Journal of Cytology and Genetics 2021 VOL. **22** (NS): 63−66 © 2021 Society of Cytologists and Geneticists, India

RESEARCH ARTICLE

KARYOTYPIC STUDY ON FOUR GALL FORMING APHID SPECIES FROM HIMACHAL PRADESH

RAGHUBIR SINGH, MEENA KUMARI* AND D. C. GAUTAM
Department of Biosciences, Himachal Pradesh University, Shimla 171 005, India
*For correspondence. Email: Email: meenakchaudhary@gmail.com

(Received 16 August 2021, revised accepted 5 September 2021)

SUMMARY In the present investigation, karyotypes of 4 species of gall forming aphids, viz., *Epipemphigus imaicus*, *Pemphigus matsumurai*, *P. laurifolia* and *P. populitransversus* have been studied. These aphid species were found infesting *Populus* sp. from Kullu, Mandi and Shimla districts of Himachal Pradesh. The diploid chromosome number in *E. imaicus* is 18. Whereas *P. matsumurai* has diploid number of 12, *P. laurifolia* and *P. populitransversus* have 2n = 20. The chromosomes were holocentric. The karyotypes have been described. Idiograms were prepared on relative length data.

Keywords: *Epipemphigus*, *Pemphigus*, gall aphid, karyotype, holocentric, chromosome.

INTRODUCTION

Aphids are among the most destructive insect pests of cultivated plants in temperate regions (Mc Gavin 1993). These are the members of superfamily Aphidoidea under the suborder Homoptera of order Hemiptera. Aphids have attracted attention of man because of the unusual phenomena such as cyclic parthenogenesis, vivipary and telescoping of generations in their life cycle (Dixon 1985).

About 5000 species of aphids are found around the world (Blackman & Eastop 2015, Favret 2015). Only 250 species are considered as pests and cause considerable damage to horticultural and agricultural crops throughout the world (Blackman & Eastop 1994). Galls are kind of swelling on the external tissue of plants, the abnormal plant structures induced by various organisms, in particular by the insects (Mani 1964). Aphids within the galls contribute relatively little to genetic variation, since all aphids within the gall are parthenogenetic offsprings of a single female (Wool 1977).

The other interesting feature in aphids is the nature of chromosomes which are considered to be holocentric. Due to holocentric nature of chromosomes, karyotype variations occur frequently as a result of fission or fusion mechanism which leads to evolution of new biotypes (Blackman & Eastop 1994). Cytogenetic mechanisms in aphids are very complex but interesting. Chromosome number and physical features of aphid species are so closely related that the evolutionary scale of any aphid species could be determined from its chromosome number.

Although chromosomes of many species of aphids from Himachal Pradesh are reported by earlier workers, there is no detailed account available on chromosomes of gall aphids from Himachal Pradesh. It is desirable to study the chromosomes of different species of gall forming aphids and to analyse their karyotypes. These studies will also help in providing the detailed information about the chromosome numbers and karyotypes of different species of gall aphids. The present paper deals with karyomorphology of 4 gall forming aphid species viz., *Epipemphigus imaicus*, *Pemphigus matsumurai*, *P. laurifolia* and *P. populitransversus*.

MATERIALS AND METHODS

The gall forming aphids were collected from galls on the leaves and twigs of *Populus* sp. plant from Kullu, Mandi and Shimla districts of Himachal Pradesh. For chromosomal study, only apterous, parthenogenetic and viviparous females were used. The embryos were taken out by puncturing the posterior end of abdomen. Then, pretreated in 0.7% sodium citrate solution for 30 min. The pretreated embryos were then fixed in 1:3 acetic acid-ethanol solutions for about 15–20 min at room temperature. After fixation, embryos were placed on a glass slide in a drop of 45% acetic acid for 3–5 min. A cover slip was put on the material. Staining of slides was done with 2% Giemsa. Well spread metaphase plates were selected and lengths of chromosomes were measured using ocular micrometer. From actual lengths, the total complement length (TCL) and relative lengths of chromosomes were calculated for each species.

OBSERVATIONS

Epipemphigus imaicus

The diploid chromosome number in this species was found to be 18 (Figs 1, 2). The mean actual length of chromosomes ranged from 0.97 µm to 5.22 µm with TCL of 44.44 µm. The idiogram of this species reveals the gradual decrease in chromosome length (Fig.3). Relative length of chromosomes ranged from 2.17 to 12.04.

Pemphigus matsumurai

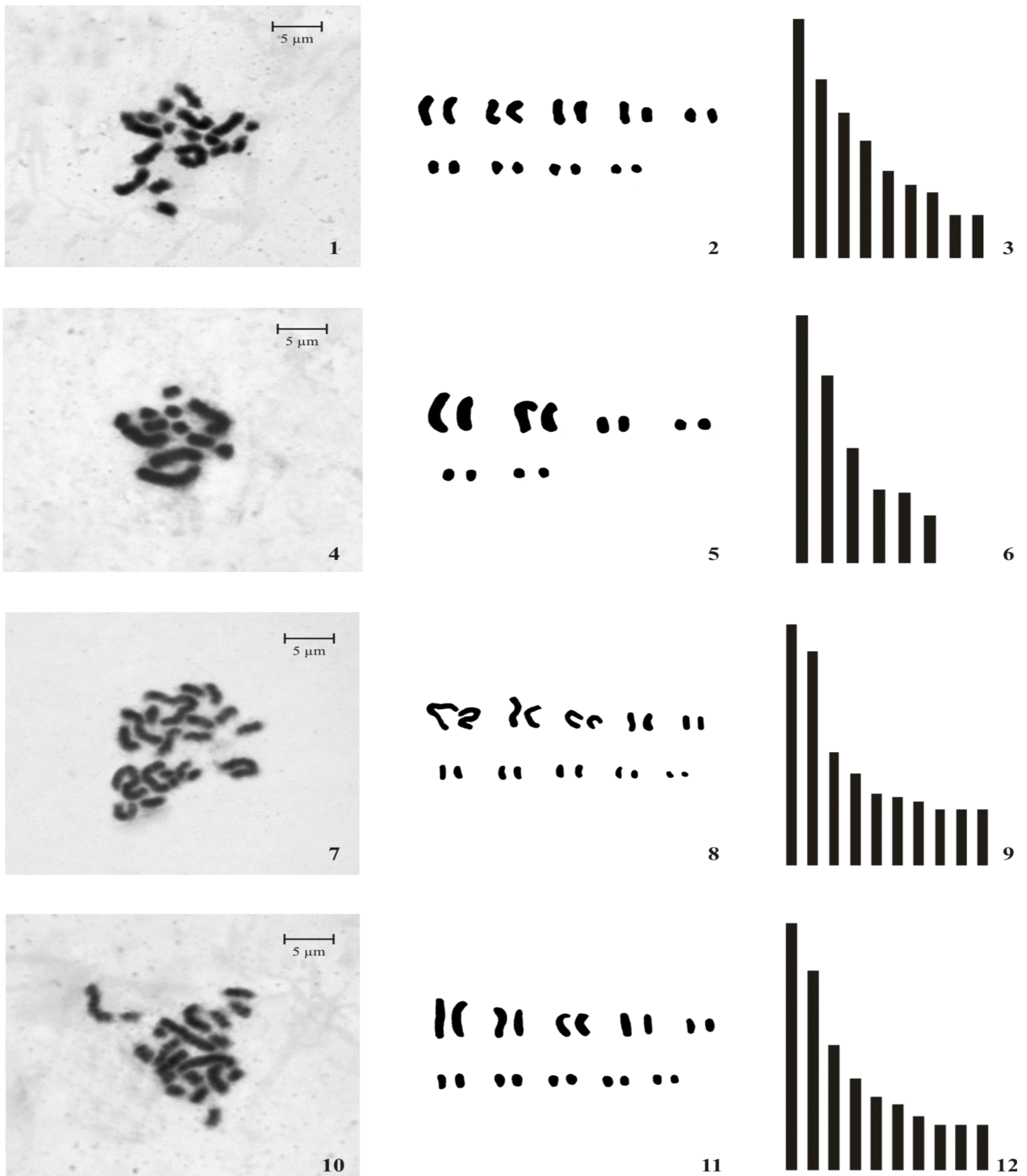
The diploid chromosome number in this species was found to be 12 (Figs. 4, 5). The mean actual length of chromosomes ranged from 1.05 µm to 5.29 µm with TCL of 32.20 µm. The idiogram reveals a gradual decrease in chromosome length (Fig. 6). Relative length of chromosomes ranged from 3.24 to 16.68.

