Accepted Manuscript

Occurrence of Azo Food Dyes and their Effects on Cellular Inflammatory Responses

Latasha Leo, Claudine Loong, Xing Lin Ho, Muhammed Faiz B. Raman, Melissa Yue Tian Suan, Wai Mun Loke

PII: S0899-9007(17)30192-2

DOI: 10.1016/j.nut.2017.08.010

Reference: NUT 10027

To appear in: *Nutrition*

Received Date: 18 April 2017

Revised Date: 2 August 2017

Accepted Date: 15 August 2017

Please cite this article as: Leo L, Loong C, Ho XL, Raman MFB, Suan MYT, Loke WM, Occurrence of Azo Food Dyes and their Effects on Cellular Inflammatory Responses, *Nutrition* (2017), doi: 10.1016/j.nut.2017.08.010.

Occurrence of Azo Food Dyes and their Effects on Cellular Inflammatory Responses

Latasha Leo[1,2], Claudine Loong[1,2], Xing Lin Ho[1,2], Muhammed Faiz B Raman[2], Melissa Yue Tian Suan[2], Wai Mun Loke[1,2]

[1] Centre for Functional Food & Human Nutrition, School of Chemical & Life Sciences, Nanyang Polytechnic, Singapore 569830.

[2] Food Science & Nutrition Group, School of Chemical & Life Sciences, Nanyang Polytechnic, Singapore 569830.

Corresponding Author:

Wai Mun Loke

Centre for Functional Food & Human Nutrition

School of Chemical & Life Sciences

Nanyang Polytechnic

180 Ang Mo Kio Ave 8

Singapore 569830.

Tel: +65 6550 1504

Cell: +65 94378166

Email: loke_wai_mun@nyp.edu.sg

Word count of text = 4,075 words

Short version of title

Azo food dyes on oxidative stress and inflammation

ABSTRACT

The study aimed to examine the occurrence of five azo food dyes, tartrazine, sunset yellow, carmoisine, allura red, and ponceau 4R in the food supply chain of Singapore and their effects on the *in vitro* synthesis of leukotriene B_4 and F_2-isoprostanes. The names of foods and beverages sold in a local supermarket which contained at least one of the five azo dyes were recorded by trained personnel. The occurrence of the azo dyes in the local food supply was computed. The synthesis of leukotriene B_4 and F_2-isoprostanes from freshly isolated blood neutrophils were measured using gas chromatography-mass spectrometry. 11.54% of the 1681 processed food items contained at least one of the five azo dyes. Tartrazine was most prevalently present in food and beverage products sold in Singapore, followed by allura red, sunset yellow, ponceau 4R, and carmoisine. The five azo dyes augmented the *in vitro* synthesis of leukotriene B_4 and F_2-isoprostanes from blood neutrophils. Tartrazine was significantly more potent in increasing leukotriene B_4 synthesis than the other dyes, which exhibited similar potencies. The five food dyes increased the formation of F_2-isoprostanes from blood neutrophils at all tested concentrations. The high prevalence of azo dyes in the food supply of Singapore and their ability on eliciting pro-inflammatory responses *in vitro* suggest potential health detrimental risk to the local population.

Keywords: azo dyes, occurrence in food, leukotriene B_4, F_2-isopprostanes

Introduction

Colourants are often added to foods and beverages during processing to improve the sensory attributes of the final products. Food colourants are usually divided into pigments from natural origins and artificially synthesised dyes. Azo dyes, tartrazine (E102, TZ), sunset yellow (E110, SY), carmoisine (E122, CS), ponceau 4R (E124, PR) and allura red (E129, AR) are common food additives approved for use in Singapore by the Agri & Veterinary Authority of Singapore. They have been receiving significant amounts of attention as they are thought to elicit pro-inflammatory responses [1-3]. Coincidentally, the five tested dyes are aryl azo compounds, bearing the Ar-N=N-Ar in which the Ar is the aromatic group [4]. These aryl azo molecules may be reductively cleaved to form aromatic amines, which may be toxic, mutagenic, and carcinogenic [5]. The acceptable daily intakes (ADI) of TZ, SY, CS, PR, and AR were established by the World Health Organisation as 7.5 [6], 2.5 [6], 4.0 [7], 4.0 [7], and 7.0 [7] mg kg^{-1} body weight, respectively. The Singapore food regulation does not have ADI or permissible upper limits for food colourants [8][8][6]. There is, at present, limited data about the occurrence of these five azo food dyes in the food supply chain of Singapore.

