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Toxicity, Genotoxicity and Ecotoxicity of Food Colourants Especially Synthetic Dyes and its Metabolites: An *in silico* Approach

ABHIJIT KARGUPTA[1], KAKOLI DUTTA[1,*], DIBAKAR ROYCHOUDHURY[1], SUBHASIS RANA[2] and SOUMENDRA NATH TALAPATRA[3]

[1]Department of Basic Science and Humanities, Institute of Engineering & Management, Sector V, Salt Lake Electronics Complex, Kolkata-700091, India
[2]Department of Basic Science and Humanities, University of Engineering & Management, New Town, Action Area-III, Kolkata-700160, India
[3]Department of Bio-Science, Seacom Skills University, Kendradangal, Bolpur-731236, India

*Corresponding author: E-mail: kakoli.dutta@iemcal.com

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In present study, *in silico* study was performed to predict rat oral acute toxicity, hepatoxicity, immunotoxicity, genotoxicity endpoints as well as ecotoxicity of daphnids and fish of different food colourants and its metabolites. A total 13 types of food colourants and 8 metabolites were selected. The prediction of rat oral acute toxicity (LD_{50}) and genotoxicity endpoints, and ecotoxicity (LC_{50}) were performed by using ProTox-II webserver and ECOSAR tool. It was obtained as 81 and 275 mg/Kg for tartrazine and green S as class 3 and rests were between class 4-6 for food colourants and 626 and 1925 mg/Kg for aminopyrazolone and 5-sulphoanthranilic acid as class 4 and rest were between 5-6 for metabolites. All the food colourants were non-hepatotoxic while four compounds were found immuno-toxic. For metabolites, only aminopyrazolone obtained hepatotoxic, but not immuno-toxic. Allura red was carcinogenic and few were mutagenic, but all were non-cytotoxic. For metabolites, few were carcinogenic but non-mutagenic and non-cytotoxic.

Keywords: *In silico*, Predictive toxicity, Genotoxicity, Ecotoxicity, Food colourants.

INTRODUCTION

Generally, the colour is used as an additives in beverages, food, pharmaceutical products and commonly called food colourant. Sometimes, a colour additive is well known organic chemical that reacts with another substance and causes formation of a colour [1-3]. However, the usage of colorants in the food approved by many regulatory authorities such as European Union (EU) followed the REGULATION (EC) No. 178/2002 of the European Parliament and of the Council on 28 January 2002, laid down on the general principles and designated food law, launching by the European Food Safety Authority in which the procedures of food safety matters declared. This was exposed to a wide range of toxicity tests such as detection of the acute, subacute and chronic toxicity, carcinogenicity, mutagenicity, teratogenicity, reproductive toxicity, bioaccumulation, bio-energy effects and immune effects based on strict legislative provisions in all developed countries [3].

In earlier studies, toxicity studies were performed for food colourants (FCs) and their metabolites in separate experimental study on rat or mice or microorganism models as *in vivo* or *in vitro* assay [3-10]. Amchova *et al*. [3] reported the toxicity and genotoxicity of food colourants and their metabolites. Majority of studies were based on toxicity and genotoxicity related to individual food colourant and its metabolite on mammals.

Interestingly, Ahmed *et al*. [11] studied the nutritional risk in children related to food safety among 6-17 year-old school-going children in Saudi Arabia. They evaluated 8 types of permitted artificial food colour additives *viz*. tartrazine, sunset yellow, carmoisine, Allura red, Indigo carmine, brilliant blue, fast green and black PN used in different food items, which was consumed by school children.

Moreover, researchers have also been studied individual food colourant or multiple food colourants through an experiment with parameter of toxicity, carcinogenicity especially on specific cancer type, teratogenicity as reproductive toxicity, *etc*. on human and/or mammals, which was observed long experimental duration, huge laboratory cost and animal harming while *in silico* approach revealed faster screening, cost-effective

and not require animal testing [12-14]. Still, experimental studies are unexplored to know overall toxicity mechanisms of food colourants and its metabolites.

