

# The Effect of Polystyrene, High-Density Polyethylene and Polyethylene terephthalate on the Consumption of Plastic by Galleria Mellonella (Wax Worms)

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### Abstract

Though the demand and utilization of plastic are constantly increasing due to its various uses, like using them as disposable containers or plastic bags, these plastics have become a huge problem for the environment due to its non-degradable nature. All these plastics have toxic substances that cause water, land and air pollution, when left to accumulate in nature. The plastics accumulating on the land and in the seas, oceans or rivers is not only affecting the habitat but also the organisms that live in that habitat, especially the marine species that tend to ingest plastic assuming that it is food, which poisons the animals. This indirectly affects the human population as people might consume these fish or other marine animals which ingested these toxins. Some plastics might be degradable but only with the use of harmful chemicals-which have a negative impact on the environment as these chemical gases cause air pollution. Therefore, the use of living organisms, like wax worms, as natural decomposers for plastic, is very essential to balance the amount of pollution using natural biodegradation. This experiment tested how effectively wax worms would consume various plastics in an average span of 24 hours. The plastics that were provided to the wax worms were Styrofoam (Polystyrene), plastic bags(High-Density Polyethylene-HDPE) and Coca-Cola Bottle (PET-Polyethylene Terephthalate). These plastics are some of the most commonly used in the form of containers, cups, disposable plates, bags and many other forms, resulting in most of the human population massively depending on these plastics on a daily basis. Determining a natural way to decompose these plastics is very essential in order to protect the environment from the negative effects of chemical degradation or plastic usage alone. This would also help in eliminating plastic that has been accumulating on land and in water bodies for a long period of time. Organisms like wax worms are one of the best ways to approach this plastic pollution issue as they can be used in large scale quantities to decompose various plastics without any sort of toxicity returning to the environment. This experiment paves the way for further research focusing on the best suitable environment for these worms in order to degrade plastic, to test whether it is feasible to handle this in a large- scale production.

### Introduction

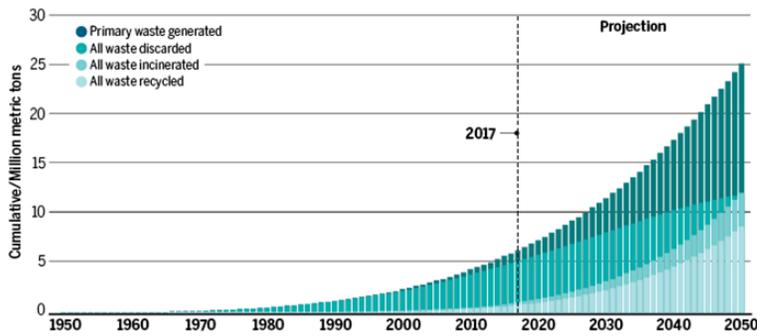
By the year 2030, approximately 26 billion metric tons of plastic will have been produced by humankind based on research conducted about plastic waste (Guglielmi 2017). All plastics that are being produced are not completely compostable or recyclable. This means that the plastics are being left on earth, accumulating on land and in bodies of water. These wastes are not only harmful to the environment, but also to the living organisms including humans. The author of "The Seven Types Of Plastic and What They Mean To Your Health" says, "... BPA, the highly toxic chemical found in plastic, is linked to obesity, cancer, and endocrine problems in fetuses and children..." (" The Seven Types of Plastics," 2017). The types of plastics vary from a range of 1-7, in which plastics from 1, 3, 6 and 7 are the more harmful ones whereas 2, 4 and 5 are safer to use. This experiment deals with the plastic numbers of 1, 2 and 6.

The plastic with the number 6 is called Polystyrene. It is a plastic that is made of monomers called styrene and is more widely known as Styrofoam. Styrofoam's carbon to hydrogen ratio is 1 and it has a chemical formula of  $C_8H_8$ . This plastic is often used in the making of disposable containers, kitchen cups, glasses, to go containers, protection for glassware and electronics, and so on. Despite its prevalence, the number 6 plastic is very difficult to recycle and if not recycled takes more than a hundred years to decompose ("The Seven Types Of Plastic," 2017).