Arachidonic acid (20:4 n-6) (AA) or all cis-5,8,11,14-eicosatetraenoic acid is a 20-carbon polyunsaturated fatty acid, found in mammalian tissues. AA can be converted via enzymatic or non-enzymatic pathways, to a range of oxygenated metabolites, collectively known as eicosanoids, which are involved in inflammatory and allergic processes [9]. AA may be oxygenated by 5-lipoxygenase (5-LO) to form leukotriene B_4 (LTB_4) [10]. LTB_4 is a potent chemoattractant capable to recruiting leukocytes, neutrophils, monocytes to the sites of inflammation and amplifying the inflammatory responses [10]. LTB_4 upregulation has been reported in various inflammatory and allergic diseases [11-17]. AA can also undergo non-enzymatic free radical-initiated peroxidation to form F_2-isoprostanes, which has been established as the stable in vivo marker of oxidative stress [18]. At present, limited data are

available on the effects of azo dye on the 5-LO-catalysed and free radical-initiated conversion of AA.

The study examined the occurrences of the five azo dyes in processed foods and beverages sold in Singapore. *In vitro* experiments were conducted to evaluate the effects of these azo dyes on AA metabolism via their effects on LTB_4 and F_2-isoprostanes synthesis from human blood neutrophils.

Materials and Methods

Chemicals & Materials

Leukotriene B_4-d_4, F_2-isoprostanes-d_4, and AA were purchased from Cayman Chemical (MO, USA). Glucose, TZ, SY, CS, PR, AR, dextran 500, phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), calcium ionophore A23187, MK-886, trypan blue, isooctane, Hank's balanced salt solution (HBSS), pyridine, toluene, isooctane, phosphate-buffered saline (PBS), 2,3,4,5,6-pentafluorophenylbromide (PFPBr), bis(trimethylsilyl)trifluoroacetamide (BSTFA), guaiacol and hydrogen peroxide were purchased from Sigma-Aldrich (MO, USA.; Acetonitrile, ethyl acetate, methanol, ethanol and sulfuric acid were from Tedia (OH, USA). Ficoll-paque was from GE Healthcare (Uppsala, Sweden).

Occurrence data collection

All the processed food and beverage items on the shelves of a local supermarket were included in this study. The items are categorised according to the supermarket shelf labels. For foods: baking needs, biscuits, breads, butter, cakes, canned fruits, canned meat, canned seafood, canned vegetables, cereals, cheese, chips, coconut products, crackers, chocolates, dairy products, dried food, fish and seafood, frozen meat, frozen snacks, ice cream, instant noodles, jellies, margarine, nuts, pre-packed noodles, puddings, sauces, soups, spreads, sweeteners, sweets, and tofu; and for beverages: Asian drinks, coffee drinks, dairy-based products, energy drinks, fruit juices, health beneficial beverages, juice drinks, soft drinks, soy-based beverages, sports beverages, syrups or fruit cordials, and tea. The name of each food or beverage item and the added azo dyes used in its production were recorded by trained research personnels. The presence of added food dyes should be listed on the ingredient list printed on the item's packaging as regulated by the Singapore Food Regulations.

Isolation of peripheral blood neutrophils

Blood neutrophils were isolated from the neutrophil/ erythrocyte pellet of fresh human whole blood after Ficoll-Paque gradient centrifugation and dextran sedimentation of red cells as previously described [19]. The whole human blood was obtained in kind by the study researchers, as such human ethics approval is not required. Cell viability was assessed using trypan blue exclusion and was typically >98%. The freshly isolated neutrophils were resuspended in HBSS at a concentration of 5×10^6 cells mL^{-1}.