P. laurifolia

The diploid chromosome number in this species was found to be 20 (Fig.7, 8). The mean actual length of chromosomes ranged from 0.82 µm to 3.80 µm with TCL of 31.79 µm. The idiogram of this species reveals a gradual decrease in first 7 pairs of chromosomes while the last 3 pairs were of the same length (Fig. 9). Relative length of chromosomes ranged from 2.69 to 11.68.

P. populitransversus

The diploid chromosome number in this species was found to be 20 (Figs.10, 11). The mean actual length of chromosomes ranged from 0.82 µm to 4.47 µm with TCL of 35.81 µm. The idiogram of this species reveals the gradual decrease in first 7



Figs 1–12: Karyotypes of aphids. 1–3. *E. imaicus.* 1. Somatic chromosomes 2. Karyotype. 3. Idiogram. 4–6. *P. matsumurai* 4. Somatic chromosomes. 5. Karyotype. 6. Idiogram. 7–9. *P. laurifolia.* 7. Somatic chromosomes. 8. Karyotype. 9. Idiogram. 10–12. *P. populitransverses.* 10. Somatic chromosomes. 11. Karyotype. 12. Idiogram.

pairs of chromosomes while the last 3 pairs were of the same length (Fig. 12). Relative length of chromosomes ranged from 2.30 to 12.43.

DISCUSSION

Of the 4 species discussed here, in *P. laurifolia* and *P. populitransversus*, the diploid chromosome number is 20, while *E. imaicus* has diploid chromosome number of 18 and *P. matsumurai* has 12. The chromosome number of 2n = 18 in *E. imaicus* is in conformity with earlier reports of Gavrilov et al. (2015), Khuda-Bukhsh (1980) and Khuda-Bukhsh & Pal (1983). Likewise the chromosome number of *P. matsumurai* is also in conformity with earlier reports of Blackman (1986), Chen & Zhang (1985), Blackman & Eastop (1994), Blackman and Eastop (2015) and Gavrilov et al. (2015). Blackman (1986) regarded 2n = 12 as the unusual chromosome number for the genus *Pemphigus*. The chromosome number of 2n = 20 for *P. laurifolia* is in conformity with earlier reports of Blackman (1986) and Gavrilov et al. (2015). Chromosome record of this species is the first report from India. The chromosome number of 2n = 20 for *P. populitransversus* is in conformity with earlier reports of Blackman & Eastop (1994) Gavrilov et al. (2015) and Harper & MacDonald (1966). Chromosome record of this species is the first report from India. The karyotypes of different species of gall aphids prove useful in understanding the adaptive and evolutionary trends in aphids.

ACKNOWLEDGEMENT

Fellowship awarded to RS by Indian Council of Medical Research, New Delhi is gratefully acknowledged.

REFERENCES

BLACKMAN R L 1986 The chromosomes of Japanese Aphididae (Homoptera) with notes on the cytological work of Orihay Shinji *Cytologia* **51** 59–83

BLACKMAN R L & EASTOP V F 1994 *Aphids on World's Trees An Identification and Information Guide* CAB International Wallingford Oxon

BLACKMAN R L & EASTOP V F 2015 *Electronic database*: *"Aphids on the world's plants"* http:// www. aphidsonworldsplants.info.

CHEN X S & ZHANG G X 1985 The karyotypes of 51 species of aphids (Homoptera: Aphidoidea) in Beijing area *Acta Zoologica Sinica* **31** 12–19

DIXON A F G 1985 *Aphid Ecology* Blackie and Sons Ltd Glasgow

FAVRET C 2015 *Aphid species* http://Aphid.SpeciesFile.org

GAVRILOV-ZIMIN I A, STEKOLSHCHIKOE A V & GAUTAM D C 2015 General trends of chromosomal evolution in Aphidococca (Insecta: Homoptera: Aphidinea: Coccinea) *Comp Cytogenet* **9** 335–422

HARPER A M & MACDONALD M D 1966 Chromosomes of gall aphids in the subfamily Eriosomatinae (Homoptera: Aphididae) *Can J Genet Cytol* **8** 788–791

KHUDA-BUKHSH A R 1980 Karyotypic studies in six species of aphids (Homoptera: Aphididae) from the Garhwal Himalayas *Entomon* **5** 247–250

KHUDA-BUKHSH A R & PAL N B 1983 Karyology of four gall forming aphids (Homoptera: Aphididae) from the Garhwal Himalayas *Entomon* **2** 109–114

MANI M S 1964 *Ecology of plant galls* Walter Junk Publishers The Hague

Mc GAVIN G C 1993 *Bugs of the World* Infobase Publishing

WOOL D 1977 Genetic and environmental components of morphological variations in gall-forming aphids (Homoptera: Aphididae: Fordinae) in relation to climate *J Animal Ecol* **46** 875–889

Journal of Cytology and Genetics 2021 VOL. 22 (NS): 67−82 © 2021 Society of Cytologists and Geneticists, India

