



## **GENOTOXIC EFFECT OF FOOD ADDITIVES AND FOOD PRODUCTS: A REVIEW**

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### **ABSTRACT**

Our modern food industry's reliance on processing and additives continues to increase. With advanced technology, this seemingly abundance of foodstuffs found in our supermarkets of today is deceiving our bodies by selling food products that are chemically altered and designed to appeal to us. The genetic toxicology of major food additives and food products used in food market now-a-days has been reviewed. Published data for revealing the genotoxicity of different food additives used in varieties of food products (Canned meat, canned fruits, Ready to eat soups, fruit juices etc.) have been summarized and discussed. Despite the great importance of the issue regarding safety of "Ready to eat food" and canned food, the number of references was surprisingly limited.

**Key words:** Food additives, Canned food, Genotoxicity, Cytotoxicity, Short term microbial bioassays, Ames assay.

### **INTRODUCTION**

With vast population base, growing middle class and strong macroeconomic environment, the food and drinks market has emerged as one of the fastest growing segments in the retail industry. Rapid lifestyle transformation, particularly among those living in urban areas, has resulted into a dramatic increase in the demand for processed or health food, packaged and ready-to-eat food products. Arrival of food multinationals and proliferation of fast food outlets have further added to the growth in this industry. With the growing dependence on these packaged foods, harmful effects caused by the excessive use of food additives are often neglected. Among the harmful effects caused by regular use of food additives and packaged and canned food are hypersensitivity, various allergic reactions, lesions and tumors in body, genotoxicity, mutagenicity etc.

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## **Food additives, packaged food and canning**

Food additives play a vital role in today's food supply. A food additive is any substance or mixture of substances, other than basic food components added to food in a scientifically controlled amount.<sup>1</sup> These additives are used mainly for following purposes: to maintain or improve nutritional quality, to maintain product quality and freshness, to aid in the processing or preparation of food, and finally to make the food more appealing. To the majority of the food additives, to be added in packaged food (canned food or ready to eat food) particular JECFA/FAO has assigned "Admissible Daily Intake Dose"-ADI, which are often temporary and emphasized the need for further genotoxic evaluation, since a number of them are reported to be genotoxic even below the ADI dose. In India, the problem is severe because in spite of the regulation and restrictions by the prevention of Food Adulteration Act of 1954, use of non-permitted food additives are still prevalent.<sup>2,3</sup> Moreover most people are unaware about the harmful effects caused by packaged food available in the market. It has been reported that certain food additives are found to be genotoxic in different test systems.<sup>4,5</sup> Further food additives, however, have been prohibited from use because of their toxicity.<sup>6</sup>

The definition of Ready to Eat food (RTE) is food being ready for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled, and can be consumed without further heat-treatment including re-heating (Microbiological Guidelines for Ready-to-eat Food, 2006). Ready to Eat food are at the risk of containing genotoxicity because they contain large amounts of food additives.

*Canning* is a method of preserving food in which the food contents are processed and sealed in an airtight container. Coating on the containers of canned food often contains bisphenol diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of BADGE. Surveys carried out in 1995-1996 by official laboratories in several European countries have revealed high migration of BADGE from the internal coating into certain canned food products.<sup>7</sup> Canned food often contains heavy metals that are proved to be genotoxic.<sup>8</sup>

## **Genotoxicity and its significance**

Genotoxicity describes the tendency of carcinogens or their bioactivation products to attack electron-rich centres in DNA, generating chemically altered bases known as DNA adducts.<sup>9</sup> Depending on their mutagenic potency and whether they elude enzymatic repair, such adducts can promote DNA misreplication and deregulations of critical transformation-associated genes.<sup>10,11</sup>

### **Utilization of different bioassays to determine genotoxic potential**

In market, a large number of food additives are being used on per day basis. Long term assays using plants and animals are not suitable for pre-screening such a large number of samples. Studies carried out for genotoxic potential of food additives are mostly based on testing the food additives by long term procedures implying test on rats, *Allium cepa*, micronucleus test, chromosomal aberration test etc. as indicated in Table 1. Among short term microbial assays, researchers have mostly employed *Salmonella*/microsome assay (Ames assay) or *Salmonella* fluctuation assay (a liquid version of Ames assay). Testing of chemicals for mutagenicity in Ames assay is based on the knowledge that a substance that is mutagenic in the bacterium in the presence of animal liver enzymes metabolizing chemicals is likely to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans.<sup>12</sup> This review also includes studies dealing with the genotoxic potential of coatings of canned food and presence of various chemicals like furan and acrylamide in canned foods, which are known to have carcinogenic potential.