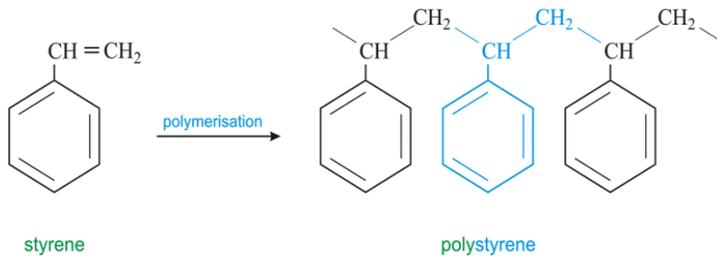
The number 2 plastic, comparatively less harmful than polystyrene, is known as High-Density Polyethylene. The most common

use of this is plastic bags. "Plastic bags typically are made from one of three basic types: high-density polyethylene (HDPE), low-density polyethylene (LDPE) or linear low-density polyethylene (LLDPE). Those thick, glossy shopping bags from the mall are LLDPE, while grocery bags are HDPE, and garment bags from the dry cleaner are LDPE" (Lajeunesse, 2004). Therefore, the most commonly found types at home would be HDPE or LDPE. The monomer for both of these plastics is ethylene which has a chemical formula of  $C_2H_4$ . Even though both of them have the same monomer, their physical properties differentiate their textures and in turn their functions. Plastic bags have also become one of the main problems regarding pollution because of the shopping bags, grocery bags and other plastic bags that people use in an everyday routine that are not always recyclable. Other than the plastic bags, LDPE(measured in mils) plastics are used for making bottles that are non-recyclable. HDPE(measured in microns), on the other hand, is also used for toiletries containers and bottles, but is recyclable.

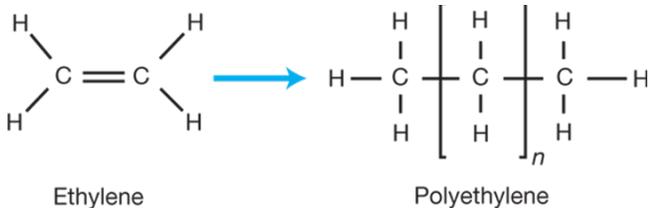
In recent years, along with the focus on plastic bags another main focus is the excess use of plastic bottles. This over accumulation of disposable water bottles is because of the soft drinks and bottled water. Companies like Coca Cola are known for producing a lot of plastics along with their soft drinks. The observations and records of the audit reveal that Coca Cola is the top Global Polluter for the second year (Nace, 2019). The reason for this is its plastic bottles. Even though the company tried to alleviate the problem by coming up with solutions like plant bottles, which are made up of sugar



**Fig. 1.** This graph projects an increase of plastic waste in the coming years, especially in 2050 increasing up to 25 million tons.



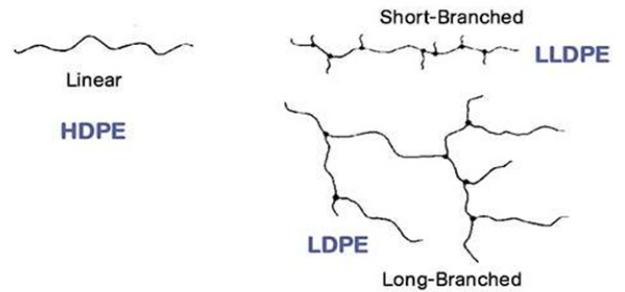
**Fig. 2.** This picture shows the structure of a styrene monomer and how the polystyrene is formed by the joining of several monomers



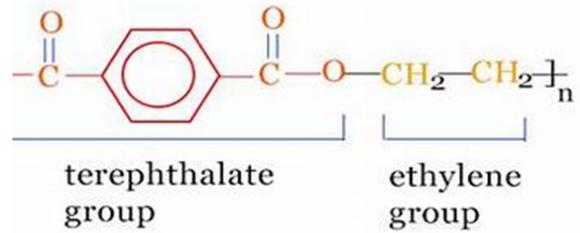
**Fig. 3.** This picture shows the ethylene monomer that joined with several other ethylene monomers to form polyethylene.

cane and other plant components, these are recyclable but not compostable. The general composition of the plastic bottle for this company is 75% of virgin PET plastic and 25% of recycled PET plastic ("What are Coca-Cola bottles and cans," n.d.). Polyethylene terephthalate (PET) is a number 1 plastic with a chemical formula of  $C_{10}H_8O_4$ . This type of plastic is used for food and beverage containers or household products. However, this plastic has its own cons of not being able to resist bacteria during a reuse. In fact, it is so irresistible to bacteria that it is harmful to use this plastic as a water bottle on a hot summer day. This plastic is recyclable but not compostable, just like any other plastic.