Effects on leukotriene B_4 synthesis

The effects of TZ, SY, CS, PR and AR on the production of LTB_4 from freshly isolated blood neutrophils were examined. Briefly, the neutrophil suspension (5×10^6 cells mL^{-1} in HBSS, 1 mL) was incubated with either TZ, SY, CS, PR or AR (final concentrations, 0, 10, 20, 50, and 100 µmol L^{-1}) and AA (final concentration, 10 µmol L^{-1}) at 37 °C for 5 min prior to 5-LOstimulation. TZ, SY, CS, PR, and AR were added using ethanol as vehicle. The cells were stimulated with calcium ionophore A23187 (final concentration, 2.5 µg mL^{-1}) at 37 °C for 15 min. Untreated cells with AA in ethanol vehicle were used as positive controls while untreated cells incubated with the leukotriene biosynthesis inhibitor MK886 (300 nmol L^{-1}) in ethanol vehicle served as negative controls [20]. The supernatant from the cell suspension was collected and stored at -80 °C before LTB_4 extraction and analysis. The release of LTB_4 from stimulated neutrophils was measured by stable isotope labelled gas chromatography-mass spectrometry (GC-MS) [19].

Effects on F_2-isoprostanes synthesis

The effects of TZ, SY, CS, PR and AR on the production of F_2-isoprostanes from freshly isolated blood neutrophils were examined. Briefly, the freshly isolated neutrophils (5×10^6 cells mL^{-1} in HBSS, 1 mL) were incubated with either TZ, SY, CS, PR or AR (final concentrations, 0, 10, 20, 50, and 100 µmol L^{-1}) and AA (final concentration, 10 µmol L^{-1}) at 37 °C for 5 min prior to stimulation. TZ, SY, CS, PR, and AR were added using ethanol as the vehicle. The cells were stimulated with PMA (final concentration, 200 nmol L^{-1}) at 37 °C for 15 min. Positive control experiments were performed by incubating neutrophils with AA before activating with PMA. Negative control experiments were carried out by incubating neutrophils with AA only. The supernatant from the cell suspension was collected and stored at -80 °C before F_2-isoprostanes extraction and analysis. F_2-isoprostanes was quantitated using stable isotope labelled GC-MS [21].

Statistical analysis

Statistical analysis of the *in vitro* results (n=5 independent experiments using different batches of freshly isolated human neutrophils) was performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Between treatment differences were analysed using ANOVA of the area under the curve (AUC) in the concentration-response results. The results analysed were considered significantly different if p-value < 0.05 based on 95% confidence interval. Error bars in the figures were presented as standard deviations (SD).

Results

Of the 1681 processed food items surveyed, 11.54% contained at least one of the five azo dyes (Table 1). TZ was the most prevalent of the five dyes and was added to 7.02% of the processed food items (Table 1). AR was used in 2.08% of the food items, followed by SY (1.19%), PR (0.71%) and CM (0.54%) (Table 1). The top three food items that contain at least one of the five dyes were puddings (100%), jellies (65.38%), and sweets (60.47%) (Table 1). Of the staple food items, pre-packed noodles (33.33%), dairy products (17.39%), bread (16.42%), fish and seafood (13.79%), frozen meat (10.87%), and biscuits (10.44%) contained at least one of the five food dyes (Table 1). Among the five food dyes, TZ was most commonly used in these staples: pre-packed noodles (100%), dairy products (10.87%), bread (11.94%), fish and seafood (10.34%), frozen meat (10.87%), and biscuits (7.14%) (Table 1). At least one of the five food dyes was added into 19.55% of the surveyed three hundred and fifty-eight beverages (Table 1). TZ (10.06%) was most commonly added, followed by SY (5.03%), E122 (3.63%), E124 (0.84%) and E129 (0.00%) (Table 1). The top three beverages that contained at least one of the five food dyes were syrups and fruit cordials (60.00%), health beneficial beverages (57.14%), and Asian drinks (35.29%) (Table 1). At least one of these food dyes was also added to popular beverages, like soft drinks (19.51%), energy drinks (16.67%), fruit juices (14.52%), and juice drinks (12.07%) (Table1).

The five azo dyes augmented the production of LTB_4 from blood neutrophils differentially at all tested concentrations (Figure 1a). TZ was significantly more potent in increasing LTB_4 production (EC_{50} = 60 µmol L^{-1}) than the other four dyes (p<0.05 using ANOVA of AUC, Figure 1a). SY, CM, PR and AR did not differ in their effects on LTB_4 production from blood neutrophils (EC_{50} = ~50 µmol L^{-1}, Figure 1a). The five food dyes increased the formation of F_2-isoprostanes from blood neutrophils at all tested concentrations

(Figure 1b). A significant difference in the effects was absent between the five food dyes (Figure 1b).