### **Evaluation of genotoxic potential by using plants and animal bioassays**

Some of the food colorants regularly used as food additives have a retard destructive effect on some vital organ functions. Therefore, large quantities and/or long periods of colorants administration should not be used as additive in man's diet or as a drink. Hassan et al.<sup>13</sup> tested tartrazine and chocolate brown color and found that they caused DNA damage in liver and kidney when tested on rat implying comet assay. Using two different cellular model systems, human lymphocytes *in vitro* and *Vicia faba* root tip meristems *in vivo*, Macioszek et al.<sup>14</sup> evaluated the potential cytological and genotoxic effects of two dyes: Quinoline Yellow (E 104) and Brilliant Black BN (E 151) by using the micronucleus and Comet assays. In both human lymphocytes and root meristem cells Brilliant Black BN showed very strong mutagenic effects, gradually rising with increasing dye concentration, in the micronucleus and Comet assays. Azo dyes, amaranth, allura red and new coccine, which are used as food color additives in Japan, were reported to cause colon specific DNA damage in mice by Shimada et al.<sup>15</sup>

Four food preservatives (sodium nitrate, sodium nitrite, potassium nitrate and potassium nitrite) and there five combinations at a concentration of 25 mM have been evaluated for genotoxicity in the somatic mutation and recombination test (SMART) of *Drosophila melanogaster* by Sarıkaya et al.<sup>16</sup> The genotoxic and toxic effects produced by the combined treatments were considerably increased, especially when the four chemicals were mixed. In a similar study carried out by Demie et al.<sup>17</sup> benzyl derivatives (benzaldehyde, benzylacetate, benzylalcohol and benzoic acid) were evaluated for their genotoxic effects and benzaldehyde was found to have significant high genotoxic effect.

**Table 1: Summary of literature on mutagenicity, genotoxicity, carcinogenicity, of different food additives using plant, animals and higher bioassays**

Study	Study design			Results
	Categories of food sample	Food sample	Bio assay employed	
Demie et al. <sup>17</sup>	Food preservatives	All benzyly derivatives	Somatic mutation and recombination test (SMART) of <i>Drosophila melanogaster</i>	Benzaldehyde was found to have significant high genotoxic effect.
Hassan et al. <sup>13</sup>	Food colors	Tatrazine and chocolate brown color	Comet assay was used using rats	Both of the food colors were found to cause DNA damage in liver and kidney in tested mice
Macioszek et al. <sup>14</sup>	Food colors	Quinilone yellow and brilliant black	Micro nucleus test and comet assay using human lympho-cytes <i>in vitro</i> and <i>Vicia faba</i> root tip meristems of <i>in vivo</i>	Brilliant Black BN showed very strong mutagenic effects, gradually rising with increasing dye concentration in both of the bioassays employed
Marathas et al. <sup>25</sup>		Eugenol	Chromosomal aberrations using V79 cells	Eugenol was found to induced Chromosomal aberration, with significant increases
Rencuzo-gullari et al. <sup>21</sup>	Artificial sweetner	Aspartame	Chromosome aberration (CA) test, sister chromatid exchange (SCE) test, micronucleus test in human lymphocytes.	Aspartame were found to induce chromosomal aberration.

