International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue2, 2014, pp141-150
http://www.bipublication.com

ANALYSIS OF GENOTOXIC AND CYTOTOXIC POTENTIAL OF CANNED FOOD PRODUCTS USING MICROBIAL BIOASSAYS

Ruchika Atri[1]*, Anuradha Singh[2], Nupur Mathur[2],
Aagosh Verma[1] and Suchitra Sharma[1]

[1]Enviornmental Molecular Microbiology Lab, [1,2]Department of Zoology,
University Of Rajasthan, Jaipur 302004 India.
Corresponding Author- ruchikaatri@gmail.com

[Received-20/03/2013, Accepted-02/04/2014]

ABSTRACT

Busier lifestyles have resulted in the increased demand for convenience food products and this trend has persistently provided a major thrust to the growth in the packaged and canned food sector in the world. Most packaged food are laden with food additives such as artificial sweeteners, salt, artificial flavors, synthetic fats, coloring agents and chemicals that alter the textures and techniques of preservation in food industries to increase the shelf life of food. With the growing dependence on these packaged and foods harmful effects caused by the excessive use of these food products are often neglected. An attempt has been made to study the genotoxic and cytotoxic potentials of commonly used canned food products. Microbial Bioassays like *Salmonella* mutagenicity assay /Ames assay (Prokaryotic assay) (with and without metabolic activation) was used to evaluate genotoxic potential and *Saccahromyces cerevisiae* Respiration Inhibition Assay (eukaryotic bioassay) were used to evaluate cytotoxic potential of canned food products. Among all the products that were tested canned Tuna fish was found to possess genotoxic potential with high number of revertants as high, and muatagenicity ratio was found to be greater than 2. Similar results were observed for cytotoxicity potentials.

Keywords: Genotoxicity, Cytotoxicity, Bioassays, Ames assay, *Saccharomyces* cerevisiae Respiration Inhibition Assay, canned food.

INTRODUCTION

Canned food is the most commonly used packaged food all over the world. The quantity of food stuffs packaged in coated cans and laminates has increased in the past decades due to their advantages like long storage time, they are easy to maintain and need less time to be cooked. Canned food is a good option for buying vegetables or fruits that are not available in a season. They are also convenient especially for storage and transportation. But with these advantages canned foods also come with disadvantages and risks. The food can lose some of its nutritional value when packaging is done, due to the high temperatures used in sterilization. Recent research and studies have reported that many of the preservative materials that are added

to canned foods have a serious effect on human health for example, the use of Butylated hydroxyanisole and Butylated hydroxytoluene as a preservative against food spoilage were found to be cytotoxic [1]. The use of nitrates and nitrites as preservative against botulism poisoning and to improve the taste and smell of canned meat has negative effects on human health. Food preservatives of this category namely Sodium nitrate, Sodium nitrite and Potassium nitrate were reported to cause mutation having deleterious effects [2].The use of certain sugars such as industrial Saccharin and Acesulfame - K as preservatives in the canned food to give sweet taste, is also posing a risk to human health as they have also been reported to have genotoxic potentials [3]. Such materials are classified by U.S. Food and Drug Administration as slow cancer materials and have a negative impact on the central nervous system of children. Recent research also indicated that the use of industrial dyes in the canned foods, are directly related to thyroid cancer and the emergence of asthma. Some companies also use chemical substances to paint cans of food and their lids from the inner side to prevent interaction of the canned food with the tin cans, and these substances are hazardous material such as Dimethyl bisphenol and Bisphenol A (BPA). BPA is a known estrogen mimicker, and can cause hormone-disrupting effects, toxicity or even neurotoxicity, low sperm counts and cancer. Many studies revealed migration of Diglycidyl ether of bisphenol A i.e., BADGE from processed and non-processed cans into the food as a function of the process treatment and the temperature of storage[4,5,6]. The epoxy resin bisphenol A diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of BADGE (BADGE-2HCl), were reported to be genotoxic and cytotoxic in many studies[7]. Food packaging that has long been considered "safe" by the FDA is now being found to be surprisingly hazardous to human health and the use of canned food products are thus a matter of controversy. At present, there is no published data on mutagenicity of canned foods. For this reason the purpose of this study was to evaluate the safety of popularly used canned foods and to evaluate their significant genotoxic and cytotoxic potentials. The bioassays used to investigate the genotoxicity of these canned food were *Salmonella* mutagenicity assay/Ames assay (prokaryotic assay) and *Saccharomyces cerevisiae* respiration inhibition assay (eukaryotic bioassay) which often give results in 24-72 hours. These will be beneficial for prescreening of large number of food products in ever growing food industry.