Discussion

More than 10% of the processed food and beverages in Singapore contained at least one of the five azo food dyes. To examine the amount of food dyes consumed from the typical diet, it is important not only to monitor the levels of their usage in food and beverages, but also just as crucial to examine their occurrence in the food supply chain. TZ was the most common azo dye added to the food and beverage products sold in Singapore. It was added to more than 50% of the processed food and beverage items surveyed in the study. The study added to the limited data available on the occurrence of TZ and the other four food dyes in food and beverage products sold in developed Asian countries such as Singapore. In most developed countries, approved azo dyes are added to enhance the colours and thereby promoting the consumers' perception and acceptance of the final food products [22]. In most cases, azo dyes do not contribute to the overall nutritive quality of the final food products [22]. As TZ confers a universal beige-to-yellow colour and can be combined with Brilliant Blue (E133) or Green S (E142) to produce various green shades, it is widely employed in food and beverage products all over the world [23]. From the study results, TZ was commonly added to stapled foods and beverages such aspre-packed noodles, dairy products, bread, fish and seafood products, frozen meat products, biscuits, fruit cordial, health beneficial beverages, and Asian drinks. The wide prevalence of TZ in foods and beverages in the loical food supply may become a health concern if its intake confers biological responses *in vivo*.

The five aryl azo dyes exerted pro-inflammatory influences via the augmentation of LTB_4 production. Up-regulation of the circulating LTB_4 has been reported in asthma [12] and

rheumatoid arthritis [13]. Various studies showed that LTB_4 may mediate in specific inflammatory diseases, such as severe persistent asthma [14], exercise- and aspirin-induced asthma [15]. LTB_4 concentrations in the sputum, blood plasma, and bronchoalveolar lavage fluid were augmented in asthmatic patients, but not in healthy subjects [14]. Increased expressions of 5-LO and leukotriene A_4 hydrolase were suggested to contribute to the increased LTB_4 production in asthmatic patients [24, 25]. LTB_4 receptor-1 is required for allergen-mediated recruitment of CD8+ T cells and was involved in the development of airway hyperresponsiveness [12, 26]. Azo food dyes, especially TZ, may aggrevate asthmatic episodes via the upregulation of LTB_4 expression. Population-based studies are needed to ascertain if azo dye intake is associated to leukotriene B_4 – induced inflammation.

Many observations indicated the presence of oxidative stress in the pathogenesis of inflammatory diseases. Exhaled breath of asthmatic patients contained significantly higher concentrations of hydrogen peroxide [27] and F_2-isoprostanes [28]. Recent finding suggesting that *S*-nitrosoglutathione relaxed the airway smooth muscles [29] associates oxidative stress and asthma. House dust exposure induced the production of hydrogen peroxide from nasal eosinophils [30]. TZ and CM had been reported to increase malondialdehyde production and down-regulate the expressions of glutathione, superoxide dismutase, and catalase in rats [1]. TZ increased the levels of hydrogen peroxide in the testes of Wistar rats [31]. Reduced activities of catalase, glutathione peroxidase, and superoxide dismutase, as well as increased level of malondialdehyde, were observed in the brain of TZ-treated rats [32]. A similar reduction in antioxidant enzyme activities was reported in the blood and livers of rats fed with TZ or CM [33]. Our results added to these previous findings that all five azo dyes were equally capable of elevating the level of oxidative stress *in vitro*, as measured by the increased synthesis of F_2-isoprostanes in human neutrophils. This finding is significant as

oxidative damage has been implicated in the pathogenesis of atherosclerosis [34] and stroke [35].

Conclusion

11.54% of the 1681 processed food items contained tartrazine, allura red, sunset yellow, ponceau 4R or carmoisine. These five azo dyes augmented the *in vitro* synthesis of leukotriene B_4 and F_2-isoprostanes from blood neutrophils. The relatively high prevalence of azo dyes in the Singapore food supply and their potency on promoting pro-inflammatory responses *in vitro* suggest potential health risk to its local population.

