Identification of specific plastics which do not release estrogenic activity causing chemicals

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Abstract

Introduction:Identification of dangerous chemicals in plastics used for reusable plastic water bottles has become a serious problem due to the nature of estrogenic chemicals effects if they are present within plastics used to contain food or water. While studies have tried to identify which chemicals in plastics cause estrogenic activity within the body, there are still many plastics which leach estrogenic chemicals that have not been identified.

Methods: Secondary data analysis was used in order to identify overall estrgenic content leached from a number of different plastics under various stress and in various solutions. Percent of leached estrogenic chemicals was compared as respective %Rme2 for specific plastics, classifying having less than 15% %Rme2 as safe for use in a plastic bottle.

Results: While there were a large number of plastics which were not passable under certain stress and in certain solutions, a number of plastics were identified as not releasing chemicals which cause estrogenic activity.

Conclusions: There are plastics that already exist which would be optimal to replace current plastics that leach dangerous amounts of estrogenic chemicals. While many plastics currently sold can release estrogenic chemicals, there are available solutions which could prevent negative health effects which come with being exposed to estrogenic activity.

Key Terms: Estrogenicity, Estrogenic Activity (EA), Bisphenol A (BPA), plastic, MCF-7, BG1Luc

Introduction

Over the past decade, much attention has been brought to the topic of what chemicals are in our plastic reusable water bottles. Bisphenol A (BPA) is a xenoestrogen, or a chemical that mimics the effects of naturally occurring estrogens (also known as having estrogenic activity or EA) which was once widely used in the production if hard plastic containers for food. Chemicals with EA have the potential to be a serious health threat due to their ability to upset the estrogen balance in the body, meaning the BPA in our plastic water bottles was actually killing us. After learning about the harmful effects of BPA, researchers began to test different plastics for BPA contents to see if the chemical was leaching into the drinking water. As a result of these efforts, many unsafe water bottles were identified and substitutes of BPA were recommended such as glass or aluminum bottles (Wagner, et al., 2011). Since then, the EPA has recognized BPA as a xenoestrogen and forced manufacturers to label whether BPA is present in the plastic of the products so that consumers could identify which water bottles would be safe to use. Several other commonly used plastic resins were found to contaminate liquids with similar levels of estrogenicity as BPA. Due to the method used to detect BPA in a plastic, there was no information on its estrogenicity. The most efficient method of identifying the safety of a plastic in regards to estrogenicity is by using a estrogen receptor mimicking bioassay, such as the MCF-7 or BG1Luc bioassays. This method is much more effective as compared to previous methods as it detects many possible estrogenic substances instead of BPA alone. Because of this drawback in the older method, it ignored every estrogenic compounds other than BPA, giving a false data on the level of estrogenicity of a plastic. (Yang, et al., 2011)

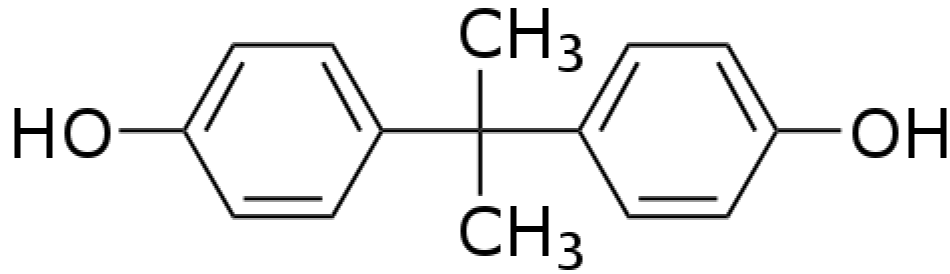


Figure 1: Chemical Structure of BPA

Estrogenic data has been collected on different plastics, but there are several important gaps of knowledge about some plastics that are still in use and sold in the market. For example, it is very likely that large number of water bottles currently being sold have a level of estrogenic content that could prove to be dangerous. Research has been done evaluating most plastics that are used commercially in order to create water bottles (Bittner, et al., 2014), but due to the lack of options for companies, unsafe water bottles continue to be sold. If the different types of plastics were organized by level of estrogenicity, the most optimal plastic could be identified.