MATERIALS AND METHODS –

Food samples

Four types of the most favorite foods (i.e. Canned Tuna Fish, Canned Tomato, Canned Spinach, and Canned Fruit Cocktail were chosen. Foods were kept in cold storage. These samples were then tested for their mutagenic and cytotoxic potential.

Ames assay (*Salmonella/*microsome reversion assay)

The tester strains of *Salmonella typhimurium*, viz. TA98 and TA100, were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. All tester strains were maintained and stored according to standard methods[8]. The strain genotypes (histidine requirement, *rfa* mutation, *uvr B*, and R-factor) were confirmed immediately after receiving the cultures and every time a new set of frozen permanents were prepared and used. The assay was carried out using the plate incorporation method described [9] and revised [10]. The samples were analyzed with and without hepatic S9 fraction. Introduction of mammalian liver enzymes into the prokaryotic system incorporates the aspect of mammalian metabolism into the *in vitro* test. Uninduced

Swiss albino mice were used to prepare the standard S9 mixture. Sodium azide (5 µg/plate) was used as positive control and sterile distilled water was used as negative control. Five doses of individual samples (2, 5, 10, 50, and 100 µl of original samples) were tested and all the plates were run in duplicates. Each set of experiments was repeated twice. Average numbers of spontaneous revertants per plate for TA98 were 52 ± 4 and for TA100 were 127 ± 17 without metabolic activation and with metabolic activation 95±6 spontaneous revertants were obtained for strain TA 98 and 155±6 spontaneous revertants for strain TA 100. The *S. typhimurium* TA 98 and TA 100 strains were grown at 37 °C, with shaking, for 10 hr to obtain a final concentration of 10^9 bacterial cells. 0.1 ml of this fresh culture was mixed with 0.2 ml of His/Bio solution, 0.1 ml of sample, 0.5 ml of buffer, or 0.5 ml of S9 mix and total volume was made up to 1.0 ml by autoclaved distilled water. This mixture was then shaken and poured on plates containing about 25 ml of minimal glucose agar medium. The test concentrations were selected from a set of standard doses for liquids. The plates were immediately covered with paper to protect photosensitive chemicals present in the test compounds. Plates were then inverted and placed in a dark incubator for 48 h at 37 °C. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria. After 48 hr, the revertant colonies on the test and control plates were counted.

S. cerevisiae Respiration Inhibition Assay

A commercial brand of dried Baker's yeast (S. cerevisiae) was used for this assay. The Baker's yeast assay [11] was carried out by preparing a 1 % (v/v) suspension of yeast in sterilized saline solution (0.85 % NaCl) as the suspending fluid. The yeast suspension was stirred for 15 min to break up yeast floc. Test samples (0.2 ml) were added to 0.8 ml of yeast suspension and incubated for 30 min at 30 °C with shaking. Different concentrations of samples (1, 2.5, 5, 25, 50, and 100 %) were analyzed; 0.001 and 0.1 % concentrations were also tested when there was more than 50 % reduction in cell growth at the 1 % concentration. To this, 0.1 ml of 2-(piodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) solution (0.2 %) and 0.1 ml of 10 % solution of yeast extract were added to each sample and the mixture incubated in the dark at 30 °C for 1 h with shaking. The reaction was stopped with 0.1 ml of 37 % formaldehyde. Tetrazolium salts act as artificial electron acceptors along the electron transport system during respiration of microbial cells, becoming reduced to form insoluble formazans; therefore, microbial respiration can be assessed using INT reduction to the photometrically measured end product, INT-formazan. The proportion of respiring cells was determined as follows: one or two loopfuls of the yeast suspension were spread on a glass slide, air dried, counterstained with 0.025 % malachite green, and blotted after 1 min; 500 cells were examined with bright field microscopy (×100) and the number of respiring cells (green cells with red INT-formazan crystals) and non respiring cells (green cells) were determined. All reagents used for both assays were of analytical grade, supplied by HiMedia Laboratories Limited and Sigma-Aldrich (Mumbai, India).

