ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (49-53), Month: July - September 2018, Available at: www.researchpublish.com

Toxicity Assessment of Microwavable Containers Provided With the Microwave

Noopur Mathur[1*], Nupur Mathur[1], Anuradha Singh[1], Pradeep Bhatnagar[2]

[1]Environmental Molecular Microbiology Lab, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India
[2]Department of Life Sciences, International College for Girls, IIS University, Jaipur, Rajasthan, India

Abstract: **Microwaves are becoming an important part of our homes and so are their containers. These containers are usually made of plastic and this is what has raised concern. . Plastic is not a single substance as it is made up of several organic and inorganic compounds. Further, additives like phthalates and Bisphenol A (BPA) are added to plastic to make them soft, mouldable and shiny. However, these compounds have been reported to be toxic and endocrine disruptors. These chemicals are found to leach out into the food from the containers upon heating. Thus, regarding the possible toxicity generated due to microwavable plastic, this study attempts toxicological analysis of these containers in order to investigate their possible toxic effects on humans. To estimate and predict their effect a bacterial short term bioassay *viz. Pseudomonas fluorescens* growth inhibition assay was used to determine the toxicological effects of the containers provide with the microwave. A comparative study was done using a microwavable plastic and a glass container. The results revealed that containers provided with microwave can be considered safe and glass can be considered as an alternative to plastic containers.**

Keywords: **Microwave containers, BPA, Phthalate, *Pseudomonas fluorescens* growth inhibition assay.**

1. INTRODUCTION

The emergence of wide range of plastic microwavable containers has helped in the convenience of modern urban lifestyle. Usually these containers are sometimes also provided with the microwave itself and are marked as microwave safe. These plastic containers contain many additives like plasticizers which make them soft and easily mouldable into different shapes. But these additives do not bind chemically to plastic molecules and can easily migrate into the food [1]. The shining and the smooth surface of the microwave plastic ware are achievable by addition of a surface coating of BPA. This compound is known to migrate into the food and is endocrine disrupter chemical [2]. The chemical leaching from plastic products are shown to increase with temperature [3].

In a study on PVC, it was found that overall migration into food simulants increased significantly during 3 min of microwave heating at full effect compared to the overall migration during microwave and conventional heating of other polymer types such as polypropylene, polyethylene and polyamide [4]. The diffusion rate of ethylene oxide also increased in PVC upon microwave heating [5] and cyclopentanone in an epoxy resin [6]. Another study found that overall migration into olive oil from a polypropylene package increased after repeated microwave heating to 400% compared to the first heating [7].

There is thus evidence that microwave heating could increase the migration of specific migrants in specific types of polymers. Possibly those migrant or polymer types that are more susceptible to absorption of microwave radiation are more prone to leach out during microwave heating. Studies have been conducted to determine migration of toxic chemicals to food or food simulants from different plastics in the past both during standard test conditions and during heating in microwave oven [8]. It was found that overall migration from polypropylene to aqueous food simulants during microwave heating for 3 min at 800 W was comparable to the overall migration during continuous heating at 80 °C for 30 min, with results in the range from 0.05 mg/dm2 to 0.14 mg/dm2 [4]. Overall migration determinations from polyethylene terephthalate (PET) into food simulants conducted during conventional heating for 1 h at 90 °C revealed overall migration values of <0.1 mg/dm2 into water, 0.2 mg/dm2 into 10% ethanol and 1.4 mg/dm2 into 3% acetic acid [9].

Polycarbonate is polymerized from the sodium salt of BPA and phosgene and sometimes unreacted BPA is left in the polymer. There have been several scientific studies investigating the release of Bisphenol A (BPA) from polycarbonate, because in the past few years it was shown that this compound could have endocrine disrupting or estrogenic properties. More recent studies have, however, concluded that in most cases the release of BPA from polycarbonate is not a result of migration of residual BPA in the polymer, but rather a result from the depolymerization during hydrolysis reactions under certain conditions [10] e. g. when the polymer is in contact with highly alkalic water [3] which produces BPA from the polymer chains. Ehlert et al.[1] found that BPA migrates into boiling water during microwave heating of water in polycarbonate baby bottles in an ordinary microwave oven.

The researchers hypothesized that environmental BPA exposure may increase susceptibility to obesity, diabetes and cardiovascular disease [11]. Biedermann- Brem and Grob[3] systematically studied the effect of temperature on the release of BPA into tap water and boiled tap water of the same water supply by heating in a microwave for 5 min. The concentration of BPA in tap water increased from < 0.0001 mg/L at 50°C to 0.0006 mg/L at boiling temperature whereas the concentration of BPA in boiled tap water having a pH of about 9.5 increased from < 0.002 mg/L at 50°C to 0.033 mg/L at boiling temperature.