REVIEW ARTICLE

GENOTOXIC AND CYTOTOXIC EFFECTS OF FOOD ADDITIVES

ALEEM YOOSUF N. AND JASMINE M. SHAH*
Department of Plant Science, Central University of Kerala, Kasaragod 671 320, India
*For correspondence. Email: jasmine@cukerala.ac.in

(Received 12 July 2021, revised accepted 5 October 2021)

SUMMARY Food additives are added to food in order to enhance qualities such as colour, flavour and storage life of the food. Many food additives are artificial and are known to affect the physiology of organisms and/or are mutagenic. Therefore, many food additives are being discontinued from the market. Various tests using different model organisms have been reported previously to validate the deleterious effects of food additives. An elaborate survey of the literature reveals that a given food additive can cause deleterious effect either by virtue of its mutagenic, apoptotic, or any other property and, this effect is not the same on all model systems. For example, an additive that is not mutagenic to a bacterium can be mutagenic to eukaryotes, and vice versa. Even the technique used to study matters. For example, cytogenetic studies can reveal ploidy changes, but not random point mutations. In this review we have profiled various food additives for their cytotoxic and mutagenic effects in various living systems, along with the techniques used to study them. This review indicates that before concluding on the safety of any food additive, it is advisable to cross-check its effects on multiple organisms and techniques. Our compilation serves as a quick reference guide to compare the deleterious effects induced by multiple food additives, along with the techniques used to study them.

Keywords: Food additive, cytotoxicity, mutagenic, mutation, genotoxic, antimitotic.

INTRODUCTION

Food additives are the non-food substances added to food products for improving flavour, colour, nutrients, texture, shelf-life of food as well as food safety measures. Around 2500 such food additives are available all over the world (Carocho & Barreiro 2014). Mankind has started using food additive from Egyptian civilization, at 5000 B.C. and use of food colourant has begun around the same period (Carocho & Barreiro 2014, Meggos 1995, Mpountoukas et al. 2010). For centuries, some food additives like salt, sugar and sulphur dioxide (in wines) have been used as preservatives (https://www.who.int/news-room/fact-sheets/detail/food-additives). In order to tackle the rising demand of processed food products, hundreds of additives were developed over the subsequent years, after the industrial revolution (Jen & Chen 2017). Nowadays, food additives have become an inevitable part of food industries. They are derivatives of plant or animal substances, or they can also be artificial; details

on types of food additives have been described previously in other reviews (Carocho & Barreiro 2014, Maga & Tu 1994, Msagati 2013). The risks and adverse effect of food additives on human and live-stock health as well as environment have been under high grade inspection for a number of years. As a consequence, a number of food additives were banned after confirming their carcinogenic, hepatotoxic, cardiotoxic and/or neurotoxic effect on body. For example, calamus, a taste enhancer derived from β–Asarone, contained in the oil extract of *Acorus calamus*, was banned in 1968 after confirmation of its carcinogenicity (Rangan & Barceloux 2009). More details in this area have been described elsewhere (Branen et al. 2001). Usually, the concentration of food additives required in the food is very less, sometimes negligible, and hence, it is often expressed in parts per million (PPM). However, the toxic potential of many food additives is detected even at this low concentration. Voluntary addition of overdose of food additives, beyond the allowed limit, or accidental adulteration may also induce physical and physiological risks including cancer (Merrill 1978, Packard & Myers 1978). Many food additives contain toxic heavy metals like mercury and cadmium and adulteration even at small quantities can lead to accumulation of these metals within the body, if consumed repeatedly. The food waste containing food additives that are consumed by other animals (like domestic animals) also are of concern as they may cause biomagnification through the food chain. Taking different model systems as biomonitoring tool, cytotoxic effect of various food additives and their potential risks were demonstrated over the years, both in plant and animal systems. Cytotoxic effects of various food additives are of various types and they lead to altered cellular growth, physical and physiological changes in organisms (Fig.1). Different strategies to examine and validate the toxic, physiological and carcinogenic risks of various food additives have been developed over the years. In this review, we have compiled the cytotoxic effects such as genotoxic, apoptotic, antimitotic, physiological and carcinogenic effects of 67 food additives and, the model organism and methods used to screen these cytotoxic effects. This compilation conveys the importance of analyzing one food additive by various methods and parameters in multiple organisms, in order to rule out any chance of it being harmful. This review thus identifies the gaps in the screening of currently used food additives.

Genotoxic potential of food additives and important parameters to be considered

Genotoxicity is a type of cytotoxicity, where damage or any type of harm is caused to the genetic material of living cells. Genotoxic damages, if not controlled, may lead to physiological changes such as delayed or lack of cell division, inefficient DNA repair and even mutations (Fig.1). Hence, all genotoxicity-causing factors need not be mutagenic, while, all mutagens are genotoxic. In animals, mutagenic effects of genotoxicity can lead to cancer as well. Mutations can be of different types, affecting one or few nucleotides at the DNA level, or can

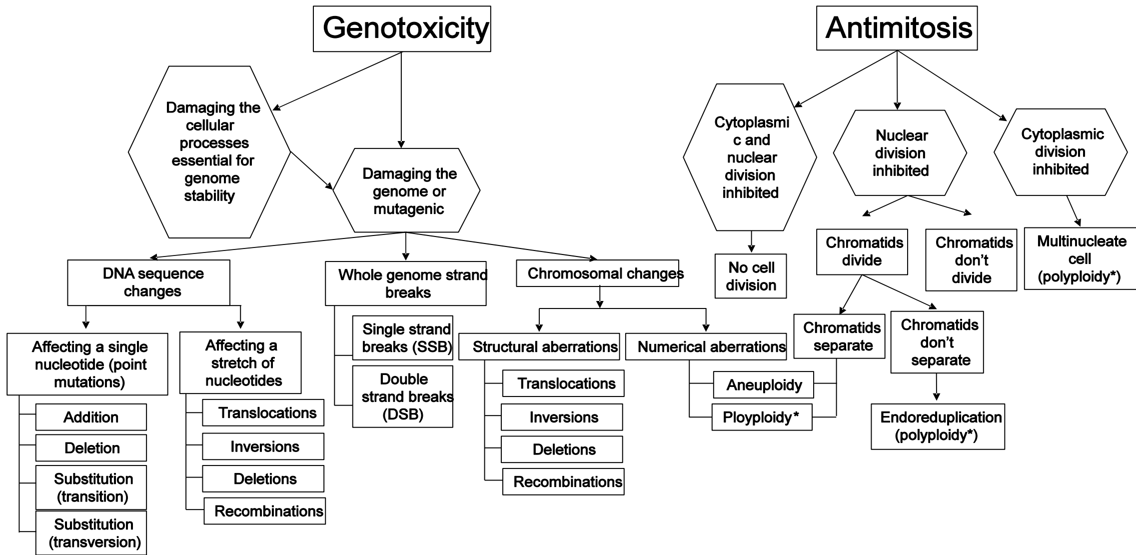


Fig. 1: Various genotoxic and antimitotic effects of food additives.

involve entire chromosome (Fig. 1). The former ones include those with single nucleotide (point mutations) and stretches of nucleotides (such as deletions, translocations, inversions). The latter types include ploidy changes, chromosomal inversions, deletions and translocations. The extent of damage may be limited to few nucleotides or can be at a massive scale as in whole genome strand breaks. Though all types of genotoxicity have the potential to trigger cancer and other physiological damages, our compilation of reports on 67 food additives revealed that most studies involve chromosomal aberrations and whole genome strand breaks, but not loci-specific mutations such as frame-shift and point mutations (Table 1).