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Study	Study design			Results
	Categories of food sample	Food sample	Bio assay employed	
Sarkaya et al. <sup>16</sup>	Food preservatives	Sodium nitrate, sodium nitrite, potassium nitrate and potassium nitrite	Somatic mutation and recombination test (SMART) of <i>Drosophila melanogaster</i>	All the food preservatives were found to cause mutations. The genotoxic and toxic effects produced by the combined treatments were considerably increased, especially when the four chemicals were mixed.
Shimada et al. <sup>15</sup>	Food colors	Allura red, Amaranth	Alkaline comet assay using mice	Both of the dyes were reported to cause colon specific DNA damage in mice
Sifa (2009)	Food preservatives	Monosodium phosphate (MSP), disodium phosphate (DSP) and trisodium phosphate	Root tips of <i>Allium cepa</i> were used	Food preservatives reduced mitotic division in <i>A. cepa</i> when compared with the respective control
Yilmaz et al. <sup>26</sup>	Food preservatives	Benzoic acid	Chromosomal aberration (CA), sister chromatid exchange (SCE) and micronucleus test	Benzoic acid significantly increased the chromosomal aberration, sister chromatid exchange and micronucleus frequency
Zengin et al. <sup>27</sup>	Food preservatives	Sodium and potassium benzoate	Micronucleus test, chromosomal aberration and also comet assay	SB and PB were found to be clastogenic, mutagenic and cytotoxic to human lymphocytes <i>in vitro</i> .

**Table 2 : Summary of literature on mutagenicity, genotoxicity, carcinogenicity, of different food additives using microbial bioassays**

Study	Study design			Results
	Categories of food samples	Food sample	Bioassay employed	
Bandy-opadhyay et al. <sup>51</sup>	Artificial sweeteners	Three low calorie sweeteners	Ames /Salmonella /microsome test using <i>Salmonella typhimurium</i> TA 97a and TA 100 strains both in the absence and presence of the S9 mix.	None could act as a potential mutagen in the Ames/Salmonella/microsome test.
Carneiro et al., (1998)	Flavor enhancer	Citral, citronellal, camphor, eucalyptol, terpineol and menthol	Mutagenicity was evaluated by the <i>Salmonella</i> /microsome assay (TA97a, TA98, TA100 and TA102 tester strains), without and with addition of an extrinsic metabolic activation system.	Terpinol was found to be mutagenic in Ames Assay. No mutagenic effect was found with (±) camphor, citral, citronellal, 1,8-cineole, and (?) -menthol
Evandri et al. <sup>38</sup>	Flavor enhancer	<i>Lavandula angustifolia</i> (lavender oil), <i>Melaleuca alternifolia</i> (tea-tree oil)	Bacterial reverse mutation assay in <i>Salmonella typhimurium</i> TA98 and TA100 strains and in <i>Escherichia coli</i> WP2 uvrA strain,	Neither essential oil had mutagenic activity on the two tested <i>Salmonella</i> strains or on <i>E. coli</i> , with or without the metabolic activation system. Conversely, lavender oil exerted strong antimutagenic activity, reducing mutant colonies in the TA98 strain exposed to the direct mutagen 2-nitrofluorene
Growther et al., (2009)	Food additives	Artificial sweeteners like saccharin, aspartame, and flavoring agents such as vanilla essence, soy sauce, chili sauce, worcestershire sauce, ice cream essence and rose syrup	Ames/ <i>Salmonella</i> / microsome assay	All the food additives were found to be mutagenic except vanilla essence and ice cream essence.

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Study	Study design			Results
	Categories of food samples	Food sample	Bioassay employed	
Ishikawa et al. <sup>55</sup>	Flavor enhancer	Kojic acid	Ames assay using <i>S. typhimurium</i> TA100 with or without S9 mix	Kojic acid was found to be mutagenic with a specific activity of around 100 revertants per mg of KA
Kayraldiz et al. <sup>56</sup>	Food preservatives	Potassium metabisulphite, potassium sulphate, sodium sulphite, potassium nitrate, sodium nitrate,	Ames assay using <i>S. typhimurium</i> TA100 with or without S9 mix	Potassium sulphate and sodium nitrate was found to have mutagenic effect on TA98 and TA100 strains of <i>Salmonella typhimurium</i> in the absence of S9 mix.
Mansour et al. <sup>47</sup>	Food colors	Acids yellow 17, violet 7 and orange 52	SOS chromotest using <i>Escherichia coli</i> PQ37, with and without metabolic activation (S-9 preparations), was used	In presence of the preparation S-9, the genotoxicity of dyes degradative products were found out.
Molinary <sup>53</sup>	Artificial sweetener	Aspartame	Ames Assay	ASP was not mutagenic in Ames test
Ozaki et al., 2002	Food colors	Gardenia yellow and its components, crocetin, gentiobiose geniposide and genipin	Ames test, rec-assay, and sister chromatid exchange (SCE)	Gardenia yellow and genipin caused damage of DNA in rec-assay. Gardenia yellow induced a significant dose-dependent increase of SCE frequency. genipin induced SCEs significantly among the components of gardenia yellow. Moreover, genipin induced a significant increase of tetraploids.
Pounikar and Dawande <sup>58</sup>	Food preservatives	Potassium nitrate	Ames assay using <i>S. typhimurium</i> TA 98 and 100	It was found to be mutagenic producing significant numbers of revertant colonies.
Shephard et al. <sup>54</sup>	Artificial sweetener	Aspartame	Ames assay using <i>Salmonella typhimurium</i> TA100 and TA98 strains after nitrosation.	ASP was found to have weak mutagenic effect.