Estrogenic chemicals are a serious threat to the body during development. Exposure to estrogenic chemicals can cause mutations in genes that can lead to cancer or mental disabilities if one is exposed to estrogenic chemicals early in life. One of the most common places that an estrogenic chemical is found is in the plastics that make up containers or vessels used to hold food, such as water bottles. For a long time, the most well known estrogenic chemical was Bisphenol A, or BPA, which received much attention from the media due to its presence in almost every plastic water bottle that was sold. But BPA was only one of a long list of chemicals that were estrogenic, meaning even plastics considered BPA free had a very likely chance of containing estrogenic chemicals that could potentially be dangerous. (Cooper, et al., 2011)

In order to solve this problem, instead of finding every chemical in every plastic and determining whether or not it has estrogenic activity, each plastic is tested individually to see which ones do not have estrogenic activity and would be suitable to be used for a water bottle or container without the risk of releasing estrogenic chemicals (Bittner, et al., 2014). The composition of chemicals and plastics that come together to create one plastic creates an unreasonably long list that, if each chemical were to be tested, would waste time identifying things that we would not need to know. The solution is to use plastics that are already economically feasible to be produced for use in water bottles and test which types release zero to very little estrogenic activity. This method would help to identify safe plastics while not wasting time identifying the potentially thousands of chemicals that could be estrogenic.

One suggested solution to this problem involves identifying which chemicals cause EA and then finding which plastics have these chemicals, similarly to the way BPA was discovered to be estrogenic and then banned. There are several major problems with this method. One, most reusable plastic bottle producing companies do not release the full chemical list of what their plastics that make up their water bottles are made of (Kabuto H, *et al*. 2004). Companies that specify that their plastic bottles are BPA free do so of their own accord in order to convince customers that their product is safe. Unless these companies would be willing to sacrifice money in order to prove that they are creating EA leaching free chemicals, then it would be nearly impossible to identify. And secondly, there are many different kinds of plastic with different chemical makeups and different combinations of chemicals. BPA is just one chemical out of potentially thousands that make up different plastics. Isolating each chemical from every single plastic and then testing those chemicals in varying situations would add up to an impossibly large amount of tests. It is much more efficient to test each plastic individually, testing for different solutions effects on the plastic and identifying whether or not it will release EA causing chemicals.

Because of the large amount of research that has been done on different chemicals and plastics that make up water bottles, a combination of all of this data could be used to determine specifically which plastics provide a safe level of estrogenicity (Bittner, et al., 2014). For example, if one researcher covered 5 different plastics in order to identify which chemicals were dangerous, that data could be combined with that of another set of data, focusing on economically viable plastics that also did not have enough estrogenic content in order to cause any serious issues. This process could be repeated until almost every plastic that is used in water bottles is identified, showing which plastics should be used.