Acknowledgments

We thank Prime Supermarket (Singapore) for their assistance in the collection of the food ingredients data. This work was supported by the Nanyang Polytechnic Student Final Year Project fund.

Author Contributions

WML designed research; MFBR and MYTS collected the data; WML conducted the experiments, LL, CL, XLH, and WML analysed data; and WML wrote the paper. WML had primary responsibility for final content. All authors read and approved the final manuscript.

References

1. Amin KA, Abdel Hameid Ii H, Abd Elsttar AH: **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* 2010, **48:**2994-2999.
2. Sayed HM, Fouad D, Ataya FS, Hassan NH, Fahmy MA: **The modifying effect of selenium and vitamins A, C, and E on the genotoxicity induced by sunset yellow in male mice.** *Mutat Res Genet Toxicol Environ Mutagen* 2012, **744:**145-153.

3. Ceyhan BM, Gultekin F, Doguc DK, Kulac E: **Effects of maternally exposed coloring food additives on receptor expressions related to learning and memory in rats.** *Food Chem Toxicol* 2013, **56:**145-148.
4. Chudgar R, Oakes J: *Dyes, azo. Kirk-Othmer encyclopedia of chemical technology.*2003.
5. Zhang G, Ma Y: **Mechanistic and conformational studies on the interaction of food dye amaranth with human serum albumin by multispectroscopic methods.** *Food Chemistry* 2013, **136:**442-449.
6. Fennema OR: *Food chemistry.*1996.
7. Joint F, Additives WECoF: **Toxicological evaluation of certain food additives and contaminants.** 1983.
8. **Singapore Food Regulations.** Agri-Food & Veterinary Authority of Singapore.
9. Funk CD: **Prostaglandins and leukotrienes: advances in eicosanoid biology.** *Science* 2001, **294:**1871.
10. Lotzer K, Funk KD, Habenich AJR: **The 5-lipoxygenase pathway in arterial wall biology and atherosclerosis.** *Biochimica et Biophysica Acta* 2005, **1736:**30 – 37.
11. De Caterina R, Mazzone A, Giannessi D, Sicari R, Pelosi W, Lazzerini G, Azzara A, Forder R, Carey F, Caruso D: **Leukotriene B_4 production in human atherosclerotic plaques.** *Biomed Biochim Acta* 1988, **47:**S182-185.
12. Gelfand EW, Dakhama A: **CD8+ T lymphocytes and leukotriene B4: Novel interactions in the persistence and progression of asthma.** *J Allergy Clin Immunol* 2006, **117:**577-582.
13. Chen M, Lam BK, Kanaoka Y, Nigrovic PA, Audoly LP, Austen KF, Lee DM: **Neutrophil-derived leukotriene B_4 is required for inflammatory arthritis.** *J Exp Med* 2006, **203:**837-842.
14. Csoma Z, Kharitonov SA, Balint B, Bush A, Wilson NM, Barnes PJ: **Increased Leukotrienes in Exhaled Breath Condensate in Childhood Asthma.** *Am J Resp Crit Care Med* 2002, **166:**1345-1349.
15. Hilberg T, Deigner H-P, Möller E, Claus RA, Ruryk A, Gläser D, Landre J, Brunkhorst FM, Reinhart K, Gabriel HHW, Russwurm S: **Transcription in response to physical stress— clues to the molecular mechanisms of exercise-induced asthma.** *FASEB J* 2005, **19:**1492-1494.
16. Shaw R, Fitzharris P, Cromwell O, Wardlaw A, Kay A: **Allergen‐Induced Release of Sulphidopeptide Leukotrienes (SRS‐A) and LTB4 in Allergic Rhinitis.** *Allergy* 1985, **40:**1-6.
17. Oyoshi Michiko K, He R, Li Y, Mondal S, Yoon J, Afshar R, Chen M, Lee David M, Luo Hongbo R, Luster Andrew D, et al: **Leukotriene B4-Driven Neutrophil Recruitment to the Skin Is Essential for Allergic Skin Inflammation.** *Immunity* 2012, **37:**747-758.
18. Sies H, Jones DP: **Oxidative Stress.** In *Encyclopedia of Stress. Volume* 3. 2nd edition. Edited by Fink G. San Diego: Elsevier; 2007: 45-48
19. Loke WM, Lam KM-J, Chong WL, Chew SE, Quek AM, Lim EC, Seet RC: **Products of 5-lipoxygenase and myeloperoxidase activities are increased in young male cigarette smokers.** *Free Rad Res* 2012, **46:**1230-1237.
20. Rouzer CA, Ford-Hutchinson AW, Morton HE, Gillard JW: **MK886, a potent and specific leukotriene biosynthesis inhibitor blocks and reverses the membrane association of 5-lipoxygenase in ionophore- challenged leukocytes.** *J Biol Chem* 1990, **265:**1436-1442.