Calculations

Data analysis for Ames test

The most common method for the evaluation of data from the mutagenicity assay is the "twofold rule" according to which doubling of spontaneous reversion rate at one or two test chemical concentrations constitutes a positive response[8]. This rule specifies that, if a test compound doubles or more than doubles the mean spontaneous mutation frequency obtained on the day of testing, then the compound is considered significantly mutagenic. Using this procedure, the following criteria were used to interpret results:

Positive— A sample was considered mutagenic if it produced a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *S. typhimurium*. A sample was considered weak mutagenic if it produced a reproducible dose-related increase in the number of revertant colonies in one or more strains but the number of revertants was not double the background number of colonies.

Negative—A sample was considered nonmutagenic if no dose-related increase in the number of revertant colonies was observed in at least two independent experiments.

Inconclusive—If a sample could not be identified clearly as mutagenic or nonmutagenic, the results were classified as inconclusive (e.g., if there was only one elevated count). For all samples that showed dose-dependent increase in the number of revertant colonies, mutagenicity ratios were calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants) [12].

Interpretation of test results for *S. cerevisiae* respiration inhibition assay

Results of Baker's yeast assay were expressed in terms of percent inhibition of respiring cells compared with negative controls. Effective concentrations resulting 20% and 50 % inhibition (EC_{20} and EC_{50}) in the percentage of respiring cells, respectively, were calculated using logistic regression.

RESULTS

The results of the *Salmonella* mutagenicity assay for four different canned food products are summarized in Table 1 as the mutagenicity ratio of average induced reversions to spontaneous reversions.

Sample	Dose (µl)	Mutagenicity Ratio TA 98		Mutagenicity Ratio TA 100	
		-S9	+S9	-S9	+S9
Canned Tuna Fish	2	+	+	+	+
	5	+	+	+	+
	10	+	+	+	+
	50	+	+	+	+
	100	+	+	+	+
Canned Tomato	2	-	-	-	-
	5	-	-	-	-
	10	+	+	+	+
	50	+	+	+	+
	100	+	+	+	+
Canned Spinach	2	+	+	+	+
	5	+	+	+	+
	10	+	+	+	+
	50	+	+	+	+
	100	+	+	+	+
Canned Fruit Cocktail	2	-	-	-	-
	5	-	-	-	-
	10	-	-	-	-
	50	+	+	+	+
	100	+	+	+	+

Table 1 Mutagenicity ratio of *Salmonella* TA 98 and TA 100 in Ames test on various canned food + ratio >2.0 indicating possible mutagenicity, − ratio < 2.0 indicating non mutagenicity

Canned Tuna Fish Canned Tuna Fish sample under study showed dose dependent significant mutagenicities with mutagenicity ratios >2.0 for all the sample doses (Table 1 Fig. 1a, and 1b). The specific mutagenic activity of Canned Tuna Fish as measured with strain TA98 (2,500–3,000 induced revertants at 100 µl dose of the sample, in the absence of S9 hepatic fraction) and with strain TA100 (2,600–3,200 induced revertants at 100 µl dose of sample, in the absence of S9 hepatic fraction) indicated that the Canned Tuna fish were highly genotoxic to be consumed. The addition of S9 mix further increased the number of revertant colonies of strain TA98 (3,100–3,400 induced revertants at 100 µl concentration of sample) and TA100 (3,600–3,900 induced revertants at 100 µl dose of sample). Fig 2(a)(b).

Canned Tomato

Mutagenicity ratios less than 2.0 were obtained with both the strains TA 98 and TA 100 at sample dose of 2 µl and 5 µl with and without metabolic activation (Table 1). At all other sample doses 10 µl, 50 µl and 100 µl the mutagenicity values greater than 2 were observed with and without metabolic activation (Table 1). Dose response curves calculated for increasing