The assessment of genotoxicity relies on the techniques used. Experiment using one or few techniques can never reveal about all possibilities of the occurrence of any genotoxic effects. For example, while alkaline comet assay examines the single and double stranded breaks in denatured DNA, neutral comet assay specifically examines the double stranded DNA breaks (Aleem Yoosuf et al. 2020, Gontijo et al. 2003,). Another technique, cytokinesis block micronucleus cytome (CBMN-Cyt) assay (An et al. 2007, Tsuboy et al. 2007) is used to evaluate aneugenic and clastogenic effect such as binucleate cells with 1–4 micronuclei (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) frequencies (Bonassi et al. 2003, Wultsch et al. 2011). Comet assays and CBMN-Cyt assay are often done simultaneously to evaluate the toxicity at genome and DNA levels. However, these cannot be used to identify loci-specific mutations, which in turn are studied by various methods such as Ames test, somatic mutation and recombination tests (SMART) and

TABLE 1: Genotoxicity of food additives.

Food additive	Organism/cells	Methodology	Aberration	Reference
Preservative				
Tert-butylhydroquinone	Human umbilical vein endothelial cells (HUVEC) and lung cells	MTT assay and flow cytometry	Fragmentation, necrosis and apoptosis	Eskandania et al.2014
Boric acid	*Allium cepa*	Mitotic squash	C-mitosis, sticky chromosome, micronuclei, multipolar anaphase and mitosis, laggards, bridges and unequal distribution.	Donbak 2002 Turkoglu 2006
	Trigonella foenum-graecum	Mitotic squash	Non-orientation at metaphase, precocious movement at metaphase, stickiness of chromosomes, anaphase laggard and chromosomal bridges.	Kumar & Srivastava 2011
Sodium metabisulphite	*A. cepa*	Mitotic squash	C-mitosis in cells with sticky abnormal anaphase micronucleated and bridges.	Rencuzogullari et al. 2001
Sodium sulphite	*Vicia faba*	Root sample pressing	Severe anaphase bridges, premature chromosome condensation heading to pycnotic nuclei and chromatin erosion.	Njagi et al. 1982
Sodium benzoate	*V. faba*	Root sample pressing	Anaphase bridges, premature chromosome condensation heading to pycnotic nuclei and chromatin erosion.	Njagi et al. 1982
Potassium nitrate	*A. cepa*	Mitotic squash	Chromosome stickiness, c-mitosis, bridges, chromosome break, multipolar stages, laggard, micronuclei and necrotic cell.	Gomurgen 2005, Pandey et al. 2014
	Drosophila melanogaster	SMART	Mutation in wings.	Sarikaya & Cakir 2005
Potassium metabisulphite	*A. cepa*	Mitotic squash	Chromosome stickiness, c-mitosis in most of the treatments, bridges, anaphase lagging chromosomes and micronuclei.	Gomurgen 2005
Sorbic acid and its sodium and potassium salts	Chinese hamster	Chromosome aberration test, assay of sister chromatid exchanges, mutation test	Sister chromatid exchanges and clastogenic chromosomal aberrations.	Hasegawa et al. 1984
Sodium nitrite	*D. melano-gaster*	SMART	Mutation in wings.	Sarikaya & Cakir 2005
	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002

(Contd)

TABLE 1: (contd).

Food additive	Organism/cells	Methodology	Aberration	Reference
Sodium nitrate	*D. melano-gaster*	SMART	Mutation in wings.	Sarikaya & Cakir 2005
Potassium nitrite	*D. melano-gaster*	SMART	Mutation in wings.	Sarikaya & Cakir 2005
Sodium benzoate	*V. faba*	Root sample pressing	Anaphase bridges, premature chromosome condensation heading to pycnotic nuclei and chromatin erosion.	Njagi & Gopalan 1982
Butylated hydroxyltoluene (BHT)	*A. cepa*	Mitotic squash	Sticky metaphase, c-mitosis, phases with break, multipolar stages, laggard, bridge, lobulated nuclei, sticky stages, binucleate cell and necrotic cell.	Pandey et al. 2014
	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, urinary bladder, urinary bladder and brain. No death, morbidity or clinical signs.	Sasaki et al. 2002
Butylated hydroxyanisol (BHA)	*A. cepa*	Mitotic squash	Sticky metaphase, c-mitosis, phases with break, multipolar stages, laggard, bridge, lobulated nuclei, sticky stages, binucleate cell and necrotic cell.	Pandey et al. 2014
	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sorbic acid	*A. cepa*	Mitotic squash	Sticky metaphase, c-mitosis, phases with break, multipolar stages, laggard, bridge, lobulated nuclei, sticky stages, binucleate cell and necrotic cell.	Pandey et al. 2014
Propyl gallate	*A. cepa*	Mitotic squash	Sticky metaphase, c-mitosis, phases with break, multipolar stages, laggard, bridge, lobulated nuclei, sticky stages, binucleate cell and necrotic cell.	Pandey et al. 2014
Sodium benzoate	*A. cepa*	Mitotic squash	C-mitosis, stickiness, unequal distribution, anaphase bridges, laggards, micronucleus, breaks, stickiness and anaphase bridges.	Turkoglu 2007
Citric acid	*A. cepa*	Mitotic squash	C-mitosis, stickiness, unequal distribution, anaphase bridges, laggards, micronucleus, breaks, stickiness and anaphase bridges.	Turkoglu 2007
Potassium citrate	*A. cepa*	Mitotic squash	C-mitosis, stickiness, unequal distribution, anaphase bridges, laggards, micronucleus, breaks, stickiness and anaphase bridges	Turkoglu 2007
Sodium citrate	*A. cepa*	Mitotic squash	C-mitosis, stickiness, unequal distribution, anaphase bridges, laggards, micronucleus, breaks, stickiness and anaphase bridges.	Turkoglu 2007

(Contd)

TABLE 1: (contd).