Phthalates are classified as endocrine disruptors (EDC) by International Programme on Chemical Safety[12] and are found to affect the endocrine system, which consequently affects vital functions in living organisms. Dibutylphthalate (DBP), bis-(2-ethylhexyl) phthalate (DEHP), benzylbutylphthalate (BBP), and di-*n*-octylphthalate (DOP), among others, are examples of phthalates [13]. Phthalates such as DBP have been associated with toxicity to the neural, reproductive and developmental systems [14] and can also cause alterations to the kidneys and the liver, fetal malformation and fertility impairments [14], [15], [16]. In humans, exposure to phthalates such as DEHP and DBP can alter human sperm motility [17].

The European Food Safety Authority (EFSA) [18], [19], [20] has established the tolerable daily intake (TDI) for phthalates at 0.01 mg/kg body weight per day for DBP, 0.5 mg/kg body weight per day for BBP, and 0.05 mg/kg body weight per day for DEHP based on toxicological studies. Thus there are number of possible toxicants which can leach out on heating or cooking food in microwave plastic containers. This study was planned to investigate whether the microwave plastic containers provided with microwave, also have possible leaching of such harmful chemicals into the ingredients present in the containers during microwave heating. To analyse this possible toxicity of gene level, prokaryotic bioassays were used.

Evaluating the toxicity by microbial assays is an important and cost effective tool as it provides the complete response of test organisms to all the compounds present in these microwaveable plastic containers. So a bacterial short term bioassay *viz. Pseudomonas fluorescens* growth inhibition assay was used to determine the toxicological effects. Being simple, quick, cost effective and relatively easy to perform, short term bioassays can assess harmfulness of toxic components leaching from plastic containers conveniently. The positive samples should be taken for higher screens to ensure safety. This study also builds up a basic groundwork to acquire more information about the prevalence and levels of mutagenic agents in the microwavable containers. A comparative study was also performed by using glass containers to give a contrasting overview of toxic potential of both types of containers

2. MATERIALS AND METHODS

Sampling:

The samples were collected from the 2 different models of LG microwave brand. LG is a very large manufacturer of microwaves and is very popular.

Following samples of microwavable containers were collected during the two samplings.

1. Microwavable idli mould
2. Microwavable cake mould
3. Glass container of Borosil company

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (49-53), Month: July - September 2018, Available at: www.researchpublish.com

The samples were collected in duplicates as sampling one and two to avoid any chances of batch contamination. Ultrapure water was taken as stimulant. These samples were treated with water by heating it in microwave till water boiled and then were kept for 5-6 hours. This water was tested in the assay to find out if any leaching of toxic chemicals that might be coated on these products occurs in the water and can be the cause of genotoxicity.

***Pseudomonas fluorescens* GROWTH INHIBITION BIOASSAY**

P. fluorescens growth inhibition assay helps in the determination of the effect of toxic substances on the growth of a pure culture of this bacterium. The tester strain *Pseudomonas fluorescens* MTCC103 equivalent to ATCC13525 was obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh, India. 15 ml of logarithmic growth phase culture of *P. fluorescens* were aseptically inoculated into 1 L of sterile nutrient broth. This test inoculum was then immediately dispensed in 25 ml volumes into 125 ml test flasks. To each flask, a 25 ml of sample was added and then the flasks were placed on a rotator shaker at 25 30°C for 10h. Five concentration levels (2%, 5%, 10%, 50% and 100%) of samples were tested and each set of experiment was repeated twice.The growth inhibition of *P. fluorescens* was determined by taking the absorbance on a spectrophotometer at 650 nm. The results were expressed as percent inhibition as compared to the negative control. Inhibition of an increase in turbidity or the % growth inhibition in the samples was compared with that of the control using the following equation:

%I= ODc - ODt / ODc X 100

Where %I is the growth inhibition, expressed as a percentage, ODt is the optical density (at 650nm) of a culture incubated with th concentration of test sample and ODc is the measured turbidity i.e. optical density of biomass at the end of the test period in the control,. The inhibition values (%I) for each concentration were plotted against the corresponding concentration (ll) in both assays. MS excel 2013 was used for drawing dose– response plots. Statistical analysis of dose–response curves to calculate EC20 and EC50 values was performed by logistic regression using a MS excel software XLSTAT 2015; Addinosoft.

3. RESULTS AND DISCUSSION

Sterile distilled water was used as negative control. After 10 hours of incubation with a log phase culture of the tester strain, the negative control showed an optical density of 0.91 and 0.93 at 620 nm and a % growth inhibition of 0.78% and 0.82% in sampling 1 and sampling 2 respectively. The optical density of negative control was used for calculation of % growth inhibition of the test samples. These values are the average of values obtained in each experiment and were used for calculations of all the samples of a particular sampling. A 0.5M solution of $ZnCl_2$ was used as positive control It showed an EC20 value 0.71% and an EC50 value of 1.34%.