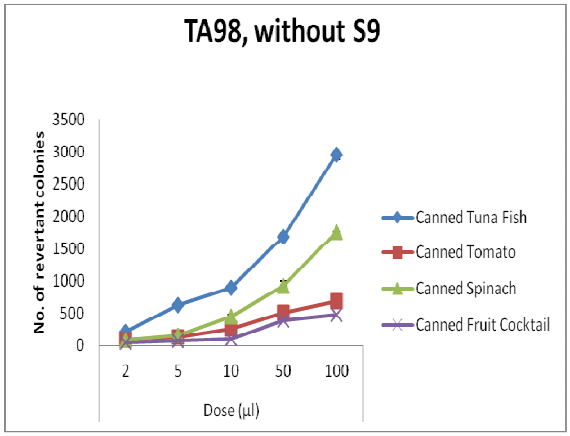
exposures to canned tomato samples revealed 600–700 and 600-900 induced revertants at 100 µl sample dose for strains TA 98 and TA 100, respectively, Fig 1(a) (b) in the absence of S9 fraction. In the presence of hepatic S9 fraction, 800-900 and 1,000-1,500 revertant colonies at 100 µl of sample dose were found for strains TA98 and TA100, respectively, Fig. 2(a)(b). All samples indicated significant mutagenicity at higher doses, with a marked increase in the number of induced revertants at 50 and 100 µl of the original concentration. However, at lower dose of samples 2 and 5µl, no mutagenicity were observed (Fig. 1a, b and 2a, b).

Canned Spinach

Mutagenicity ratio greater than 2.0 was found for all the doses tested (Table 1). In the absence of S9 fraction Dose response curves calculated for increasing exposures to canned spinach samples revealed 1,500–2000 and 1200-1800 induced revertants per 100µl of sample dose for strains TA 98 and TA100, respectively (Fig. 1 a & b). The addition of S9 mix further increased the number of revertant colonies of strain TA 98 (1,800–2,500 induced revertants per 100 µl dose of sample) and TA100 (2,000–2,600 induced revertants per 100 µl of sample dose (2 a & b).

Canned Fruit Cocktail

For both the strains TA 98 and TA 100 lower sample dose i.e. at 2 µl, 5 µl and 10 µl, the muatagenicity ratios were found to be less than 2, with and without metabolic activation (Table 1).This indicates no mutagenicity acitivity of the sample at these dose. With both the strains mutagenicity was observed at dose levels of 50 and 100 µl. Dose response curves for increasing dose of canned fruit cocktail samples revealed them to be less genotoxic. With both the strain of *Salmonella* without S9 mix, at 100 µl sample dose the mutagenic activity was observed to be very low, with TA 98 300-500 induced revertants and with TA 100 400-500 induced revertants were observed (Fig.1 a & b). With addition of hepatic fraction the number of induced revertants obtained was higher, 600-800 induced revertants per 100 µl of sample for strain TA 98 (Fig. 2 a) and 700-900 per 100 µl of the sample with strain TA 100 (Fig. 2b).



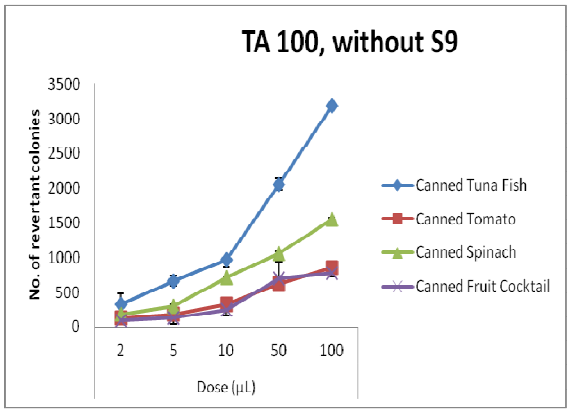
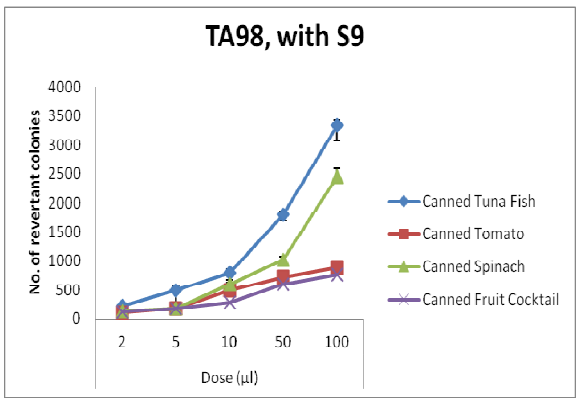