Food additive	Organism/cells	Methodology	Aberration	Reference
Benzoic acid and its sodium salt	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
five p-hydroxybenzoic acid esters	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sodium dehydroacetic acid	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sorbic acid and its potassium salt	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Fungicide				
Biphenyl o-phenylphenol	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sodium o-phenylphenol	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, urinary bladder, lung, liver and kidney. No death, morbidity or clinical signs.	Sasaki et al. 2002
Thiabendazole	Different mouse organs	Comet assay	DNA damage in all the organs. No death, morbidity or clinical signs.	Sasaki et al. 2002
Antimicrobial, Buffer, Stabilizer, Emulsifier				
Sodium phosphate	*A. cepa*	Mitotic squash	Stickiness, anaphase bridges, C-mitosis and micronuclei.	Turkoglu 2009
Antioxidant				
Erythorbic acid and its sodium salt	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Gallic acid n-propyl ester	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Dye				
Amaranth	Human peripheral blood cells	In vitro SCE assay, electrophoretic mobility shift assay, spectroscopic titration, PCR	SCE, decrease in mitotic index.	Mpountoukas et al. 2010
	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, gastrointestinal organ. No death, morbidity or clinical signs.	Sasaki et al. 2002

(Contd)

TABLE 1: (contd).

Food additive	Organism/cells	Methodology	Aberration	Reference
Erythrosine B (Cherry-pink food dye)	Human peripheral blood cells	In vitro SCE assay, electrophoretic mobility shift assay, spectroscopic titration, PCR	Sister chromatid exchanges (SCE) and decrease in mitotic index.	Mpountoukas et al. 2010
	HepG2, HB-8065, hepatocellular carcinoma	Comet assay, cytokinesis block micronucleus cytome (CBMN-Cyt) assay	Binucleate cell, micronuclei, nucleoplasmic bridges and nuclear buds.	Chequer et al. 2012
	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon, and urinary bladder at 3 h; no DNA damage was evident at 24 h. No death, morbidity or clinical signs.	Sasaki et al. 2002
Tatrazine	*A. cepa*	Mitotic squash	Bridges in anaphase and telophase and micronucleated cells. Breaks, bridges, stickiness and binucleate cells.	Gomes et al. 2013 Lerda 2017
	Swiss albino mice - *Mus musculus*	Haematological and serological parameters	Significant decrease in body weight in parameters like Hb, haematocrit %, TEC, TLC and polymorph count. Increase in lymphocytes count, MCV and MCH	Sharma et al. 2009
	Human peripheral blood cells	In vitro SCE assay, electrophoretic mobility shift assays spectroscopic titration, PCR	Sister chromatid exchanges (SCE) and decrease in mitotic index.	Mpountoukas et al. 2010
	Albino rat	Biochemical parameters related to renal, hepatic function and oxidative stress biomarkers	Adversely affect the physiological function of the body by altering the biochemical markers in vital organism like kidney and liver.	Amin et al. 2010
	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, gastrointestinal organ. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sunset Yellow	*A. cepa*	Mitotic squash	Bridges in anaphase and telophase and micronucleated cells.	Gomes et al. 2013
	A. cepa	Mitotic squash	Decrease in mitotic index and increase chromosomal aberrations in a dose- and time-dependent manner.	Yoosuf et al. 2020
	A. cepa	Comet assay	DNA damage with lesser concentration and higher exposure time.	Koc & Pandir (2018)
	A. cepa	Comet assay	No DNA damage with higher concentration and lesser exposure time.	Aleem Yoosuf et al. 2020

(Contd)

TABLE 1: (contd).

Food additive	Organism/cells	Methodology	Aberration	Reference
	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
	T. foenum-graecum root meristem	Mitotic squash	Unorientation at metaphase, Precocious movement at metaphase, sticky metaphase and anaphase, anaphase laggard and chromosomal bridges.	Kumar & Srivastava 2011
	Brassica campestris	Mitotic squash	Stickiness, micronuclei, uniorientation, laggards and chromatin bridges.	Dwivedi & Kumar 2015
Allura Red	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, gastrointestinal organ. No death, morbidity or clinical signs.	Sasaki et al. 2002
New coccine	Different mouse organs	Comet assay	DNA damage in the colon, glandular stomach, urinary bladder, liver, kidney, urinary bladder, and lung. No death, morbidity or clinical signs.	Sasaki et al. 2002
Phloxine	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon, and urinary bladder at 3 h; no DNA damage was evident at 24 h. No death, morbidity or clinical signs.	Sasaki et al. 2002
Rose Bengal	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon, and urinary bladder at 3 h; no DNA damage was evident at 24 h. No death, morbidity or clinical signs.	Sasaki et al. 2002
Acid Red	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Fast Green	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Brilliant Blue	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Indigo carmine	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sweetener				
Acesulfame K	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Aspartame	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002

(Contd)

TABLE 1: (concluded).

Food additive	Organism/cells	Methodology	Aberration	Reference
Sodium cyclamate	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon, kidney and urinary bladder. No death, morbidity or clinical signs.	Sasaki et al. 2002
Glycyrrhizin	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Saccharin	Different mouse organs	Comet assay	DNA damage in the colon. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sodium saccharin	Different mouse organs	Comet assay	DNA damage in the glandular stomach and colon. No death, morbidity or clinical signs.	Sasaki et al. 2002
Stevia	Different mouse organs	Comet assay	No significant DNA damage in any of the organs studied. No death, morbidity or clinical signs.	Sasaki et al. 2002
Sucralose	Different mouse organs	Comet assay	DNA damage in the glandular stomach, colon and lung. No death, morbidity or clinical signs.	Sasaki et al. 2002
Flavour and aroma synthetic additive				
Passion fruit flavouring	*A. cepa*	Mitotic squash	Colchicine metaphase, chromosome bridges and micronuclei.	Nunes et al. 2017
Vanilla flavouring	*A. cepa*	Mitotic squash	Colchicine metaphase, chromosome bridges and micronuclei.	Nunes et al. 2017

plant-based mutation detector systems, depending on the organism understudy. For example, in Ames test or *Salmonella* mutagenicity assay amino acid-dependent strains of *Salmonella* having various gene mutations in histidine operon are used. These strains cannot survive until they get histidine from outside or, until the organism gets reverted to histidine independent. The mutation in histidine operon acts as the 'substrate' for the action of mutagen, and induce reverting potential to become histidine independent (Sijila & Shah 2016, Zeiger 2019). The mutagen can induce the reverting potential in a dose-dependent manner (Tejs 2008). The SMART in *Drosophila melanogaster* is used to study homologous recombinations (Sarikaya & Cakir 2005). Each technique reveals a different aspect. Thus, Ames test, that can identify single nucleotide changes, cannot reveal other consequences such as ploidy changes. Similarly, a frame-shift mutations identified using Ames test could actually be a reflection of a damage of much larger scale such as whole genome strand break. Thus, conclusions on the safety parameters of any food additive need to be made after cross-checking with multiple techniques. Our compilation on 67 food additives, along with the techniques (Table 1) used thus reveals the presence of gaps and indicating the requirement of more analyses in this area.