Moreover, few experimental ecotoxicological studies have been conducted on fish related to individual food colourants [15-17] but ecotoxicological level especially daphnids and fish toxicity study related to food colourants is lacking. An *in silico* study was attempted to predict rat oral acute toxicity, hepatoxicity, immunotoxicity, genetic toxicity endpoints *viz*. carcinogenicity, mutagenicity and cytotoxicity as well as acute toxicity of daphnids and fish of different food colourants and its metabolites.

EXPERIMENTAL

Selection of food colourants and its metabolites: Different types of food colourants especially synthetic dyes were selected and separately searched the metabolites of each food colourants. Among food colourants, a total 13 types were selected for present predictive study and 8 metabolites were obtained from literature and the 2D structure of selected compounds are exhibited in Fig. 1, which were retrieved from ProTox-II tool.

***In silico* study for toxicity and genotoxicity of food colourants and its metabolites:** The ProTox-II webserver developed by Banerjee *et al*. [14] in which we studied four different *in silico* phases such as (i) rat oral acute toxicity as median lethal dose (LD_{50}) prediction model as per six different toxicity classes such as Class 1: fatal if swallowed ($LD_{50} \leq 5$), Class 2: fatal if swallowed ($5 < LD_{50} \leq 50$), Class 3: toxic if swallowed ($50 < LD_{50} \leq 300$); Class 4: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class 5: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class 6: non-toxic ($LD_{50} > 5000$) [12]; (ii) organ toxicity model for hepatotoxicity prediction; (iii) immunotoxicity model and (iv) genotoxicity endpoints (carcinogenicity, mutagenicity and cytotoxicity model) endpoints.

***In silico* study for ecotoxicity of food colourants and its metabolites:** For ecotoxicity especially daphnids and fish acute toxicity testing, the software was used namely Ecological Structure-Activity Relationship Model (ECOSAR) Version 1.11 developed by Mayo-Bean *et al*. [18]. The acute toxicity prediction as median lethal concentration (LC_{50}) values were determined for food colourants and its metabolites for daphnids and fish separately.

RESULTS AND DISCUSSION

In the predictive study, the results of different food colourants and its metabolites were obtained for the rat oral acute toxicity (LD_{50}) values (mg/Kg) along with activity (A) or inactivity (I) on liver toxicity, immunotoxicity, genetic toxicity end points such as carcinogenicity, mutagenicity, and cytotoxicity.

In Table-1, the rat oral acute toxicity as median lethal dose (LD_{50}), predicted toxicity classes between (3-6) and prediction accuracy in percentage % for food colourants. In case of LD_{50} values (mg/Kg), lower value was obtained as 81 mg/Kg and 275 mg/Kg for tartrazine and Green S as class 3. Rest food colourants were obtained LD_{50} values of about 1264, 1350 and 2000 mg/Kg as class 4, 3600 mg/Kg as class 5 and 8000 and 10000 mg/Kg as class 6.

TABLE-1
PREDICTION OF ORAL ACUTE TOXICITY, CLASS, AND ACCURACY OF DIFFERENT FOOD COLOURANTS

Compounds name	Rat oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
Tartrazine	81	3	100.00
Quinoline yellow	2000	4	68.07
Sunset yellow	2000	4	72.90
Azorubine	8000	6	100.00
Ponceau 4R	8000	6	72.90
Erythrosine	1264	4	72.90
Allura red	10000	6	72.90
Patent blue	2000	4	68.07
Indigo carmine	3600	5	23.00
Brilliant blue FCF	2000	4	69.26
Green S	275	3	68.07
Brown HT	1350	4	23.00
Brilliant black	2000	4	72.90

Class 3: toxic if swallowed ($50 < LD_{50} \leq 300$); Class 4: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class 5: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class 6: non-toxic ($LD_{50} > 5000$)

In Table-2, the rat oral acute toxicity as median lethal dose (LD_{50}), predicted toxicity classes between (4-5) and prediction accuracy in percentage % for the metabolites of food colourants. In the case of LD_{50} values (mg/Kg), lower value was obtained as 626 mg/Kg and 1925 for aminopyrazolone and 5-sulphoanthranilic acid as class 4. Rest metabolites were obtained LD_{50} values of about 2344, 3710, 3770, and 5000 mg/Kg as class 5 and 11500 mg/Kg as class 6.