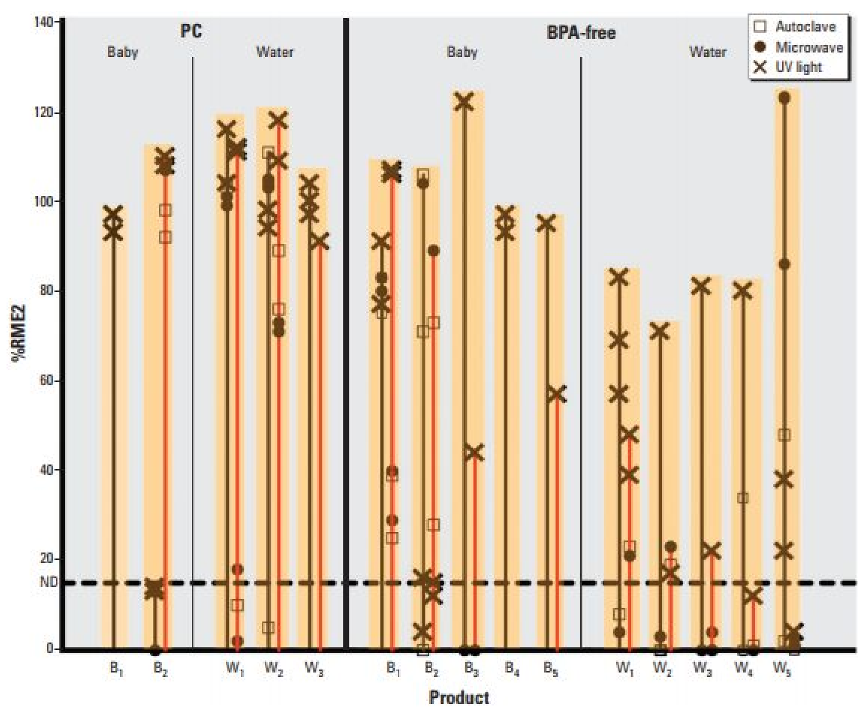


Figure 2: Representation of %Rme2 of separate plastics used in baby bottles and water bottles. Autoclave microwave and UV light stresses were used with a sample of plastics both contain BPA and without BPA. MCF-7 bioassay was used in order to measure %Rme2 (Yang *et al.* 2001)

Estrogenic chemicals can be a serious threat during development. If a child is exposed to estrogenic chemicals during development, there is a chance that it could increase the likelihood of developing cancer later in life. This study could easily prevent this be identifying which plastics were safe as well as economically viable, companies could continue to benefit while guaranteeing the safety of those who wish to use a plastic bottle. Currently, you are able to purchase a water bottle that has no indication that it could potentially harm you, meaning an uninformed parent could give their child a water bottle that was leading to them developing diseases that have the potential to kill them. Efforts being made to prevent this are much less effective than what could be done, and this change could help to create a situation in which available plastics are implemented more effectively in order to prioritize safety. (Jones, et al., 1995)

Anyone that wishes to purchase a plastic water bottle and use it for drinking is put at risk of being exposed to estrogenic chemicals that could be leaching from the plastics that make up the bottle. Especially in infants, this can be very dangerous and lead to health problems. While we have discovered that several chemicals that make up plastics are estrogenic, it is very likely that a large amount are still left unidentified within the plastics that we have created (Yang, et al., 2011). Chemicals can take different effects when they are combined with other chemicals, meaning that some chemicals that would be safe on their own could become unsafe when added to another in order to produce a different kind of plastic. Due to the extent of research that would need to be done on each scenario in which a chemical is implemented into a plastic, it is unrealistic to consider identifying each chemical that can cause estrogenic activity. In order to get around this problem, each plastic that is able to be used in a water bottle should be tested, finding the estrogenic content of that combination of chemicals. This bypasses the need to identify each chemical that can cause estrogenic activity, as there are potentially thousands of chemicals that would need to be tested individually as well as in combinations with every other chemical. (Wagner, et al., 2011)

In order to achieve accurate results over a large array of studies and methods for assessing EA leached from a plastic, certain factors were considered when deciding which data points and which studies to use. Because plastics being tested were plastics being studied for their use as hard reusable plastic water bottles, they would need to be able to withstand what basic stresses a plastic water bottle would go through with ordinary use. For example, exposure to regularly amounts of sunlight in the form of UV rays has been proven to have profound effects on the levels of EA leached from a plastic (Routledge et al. 1996). Studies which did not account for a wide range of stresses that would be expected of a reusable water bottle were disregarded as they did not report a full set of data that could be analyzed and left out key data points that would greatly affect the overall estrogenicity of the plastic.

Another key aspect of testing for EA leached from specific plastics is the solutions plastic samples are added to in order to assess estrogenicity. Another group of research papers that must be disregarded used only one type of solution such as saline or deionized water. Research done by George D Bittner *et al.* in 2014 showed leaching of EA from plastic samples differed greatly between solutions of saline, 100% EtOH, 50% EtOH, 10%EtOH, and deionized water. Studies which utilized a multitude of solutions to test each sample in provided a wide set of data. When A study took a plastic and tested it with only one kind of solution, it neglects the possibility that that same plastic could release deadly amounts of estrogenic chemicals in another solution. Each kind of solution has the potential to have a different effect on the sample, and when a reusable plastic water bottle will be expected to contain a number of different fluids with different possible chemical makeups, testing needs to be done in order to determine it will not affect the plastics estrogenicity and whether or not it will leach EA.