Fig. 1(a)(b) Dose–response curve for Canned food samples with strain TA 98 and TA 100 in absence of hepatic fraction



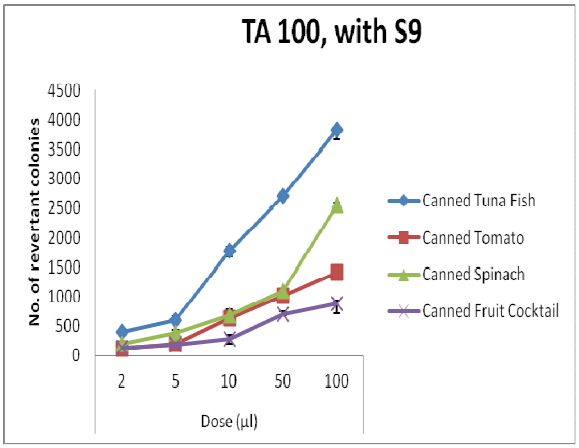


Fig. 2(a)(b) Dose–response curve for Canned food samples with strain TA 98 and TA 100 in presence of hepatic fraction

***S. cerevisiae Respiration Inhibition* Assay** When the samples were analyzed with the *S. cerevisiae* respiration inhibition assay, responses observed were similar to that of Ames assay. The results demonstrated that canned Tuna Fish was highly cytotoxic among all the tested canned products with EC_{20} values calculated to be 0.01 and EC_{50} values was found to be 0 .025. Among the canned vegetables canned spinach was found to be highly cytotoxic with low values of EC_{20} (0.14%) and EC_{50} (2.095%).

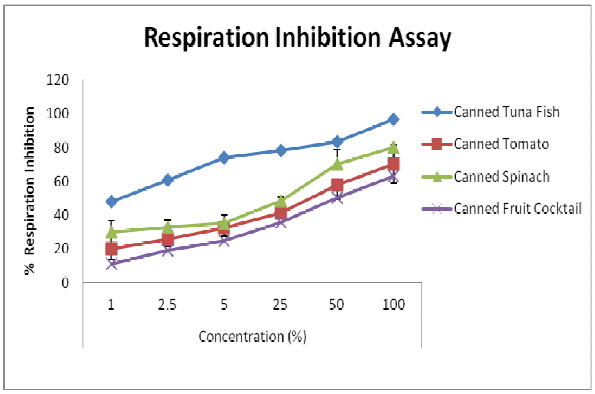


Fig. 3 Concentration–response curves for canned food samples in Baker's yeast respiration inhibition assay

Concentration–response curves clearly show that all canned samples, at higher concentrations are found to be cytotoxic, although at lower concentrations, their cytotoxic potential differed slightly. (Fig. 3).

DISCUSSION

Canned foods are popular food sources all over the world [13], because they are inexpensive and affordable [14, 15] such as canned Tuna and canned tomato, canned spinach and canned fruit cocktail. For most people, the main route of exposure to toxic elements is through the diet. Food safety is a worldwide major public concern, and the increasing worry about food safety has stimulated research regarding the risk associated with the consumption of canned food [16, 17]. Consequently information concerning dietary intake is of utmost importance in being able to assess risks to human health. In the present study an attempt to study the genotoxic and cytotoxic potentials of most commonly used canned food products was made and they all were found to possess genotoxicity and cyotoxicity at higher doses. There can be many reasons for this perspective. Genotoxicity and cytotoxicity can be the result of the manufacturing procedure, the equipments used during the process, packaging and storage procedures, excess use of food preservatives and flavor enhancers etc. Use of bisphenol- A, Aluminum and tin coating can be the cause of their cytotoxic and genotoxic potentials. Bisphenol-A is used for the manufacture of epoxy resins bisphenol A diglycidyl ether (BADGE) which is used for lacquer coatings on food cans and food storage vessels[18,19]. There are documented studies about potential migration of BPA leaching from epoxy resin coatings to food in metal cans [20]. When BADGE and its hydrolysis product BADGE2H2O, and BADGE.2HCl, were investigated both were found to induce both cytotoxic and genotoxic effects *in vitro* in cultured human lymphocytes. Due to the use of BADGE in food packaging materials and its degradation to hydrolysis products, it may

represent a potential risk to consumers. These risks could be reduced *in vivo* because the potential detoxification of the human cells could be similar to the detoxification effect observed by S9 fraction treatment.