In case of hepatoxicity, all the food colourants were obtained inactive while four compounds such as Azorubine, patent blue, Green S and Brown HT were immunotoxic as active. For metabolites, only aminopyrazolone obtained hepatotoxic as active and rests were obtained inactive while all the metabolites were obtained inactive for immuno-toxicity (Table-3).

In case of genotoxicity end points, all the food colourants were obtained carcinogenic inactive except Allura red as obtained active. For mutagenicity prediction, quinoline yellow, patent blue and brilliant blue FCF were obtained mutagenic as active. For cytotoxicity prediction, all the food colourants were obtained inactive as non-cytotoxic (Table-4).

In case of genotoxicity end point, among all the metabolites, there compounds *viz.* aminopyrazolone, triiodofluorescein and cresidinesulfonic acid were obtained carcinogenic active. For mutagenicity prediction, all the compounds were obtained mutagenic and cytoxic inactive (Table-5).

The present predictive acute toxicity (LC_{50}) results (ppm) were obtained lower value, which showed as highly toxic food colourants *viz*. erythrosine and quinoline yellow while toxic as Azorubine on daphnids and fish as per ECOSAR tool. Rest food colourants were obtained comparatively non-toxic (Table-6). The present predictive acute toxicity (LC_{50}) results (ppm) were obtained lower value only for metabolite namely triiodofluorescein, which showed higher toxicity compared to other metabolites, which showed non-toxicity on daphnids and fish as per ECOSAR tool (Table-6).