There are many different tools for assessing the level of EA present in a solution, but only some are produce reliable and accurate data. The most prominent method for assessing estrogenicity of plastics is in the form of robotized MCF-7 cell proliferation assays and BG1Luc4E2 cells. MCF-7 assays bind to chemicals with EA through estrogen receptors, which in turn leads to the proliferation of the MCF-7 cells which can then be measured in order to determine levels of EA causing chemicals leached. BG1Luc assays respond to EA causing chemicals with the induction of luciferase, which again, can be measured in order to determine estrogenicity. Both of these methods are utilised in many studies regarding estrogenicity of plastics and have been well established in a manner of different studies. (Meeker et al. 2009)

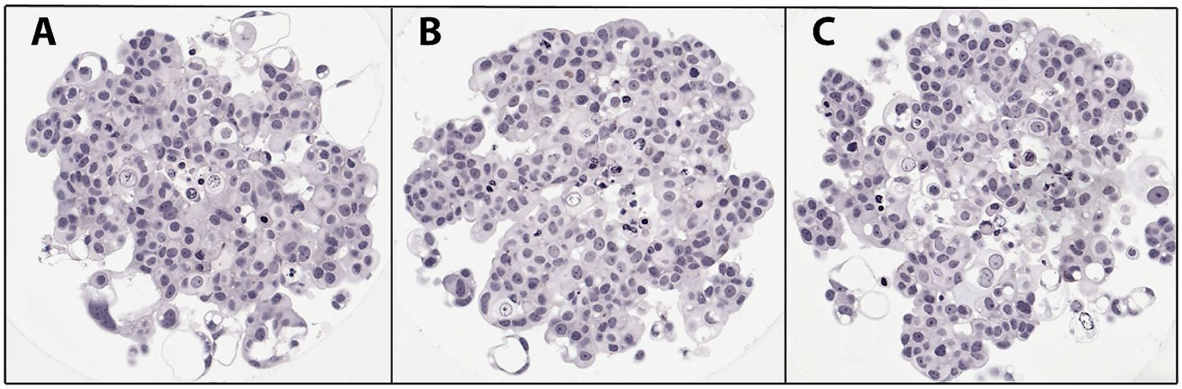


Figure 3: MCF-7 bioassay cells from a breast cancer patient.

Methods

Data regarding EA of specific plastics was conducted via secondary data analysis. Various sources of finding research papers regarding the topic were used such as Ebscohost, Google Scholar as well as California State University Channel Islands' library database. Research papers and studies found from these sources were used in order to compile a hypothesis as well as conduct data analysis that lead to the conclusion reached. Studies were reviewed that showed the extent at which BPA has been eradicated, the effects of chemicals which have EA, as well as data regarding levels of chemicals which have EA in plastic bottles and similar food containers. Articles used in this research paper were all peer reviewed studies that have been done in recent years in order to ensure reliable data. Secondary data analysis as appropriate for this research because it allowed the comparison of different data points from different studies to be compared which allowed for a wider scope of research than if it were a first hand primary research study. This allowed the hypothesis to be more efficiently tested and a more accurate conclusion to be reached.

Data regarding levels of EA in different plastics was taken from peer reviewed scholarly articles that utilised a sound method of research that would allow for accurate results to be reached. The two main methods of measuring levels of EA involve the use of roboticised estrogen receptor mimicking cell proliferation bioassays, MCF-7 and BG1Luc (Figure 3). These two bioassays have been used for decades in order to accurately assess the levels of EA and are currently under review for international use by the ICCBAM/NTP (Interagency Center for the Evaluation of Alternative Toxicological Methods/National Toxicology Program). These assays work by allowing estrogen mimicking compounds to bind to estrogen receptors and activating the transcription of estrogen responsive genes, which causes the MCF-7 cells to proliferate. EA measurements are recorded as %Rme2, or as a percentage of the maximum cells proliferate in response to 17B-estradiol, the positive control. This method of calculating EA of a plastic rarely produces a false positive result and has been tested many times before in order to assess estrogenicity. The several studies whose data are analyzed used this method, allowing various the %Rme2 of each plastic to be compared and identified.