In *in vitro* chromosomal aberration (CA) tests have come to play a central role in testing for mutagenic/carcinogenic potential of chemicals in most countries.<sup>18</sup> It has proved to be a useful and sensitive test for detection of genotoxic agents. The damage is scored by microscopic examination of chromosomes in mitotic metaphase cells. Tests are carried out with and without extrinsic metabolic activation.<sup>19</sup> A micronucleus assay using cultured cells has been developed.<sup>20</sup> This assay is more easily scored than the chromosome aberration assay and utilizes relatively small amounts of test article; thus, requiring less time to make an assessment of mutagenic potential of a chemical. Therefore, this assay has been widely used as an alternative means to screen for mutagens. The genotoxic effects of the low-calorie sweetener aspartame was investigated by Rencuzogullari et al.<sup>21</sup> using chromosome aberration (CA) test, sister chromatid exchange (SCE) test, micronucleus test in human lymphocytes. Aspartame induced CAs at all concentrations (500, 1000 and 2000 mg/mL) and treatment periods (24 and 48 h) dose dependently, while it did not induce SCEs. Olney et al.<sup>22</sup> reported that ASO may be carcinogenic in Sprague-Dawley rats. However, Jeffrey and Williams<sup>23</sup> reported that ASP did not induce DNA damage in rat hepatocytes and was not clastogenic in mice when given orally.<sup>24</sup> Marathas et al.<sup>25</sup> evaluated the genotoxicity of eugenol in V79 cells using chromosomal aberrations (CAs), with and without rat liver biotransformation (S9). The chromosomal aberration (CA), sister chromatid exchange (SCE) and micronucleus test (MN) were employed to investigate the *in vitro* effect of antimicrobial food additive benzoic acid on human chromosomes by Yilmaz et al.<sup>26</sup> The results of used assays showed that benzoic acid significantly increased the chromosomal aberration, sister chromatid exchange and micronucleus frequency. Zengin et al.<sup>27</sup> also employed micronucleus test, chromosomal aberration and also comet assay to evaluate genotoxic effect of sodium benzoate and potassium benzoate and found that SB and PB are clastogenic, mutagenic and cytotoxic to human lymphocytes *in vitro*.

### **Genotoxicity in canned and ready to eat food products**

Very few articles are available revealing the genotoxic effect of canned and ready to eat food. Epoxy-based solution coatings are used for lacquer coatings on food cans and food storage vessels.<sup>28,29</sup> Bisphenol A diglycidyl ether (BADGE) is an diepoxy resin obtained by a condensation reaction between epichlorohydrin and bisphenol A. The epoxy resin bisphenol A diglycidyl ether (BADGE), its hydrolysis products and a chlorohydrin of BADGE (BADGE2HCl), were examined for their genotoxicity in the micronucleus test (MNT) with human peripheral blood lymphocytes *in vitro*, in presence and in absence of an exogenous metabolizing system S9 rat liver by Suarez et al.<sup>30</sup> These compounds are able to induce both cytotoxic and genotoxic effects, as revealed by the increases observed in

