PRINT: ISSN 0972-3757 ONLINE: 2456-6360

Int J Hum Genet, 4(4): 277-280 (2004)
DOI: 10.31901/24566330.2004/04.04.09

Genotoxicity Testing of the Food Colours Amaranth and Tartrazine

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KEYWORDS Mutagenic; chromosomal aberrations; monoazo dyes

ABSTRACT Colour, a vital constituent of food, is indispensable to the modern day consumer as a means for the rapid identification and ultimate acceptance of food. A number of reviews concerning the toxicology of natural and synthetic dyes, especially those used in food, have appeared since the dyestuffs became potential suspects for causing cancer. A large number of natural or synthetic dyes have been removed from both national and international lists of permitted food colours because of their mutagenic or carcinogenic activity. To the majority of the food additive 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 below the ADI dose. In India, the problem is severe because in spite of regulation and restrictions by the Prevention of Food Adulteration Act of 1954, use of non-permitted food colours is prevalent. We have tested the mutagenic and genotoxic effects of amaranth, and tartrazine utilizing Ames mutagenicity assay and in vivo mouse bone marrow assay. *Salmonella typhimurium* TA97a, TA98 and TA100were used for Ames Mutagenicity Assay without metabolic activation. The dyes were dissolved in sterile double distilled water at different concentrations (10,100,250,500 and1000 µg /plate) For genotoxicity testing four animals per dose were administered intra peritoneally with the different doses of the amaranth or tartrazine (50,100 and 200 mg/kg body weight). The results show that with in the restriction of the protocol followed, the dyes were found to be non mutagenic and non genotoxic

INTRODUCTION

Amaranth (Food Red No.2), and Tartrazine (FD & C Yellow No.5), are two monoazo dyes used as food additives in many countries. These dyes are popularly used as colourants in food drugs and industrial manufacturing products, intended for human consumption. Tartrazine is permitted in India while amaranth is not. But illegal use of it still continues. The global production of food dyes is more than 8000 tons per year, while India produces 2% of the World production among which Tartrazine only accounts for 40% of the whole Indian production. Epidemiological evidences suggest that dyes may be carcinogenic under certain circumstances (Anonymous 1983; Bonin and Baker 1980; Price et al. 1978). A comprehensive review of the genotoxicity of food, drug, and cosmetic dyes was published by Coombs and Haveland-Smith (1982); Giri (1991). From these and other reports, it is apparent that for many dyes, considerable inconsistency exists not only between bioassay and various short-term results, but also among the various short-term tests.

Amaranth is one of the most widely tested dyes, yet a consistent characterization of its genotoxicity has not emerged, and its status as a carcinogen remains equivocal. Tartrazine is an approved dye (Preston et al. 1987) which has been shown not to be carcinogenic or genotoxic in most short-term tests, including the Ames assay(reviewed by Coombs and Haveland-Smith 1982; Giri 1991). Literature survey on the food colours revealed that majority of the toxicity tests performed were antiquated. Recently, diagonostic techniques are becoming increasingly sensitive and are disclosing new potential risks where none were suspected before (FAO/WHO 1975; Maron and Ames 1983; Sasaki et al. 2002). Thus a preliminary investigation was undertaken in our laboratory to study the induction of chromosomal abnormalities in the mouse bone marrow cells and in different histidine dependent mutant strains of *Salmonella typhimurium* by amaranth and tartrazine.

MATERIAL AND METHODS

Bacterial Strains Used: Salmonlla typhimurium TA97a, TA98 and TA100were used for Ames Mutagenicity Assay (Maron and Ames 1983).

Preparation of Test Chemicals: The dyes

were dissolved in sterile double distilled water at different concentrations (10,100,250,500 and1000 µg /plate)

Experiment: The plate incorporation test was performed following the method of Maron and Ames (1983). The plates were inverted within an hour and placed in a dark vented incubator at 37°C for 48 hours. Positive controls (for TA97a and TA98, 20 µg/plate nitro phenylene diamine and for TA100, 1.5 µg/plate sodium azide) and negative controls were maintained concurrently for all the experiments. Three plates were used for each set. After 48 hours of incubation, the revertant colonies were counted.