"Every year, around 80 million tonnes of polyethylene, a material which is extremely tough and difficult to degrade, are produced around the world ... Currently, the very long processes of chemical degradation, which require the use of corrosive liquids such as nitric acid can take up to several months"(Spanish National Research Council [CSIC], 2017). The council addressed the problems during the degradation of plastics like polyethylene, especially the Low-Density type. However, an unintentional discovery by Federica Bertocchini paved the way for a solution to degrade plastics in a natural way. The discovery showed that a type of worm that typically feeds on wax, called the wax moth or Galleria



**Fig. 4.** The picture shows the different structures of HDPE, LDPE and LLDPE plastics.



**Fig. 5.** This picture shows the terephthalate group and the ethylene group that join together to form the PET plastic's structure.



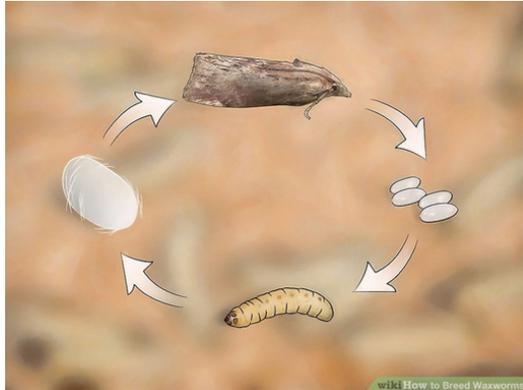
**Fig. 6.** The picture shows the structure of a wax worm during its larval stage.

Mellonella, can feed on plastic, especially polyethylene. The possible explanation for this occurrence was that wax was a polymer with a similar carbon backbone as polyethylene and that wax worms had the enzyme to digest the plastic as well (Arnold 2017). Further, experiments showed that 100 wax worms are capable of eating 92 mg of plastic in 12 hrs (CSIC, 2017).

Galleria Mellonella are typically found in bee hives. They feed on the wax around the beehive which is not beneficial to the bees at all, as they tend to feed on the structure of the beehive and occupy a lot of space for their living which might in turn kill the bees. "In the wild, the worms live as parasites in bee colonies" ("Caterpillar Found Eating Shopping Bags," n.d.). The spread of these insects to different areas is often controlled by climatic conditions, as they 50-60 °F or 10-15 °C, but they are commonly found in many places. The larvae are case bearing and have small fringe winged moths which prefer darker places to live. They generally feed on the midriff of the hive and the cast skins of the larvae, honey or propolis (Paddock, M. S, State Entomologist & Entomologist in charge, 1918). However, they cannot feed on pure wax.

The life cycle of a larvae starts out with a female moth laying an egg. "The eggs are elliptical and pearly white in color" (Paddock et al., 1918). Once the larvae hatch, they start feeding on the food in

the hive and grow. This stage in this life cycle is the stage where the wax moth feeds the most. Once they are ready for the next stage they spin silk threads around themselves and move onto the pupal stage of their life. After they turn into moths, they mate with a male or a female and the female lays eggs. The moths, after the pupal stage, are very active during the nighttime with no breeze when compared to day times (Paddock et al., 1918). The environment they live in and the food they eat determines the growth of the population for the wax moth. But the wax moths have better growth when they are in their natural environment, than when they are held captive. This experiment observes how the wax worms feed on the plastics of Polystyrene, HDPE and PET plastic.



**Fig. 7.** The figure shows the life cycle wax moth, which starts from eggs and then progressing into larvae. It grows and forms into a pupa and then becomes a moth.