In the *P. flourescens* growth inhibition assay % respiration inhibition increased with the increase in concentration of the sample resulting in the dose response profiles shown in Fig.1. The microwavable idli mould was found to be slightly cytotoxic in this assay. The EC20 values were 68.39% and 80.71% for sampling 1 and 2 respectively. However the EC50 values were as high as 128.47% and 146.06%. At 100°C, the % growth inhibition shown by this sample was 30.75% for sampling 1 and 34.32% for sampling 2 (Table. 1). These EC20 and EC50 values were obtained using logistic regression of killed cells by the log of sample concentration (%).

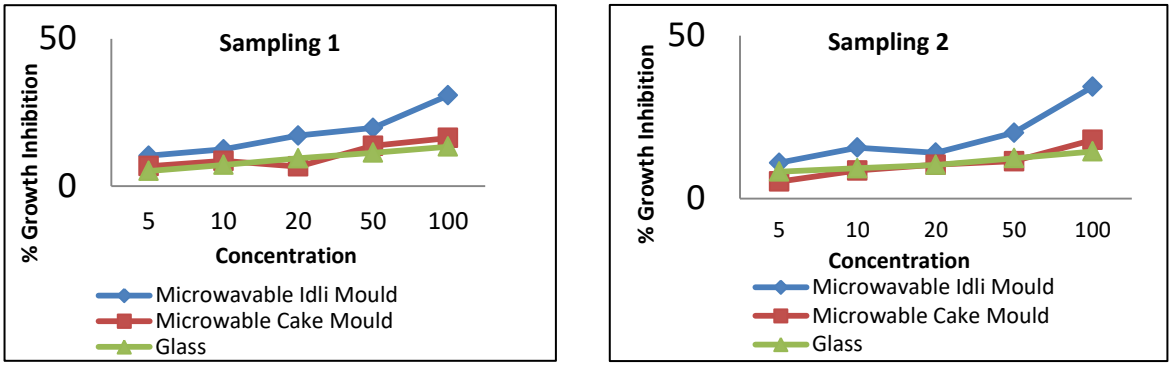
TABLE 1: EC20 and EC50 values of microwavable plastic containers and glass using *Pseudomonas flourescens* growth inhibition assay for sampling 1 and 2

S. No.	Sample	EC_{20} (%)		EC_{50} (%)	
		Sampling 1	Sampling 2	Sampling 1	Sampling 2
1	Microwavlable Idli Mould	68.30%	80.71%	-	-
2	Microwavlable Cake Mould	-	-	-	-
3	Glass	-	-	-	-

The microwavable cake mould however did not show any toxicity in the assay. The EC 20 and EC50 values were higher than 100% for both the samplings. The % growth inhibition at 100% was 16.52% and 18% for sampling 1 and sampling 2 respectively.(Figure 1). The glass was the not toxic among the three samples tested in this category according to this assay. Both EC20 and EC50 values were higher than100°C for both the samplings. The % growth inhibition at 100°C was 13.40% for sampling 1 and 14.21% for sampling 2.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (49-53), Month: July - September 2018, Available at: www.researchpublish.com

Fig 1: Dose response curves of microwavable plastic containers and glass using *P. flourescens* growth inhibition assay during sampling 1 and 2



A study by using mud snail *Potamopyrgus antipodarum* reproduction test was also found to be to effective in plastic toxicity testing. In this test female snails are used which reproduce by cloning themselves and reproductive output significantly increases in the presence of (xeno) estrogens [21], [22]. In a comparative migration study between plastic PET mineral bottles and glass by Wagner and Oehlmann [23], showed that reproductive activity of snails significantly increased when they were cultured in PET suggesting the release of some estrogen receptor active substance but there was no effect seen in snails cultured in glass correlating with the results obtained.

The results have shown that the containers provided with microwave can be considered to be safe. They did not show toxicity in *P. flourescene* assay and the LG brand containers are safe to use. The glass container was found to be safe for use and can be considered as an alternative to plastic containers.

4. CONCLUSION

The microbial bioassays have grown steadily in recent years and are a useful tool in chemical toxicity assessment. These rapid, reproducible and cost effective bacterial assays are useful for screening and assessment as they do not require a prior knowledge of toxicant identity and/or physico – chemical properties. There is a growing interest in short term microbial tests due to the fact that despite the existence of different toxicity for various organisms of different species, a substance that is toxic for an organism often demonstrates similar toxic effects on the other organisms. Therefore, the plastic containers provided with microwave are safe for cooking or heating of food products. Glass is also found to be safe and can be considered alternative for plastic.