Another aspect is that the effect of food additives may vary with cells, concentration and exposure time, based on the model organisms. For example, sodium salt of sorbic acid induced sister chromatid exchanges (SCE) and clastogenic chromosomal aberrations, the intensity of which was proportional to the dose of the additive. However, sorbic acid alone or its potassium salt caused chromosomal aberration only during high dose-exposure (Hasegawa et al. 1984). Unlike the above example, sodium nitrite induced non dose-dependent aberrant metaphases (Luca et al. 1987). Interestingly, sodium nitrite as well as other food preservatives such as sodium nitrate, potassium nitrate and potassium nitrite induced a dose related increase of another mutation type, somatic homologous recombination (Sarikaya & Cakir 2005). Coming to the period of exposure, reports of ST reveals that though SY induced a clastogenic, nonclastogenic (structural) and aneugenic aberrations in *A. cepa* in a dose- and time-dependent manner, the whole genome strand breaks were induced in a time-dependent manner and, high doses if applied for short duration failed to induce strand breaks (0.2% dose at 24 h) (Aleem Yoosuf et al. 2020). At the same time, even low doses (25 ppm) for longer duration (72 h) induced strand breaks in *A. cepa* (Koc & Pandir 2018). However, SY caused chromosomal aberrations at low concentration and low exposure time (Aleem Yoosuf et al. 2020). The organism under study also matters. Thus, SY that induced strand breaks in *A. cepa,* did not induce the same in any of the eight mouse organs studied (Sasaki et al. 2002) (Table 1). Thus, before declaring a food additive as "safe for consumption", we suggest that multiple screenings at various parameters be made imperative. Our compilation on food additives (Table 1) can be used to make more such comparisons and gaps in research need to be identified and sealed, importantly for the purpose of food safety.

Antimitotic, apoptotic, physiological and carcinogenic damages by food additives

Antiproliferative or antimitotic potential coin for the inhibitory action of the chemicals on phenomenon of cell cycle, and apoptosis or programmed cell death coin for the series of events which leads to the death of a cell due to injury or natural aging. Antimitotic potential of food additive can inhibit nuclear division, chromatid division and cytoplasmic division independently or simultaneously and their consequences are different (Fig.1). While antimitotic and apoptotic terminologies imply changes at cellular and subcellular levels, physiological and carcinogenic damages are conditions at an organismal level. Additives which cause genotoxicity or are antimitotic or antiapoptotic, may or may not lead to physiological changes. Certain toxic substances can also lead to physiological changes. Cancer is a type of physiological change, but all physiological changes are not carcinogenic. Any food additive, genotoxic, antimitotic, or apoptotic, if causes cancer, is declared as carcinogenic. The possible role of carcino-genicity of food additives is an area of great public interest. Cancer is a condition of a body in which the cells of a particular tissue undergo uncontrolled and rapid growth, have

TABLE 2: Cytotoxicity, carcinogenicity, apoptosis and other physiological risks induced due to various food additives.

Food additive	Organism	Methodology	Effect	Reference
Antioxidant				
Butylated hydroxyanisol (BHA)	Mice	Observing tumor nodules	Lung tumors.	Clapp et al. 1973
	Cultured heart cells of rat	Culturing of myocardial cells and endothelioid, analysis of beating activity, quantity of LDH and morphology	LDH heart beating rate depression, cell lysis and injury on myocardial cells.	Leslie et al. 1978
Butylated hydroxyanisol (BHA)	*Vibrio fischeri*	Bioluminescence test	Inhibition of bioluminescence.	Jos et al. 2005
	Chlorella vulgaris	Growth inhibition analysis	Inhibition of the growth.	Jos et al. 2005
	A. cepa *Daphnia magna*	Mitotic squash Immobilization of the cladoceran test	Antimitotic effect, root growth inhibition and immobilization of the cladoceran.	Jos et al. 2005
	Vero monkey cells	In vitro analysis	Loss in total protein content, neutral red uptake, MTT metabolization and LDH activity.	Jos et al. 2005
	RTG-2 salmonid fish	In vitro analysis	Loss of cells, induction of cellular pleomorphism, hydropic degeneration, loss of cells and induction of apoptosis.	Jos et al. 2005
	Rat	Culturing of myocardial cells and endothelioid, analysis of beating activity, quantity of LDH and morphology	LDH heart beating rate depression, cell lysis and injury on myocardial cells.	Leslie et al. 1978
			Incidences of forestomach papilloma and colon tumor.	Hirose et al. 1997
Caffeic acid	Male F344 rats	In vivo carcinogenic Study	Incidences of forestomach papilloma and colon tumor.	Hirose et al. 1997
Sesamol 4- methoxyphenol (4-MP)	Male F344 rats	In vivo carcinogenic Study	Incidences of forestomach papilloma and colon tumor.	Hirose et al. 1997
Catechol	Male F344 rats	In vivo carcinogenic study	Incidences of forestomach papilloma and colon tumor.	Hirose et al. 1997

(Contd)

TABLE 2: (concluded).

Food additive	Organism	Methodology	Effect	References
Preservative				
Sodium nitrate	Wistar rats, Swiss mice and Chinchilla rabbit	In vitro and In vivo cytogenetic damage assay	Micronucleus induction, aberrant metaphases, micronucleated polychromatic erythrocytes and a light bone marrow depression.	Luca et al. 1987
Acetic Acid	*Escherichia coli*	Growth experiments	Inhibition of growth rate.	Roe et al. 2002
Dye				
Erythrosine	*Bacillus subtilis*	Multigene sporulation assay	No excision repair-proficient (168) and deficient (hcr-9) strains.	Lakdawalla & Netrawali 1987
Apple green	TA98 and TA100 strains of *Salmonella typhimurium.*	Ames test	Moderately mutagenic at higher concentration.	Kaur et al. 2010
Metanil yellow	Albino rat (*Rattus norvegicus*)	Histopathological and ultrastructural changes	Disruption of gastric folds and bowman's capsule, profuse secretion of mucus, necrosis columnar epithelial cells and gastric glands, mucosal folds and columnar epithelial cells, damage to intestinal villi and columnar epithelial cells, disrupted brush border and lamina propria, degeneration of hepatocytes, diminish in cytoplasmic content, pycnosis of nuclei, damage to central vein regions, necrosis of tubular epithelium and swelling of epithelial cells of renal tubules.	Sarkar & Ghosh 2012
Carmoisine	Albino rat	Biochemical parameters related to renal, hepatic function and oxidative stress biomarkers	Adversely affect the physiological function of the body by altering the biochemical markers in vital organism like kidney and liver.	Amin et al. 2010
Sunset Yellow	Zebrafish (*Danio rerio*)	Developmental profile	Cuts hatching rate, morphometry, eye diameter and heart rate, cardiac edema, yolk sac edema, Spinal curvature, tail distortion and cellular apoptosis.	Joshi & Pancharatna 2018
Fast Green	Rat	Blood sampling and processing, biochemical blood indices, hematological parameters	Decrease serum glucose level, serum triglycerides, cholesterol, total protein, albumin and globulin values, and increase urea, uric acid and creatinine.	Ashour & Abdela 2009
Tartrazine	Zebrafish (*Danio rerio*)	Developmental toxicity assay	Cuts in heart rate, cardiac edema, yolk sac edema, tail distortion and ceased development resulting in mortality.	Joshi & Katti 2018