Chromosome Aberration Assay: The studies were conducted on male Swiss albino mice, 8-10 weeks old and weighing 20-25 g. They were maintained under conditions of ambient room temperature and relative humidity. A commercial diet and water were provided *ad lib.* The bone marrow chromosome preparations were made according to Preston et al. (1987) with slight modifications.

Four animals per dose were administered intra peritoneally with the different doses of the amaranth or tartrazine (50,100 and 200 mg/kg body weight). The selection of dose was based on the permitted dose of the dyes being 100mg/kg of the prepared food. The animals were killed after 18 hr. Positive control (cyclophosphamide CP 20 mg/ kg body weight) and vehicle control (distilled water) sets were maintained and the animals were subsequently killed. For bone marrow chromosome analysis, animals were injected with 0.1 ml colchicine solution (4mg/10ml distilled water /10g body weight, 90 minutes before they were killed (Tice and Ivett 1985). Bone marrow cells were routinely processed by the standard procedure and slides were coded and stained in diluted Giemsa (Preston et al. 1987).

Scoring of Slides and Statistical Analyses: For chromosomal aberration analysis, four animals were used per point. Hundred well spread metaphase plates were scored per animal (400 metaphase plates per treatment set) at random. The types of aberrations were scored and recorded strictly in accordance with the method of Tice and Ivett (1985). All aberrations (chromatid gaps, isochromosome gaps, chromatid breaks and rearrangements) were considered equal- regardless of the number of breakages involved. The percentages of damaged cells (% DC) and chromosomal aberrations per cell (CA/cell) values were calculated excluding gaps.

Statistical Analysis: ANOVA test was performed at 0.05 level (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

The results of testing the two food colours in the *in vitro* and *in vivo* are shown in tables 1 and 2. Mutagenicity of the two food dyes in *in vitro* Ames *Salmonella* mutagenicity test (without metabolic activation) is presented in the table 1. The different concentrations of the dyes namely amaranth, and tartrazine was prepared in double distilled water and the concentrations ranged from 10-to1000-µg/ plates. The three strains of *Salmonella* TA97a, TA 98, and TA100 were used and the his^+ revertant colonies and micro colonies in the background lawn were observed.

Compared to the negative control the number of revertant colonies in the strain TA 97a, and TA100 was not high in the two dyes. There was no dose related increase over the control set. This indicates that in the *in vitro* Ames *Salmonella* mutagenicity test, the dyes failed to induce point mutation as frame shift mutation in TA97a and base pair mutation in TA100. However, ANOVA test shows that in the tester strain TA 98 there was significant increase in the number of revertant colonies for both the dyes tested. The positive compounds for the respective strains gave mutagenic responses as expected. A multiple fold increase in the number of revertant colonies over negative control plates and the dyes was observed.

The data on the types of chromosomal aberrations (CA) and the percentage of damaged cells (% DC) are given in table 2. The types of aberrations were classified according to Tice et al. (1985). The aberrations scored were mainly found to be of chromatid breaks, while in animals treated with the positive compound (cyclophosphamide) both chromatid and chromosome type of aberrations were recorded. ANOVA test showed that the frequency of aberrant cells and the number of breaks per cell were not significantly high.

The present negative findings are consistent with the preponderance of the previous genotoxicity tests performed on these two dyes. Amaranth is one of the most widely tested dyes, yet a consistent characterization of its genotoxicity has not emerged, and its status as a

carcinogen remains equivocal. At present it is banned in most of the countries except Japan. There are numerous reports of non-mutagenicity of amaranth (Garner and Nutman 1977; Ishidate and Odashima 1977; Muzzal and Cook 1979; Prival et al. 1988; Sugimura et al. 1976) with a positive mutagenic effect (FAO/WHO 1975) and clastogenic effect in mice (Vaidya and Godbole 1978). Recently Kawaguchi *et al* [2] have elegantly demonstrated that amaranth induced dose-related DNA damage in stomach, colon and/or urinary bladder.

