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# Chronic toxicity and carcinogenicity study of dietary gardenia blue in Sprague Dawley rats

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ABSTRACT

In this combined chronic toxicity/carcinogenicity study of gardenia blue as a natural food color additive, Sprague Dawley rats were administered 0.5%, 2.5%, or 5.0% gardenia blue via the feed or carrier diet (0.0% gardenia blue) for 12 (chronic toxicity cohort) or 24 (carcinogenicity cohort) months. No abnormal clinical, ophthalmological, neurotoxicity or clinical pathology changes were attributed to treatment, and there was no increase in mortality due to gardenia blue exposure. The only treatment-related change was grossly observed blue discoloration of the stomach, intestines, and mesenteric lymph nodes as well as reversible dark discoloration of the kidneys all without associated histopathology. The no-observed-adverse-effect level (NOAEL) for gardenia blue exposure via the diet for one or two years was determined to be 5.0% (2175.3 mg/kg body weight/day in male rats and 3075.4 mg/kg body weight/day in female rats).

## 1. Introduction

Because of health-conscious consumer fears about the safety of synthetic food colorants commonly used in the food industry, there is interest in colorants derived from natural sources that are likely to be generally regarded as nontoxic and environmentally friendly (Gao et al., 2021; Li et al., 2021). The resources of blue colorants are most difficult to find naturally (Landim Neves et al., 2021). Gardenia blue is a dark blue food colorant widely used in Japan (Tsutsumiuchi et al., 2021) and has high stability compared to other natural pigments with a coloring capacity comparable to synthetic dyes (Li et al., 2021). Gardenia blue is made from naturally derived raw materials obtained from the fruit of *Gardenia jasminoides* (Newsome et al., 2014). *Gardenia jasminoides* fruit contains geniposide that following reaction with beta-glucosidase forms genipin that can then react with peptides, amino acids, or proteins to form a blue pigment (Li et al., 2021). Although natural food additives derived from natural resources, including natural food colorants, are usually regarded as safe and non-toxic for humans among health-conscious consumers, there are occasional major safety concerns, including concerns about carcinogenicity, associated with some botanical ingredients, with early regulatory prohibition of some natural additives such as calamus, safrole, and madder (Do and Kwon, 2022). Consequently, it is essential to adequately test such natural additives for potential toxicity and carcinogenicity.

Gardenia blue has been tested in the past in toxicity studies, and several genotoxicity studies showed that gardenia blue is non-mutagenic and of no genotoxic concern for humans (Do and Kwon, 2022; Hobbs et al., 2018). However, there are reports on liver toxicity of the Gardenia

*Abbreviations:* APTT, activated partial thromboplastin time; CPN, Chronic progressive nephropathy; FDA, Food and Drug Administration; FOB, functional observational battery; GALT, gut-associated lymphoid tissue; GLP, Good Laboratory Practice; ILS, Integrated Laboratory Systems; NOAEL, no-observed-adverse-effect level; OECD, Organization for Economic Co-operation and Development; SD, Sprague Dawley.

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jasminoides fruit (Cui et al., 2017) and possible hepatotoxic effects of genipin were observed in both *in vitro* and *in vivo* studies (Li et al., 2019a, 2019b; Xu et al., 2018). Therefore, there is a need for properly designed toxicity and carcinogenicity studies that adhere to the strict regulatory standards using materials of acceptable purity.

The aim of this study was to assess chronic toxicity and carcinogenic potential of gardenia blue when administered orally to Sprague Dawley (SD) rats. The study was performed in anticipation of the worldwide marketing of gardenia blue as a food colorant after authorization by the U.S. Food and Drug Administration (FDA) and the European Union, and a positive safety opinion from JECFA, the FAO/WHO Joint Expert Committee on Food Additives. No adverse effects were identified in SD rats following 12 and 24 months of dietary exposures up to 5% gardenia blue in SD rats.

## 2. Materials and methods

### 2.1. Regulatory compliance

This study was conducted by Integrated Laboratory Systems (ILS) (Research Triangle Park, NC, USA), in accordance with all applicable ILS standard operating procedures and U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations 21 CFR Part 58 (FDA, 2018). This study was also designed to satisfy the Organization for Economic Co-operation and Development (OECD) Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies (OECD, 2009).

### 2.2. Test article

Gardenia blue was obtained from San-Ei Gen, F.F.I., Inc., Osaka, Japan, as a dark blue powder, containing 32.3% gardenia blue color, 62.7% dextrin, 3.2% water, and 1.8% other components. Genipin was not detected in the powder. Purina Certified 5002 meal diet containing gardenia blue at dose concentrations of 0.5%, 2.5%, and 5.0% was prepared at RTI International (Research Triangle Park, NC, USA).

### 2.3. Animal husbandry and maintenance

Hsd: SD rats, 4–6 weeks of age, were obtained from Envigo Laboratories (Frederick, MD, USA), and housed in polycarbonate cages with micro-isolator top. Purina Certified 5002 Meal Diet (Ralston Purina Co., St. Louis, MO, USA) and reverse osmosis tap water were provided *ad libitum*. Heat-treated hardwood bedding (Northeastern Products Corp., Warrensburg, NY, USA) was changed at least once per week. A 12/12-h light/dark cycle was maintained throughout the study period. SD rats were chosen for the study since they are a standard and accepted species for toxicity studies. All procedures followed U.S. Department of Agriculture (USDA) *Animal Care: Animal Welfare Act and Animal Welfare Regulations* 9 CFR 1–4 (USDA, 2017). Animals were handled and treated according to the *Guide for the Care and Use of Laboratory Animals* (NRC, 2011).