In the present study among all the canned food canned Tuna fish was found to be highly genotoxic and cytotoxic(Fig.1 a,b and 2 a,b). Tuna was recognized as a predator able to concentrate large amounts of heavy metals[21]. The ingestion of food is an obvious means of exposure to heavy metals, not only because many metals are natural components of foodstuffs but also because of environmental contamination and contamination during processing. Literature has reported extensively the accumulation of Hg in marine food thus contaminating canned marine foods such as tuna and sardine [22, 23]. Likewise, the accumulation of Pb and Cd in fresh and canned fish has been reported [24]. Cd, Pb, and Hg are toxic metals. These metals have been associated with acute and chronic health symptoms [25]. They are harmful at low concentration and they are not easily biodegradable [26]. The potential genotoxicity and cytotoxicity of lead have been demonstrated after acute exposures lead has also reported to induce DNA damage that increased with increasing exposure time [27]. Lead has also been reported to possess the potential to induce oxidative stress and DNA damage even at very low concentrations (0.2 mg Pb L−1) [28],similarly other heavy metals like cadmium, chromium and mercury etc. have been reported to have genotoxicity and cytotoxicity [29].In the present study Fig 1(a),(b) Fig2 (a),(b), Fig 3 depicts that at higher concentration canned all canned food samples were proved to be cytotoxic and genotoxic this can also be due to the presence of heavy metals in them as there are studies which revealed higher heavy metal concentrations in canned vegetable mix, higher than a prescribed limit[30]. There can be one more prominent reason for genotoxic and cytotoxic potentials of canned food as proved in our study Fig.1(a)(b), Fig2 (a)(b), and Fig. 3 Canned food often contains food additives like food preservatives and flavor enhancers which are required to increase the shelf life of canned food. Many of the food additives used in canned food have proved to be genotoxic and cytotoxic[31,32]. The food additives monosodium phosphate (MSP), disodium phosphate (DSP) and trisodium phosphate (TSP) have been reported to induce chromosome stickiness, c-mitosis and anaphase bridges in *A. cepa* which proved that these food additives caused chromosomal aberrations and hence they are genotoxic [33]. Canned food often contains sulphates, sulphites and nitrates where they are used as antimicrobial, antioxidant and reducing dicolourable substances. Sodium nitrate, and potassium sulphate have proved to be genotoxic by[34]. As in our study canned spinach was found to be more genotoxic and cytotoxic than canned tomato food colors used can be the main explanation for this result. Colors are often added to increase the appealing value of canned food and many of them have proved to be genotoxic.

As proved by our study that regularly used canned products contain genotoxic and cytotoxic potentials, the reported studies proved that there can be many reason for this. The present study also highlighted the importance of short-term microbial bioassays (both prokaryotic and eukaryotic bioassays) as monitoring tools for pre-screening the canned food for genotoxicity and cytotoxicity in ever growing food industry. Furthermore, based on the strong mutagenic responses observed in the *in vitro* tests utilized here, it is also recommended that *in vivo* animal studies should also be performed to test these regularly used canned food samples in order to determine the potential relevance of the toxic effects of canned foods.

CONCLUSION

Based on the results obtained by this study regarding the genotoxicity and cytotoxicity of most popularly used canned food it can be concluded that in the light to clarify the real situation of consumer exposure and for the evaluation of its genotoxic risk, further screening of different canned food from the market should be conducted. A better selection of the fresh material, including an analysis for toxic elements prior to processing and canning, could surely improve the situation. Besides stringent government control on the excess use of food additives and establishment of packaging and processing guidelines will help to lessen the worse situation. Short-term microbial bioassays are of particular importance in characterizing the genotoxic and cytotoxic potential of canned food as these give results in 24-72 hours. The growing interest in these tests is due to the fact that despite the existence of different mechanisms of toxicity and sensitivities of different organisms, a substance that is toxic in one organism often can be used as an indicator of toxicity to other organisms [35]. Therefore, evaluation of biological effects using a rapid, simple, sensitive, and cost-effective method could indicate specific information on genotoxicity and allow the incorporation of toxicity parameters in the regulatory framework [36].