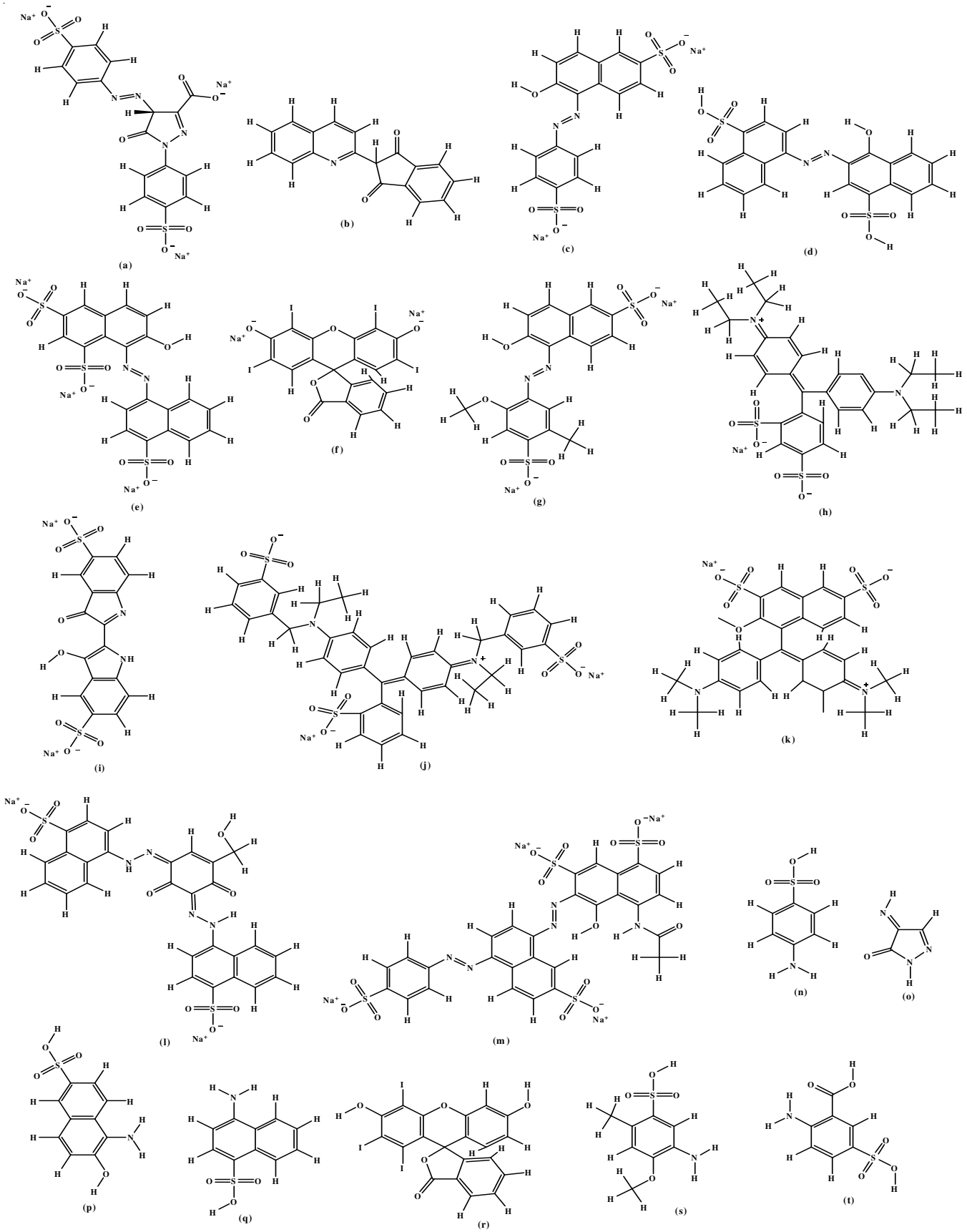

Fig. 1. Two-dimensional structure of food colorants and its metabolites (a = Tartrazine; b = Quinoline yellow; c = Sunset yellow; d = Azorubine; e = Ponceau 4R; f = Erythrosine; g = Allura red; h = Patent blue; i = Indigo carmine; j = Brilliant blue FCF; k = Green S; l = Brown HT; m = Brilliant black; n = Sulfanilic acid; o = Aminopyrazolone; p = 1-Amino-2-naphthol-6-sulfonic acid; q = Naphthionic acid; r = Triiodofluorescein; s = Cresidinesulfonic acid; t = 5-sulphoanthranilic acid)

TABLE-2
PREDICTION OF ORAL ACUTE TOXICITY, CLASS AND ACCURACY OF DIFFERENT METABOLITES OF FOOD COLOURANTS

Compounds	Metabolites	Rat oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
Tartrazine	Sulfanilic acid	3770	5	100.00
	Aminopyrazolone	626	4	54.26
Quinoline yellow	Enamine	5000	5	68.07
Sunset yellow	1-Amino-2-naphthol-6-sulfonic acid	11500	6	70.97
Azorubine	Naphthionic acid	5000	5	100.00
Ponceau 4R	Naphthionic acid	5000	5	100.00
Erythrosine	Triiodofluorescein	2344	5	69.26
Allura red	Cresidinesulfonic acid	3710	5	68.07
Patent blue	–	–	–	–
Indigo carmine	5-Sulphoanthranilic acid	1925	4	69.26
Brilliant blue FCF	–	–	–	–
Green S	–	–	–	–
Brown HT	Naphthionic acid	5000	5	100.00
Brilliant black	–	–	–	–

Class 3: toxic if swallowed ($50 < LD_{50} \leq 300$); Class 4: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class 5: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class 6: non-toxic ($LD_{50} > 5000$)