## Methods

### Experimental Design

**Independent variable.** The independent variable in this experiment was the type of plastic used, which are High-Density polyethylene (Plastic Bags), Polystyrene (Styrofoam) and Polyethylene Terephthalate (Coca-Cola bottles).

**Dependent Variable.** The Dependent variable in this experiment is the amount of plastic that was consumed by the wax worms during the experiment. This is done through gravimetric analysis or measuring the mass of the plastics.

**Standardized Variables.** Some of the Standardized variables used in this experiment are the electronic balance that was used to measure the mass of the plastics, the glass jars that were used to keep the wax worms and the number of wax worms present in each of the glass jars, which was 300.

**Control Groups.** There are no control groups that were used in this experiment.

### Hypothesis

If *Galleria Mellonella* were to be fed a small amount of plastic bag (HDPE), Coca-Cola bottle plastic, or Styrofoam (Polystyrene), then the *Galleria Mellonella* would consume the most amount of the plastic bag because there have been studies shown through labs where actual scientists where *Galleria Mellonella* that has been placed into plastic bags- which has been known for having similar structure to the wax they consume-has consumed approximately 0.092 g of the plastic bag each day with 100 *Galleria Mellonella*.

Also, the Coca-Cola bottle and Styrofoam has a higher plastic number meaning that it is much harder; being that the Coca-Cola bottle with a number of 1-with the material of PET, which is the hardest to breakdown due to the higher density-and the Styrofoam with the number of 6.

### Replication and Sample Size

In the experiment, each set of three plastics were placed in the jars for 24hrs. This process was repeated 3 more times.

### Materials

- 900 *Galleria Mellonella*
- 3 clear glass jars
- 1 Coca-Cola 2 Liter Plastic Bottle
- 2 Styrofoam Trays with the thickness of 0.25 cm
- 1 Regular grocery Plastic Bag which is made of HDPE plastic (Fry's bag used in this experiment)
- Blade knife
- Electronic balance

### Procedures

1. Gather all materials on the Materials List.
2. Cut the Coca Cola bottle into 4 pieces with an approximate mass of 8.686g.
3. Cut the plastic bag and the styrofoam sheet into 4 pieces with an approximate mass of 1.248g and 0.58g respectively.
4. Record the masses of all these pieces in a table as the data before the experiment.
5. Divide the *Galleria Mellonella* into three equal numbers of 350 and place each batch in a glass jar.
6. Put a plastic bag piece in one of the jars, a coke bottle plastic piece in another and styrofoam piece in the third jar. Then cover the top with an aluminium foil with holes poked for gas exchange.
7. Place these jars in a temperature that is between 50-60 °F for 24 hours. Replace the plastics in each of the jars with a new piece and repeat steps 5 and 6 for another three days.
8. Record the masses of all the pieces after the experiment and calculate the amount that was eaten by the worms.



**Fig. 8.** This picture shows the setup of the experiment and the way that each type of plastic was placed in a different glass jar with 300 worms in each glass jar.

### Statistical Methods

In order to find the mass of the amount of plastic that was eaten by the waxworm, the mass of the plastic after the trial was subtracted from the mass of the plastic before the trial. The masses of the four pieces of plastic were averaged.



**Fig. 11.** This image shows the jar with worms that was feeding on styrofoam.



**Fig. 12.** This picture shows the jar containing Coca-Cola or PET plastic.



**Fig. 13.** The image shows the jar which had the plastic bag (HDPE) with the worms.

## Results

### Data Tables

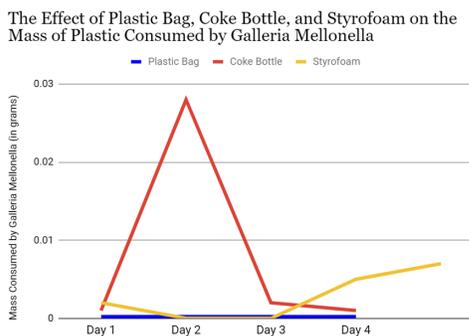
The Difference of the Mass of Each Plastic Consumed			
	Plastic bag	Coke Bottle	Styrofoam
Before	0.58 g	8.686 g	1.258 g
After	0.591 g	8.656 g	1.251 g
Difference	-0.02 g	0.03 g	0.007 g

**Fig. 9.** The table here shows the before and after difference of the plastic consumed by the Galleria Mellonella. Shown in the Plastic bag column, there was actually an increase instead of decrease in plastic consumed due to the fact that the Galleria Mellonella in that jar did not consume any of the plastic that was placed in it.