increased genome level, form tumors and spread across the body. The 'Delaney clause', which is included in the Food Additives Amendment (1958) and the Colour Additives Amendment (1960), states that 'if a substance was found to cause cancer in man or animal, then it could not be used as a food additive' (Jukes 1979). Since most potential mutagens are carcinogens, Ames test is generally used to screen food additives (Kaur et al. 2011). However, Ames test is done on a prokaryotic system and cancer is an eukaryotic phenomenon of certain higher animals. Thus, more tests are required to rule out the possibility of an additive to be carcinogenic. Similar to genotoxic effect of food additives, various parameters of dosage, treatment time, and model organism need to be assessed while analyzing antimitotic, apoptotic, toxic or carcinogenic effect of food additives. Details on the same are also compiled in this review (Tables 1, 2).

In India, Food Safety and Standards Authority of India (FSSAI) is the competent authority responsible for regulating and supervising the food safety. It is an independent body under Ministry of Health and Family Welfare, Government of India since 2006. This authority will decide as to what food additives and at what concentrations are permissible (Food Safety and Standards Authority of India 2011 (www.fssai.gov.in).

Conclusions

Nowadays, food additives have become an inevitable part of our food style. The increasing world population has created a demand to enhance the food production which compel people use these chemicals. The authorities should examine the food additives available in our market and ensure the safety of consumers, and at the same time with the help of society should conduct awareness among the common people as well as young generation about the genotoxic effects of the food additives in fast foods, and spread the message of *'no to fast foods and yes to vegetables and fruits rich in anti-oxidants and carotenoids'*.

There are studies that pointed toward certain foods that can act as nutraceuticals which can prevent or, conversely, contribute to certain types of cancer. And among the different types of food additives, there are certain additives without any harm to our body, and rather with beneficial effects too (for example, vitamin C and E are used as food preservative). There is an urgent need for more research assessing the nutraceutical effect of more fruits and vegetables that prevent the cytotoxicity at the cellular and molecular levels.

REFERENCES

AMIN K A, HAMED H A & ELSTTAR A H A 2010 Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats *Food Chem Toxicol* **48** 2994–2999

AN Y, JIANG L, CAO J, GENG C & ZHONG L 2007 Sudan I induces genotoxic effects and oxidative DNA damage in *HepG2* cells *Mut Res* **627** 164–170

ASHOUR A A & ABDELA A 2009 Role of fast green on the blood of rats and the therapeutic action of vitamins C or E *Int J Integr Biol* **6** 1–6

BONASSI S, NERI M, LANDO C, CEPPI M, LIN Y P, CHANG W P, HOLLAND N, KIRSCH-VOLDERS M, ZEIGER E & FENECH M 2003 Effect of smoking habit on the frequency of micronuclei in human *lymphocytes*: results from the human micronucleus project *Mut Res* **543** 155–166

BRANEN A L, DAVIDSON P M, SALMINEN S & THORNGATE J 2001 *Food additives* Taylor & Francis New York

CAROCHO M & BARREIRO M F 2014 Adding molecules to food pros and cons: A review on synthetic and natural food additives *Comp Rev* **13** 377–399

CHEQUER F M D, VENANCIO V P, BIANCHI M L P & ANTUNES L M G 2012 Genotoxic and mutagenic effects of erythrosine B a xanthene food dye on *HepG2* cells *Food Chem Toxicol* **50** 3447–3451

CLAPP N K, TYNDALL R L & CUMMING R B 1973 Hyperplasia of hepatic bile ducts in mice following long-term administration of butylated hydroxytoluene *Fd Cosmet Toxicol* **11** 847–849

DONBAK L 2002 The cytogenetic effect of the food additive boric acid in *Alium cepa* L *Cytologia* **67** 153–157

DWIVEDI K & KUMAR G 2015 Genetic damage induced by a food colouring dye (Sunset yellow) on meristematic cells of *Brassica campestris* L *J Env Pub Health* **2015** 1–5

EFSA 2011 Panel on food additives and nutrient sources added to food (ANS) scientific opinion on the re-evaluation of erythrosine (E 127) as a food additive *EFSA J* **9** 1854

ESKANDANIA M, HAMED H & JAFAR E N D 2014 Cytotoxicity and DNA damage properties of tert-butylhydroquinone (TBHQ) food additive *Food Chem* **153** 315–332

FURUMIYA K & MIZUTANI T 2008 Inhibition of human *CYP3A4 UGT1A6* and *P glycoprotein* with halogenated xanthene food dyes and prevention by superoxide dismutase *J Toxicol Env Health* **71** 1307–1313

GANESAN L, MARGOLLES-CLARK E, SONG Y & BUCHWALD P 2011 The food colorant erythrosine is a promiscuous protein–protein interaction inhibitor *Biochem Pharmacol* **81** 810–818

GOMES K M S, OLIVEIRA M V G A, CARVALHO F R D S, MENEZES C C & PERON A P 2013 Cytotoxicity of food dyes sunset yellow (E-110) bordeaux red (E-123) and tatrazine yellow (E-102) on *Allium cepa* L root meristematic cells *Food Sci Tech* **33**: 218–223

GOMURGEN A N 2005 Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L *Cytologia* **70** 119–128

GONTIJO A M, GREEN C M & ALMOUZNI G 2003 Repairing DNA damage in chromatin *Biochimie* **85** 1133–1147

HASEGAWA M M, NISHI Y, OHKAWA Y & INUI N 1984 Effects of sorbic acid and its salts on chromosome aberrations sister chromatid exchanges and gene mutations in cultured chinese hamster cells *Food Chem Toxicol* **22** 501–507

HIROSE M, TAKESADA Y, TANAKA H, TAMANO S, KATO T & SHIRAI T 1998 Carcinogenicity of antioxidants BHA caffeic acid sesamol 4-methoxyphenol and catechol at low doses either alone or in combination and modulation of their effects in a rat medium-term multi-organ carcinogenesis model *Carcinogenesis* **19** 207–212

JAHAN–TIGH R R, RYAN C, OBERMOSER G & SCHWARZENBERGER K 2012 Flow cytometry *J Invest Dermatol* **132** 1–6