Results

Table 1: Respective Rme2 values of specific plastics numbered 1 through 17 of saline and EtOH solutions tested with microwave, UV light , and autoclave stresses (Bittner et al. 2014)(Chun et al. 2011)

Plastic #	Type of Resin	Saline Microwave	EtOH Microwave	Saline UV	EtOH UV	Saline Autoclave	EtOH Autoclave
1	LDPE	5	7	0	4	4	30
2	LDPE	3	7	26	3	-1	27
3	PET	100	3	31	2	47	1
4	LDPE	2	3	0	0	4	5
5	HDPE	6	-4	2	-2	-1	-3
6	PPHO	0	-4	3	2	-6	-3
7	PPCO	3	7	-7	-6	-6	-3
8	Random Bottle	3	23	71	17	-1	19
9	Random Bottle	4	21	98	39	8	23
10	Random Bottle	-7	-5	81	22	0	4
11	Random Bottle	34	-2	80	12	-1	1
12	PETG Baby Bottle	0	-2	122	44	0	1
13	PETG Baby Bottle	-8	17	61	111	0	15
14	PS	3	3	17	45	76	0
15	COC 3	9	7	20	20	0	6
16	COC p18	4	1	9	11	1	-2
17	COC p19	6	2	6	-2	4	2

Table 1 shows that there were large amounts of plastics which had a %Rme2 greater than 15%. Data was organized by numbering each plastic and organizing them by type of plastic resin

(LDPE, PET PPHO, etc.) Then, depending on which solutions the plastic was tested in (Saline, EtOH, Deionized water) as well as any types of stress that the plastic was tested under (Microwave, Autoclave, UV rays, etc), each %Rme2 was divided into its respective area. (Figure 1) Because each study tested the plastics in different ways, plastics that were included all had to be tested with similar methods under the same type or nature of stress and solution combinations. Plastics included in this study were those that had been adequately experimented with and all possible data had been retrieved from them.

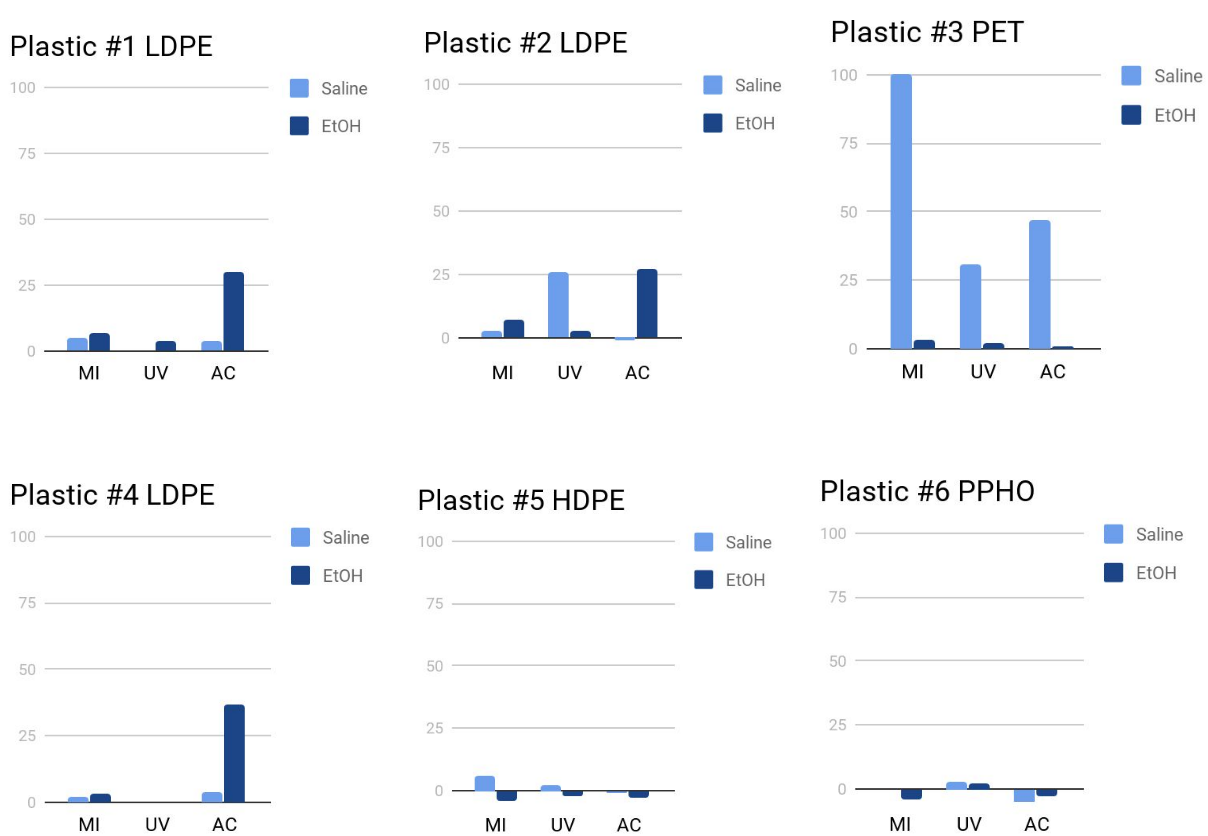
17 plastics in total met the different testing criteria. 2 solutions were used to assess EA, Saline and EtOH. Consistent amounts of each plastic in each solution allows the results of each plastic to be compared consistently. In order to stress the plastics in similar ways to which a reusable plastic water bottle would be expected to, 3 different stresses were used. For normal sunlight exposure over extended periods of time, a UV stress was used. For expected washing machine use, samples were autoclaved at high temperatures in order to simulate the movements and high temperatures of a machine wash. The third stress was intended to assess whether use in a microwave, which is common for plastic containers, would have any serious effects on the EA leached from a plastic.