cytokinesis block proliferation index (CBPI) and in micronuclei (MN) frequencies, respectively. The first reported data on acrylamide in foods available on the market were developed by the Swedish National Food Administration using liquid chromatography/tandem mass spectroscopy (LC/MS/MS). Since then varieties of baby foods have been found to have acrylamide by Rosen<sup>31</sup> and Alexy et al.<sup>32</sup> The genotoxic, mutagenic and carcinogenic potentials of acrylamide have been studied extensively. Nevertheless, there is sufficient evidence in the literature that both acrylamide and its metabolite glycidamide are mutagenic and clastogenic in mammalian cells.<sup>33,34</sup> Data suggest that mice are more vulnerable to acrylamide tumorigenicity. Farag et al.<sup>35</sup> carried a recent study to reveal genotoxicity of brown parts of some ready to eat meals. In their study, they take brown roasted poultry, brown or black part of grilled fish, rusted brown layer of local breads and normal kids' candy as their samples and employed cytokinesis-blocked micronucleus assay on human lymphocytes. The result showed increased frequency of micronuclei formation in humans lymphocytes *in vitro* from most of the tested samples.

Plants are also the essential members of the ecosystems; they are in general more sensitive to environmental stress than other systems using biomonitors.<sup>36</sup> Mutagenic activity of chemicals has been analyzed with different plant systems such as *Allium cepa* (onion) and *Vicia faba* (broadbean). With these plant systems, chromosomal aberration assays, mutation assays and cytogenetic tests have been performed.<sup>37-40</sup> The mitotic index and replication index are used as indicators of adequate cell proliferation<sup>41</sup>, which can be measured using *Allium cepa* (onion). This test combines two test targets, toxicity and genotoxicity. Toxicity is easily measured by observation of root growth inhibition and mitotic index, and genotoxicity is detected by frequency of chromosomal aberrations. Fiskesjo<sup>42</sup> suggested that positive results in the *Allium* test should be considered as a warning and also an indication that the tested chemical may be a risk to human health and to our environment.

### **Short term microbial bioassay- A new approach for evaluating genotoxicity**

The main disadvantages associated with animal and plant bioassays are: problems with standardization of the organisms, requirements for special equipment and skilled operators, long duration of the assay and lack of reproducibility. Therefore, evaluation of biological effects using a rapid, simple, sensitive and cost effective method could indicate specific information on toxicity and ecotoxicity and allow incorporation of toxicity parameters in the regulatory framework.<sup>43</sup> Short term microbial bioassays do not require prior information about chemical composition and can effectively, economically and rapidly assess the genotoxicity.<sup>44</sup> Bacterial bioassays are relatively quick and simple. The growing interest in these tests is 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 other organisms.<sup>45</sup> The Salmonella/microsome assay with *Salmonella typhimurium* is considered by many researchers as the most sensitive one for a wide array of substances when compared to other bacterial assays.<sup>46</sup> This assay is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. The test employs several histidine dependent Salmonella strains each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage via different mechanisms (Mortelmans and Zeiger, 2000). Thus, the Ames test being simple, quick and relatively easy to perform can be used as an initial screening test.

The *Escherichia coli* WP2 tryptophan reverse mutation assay detects trp<sup>-</sup> to trp<sup>+</sup> reversion at a site blocking a step in the biosynthesis of tryptophan prior to the formation of anthranilic acid. The different WP2 strains all carry the same AT base pair at the critical mutation site within the trpE gene. The assay is currently used by many laboratories in conjunction with the Ames Salmonella assay for screening chemicals for mutagenic activity. In general the WP2 strains are used as a substitute for, or as an addition to Salmonella strain TA102 which also carries an AT base pair at the mutation site.<sup>46</sup>

In addition to these, test genotoxicity of food colors was also evaluated by SOS chromo test using *Escherichia coli* PQ37.<sup>47</sup> The SOS chromotest originally was developed by Quillardet et al.<sup>48,49</sup> The test detects induction of the SOS genes, fused with lacZ reporter genes, which are involved in DNA repair in *Escherichia coli* K12 bacteria. The capacity of the Ames test to identify carcinogens is higher than that of the SOS chromotest. However, because the number of false positive compounds was lower in the SOS chromotest, the specificity, i.e., the capacity to discriminate between carcinogens and non-carcinogens of the SOS chromotest, appeared higher than that of the Ames test. Thus, the results of the SOS chromotest and of the Ames test are known to complement each other.<sup>50</sup>