JEN J J S & CHEN J 2017 *Food Safety in China: Science Technology Management and Regulation* John Wiley & Sons Chichester

JOS A, REPETTO G, RIOS J C, PESO A D, SALGUERO M, HAZEN M J, MOLERO M L, FERNANDEZ-FREIRE P, PEREZ-MARTIN J M, LABRADOR V & CAMEAN A 2005 Ecotoxicological evaluation of the additive butylated hydroxyanisole using a battery with six model systems and eighteen end points *Aquat Toxicol* **71** 183–192

JOSHI V & PANCHARATNA K 2018 Food colorant sunset yellow (E110) intervenes developmental profile of zebrafish (*Danio rerio) J Appl Toxicol* **39** 1–11

JUKES T H 1979 Carcinogens in food and the Delaney Clause *JAMA* **241** 617–619

KAUR M, ARORA S & KATNORIA J K 2011 Evaluation of mutagenic potential of food dye (Apple green) *Indian J Sci Technol* **3** 1208–1209

KOC K & PANDIR D (2018) All aspect of toxic effect of brilliant blue and sunset yellow in *Allium cepa* roots *Cytotechnology* **70** 449–463

KUMAR G & SRIVASTAVA N 2011 Genotoxic effects of two commonly used food additives of boric acid and sunset yellow in root meristems of *Trigonella foenum–graecum Iran J Env Health Sci Eng* **8** 361–366

KUMAR P, NAGARAJAN A & UCHIL P D 2018 Analysis of cell viability by the MTT assay *Cold Spring Harb Protoc* **2018** 469–471

LAKDAWALLA A A & NETRAWALI M S 1988 Erythrosine a permitted food dye is mutagenic in the *Bacillus subtilis* multigene sporulation assay *Mut Res* **206** 171–176

LERDA D 2017 The effects of tartrazine in *Allium cepa* L *J Food Nutr* **3** 1–5

LESLIE S W, GAD S C & ACOSTA D 1978 Cytotoxicity of butylated hydroxytoluene and butylated hydroxyanisole in cultured heart cells *Toxicology* **10** 281–289

LUCA D, LUCA V, COTOR F & RAILEANU L 1987 In vivo and in vitro cytogenetic damage induced by sodium nitrite *Mut Res* **189** 333–339

MAGA J A & TU A T 1994 *Food additive toxicology* Marcel Dekker New York

MEGGOS H 1995 Food colours: an international perspective *Manufact Confect* **75** 59–65

MERRILL R A 1978 Regulating carcinogens in food: A legislator's guide to the food safety provisions of the federal food drug and cosmetics act *Mich L Rev* **77** 171–250

MPOUNTOUKAS P, PANTAZAKI A, KOSTARELI E, CHRISTODOULOU P, KARELI D, POLILIOU S, MOURELATOS C, LAMBROPOULOU V & LIALIARIS T 2010 Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth erythrosine and tartrazine *Food Chem Toxicol* **48** 2934–2944

MSAGATI T A M 2013 *The Chemistry of food additives and preservatives* Wiley-Blackwell Oxford

NJAGI G D E & GOPALAN H N 1982 Cytogenetic effects of the food preservatives sodium benzoate and sodium sulphite on *Vicia faba* root meristems *Mut Res* **102** 213–219

NUNES R D, SALES I M, SILVA S I, SOUSA J M & PERON A P 2017 Antiproliferative and genotoxic effects of nature identical and artificial synthetic food additives of aroma and flavour *Braz J Biol* **77** 150–154

PACKARD V S & MYERS S 1978 *Food additives* Agriculture extension services University of Minnesota https://hdl.handle.net/11299/205257

PANDEY H, KUMAR V & ROY B K 2014 Assessment of genotoxicity of some common food preservatives using *Allium cepa* L as a test plant *Toxicol Rep* **1** 300–308

RANGAN C & BARCELOUX D G 2009 Food additives and sensitivities *Dis Mon* **55** 292–311

RENCUZOGULLARI E, KAYRALDIZ A, ILA H B, ÇAKMAK T & TOPAKTAF M 2001 The cytogenetic effects of sodium metabisulfite a food preservative in root tip cells of *Allium cepa* L *Turk J Biol* **25** 361–370

ROE AJ, O'BYRNE C, MCLAGGAN D & BOOTH I R 2002 Inhibition of *Escherichia coli* growth by acetic acid: a problem with methionine biosynthesis and homocysteine toxicity *Microbiology* **148** 2215–2222

SARIKAYA R & CAKIR S 2005 Genotoxicity testing of four food preservatives and their combinations in the *Drosophila* wing spot test *Env Toxicol Pharmacol* **20** 424–430

SARKAR R & GHOSH A A 2012 Metanil yellow - an azo dye induced histopathological and ultrastructural changes in albino rat (*Rattus norvegicus*) *Bioscan* **7** 427–432

SASAKI Y F, KAWAGUCHI S, KAMAYA A, OHSHITA M, KABASAWA K, IWAMA K, TANIGUCHI K & TSUDA S 2002 The comet assay with 8 mouse organs: results with 39 currently used food additives *Mut Res* **519** 103–119

SHARMA G, GAUTAMAND D & GOYAL R P 2009 Tartrazine induced haematological and serological changes in female Swiss albino mice *Mus musculus Pharmacology* **3** 774–778

SIJILA J & SHAH J M 2016 Quantitative scoring of mutations using plants *Abrahamia* **2** 5–11

TEJS S 2008 The Ames test: a methodological short review *Env Biotechnol* **4** 7–14

TSUBOY M S, ANGELI J P, MANTOVANI M S, KNASMULLER S, UMBUZEIRO G A & RIBEIRO L R 2007 Genotoxic mutagenic and cytotoxic effects of the commercial dye CI Disperse Blue 291 in the human hepatic cell line *HepG2 Toxicol In Vitro* **21** 1650–1655

TURKOGLU S 2007 Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L *Mut Res* **626** 4–14

TURKOGLU S 2009 Genotoxic effects of mono- di- and trisodium phosphate on mitotic activity DNA content and nuclear volume in *Allium cepa* L *Caryologia* **62** 171–179

WULTSCH G, MISIK M, NERSESYAN A & KNASMUELLER S 2011 Genotoxic effects of occupational exposure measured in lymphocytes of waste-incinerator workers *Mut Res* **720** 3–7

ALEEM YOOSUF N, JOYOUS JOSEPH T & JASMINE M SHAH 2020 Mutagenicity assessment of sunset yellow on chromosomal aberrations and whole genome DNA strand breaks in *Allium cepa J Cytol Genet* **21** (NS) 121–129

ZEIGER E 2019 The test that changed the world: The Ames test and the regulation of chemicals *Mut Res* **841** 43–48