The Effect of Plastic Bag, CocaCola bottle, and Styrofoam on the Mass of Plastic Consumed by Galleria Mellonella			
	Plastic Bag	Coke Bottle	Styrofoam
Day 1	0 g	0.001 g	0.002 g
Day 2	0 g	0.028 g	0 g
Day 3	0 g	0.002 g	0 g
Day 4	0 g	0.001 g	0.005 g
Total	0 g	0.032 g	0.007 g
Average (per day)	0 g/day	0.008 g/day	0.00175 g/day

**Fig. 10.** This table shows the amount of plastic the Galleria Mellonella had consumed each day with the total and average of each day. The Coca-Cola plastic had the highest average of 0.008 g/d and the highest total of 0.032 g.

### Graph



This graph shows how much the Galleria Mellonella consumed over the course of 4 days that the experiment was conducted in. The plastic bag had absolutely no change whatsoever and none

of the plastic was consumed. The Coca-Cola bottle, however, had the highest in terms of consumption of a total of 0.028g on Day 2. Parts of the styrofoam were consumed, however, it still isn't enough compared to the 0.028g on Day 2 for the Coca-Cola plastic.

### Data Analysis

The data collected showed that the Galleria Mellonella did not consume any of the plastic bags (HDPE); rather, the Galleria Mellonella consumed the Coca-Cola plastic and Styrofoam instead. From the data of the plastic bag collected, the mass of the total end result had actually increased by 0.02 g from 0.58 g to 0.591 g. On the other hand, the Coca-Cola plastic had the highest difference of 0.03 g of plastic from the initial mass of 8.686 g and result mass of 8.656 g; Styrofoam has the mass difference of 0.007 g with the initial weight of 1.258 g and end mass of 1.251 g. Overall, there was an average of consuming 0.00975 g of plastic each day and an average of 0.008 g for the Coca-Cola PET plastic along with 0.00175 g of polystyrene each day. From the graph, it shows a peak of 0.028 g of PET plastic consumed on Day 2. However, polystyrene has a trend of consuming 0.002g on Day 1 with 0.005 g in Day 4 and none in both Day 2 and Day 3. On the other hand, the HDPE plastic had consistently had nothing being consumed by the Galleria Mellonella.

### Discussion

This experiment was to determine if Galleria Mellonella could eat the common polluted plastics and observe how much of plastic and what kinds of plastic the Galleria Mellonella would consume. This supported the purpose of this experiment which was to find a solution to reduce the amount of plastic accumulating on earth. The experiment fed PET, High-Density Polyethylene and Polystyrene plastics to the wax worms and recorded the amount of change in the mass of the plastics every 24 hrs, to determine the amount that was consumed by the worms. The results of this experiment showed that the worms ate the PET plastic the most, followed by styrofoam and the HDPE plastic. The wax worms ate 0.032g of PET in total with an average of 0.008g per day. The average of the Styrofoam was 0.007g per day and 0g per day for HDPE. But, the data collection process had possible errors which might have led to errors in numbers. One possible error that might have occurred is that the Galleria Mellonella had some sort of behavior changes towards the plastic that was given. This might have led to the worms

ignoring the plastic bag which was expected to be eaten by the worms and instead of feeding on the PET plastic. Another possible error was that the mass and size of each plastic were completely inconsistent, causing the *Galleria Mellonella* to have a different reaction towards the plastic and the way it was placed into the jar. Besides the behavior and result on the mass-consumed, there also had an error of exact timing to extract the plastics at the same time. However, the data collected was not sufficient enough to fully prove that the data is always consistent.