Food additives namely, the artificial sweeteners like saccharin (sweetex), aspartame (sugar free), and flavoring agents such as vanilla essence, soy sauce, chili sauce, worcestershire sauce, ice cream essence and rose syrup were screened for their mutagenic activity utilizing Ames/ Salmonella/ microsome assay by Growther et al.<sup>2</sup> *Salmonella typhimurium* strains such as TA 98 and TA 100, with and without metabolic activation were used. The results showed that all the food additives were found to be mutagenic except vanilla essence and ice cream essence. Bandyopadhyay et al.<sup>51</sup> carried out the mutagenicity of the three low-calorie sweeteners in the Ames /Salmonella /microsome test and their

genotoxic potential by comet assay in the bone marrow cells of mice. The standard plate-incorporation assay was carried with the three sweeteners in *Salmonella typhimurium* TA 97a and TA 100 strains both in the absence and presence of the S9 mix. The comet parameters of DNA were increased in the bone marrow cells due to the sweetener-induced DNA strand breaks, as revealed by increased comet-tail extent and percent DNA in the tail. However, none could act as a potential mutagen in the Ames/Salmonella /microsome test. Butchko et al.<sup>52</sup> reviewed a study on safety of ASP and reported that ASP is safe. Molinary<sup>53</sup> reported that ASP was not mutagenic in Ames test and had no genotoxic effect in dominant-lethal and host mediated assay. However, Shephard et al.<sup>54</sup> reported that ASP has a weak mutagenic effect in *Salmonella typhimurium* TA100 and TA98 strains after nitrosation.

Three lots of kojic acid (KA) which were produced for use as a reagent, food additive and in cosmetics were shown to be mutagenic in *S. typhimurium* TA100 with or without S9 mix, with a specific activity of around 100 revertants per mg of KA.<sup>55</sup>

The mutagenic activity of five food additives ( $K_2S_2O_5$ : potassium metabisulphite, KMB;  $K_2SO_4$ : potassium sulphate, KS;  $Na_2SO_3$ : sodium sulphite, SS;  $KNO_3$ : potassium nitrate, KN;  $NaNO_3$ : sodium nitrate, SN) were investigated using histidin auxotrophs TA98 and TA100 strains of *Salmonella typhimurium* in the presence or absence of S9 mix.<sup>56</sup> Potassium sulphate and sodium nitrate was found to have mutagenic effect on TA98 and TA100 strains of *Salmonella typhimurium* in the absence of S9 mix.

Maltol has a caramel-butterscotch odour and is used as a food additive to impart flavour to bread and cakes. When maltol was irradiated with either UVA (a black light, 320–400 nm, 230  $\mu W/cm^2$ ) for 5-30 min or UVC (a germicidal lamp, 610  $\mu W/cm^2$ ) for 3 min in sodium phosphate buffer (pH 7.4) prior to the exposure of bacterial cells, it was mutagenic to *Salmonella typhimurium* TA100, TA104 and TA97.<sup>57</sup> Pounikar et al.<sup>58</sup> carried out Ames test on potassium nitrate, a food additive and revealed its mutagenic activity against strain of *Salmonella typhimurium*. This revealed that food additives are mutagenic in bacteria and could be said to possess carcinogenic potentials.

## CONCLUSION

Going through the published literature, many studies were found revealing the genotoxic effects of different food additives like food colors, food preservatives and flavor enhancers. However, the number of studies regarding genotoxicity of canned food and “Ready to eat food” is limited and needs more attention and concern. The increasing number of researches in recent years in this field, are rendering this issue internationally important.

Bioassays have proven to be of significance in envisaging the genotoxic and mutagenic potential of food additives and different food products. Among the bioassays, the popularity of bacterial assays is based on the fact that bacterial bioassays are relatively simple, cost effective and quick giving results within 24 hours. Although the results obtained with animal and plant bioassays are also of considerable importance but as already mentioned, there are disadvantages associated with animal and plant bioassays such as problem with standardization of the organisms, requirements for special equipment and skilled operators, long duration of the assay and lack of reproducibility. The bacterial assays definitely are more attractive as they are simple, rapid and cost effective. The growing interest in these tests is 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, evaluation of biological effects using these microbial bioassays could indicate specific information on genotoxicity.

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