Tartrazine is a PFA (1954) approved food dye, which has been shown not to be carcinogenic or genotoxic in most short-term tests, including the Ames assay (reviewed by Coombs and Haveland-Smith 1982; Giri 1991). A clastogenic effect of the dye in lymphocytes has been observed (Tice and Ivett 1985), which contrasts with another report of its lack of clastogenicity in CHO cells (Au and Hsu 1979). In addition, the negative response in the Ames/Salmonella mutagenicity assay can be supported by the fact that the azo bond of this dye is not well reduced by *Salmonella* bacteria (Combes and Haveland-Smith 1982). The negative results obtained for amaranth and tartrazine in the *in vivo* and invitro assay are in line with those reported by the majority of the studies, and provide an important confirmation of its lack of genotoxicity. More extensive assessment of these azo dyes is warranted to study the DNA damaging potential of these two dyes on the

Table 1: Mutagenicity of food colours in tester strains of *Salmonella typhimurium*

Test Chemicals	*Dose µG/plate*	*Mean of the No. Revertant Colonies ± S.D.*		
		TA97a	*TA98*	*TA100*
Amarnath	10	120.33 ± 15.50	222.0 ± 49.79	103.7 ± 9.07
	100	115.67 ± 17.78	205.7 ± 4.04	92.7 ± 16.17
	250	92.33 ± 12.50	170.7 ± 68.72	92.0 ± 27.73
	500	120.67 ± 22.94	178.3 ± 30.29	83.7 ± 7.095
	1000	122.33 ± 24.82	195.7 ± 36.23	109.0 ± 10.19
	10	134.00 ± 42.51	49.3 ± 13.7	98.0 ± 7.55
Tartrazine				
	100	126.70 ± 19.55	64.7 ± 4.04	110.7 ± 12.5
	250	131.30 ± 18.50	60.0 ± 3.46	105.0 ± 15.13
	500	118.00 ± 9.54	57.7 ± 21.2	125.0 ± 5.00
	1000	113.30 ± 25.50	26.7 ± 3.21	115.0 ± 13.53
'-'Ve Control (H_2O)	0	122.30 ± 9.61	21.3 ± 9.02	121.0 ± 3.61
NPD	20	827.70 ± 106.53	522.0 ± 50.48	-
SA	1.5	-	-	1624.67 ± 89.76

NPD= Nitrophynylenediamine
SA= Sodium azide
S.D.= Standard Deviation
Anova Value of TA97a (Amaranth— 2.11, ns and Tartrazine —1.256, ns)
TA 98 (Amaranth—10.19* and Tartrazine –7.96*)
TA100 (Amaranth—2.196, ns and Tartrazine—2.74,ns)
P< 0.01 (5.06)

Table 2: Chromosomal aberrations of mice bone marrow cells following treatment with different doses of Amaranth, Tartrazine

Test Chemicals	*Dose*	*Type of Aberrations*					*%DC*[a]	*CA/Cell*[b]
	Mg/kg	*G'*	*G"*	*B'*	*B"*	*RR*	*Mean ± SEM*	*Mean ± SEM*
	50	7	1	2	-	-	2.00±1.00	0.02±0.01
Amaranth	100	10	1	5	-	-	3.50±0.50	0.03±0.00
	200	8	2	6	2	-	4.00±0.81	0.04±0.01
	50	10	1	7	-	-	3.50±0.50	0.03±0.00
Tartrazine	100	17	1	7	1	-	4.00±1.15	0.04±0.01
	200	11	2	8	1	-	4.50±0.50	0.04±0.00
Distilled water	0	9	-	4	-	-	2.00±0.81	0.02±0.01
CP	20	10	1	94	26	4	17.00±1.08	0.59±0.04

Abbreviations: G' G'' = Chromatid and chromosome gaps, B' B'' = Chromatid and chromosome breaks, RR = Rearrangements, a= Percentage of damaged cells, b = Chromosome aberration /cell, x =Mean of four animals and, 50 cells scored/animals, SEM = Standard Error of Mean, CP- Cyclophosphamide.

bone marrow cells using the Comet assay (alkalime single cell gel electrophoresis) as tartrazine (Au and Hsu 1979) and amaranth (Tsuda et al. 2001**)** could induce dose related DNA damage in rats /mice at the doses close to those of ADI's recommended.

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