21. Mori TA, Croft KD, Puddey IB, Beilin LJ: **An improved method for the measurement of urinary and plasma F2-isoprostanes using gas chromatography-mass spectrometry.** *Anal Biochem* 1999, **268:**117-125.
22. Msagati TA: *The chemistry of food additives and preservatives.* John Wiley & Sons; 2012.
23. Emerton V, Choi E: *Essential guide to food additives.* Leatherhead Food International; Royal Society of Chemistry; 2008.
24. Pacheco Y, Hosni R, Chabannes B, Gormand F, Moliere P, Grosclaude M, Piperno D, Lagarde M, PERRIN‐FAYOLLE M: **Leukotriene B4 level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta‐2 agonist therapy.** *Eur J Clin Invest* 1992, **22:**732-739.
25. Zaitsu M, Hamasaki Y, Matsuo M, Ichimaru T, Fujita I, Ishii E: **Leukotriene synthesis is increased by transcriptional up-regulation of 5-lipoxygenase, leukotriene A4 hydrolase, and leukotriene C4 synthase in asthmatic children.** *J Asthma* 2003, **40:**147-154.
26. Miyahara N, Takeda K, Miyahara S, Taube C, Joetham A, Koya T, Matsubara S, Dakhama A, Tager AM, Luster AD, Gelfand EW: **Leukotriene B4 Receptor-1 Is Essential for Allergen-Mediated Recruitment of CD8+ T Cells and Airway Hyperresponsiveness.** *J Immunol* 2005, **174:**4979-4984.
27. Emelyanov A, Fedoseev G, Abulimity A, Rudinski K, Fedoulov A, Karabanov A, Barnes PJ: **Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients.** *CHEST Journal* 2001, **120:**1136-1139.
28. Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ: **Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients.** *American journal of respiratory and critical care medicine* 1999, **160:**216-220.
29. Fang K, Johns R, Macdonald T, Kinter M, Gaston B: **S-nitrosoglutathione breakdown prevents airway smooth muscle relaxation in the guinea pig.** *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2000, **279:**L716-L721.
30. Ogasawara H, Yoshimura S, Kumoi T: **Hydrogen peroxide generation by eosinophils in allergic rhinitis.** *Auris Nasus Larynx* 1991, **18:**133-143.
31. Visweswaran B, Krishnamoorthy G: **Oxidative stress by tartrazine in the testis of Wistar rats.** *J Pharm Biol Sci* 2012, **2:**44-49.
32. Gao Y, Li C, Yin H, An X, Jin H: **Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved.** *J Food Sci* 2011, **76:**T125-T129.
33. El-Wahab HMFA, Moram GSE-D: **Toxic effects of some synthetic food colorants and/or flavor additives on male rats.** *Toxicol Industr Health* 2013, **29:**224.
34. Stocker R, Keaney JF: **Role of oxidative modifications in atherosclerosis.** *Physiol Rev* 2004, **84:**1381-1478.
35. Sanchez-Moreno C, Dashe JF, Scott T, Thaler D, Folstein MF, Martin A: **Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke.** *Stroke* 2004, **35:**163-168.

Tables

Table 1. (a) Food and (b) beverage categories sold in a local Singapore supermarket that contain added Tartrazine, Sunset Yellow, Carmoisine, Ponceau 4R, and/ or Allura Red and their occurrences in each category.