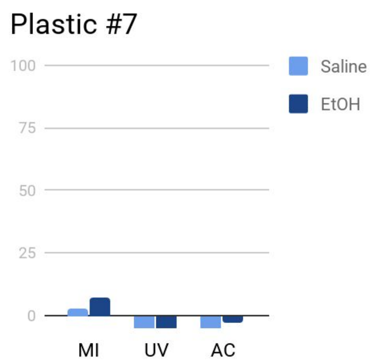
Table 2: Average values of %Rme2 of each plastic under microwave UV rays and autoclave stresses. (Bittner et al. 2014)(Chun et al. 2011)

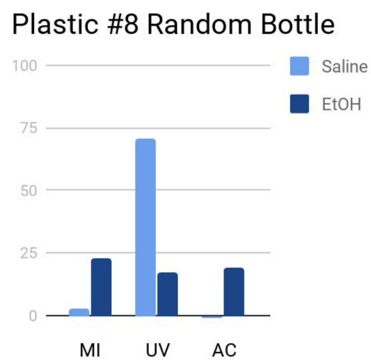
	Mean %Rme2 Overall	Mean %Rme2 Microwave	Mean %Rme2 UV Rays	Mean %Rme2 Autoclave
All Plastics	14.16	7.382	27.71	7.38
Plastic #1	8.333	6	2	17
Plastic #2	10.83	5	14.5	13
Plastic #3	30.67	51.5	16.5	24
Plastic #4	2.33	2.5	0	4.5
Plastic #5	0.3333	1	0	0
Plastic #6	1	2	2.5	0
Plastic #7	2	5	0	0
Plastic #8	22	13	44	9
Plastic #9	27.5	1	83	0
Plastic #10	15.83	6	51.5	2
Plastic #11	20.67	16	46	0
Plastic #12	32.17	12.5	68.5	31
Plastic #13	32.67	4.5	86	7.5
Plastic #14	24	3	31	38
Plastic #15	10.33	8	20	3
Plastic #16	24	2.5	10	0
Plastic #17	3.167	4	2	3

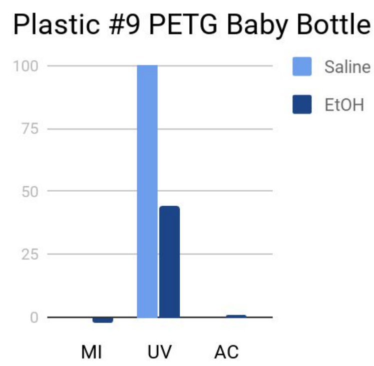
Each solution and stress had varying effects on EA detected for each plastic. According to a study done done by Chun Z. Yang et al., plastics with a %Rme2 less than 15% can be considered free from EA. When the plastics were tested with the microwave stress, 85% had a %Rme2 less than 15%, between being tested with saline solution as well as with EtOH solution. When plastics were put under the UV stress, only 50% of the samples had a %Rme2 less than 15% Rme2, showing that one of the most common stress that a plastic bottle would be expected to go through leads to high amounts of leaching of EA causing chemicals, which eliminated over half of the plastics from being suitable as a water bottle.