COMPETING INTERESTS

All authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Ruchika Atri was the main investigator, designed and performed the study and drafted the manuscript. Anuradha Singh and Nupur Mathur supervised the study. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors are thankful for the financial support provided by UGC Major Project entitled "Genotoxicity evaluation of wastewaters discharged from hospitals in Jaipur city" (File No. 40-113/ 2011).

REFERENCE

1. Ames, B.N, McCann, J, Yamasaki E,(1975), Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutat. Res.Vol- 31, issues 1-2, 347–364.
2. Ashraf, W, (2006), Levels of selected heavy metals in tuna fish. Arab Journal of Science and Engineering. Vol- 3, issue 1, pg 89-92.
3. Bakircioglu, D, Kurtulus, Y. B, Ucar, G,(2011),Determination of some trace metal levels in cheese samples packed in plastic and tin containers by ICP-OES after dry wet and microwave digestion, Food and chemical Toxicology. Vol-49,pg 202-207.
4. Bitton, G, Koopman, B, Wang, H. D(1984) Baker's Yeast Assay Procedure for Testing Heavy Metal Toxicity, Bull Environ. Contam. Toxicol. Vol- 32, 80-84.
5. D,Mello, JPF,(2003) Food safety: Contaminants and Toxins. Wallingford, Oxon, UK, Cambridge, CABI Publishing.
6. Ganjavi, M, Ezzatpanah, H, Ginvianrad, M. H, Shams, A(2010), Effect of canned tuna fish processing steps on lead and cadmium contents of Iranian tuna fish. Food Chemistry. Vol- 118, issue 3, 525-528.
7. Grillo, C.A, Dulout, F.N,(1995), Cytogenetic evaluation of butylated hydroxytoluene, Mutation Research/Genetic Toxicology. Vol- 345, issue 1-2,pg 73–78.
8. Growther, L, Parimala, R , Karthiga, G , Vimalin, H.J, Kalimuthu, K, Sangeetha, K.B, Food Additives And Their Mutagenicity, The Internet Journal of Nutrition and Wellness 2009 , 7 issue- 2.
9. Hammarling, L, Gustavsson, H, Svensson, K, Oskarsson, A, (2000), Migration of bisphenol-A-diglycidyl ether (BADGE) and its reaction products in canned foods, Food Additives and Contaminants. Vol-17, issue 11, pg- 937–94.
10. Kaiser, KLE,(2006), Correlations of Vibrio ,Fischeri bacteria test data with bioassay data for

other organisms, Environ. Health Perspec. Vol-106 issue 2, pg 583-591.