TABLE-3
PREDICTION OF HEPATOTOXICITY AND IMMUNOTOXICITY END POINTS OF DIFFERENT FOOD COLOURANTS AND ITS METABOLITES

Compounds	H	P	I	P	Metabolites	H	P	I	P
Tartrazine	Inactive	0.60	Inactive	0.99	Sulfanilic acid	Inactive	0.74	Inactive	0.99
					Aminopyrazolone	Active	0.66	Inactive	0.97
Quinoline yellow	Inactive	0.57	Inactive	0.91	Enamine	Inactive	0.64	Inactive	0.99
Sunset yellow	Inactive	0.64	Inactive	0.99	1-amino-2-naphthol-6-sulfonic acid	Inactive	0.63	Inactive	0.62
Azorubine	Inactive	0.63	Active	0.54	Naphthionic acid	Inactive	0.67	Inactive	0.87
Ponceau 4R	Inactive	0.64	Inactive	0.93	Naphthionic acid	Inactive	0.67	Inactive	0.87
Erythrosine	Inactive	0.62	Inactive	0.83	Triiodofluorescein	Inactive	0.64	Inactive	0.60
Allura red	Inactive	0.60	Inactive	0.84	Cresidinesulfonic acid	Inactive	0.66	Inactive	0.99
Patent blue	Inactive	0.71	Active	0.94	–	–	–	–	–
Indigo carmine	Inactive	0.55	Inactive	0.97	5-sulphoanthranilic acid	Inactive	0.58	Inactive	0.99
Brilliant blue FCF	Inactive	0.71	Inactive	0.81	–	–	–	–	–
Green S	Inactive	0.61	Active	0.96	–	–	–	–	–
Brown HT	Inactive	0.60	Active	0.72	Naphthionic acid	Inactive	0.67	Inactive	0.87
Brilliant black	Inactive	0.59	Inactive	0.98	–	–	–	–	–

H = Hepatotoxicity; I = Immunotoxicity; P = Probability

TABLE-4
PREDICTION OF GENOTOXICITY END POINTS OF DIFFERENT FOOD COLOURANTS

Compounds	Carcinogenicity	Probability	Mutagenicity	Probability	Cytotoxicity	Probability
Tartrazine	Inactive	0.71	Inactive	0.85	Inactive	0.67
Quinoline Yellow	Inactive	0.52	Active	0.73	Inactive	0.60
Sunset Yellow	Inactive	0.99	Inactive	0.92	Inactive	0.76
Azorubine	Inactive	0.64	Inactive	1.00	Inactive	0.78
Ponceau 4R	Inactive	0.99	Inactive	0.92	Inactive	0.76
Erythrosine	Inactive	0.64	Inactive	0.83	Inactive	0.68
Allura Red	Active	0.70	Inactive	0.90	Inactive	0.76
Patent Blue	Inactive	0.66	Active	0.76	Inactive	0.59
Indigo Carmine	Inactive	0.64	Inactive	0.75	Inactive	0.68
Brilliant Blue FCF	Inactive	0.77	Active	0.72	Inactive	0.58
Green S	Inactive	0.56	Inactive	0.76	Inactive	0.57
Brown HT	Inactive	0.53	Inactive	0.60	Inactive	0.69
Brilliant Black	Inactive	0.79	Inactive	0.94	Inactive	0.73

According to Demirkol *et al*. [19], tartrazine dye exposure observed toxicity on CHO cells and this food colourant also showed genotoxicity in rats [20] but present study did not obtain carcinogenic, mutagenic and cytotoxic active. On the other hand, 13 week sub-chronic toxicity study with tartrazine caused alterations in hepatic and renal parameters due to the generation of free radicals leading to oxidative stress [21]. In present study, aminopyrazolone metabolite was predicted hepatoxic and this may be occurred as per metabolism of tartrazine long-term exposure. In present findings, food colourant dye

TABLE-5
PREDICTION OF GENETIC TOXICITY END POINTS OF DIFFERENT METABOLITES OF FOOD COLOURANTS

Compounds	Metabolites	Carcinogenicity	Probability	Mutagenicity	Probability	Cytotoxicity	Probability
Tartrazine	Sulfanilic acid	Inactive	0.70	Inactive	0.93	Inactive	0.76
	Aminopyrazolone	Active	0.69	Inactive	0.52	Inactive	0.78
Quinoline yellow	Enamine	Inactive	0.79	Inactive	0.51	Inactive	0.64
Sunset yellow	1-Amino-2-naphthol-6-sulfonic acid	Inactive	0.65	Inactive	0.68	Inactive	0.73
Azorubine	Naphthionic acid	Inactive	0.72	Inactive	0.88	Inactive	0.82
Ponceau 4R	Naphthionic acid	Inactive	0.72	Inactive	0.88	Inactive	0.82
Erythrosine	Triiodofluorescein	Active	0.69	Inactive	0.91	Inactive	0.68
Allura red	Cresidinesulfonic acid	Active	0.50	Inactive	0.66	Inactive	0.75
Patent blue	–	–	–	–	–	–	–
Indigo carmine	5-Sulphoanthranilic acid	Inactive	0.78	Inactive	0.86	Inactive	0.71
Brilliant blue FCF	–	–	–	–	–	–	–
Green S	–	–	–	–	–	–	–
Brown HT	Naphthionic acid	Inactive	0.72	Inactive	0.88	Inactive	0.82
Brilliant black	–	–	–	–	–	–	–