Figure 2: Plastics #1 through #9 %Rme2 of saline and EtOH solutions put under three different stresses, microwaving, UV light and autoclaving.









Discussion

The results of this secondary data analysis demonstrated the possibilities of accessing safe water bottles through identifying specific plastics for their overall estrogenicity. Unsuitable plastics were identified through utilising multiple solutions to test water bottle plastics in, as well as various stresses in order to stimulate the plastics in similar ways they would be expected to with normal use as a water bottle. Organized data sets from various different studies compared well with each other due to the easy relation of a 17B Estradiol base %Rme2 values. Multiple plastics that released low levels of EA were also identified, meaning that some plastics already on the market are viable for use as a replacement for current less safe plastics. This proves that it is possible to find a plastic that is suitable for use in manufacture products for infants and children without risk of contaminating with EA causing plastics.

While most plastic samples released levels of estrogenicity greater than what is considered safe, there were some samples which across each solution and every combination of stresses which did not leech estrogenic chemicals. Stresses such as microwaving, autoclaving and UV rays are proven to increase the amount of leaching of plastics, and are necessary in order to determine whether or not the plastic can function overall in water bottle. Some samples showed low EA on almost none of the stresses and in none of the solutions, meaning they are a much worse case of an estrogenic plastic. But in other cases, plastics only had one or two cases in which the stress gave a %Rme2 over 15% while the rest of them showed clear from estrogenicity. These plastics need to be treated the same as plastics with no positive results from each assessment, as even though it may not be a result of every situation, the possibility that estrogenic chemicals could be leaching from the plastic of the bottle means that it is unsafe to use.

Conclusion

Overall, data shows that there are available plastics that function under basic circumstances without releasing dangerous levels of EA. Plastics #4, #5, #6, #7, #16, and #17 all did not have EA released that was over 15%Rme2 under any of the available stresses as well as in saline or EtOH solutions. This means that in any scenario for use as a reusable plastic container, these specific plastics will not have a chance of releasing a dangerous amount of EA causing chemicals. The possibility for exposure to estrogenic chemicals could be drastically reduced if companies were to utilise theses plastics instead of others that release high amounts of EA.

Despite numerous plastics still in use currently releasing large and potentially dangerous amounts of EA, there are options available that are now identified as releasing little to no EA causing chemicals. This is extremely important because it allows improvement for the overall wellbeing of those who use reusable plastic water bottles as well as an easy transition for companies that produce these water bottles, as the plastics tested are plastics already produced for water bottles already and can simply replace current plastics.

The largest problem of identifying estrogenicity of plastics lies in the lack of focus on testing specific plastics and researching which is most optimal in regards to overall safety. Focus is more heavily placed upon discovering which chemicals cause EA than on individual plastics, which causes problems when the chemical makeup of a plastic is unknown, leaving gaps in knowledge on whether plastic bottles being used are safe or not. Through testing different solutions and stresses individual specific plastics, each water bottle can be determined whether it is safe or not. While the number of plastics identified in this study are small, until more experimenting is done regarding different stresses combined with different solutions, no more data can be compiled. But this data is still very valuable, as it proves that there are already plastics available that do not release dangerous amounts of chemicals which contain EA, and are already currently available to be produced. By replacing plastics used to create plastic bottles now with plastics that have been proven to be free of EA causing chemicals, cancer risk could be reduced and solve the EA problem.

Further Work

Due to the large number of plastics currently used to produce plastic water bottles, it is difficult to test every brand and type of plastic for the possibility of leaching chemicals with EA, especially due to the necessity if testing with different stresses to ensure safety. More data is needed for a wider scope of plastics identified. Additional research done on a larger scope of plastics could greatly reduce the amount of gaps in knowledge in which plastics would be safe to use for water bottles.

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