	Total number of items	Items containing at least one of the five food dyes (%)	Items containing Tartrazine (%)	Items containing Sunset Yellow (%)	Items containing Carmoisine (%)	Items containing Ponceau 4R (%)	Items containing Allura Red (%)
(a) Food items							
baking needs	55	3.64	0.00	0.00	0.00	3.64	0.00
biscuits	182	10.44	7.14	2.20	0.00	0.00	1.10
breads	67	16.42	11.94	2.99	1.49	0.00	0.00
butter	12	0.00	0.00	0.00	0.00	0.00	0.00
cake	35	22.86	14.29	0.00	0.00	2.86	5.71
canned fruits	44	0.00	0.00	0.00	0.00	0.00	0.00
canned meat	40	0.00	0.00	0.00	0.00	0.00	0.00

canned seafood	58	0.00	0.00	0.00	0.00	0.00	0.00
canned vegetables	82	1.22	1.22	0.00	0.00	0.00	0.00
cereals	37	8.11	2.70	2.70	0.00	0.00	2.70
cheese	45	0.00	0.00	0.00	0.00	0.00	0.00
chips	73	15.07	12.33	0.00	0.00	0.00	2.74
chocolate	82	20.73	7.32	6.10	0.00	0.00	7.32
coconut products	5	0.00	0.00	0.00	0.00	0.00	0.00
crackers	32	40.63	25.00	12.50	0.00	3.13	0.00
dairy products	46	17.39	10.87	0.00	0.00	4.35	2.17
dried foods	2	0.00	0.00	0.00	0.00	0.00	0.00
fish and seafood	29	13.79	10.34	0.00	0.00	3.45	0.00
frozen meat	46	10.87	10.87	0.00	0.00	0.00	0.00
frozen snacks	62	6.45	4.84	0.00	0.00	0.00	1.61

ice cream	39	12.82	12.82	0.00	0.00	0.00	0.00
instant noodles	79	5.06	5.06	0.00	0.00	0.00	0.00
jellies	26	65.38	38.46	7.69	3.85	3.85	11.54
margarine	19	0.00	0.00	0.00	0.00	0.00	0.00
nuts	32	25.00	18.75	0.00	0.00	0.00	6.25
prepacked noodles	12	33.33	33.33	0.00	0.00	0.00	0.00
pudding	12	100.00	58.33	8.33	0.00	0.00	33.33
sauces	124	2.42	0.00	0.00	0.00	0.81	1.61
soups	76	0.00	0.00	0.00	0.00	0.00	0.00
spreads	75	12.00	8.00	0.00	4.00	0.00	0.00
sweeteners	13	0.00	0.00	0.00	0.00	0.00	0.00
sweets	43	60.47	20.93	2.33	9.30	6.98	20.93
tofu	21	0.00	0.00	0.00	0.00	0.00	0.00
Overall	1681	11.54	7.02	1.19	0.54	0.71	2.08

Beverages

Asian Drink	34	35.29	17.65	8.82	8.82	0.00	0.00
Coffee Drink	6	0.00	0.00	0.00	0.00	0.00	0.00
Dairy-based beverages	28	3.57	3.57	0.00	0.00	0.00	0.00
Energy Drink	6	16.67	16.67	0.00	0.00	0.00	0.00
Fruit Juices	62	14.52	8.06	6.45	0.00	0.00	0.00
Health Beneficial Beverages	7	57.14	14.29	28.57	14.29	0.00	0.00
Juice Drinks	58	12.07	3.45	3.45	3.45	1.72	0.00
Soft Drinks	41	19.51	12.20	2.44	2.44	2.44	0.00
Soy-based beverages	22	4.55	4.55	0.00	0.00	0.00	0.00
Sports Beverages	28	25.00	14.29	7.14	3.57	0.00	0.00
Syrups / Fruit	30	60.00	30.00	10.00	16.67	3.33	0.00

Cordials							
Tea	36	5.56	2.78	2.78	0.00	0.00	0.00
Overall	358	19.55	10.06	5.03	3.63	0.84	0.00

[1] The presence of food dye in each processed food and beverage product category was computed as the percentage of the number of items which contain at least one of the five food dyes (Tartrazine, Sunset Yellow, Carmoisine, Ponceau 4R, and Allura Red) to the total number of the items in the same category.