TABLE-6
PREDICTION OF ECOTOXICITY ON DAPHNIDS AND FISH FOR DIFFERENT FOOD COLOURANTS AND ITS METABOLITES

Compounds	Daphnids	Fish	Metabolites	Daphnids	Fish
	LC_{50} (ppm)	LC_{50} (ppm)		LC_{50} (ppm)	LC_{50} (ppm)
Tartrazine	54288.69	124000.0	Sulfanilic acid	257000	659000
			Aminopyrazolone	1630000	4680000
Quinoline yellow	1.604	2.303	Enamine	266.27	488.07
Sunset yellow	621.44	1156.65	1-Amino-2-naphthol-6-sulfonic acid	89744.23	216000
Azorubine	68.33	114.09	Naphthionic acid	32429.13	74690.62
Ponceau 4R	521.65	950.63	Naphthionic acid	32429.13	74690.62
Erythrosine	0.003	0.003	Triiodofluorescein	0.023	0.026
Allura red	199.02	349.54	Cresidinesulfonic acid	93132.72	226000
Patent blue	1870000	4480000	–	–	–
Indigo carmine	232000	567000	5-Sulphoanthranilic acid	183000	457000
Brilliant blue FCF	247000	530000	–	–	–
Green S	2330000	6290000	–	–	–
Brown HT	81801.50	188000	Naphthionic acid	32429.13	74690.62
Brilliant black	436.63	775.58	–	–	–

Green S was predicted toxicity with lower LC_{50} value, which is supported by Clode *et al*. [22] that >500 ppm no observed effect in rat model after short-term exposure. In present predictive results, food colourants *viz*. Azorubine, patent blue, Green S and Brown HT were obtained immune-toxic, which is observed that sometimes Azorubine caused skin and respiratory allergic reactions [23], patent blue and Brown HT also observed allergic manifestation among people [24-26]. All the food colourants were obtained carcinogenic inactive except Allura red, which is supported by Silva *et al*. [26]. Among different studied food colourants, quinoline yellow, patent blue and brilliant blue FCF were obtained mutagenic active, which is supported by other investigators [26-28]. Among all the metabolites, there compounds *viz*. aminopyrazolone, triiodofluorescein and cresidinesulfonic acid were obtained carcinogenic active, while all compounds were obtained mutagenic and cytotoxic inactive. As tartrazine is well known genotoxic agent, which causes DNA damage [20] and this mutagenic effect may lead to continue its metabolite namely aminopyrazolone while the metabolite of Allura red may be carcinogenic potential because the parent compound is carcinogens at higher concentration [26].

Erythrosine and quinoline yellow while toxic as Azorubine on daphnids and fish as per ECOSAR tool. According to Gupta *et al*. [16], erythrosine observed toxicity in zebrafish with long-term exposure. From the present *in silico* study, it is observed that still many *in vivo* and *in vitro* study is waiting to know the toxic effect at ecosystem level because these food colourants may be exposed to aquatic biota.

Conclusion

It is suggested from the present predictive results that few food colourants are harmful to animals and scattered information on toxicity, genotoxicity and ecotoxicity can be a new research interest. Moreover, the present *in silico* study of predictive toxicity, hepatotoxicity, immunotoxicity and genotoxicity by using ProTox-II webserver and ecotoxicity prediction on daphnids and fish by ECOSAR tool can be suitable research findings, which help further experimental assay *viz. in vitro* and *in vivo* test on biota. Due to toxicity level at class 3 for tartrazine and Green S for food colourants and the metabolites namely aminopyrazolone and 5-sulphoanthranilic acid as class 4, the usage of these compounds should be more concerned. The usage of Allura red was also required to check recommended

concentration as food colourant. In future, this predictive finding is suggested further experimental analysis to validate the present predictions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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