in systems.

When it comes to food dishes, I would be comfortable offering dry, non-greasy foods in 3D-printed dishes of food-safe PLA, PETG, and non-food safe certified Polysmooth brand PVB. However, due to the ABS samples all losing mass after chemical smoothing, I would avoid ABS for food contact until the stability of different filament brands is more well understood. For food dishes or dispensers, watertight printing should reduce the penetration of moisture and particles into the plastic body, as well as regular sanitizing washes as the rough texture of 3D prints collect grime compared to injection molded plastics.

Chemical smoothing would further reduce the penetration of grime and water into the plastic in a food context, however, checking with a chemist to discuss the risks of solvents remaining in the plastic body is important. I talked to four polymer chemists before my chemical smoothing. All four assured me that heat-stable additives and base plastic in 3D filament masterbatch would be very stable and not break down. The prediction was that the solvent would leave the plastic behind as it evaporated as a volatile gas. Then, the ABS masterbatch that was chemically smoothed all lost mass during washing, which was not anticipated by any of the chemists, leaving me not entirely trusting of the process.

For animal chewing contact, my suggestion is PLA. PLA becomes weak over time in water, as it is broken down, hydrolyzed, by water. This weakening requires replacement as the parts weaken after repeated washing or time in a wet or humid environment. PLA is not appropriate for long-term submersion, as in this author's informal tests PLA became papery and delicate after about six months of immersion in an outdoor fishpond. Despite less durability, the ability of PLA to be digested by the body may suggest it is the most appropriate filament for animals that chew on their cage furniture. In a fly study, larvae viability and development appear affected only by very high, doses, when fed PLA nanoparticles as a dose of 0.5mg per ml

(Legaz et al., 2016). PLA, the base of PLA masterbatch, is considered so safe that it is the “material of choice” for biomedical implants, cell culture, and other uses (Saini, Arora & Kumar, 2016). Though, prolonged exposure to PLA-related chemicals can accumulate in animal organs and cause behavior change, (Chagas, 2021) so the goal should not be to expose the animals to plastic dust or encourage the ingestion of plastics.

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