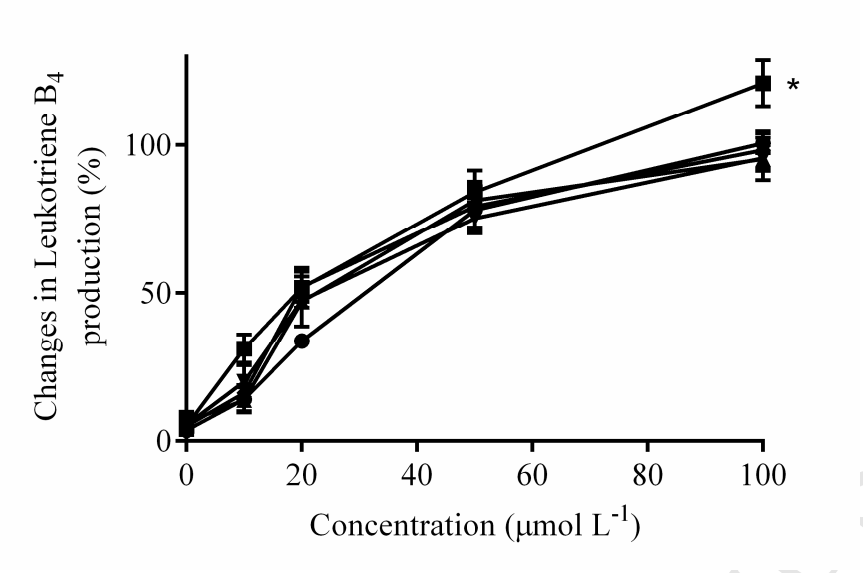
[2] The occurrence of the specific food dye in each processed food and beverage product category was computed as the percentage of the number of items which contain the food dye to the total number of the items within the same category.

Figure Legends

Figure 1. Change (%) in the (a) leukotriene B_4 and (b) F_2-isoprostanes production in freshly isolated human blood neutrophils by Tartrazine (■), Sunset Yellow (●), Carmoisine (♦), Ponceau 4R (▲) and Allura Red (▼) at concentrations up to 100 µmol L^{-1} (N=5). * $p < 0.05$ vs all other food dye using the ANOVA comparison of the area under the curve.

Figures

(a)



(b)

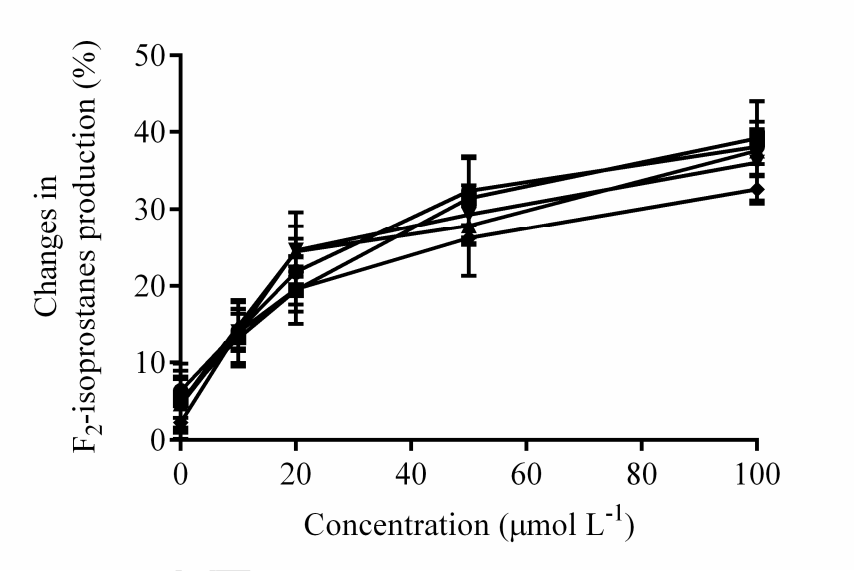


Figure 1

Highlights

- 11.54% of processed food items contained at least one azo dye.
- Tartrazine was the most prevalent dye present in the food and beverage products.
- Azo dyes augmented *in vitro* synthesis of leukotriene B_4 from blood neutrophils.
- Azo dyes augmented *in vitro* synthesis of F_2-isoprostanes from blood neutrophils.