The results showed that the wax worms did not consume the HDPE plastic bag at all, showing that they might have had behavioral changes and thus favored the consumption of the PET plastic more. The hypothesis was refuted because they consumed PET plastic the most instead of the HDPE. The hypothesis had a possible reasoning based on the structure of wax that they usually consume, which also ended up being refuted. The similarity between all the structures of the plastics was the presence of carbon backbone, which provides a fair chance for all of these plastics to be consumed by the wax worms. This also explains that the behavior of the wax worms towards the plastic was also an important factor besides the structure, determining the food they consume. Out of the three plastics used, the wax worms consumed the PET plastic more and thus, future research should focus on the consumption of PET plastics by wax worms.

## Conclusion

The hypothesis-if *Galleria Mellonella* were to be fed a small amount of plastic bag (HDPE), Coca-Cola bottle plastic, or Styrofoam (Polystyrene), then the *Galleria Mellonella* would consume most amounts of the plastic bags (due to the studies that had been made by skilled experts)-was refuted from the data collected through this experiment. The *Galleria Mellonella* ended up only consuming the Coca-Cola plastic and Styrofoam. In total, the *Galleria Mellonella* had consumed approximately 0.039 g of plastic. 0.007 g of Styrofoam was consumed along with 0.032 g of Coca-Cola plastic. Compared to the other research that was done similarly, 100 *Galleria Mellonella* had consumed approximately 0.092 g of polyethylene within 12 hours; this being that the data that was collected in our data had not even added up to half of their polyethylene within 24 hours. This could have resulted from various reasons: the environment was too crowded, there were behavior changes after receiving the plastics, insufficient days to fully observe more accurate data through averaging.

For greater understanding of the studies of biodegrading polluted plastics, there are other ways to study the biodegradation of plastics. One of which could be through observation of the *Galleria Mellonella*'s behavior towards each one. Another way is through testing specific types of plastic bags or even the color of each plastic. Since the PET plastic worked the best, it can be tested that other Coca-Cola plastics be fed to *Galleria Mellonella*. Further experiments can also test what environmental conditions are the most effective for the worms. Most of all, there could be more studies towards the gut bacteria or any other studies towards the microbiology and chemistry behind how their stomach has the ability to digest all of the plastics without having health issues.

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Fig 1: *By 2050, we'll have produced 26 billion tons of plastic waste.* (n.d.). photograph. Retrieved from <https://www.sciencemag.org/sites/default/files/>

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Fig 2: *Chemistry Glossary: Search Results for Monomer.* (n.d.). photograph. Retrieved from <https://www.periodni.com/gallery/polystyrene.png>

Fig 3: *Making Plastics from Monomer to polymer.* (n.d.). photograph. Retrieved from [https://www.aiche.org/sites/default/files/images/cep/inline/2015-09-01-Making-Plastics-From-Monomer-to-Polymer/images/Figure\\_1.png](https://www.aiche.org/sites/default/files/images/cep/inline/2015-09-01-Making-Plastics-From-Monomer-to-Polymer/images/Figure_1.png)

Fig4: *Density and Molecular Weight in polyethylene: Plastic Technology.* (n.d.). photograph. Retrieved from <https://d2n4wb9orp1vta.cloudfront.net/cms/0612pt-KHmaterials.jpg;width=550;quality=60>

Fig5: *Polyethylene Terephthalate|Chemistry@TourVista.com* . (n.d.). photograph. Retrieved from [http://images.tourvista.com/cms/images/44/pet\(1\).png](http://images.tourvista.com/cms/images/44/pet(1).png)

Fig6: *Wax Worms (Galleria Mellonella).* (n.d.). photograph. Retrieved from <http://www.recorpinc.com/images/live-pet-food/waxworm.jpg>

Fig7: *How to breed Wax Worms(With pictures) - WikiHow.* (n.d.). photograph. Retrieved from <https://www.wikihow.com/images/thumb/d/d9/Breed-Wax-worms-Step-16.jpg/aid1626228-v4-728px-Breed-Waxworms-Step-16.jpg>

Fig8: Chintala, S. A. (n.d.). *WaxWorms with three different plastics.* photograph, Chandler.

Fig11: Chintala, S. A. (n.d.). *The jar of wax worms with styrofoam.* photograph, Chandler.

Fig12: Chintala, S. A. (n.d.). *The wax worm jar with Coca Cola plastic.* photograph, Chandler.

Fig13: Chintala, S. A. (n.d.). *The wax worm jar with plastic bag.* photograph, Chandler.