REFERENCES

[1] K. A. Ehlert, C. W. E. Beumer, and M. C. E. Groot. Migration of bisphenol a into water from polycarbonate baby bottles during microwave heating. Food Addit Contam, 25(7):904–910.2008.

[2] The National Toxicology Program (NTP). Center for the Evaluation of Risks to Human Reproduction (CERHR), NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A, November http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf . 2007.

[3] S. Biedermann-Brem and K. Grob . Release of bisphenol a from polycarbonate baby bottles: water hardness as the most relevant factor. Eur Food Res Technol, 228(5) :679–684. 2009.

[4] M. J. Galotto and A. Guarda. Comparison between thermal and microwave treatment an the overall migration of plastic materials intended to be in contact with foods. Packag Technol Sci, 12(6):277–281. 1999.

[5] C. Gibson, I. Matthews, and A. Samuel. Microwave enhanced diffusion in polymeric materials. J Microw Power Electromagn Energy, 23(1):17–28.1988.

[6] C. Antonio and R.T. Deam Can "microwave effects" be explained by enhanced diffusion Phys Chem Chem Phys, 9:2976–2982 . 2007.

[7] K. Melski, J. Zabielski, and H. Kubera. Model study on intensified migration of volatile substances from food contacting plastic materials during repeated microwaving. Electron J Pol Agric Univ, 6(1).2003.

[8] M. J. Galotto and A. Guarda A. Suitability of alternative fatty food simulants to study the effect of thermal and microwave heating on overall migration of plastic packaging. Packag Technol Sci, 17(4):219–223.2004.

[9] M. T. De A. Freire, A.P. Damant, Castle and F. G. R. Reyes. Thermal stability of polyethylene terephthalate (pet):oligomer distribution and formation of volatiles. Packag Technol Sci, 12(1):29–36.1999.

[10] P. Mercea. Physicochemical processes involved in migration of bisphenol a from polycarbonate. J Appl Polym Sci, 112(2):579–593.2009.

[11] I. A. Lang, T. S. Galloway, A. Scarlett ,*et al*.. Association of Urinary Bisphenol a Concentration with Medical Disorders and Laboratory Abnormalities in Adults, JAMA, September 17, 300(11), 1303-1310.2008.

[12] International Programme on Chemical Safety. Available online: http://whqlibdoc.who.int/hq/2002/WHO_PCS_EDC_02.2.pdf(accessed on 3 November2012).

[13] D. M. Bila and M. Dezotti. Endocrine disrupters in the environment: Part 1—Effects and consequences. Quim. Nova , 30, 651–666. 2007.

[14] H. Xia, Y. Chi, X Qi, M. Su, Y. Cao, P. Song, X Li, T. Chen, A Zhao, Y Zhang, Y. Cao, X. Ma and W. Jia. Metabolomic evaluation of di-n-butyl phthalate-induced teratogenesis in mice. Metabolomics 7, 559–571.2011.

[15] F. A. Arcadi, C. Costa , C. Imperatore , A. Marchese , A. Rapisarda , M. Salemi , G. R. Trimarchi and G. Costa. Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the long-evans rat. Food Chem. Toxicol. 36, 963–970. 1998.

[16] J. S. Schmidt, K. Schaedlich, N. Fiandanese, P. Pocar and B. Fischer. Effects of Di(2-ethylhexyl) Phthalate (DEHP) on Female Fertility and Adipogenesis in C3H/N Mice. Environ. Health Perspect., 120, 1123–1129.2012.

[17] B. Fredricsson , L. Moller, A. Pousette and R. Westerholm. Human sperm motility is affected by plasticizers and diesel particle extracts. Pharmacol. Toxicol. 72, 128–133.1993.

[18] European Food Safety Authority. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to di-Butylphthalate (DBP) for use in food contact materials. EFSA J., 242, 1–17. 2005.

[19] European Food Safety Authority. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to Butylbenzylphthalate (BBP) for use in food contact materials. EFSA J. 241, 1–14.2005.

[20] European Food Safety Authority. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. EFSA J., 243, 1–20.2005.

[21] M. Duft, C. Schmitt, J. Bachmann, C. Brandelik , U. Schulte-Oehlmann , J. Oehlmann. Prosobranch snails as test organisms for the assessment of endocrine active chemicals–an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*. Ecotoxicology 16:169–82. 2007.

[22] M. Duft, U. Schulte-Oehlmann, L. Weltje , M. Tillmann, J. Oehlmann. Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. Aquat Toxicol 64:437–49.2003.

[23] M. Wagner and J. Oehlmann. Endocrine Disruptors in Bottled Mineral Water: Total Estrogenic Burden and Migration from Plastic Bottles, Environ. Sci. Pollut. Res., 16, 278-286.2009.