11. Kayraldiz, A, Kaya, F. F, Canimoglu, S, Rencuzogullari, E,(2006), Mutagenicity of five food additives in Ames/Salmonella/microsome test, Annals of Microbiology. Vol-56, issue 2, pg-129-133.
12. Khansari, E.E, Ghazi-Khansari, M, Abdollahi, M, (2005), Heavy metal content of canned tuna fish, Food Chemistry. Vol-93, issue 2, pg 293-296.
13. Korfali, S.I, Weam, A.H, (2013),Essentail and Toxic Metals in Lebanese Marketed Canned Food: Impact of Metal Cans, Journal of Food Research. Vol- 2, issue 1, pg 19-30.
14. Kumar, P, Panneerselvam N, (2007), Cytogenic Studies of food preservative in Allium Cepa root meristem cells, Medicine and Biology, vol- 14, issue 2,pg 60 – 63.
15. Leepipatpiboon, N, Sae-Khow, O, Jayanta, S,(2005), Simultaneous determination of bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether, and their derivatives in oil-in-water and aqueous-based canned foods by high-performance liquid chromatography with fluorescence detection, Journal of Chromatography A.Vol-1073, issue 1-2, 331–339.
16. Maron, D.M, Ames, B.N,(1983), Revised method for Salmonella mutagenicity test. Mutat. Res.Vol-113, issue 3-4, 175-215.
17. Mathur, N, Bhatnagar, P, Bakre, P, (2005), Assessing mutagenicity of textile dyes from Pali(Rajasthan) using Ames bioassay, Applied Ecology and Enviornmental Research. Vol-4, issue 1,pg 111-118.
18. Mol, S,(2011), Determination of trace metals in canned anchovies and canned rainbow trouts, Food and Chemical Toxicology, Vol- *49*, issue 2, 348-351.
19. Monteiro, V, Cavalcante DGSM , Vilela MBFA, Sofia, S.H, Martinez, C.B.R(2011), In vivo and in vitro exposures for the evaluation of the genotoxic effects of lead on the Neotropical freshwater fish Prochilodus lineatus, Aquatic Toxicology. Vol-104, issue 3-4, pg 291– 298.
20. Mortelmans, K, Zeiger, E, (2000), The Ames Salmonella/microsome mutagenicity assay. Mutation Research. Vol- 455, issues 1-2, 29–60.
21. Nagwa, RAH, Magda, AME, Atef AAH, Elham AAAH, (2011), Relative Mutagenecity of Some Food Preservatives on Plant Cells, Aust. J. Basic Appl. Sci. Vol-5, issue 12, pg 2817-2826.
22. Parvez, S, Venkataraman, C, Mukherji S, (2006), A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals, Environment International. Vol- 32, issue 2, pg 265–268.
23. Radwan, M.A, Salama, A.K(2006), Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology. Vol- 44, issue 8, pg 1273-1278.
24. Ruelas-Inzunza, J, Patiño-Mejia, C, Soto, J, Barbra-Quintero, G, Spanopoulos-Hernandez, M,(2011), Total mercury in canned yellow tuna Thunnus albacares marketed in northwest Mexico, Food and Chemical Toxicology.Vol- 49, issue 12,pg 3070-3073.
25. Sarikayaa, R, Cakirb, S,(2005), Genotoxicity testing of four food preservatives and their combinations in the *Drosophila* wing spot test, Environ Toxicol Pharmacol, Vol-3, issue 424-30.
26. Shiber, J, (2011), Arsenic, cadmium, lead and mercury in canned sardines commercially available in eastern Kentucky, USA. Marine Pollution Bulletin. Vol- 62, issue1, 66-72.
27. Simoneau, C, Theobald, A, Hannaert, P, Roncari, P, Roncari, A, Rudolph, T, Anklam, E,(1999), Monitoring of bisphenol-A-diglycidyl-ether (BADGE) in canned fish in oil, Food Additives and Contaminants, Vol-16, issue 5, pg-189–195.
28. Storelli, M.M, Barone, G, Cuttone, G, Giungato, D, & Garofalo, R,(2010), Occurrence of toxic metals (Hg, Cd, and Pb) in fresh and canned tuna: Public health implications, Food and Chemical Toxicology. Vol-48, issue 11,pg 3167-3170.
29. Storelli, M.M, Barone, G, Cuttone, G, Giungato, D, Garofalo, R, (2010), Occurrence of toxic metals (Hg, Cd, and Pb) in fresh and canned tuna: Public health implications. Food and Chemical Toxicology. Vol- 48, issue 11, pg 3167-3170.
30. Suarez, S, Sueiro, R,A, Garrido, J, (2000), Genotoxicity of the coating lacquer on food cans, bisphenol A diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of

BADGE, Mutation Research, Vol- 470, issue 2, pg-221–228.

31. Tice, P.A(1988), Pira project on migration of monomers and overall migration, Food Addit. Contam. Vol-5, issue 1, pg 373–380.
32. Tice, P.A, McGuiness, J.D, (1987), Migration from food plastics. Part I. Establishment and aims of the Pira project. Food Addit. Contam. Vol- 4, issue 3, 267–276.
33. Turkoglu, S, (2007), Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat. Res. Genet. Toxicol. Environ. Mutagen. Vol-626, issue 1-2, pg 4-14.
34. Unyayar, S, Celik, A, Cekic, F.O. and Gozel, A, (2006), Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in Allium sativum and Vicia faba, Mutagenesis. Vol- 21, issue 1, pg 77-81.
35. Vandenberg, L.N, Hauser, R, Marcus, M, Olea, N, Welshons, W,(2007), Human exposure to bisphenol A, Reprod Toxicol. Vol-24, issue 2, pg 139-77.
36. Wang, M.Z, Jia, X,Y,(2009), Low levels of lead exposure induce oxidative damage and DNA damage in the testes of the frog Rana nigromaculata, Ecotoxicology, Vol- 18, issue 1, pg 94–99.