### 2.4. Experimental design

#### 2.4.1. Route of administration

The oral route of administration is consistent with anticipated dietary human exposure and is in accordance with OECD Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies (OECD, 2009). Doses were chosen based on toxicological data for rats following feed exposure in a range-finding study (data not shown).

#### 2.4.2. Chronic toxicity study

Eighty male and 80 female SD rats were allocated to 1 of 4 designated groups (Groups 1–4, 20 animals per sex per group). The animals were exposed to 1 of 3 exposure levels of gardenia blue (0.5%, 2.5%, or 5.0%) or the carrier diet for at least 12 months. Clinical observations, body weights, and food consumption measurements were collected weekly and cage-side observations were performed at least daily. Ophthalmological examinations were performed prior to gardenia blue exposure and again prior to termination. After at least 6 months of exposure, neurotoxicity screening, including a functional observation battery (FOB) and motor activity assessment, was performed. Blood and urine were collected from all animals (20/sex) in each group after 3, 6, and 12 (at termination) months of exposure for clinical pathology assessment (hematology, clinical chemistry, and coagulation) and urinalysis.

#### 2.4.3. Carcinogenicity study

Two hundred male and 200 female SD rats were allocated to 1 of 4 designated treatment groups (Groups 5–8, 50 animals per sex per group). The animals were scheduled to be exposed to 1 of 3 exposure levels of gardenia blue (0.5%, 2.5%, or 5.0%) or the carrier diet for 24 months. However, termination of male rats occurred after approximately 23.5 months of gardenia blue exposure, when approximately 25% of male rats in the control group were left surviving; female rats were exposed for the full 24-month period. Clinical observations, body weights, and food consumption measurements were collected weekly and cage-side observations were performed at least daily. Blood and urine were collected from surviving animals at termination for clinical pathology assessment (hematology, clinical chemistry, and coagulation) and urinalysis.

### 2.5. Termination

Animals were euthanized by $CO_2$ asphyxiation and death was confirmed by exsanguination. A complete gross necropsy was conducted on all rats, including external surfaces, orifices, cranial, thoracic, and abdominal cavities, carcass, all organs, and gross lesions. The following organs were weighed: adrenal (paired), brain, epididymides (paired), heart, kidneys (paired), liver, ovaries (paired), spleen, testes (paired), thyroid with parathyroid, and uterus with cervix.

### 2.6. Pathology

Formalin-fixed tissues preserved for histopathological evaluation included adrenals, aorta, brain (cerebrum, cerebellum and medulla oblongata/pons), bone and bone marrow (sternum and femur), cecum and colon, uterus and cervix, coagulating gland, epididymides, esophagus, eyes, Harderian glands, heart, intestines (small only including Peyer's patches), kidneys, lacrimal gland, liver, lungs with trachea, lymph nodes (mesenteric and mandibular), mammary glands, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, and lumbar divisions), spleen, stomach, testes, tongue, thymus, thyroid/parathyroid(s), urinary bladder, and vagina.

Samples from all tissues harvested from the control and the high-dose animals, all unscheduled animals (found dead or euthanized prior to scheduled necropsy), and any lesions, were trimmed of any adherent tissue, histologically processed, embedded, sectioned at a thickness of 5 μm, and stained with hematoxylin and eosin (H&E). Tissues from animals in intermediate groups were also trimmed of any adherent tissue, histologically processed, and embedded. H&E-stained slides were evaluated using light microscopy. Lesions were graded as to severity, regardless of group. Lymphoid tissues (thymus, spleen, bone marrow, lymph nodes, and gut-associated lymphoid tissue [GALT]) were microscopically evaluated using the enhanced histopathology method (Elmore, 2012; Haley et al., 2005).

### 2.7. Statistical analysis

Group mean and standard deviations were calculated and analyzed using Statistical Analysis System (version 9.2; SAS Institute, Cary, NC, USA). Homogeneous data were analyzed using a one-way ANOVA and

treated groups were compared to the appropriate control group using Dunnett's test. Dose-dependent changes were evaluated using a linear regression model. If the data could not be transformed to be homogeneous, data were evaluated using the non-parametric Dunn's test and dose-dependent changes analyzed via the non-parametric Jonckheere's trend test.

FOB data were analyzed by the analysis of variance test, Dunnett's test, the Kruskal-Wallis test, and/or Dunn's test. Endpoints in the FOB which used graded or count scales were analyzed using a non-parametric strategy. Data from the motor activity test were analyzed using an ANOVA with Repeated Measures. Survival curves were estimated using the Kaplan and Meier procedure separately for each sex of rat. Animals found dead from reasons other than natural causes and those animals surviving to the terminal time point were censored from the survival analysis.

## 3. Results

### 3.1. 12-Month chronic toxicity cohort

#### 3.1.1. Survival

All animals survived until scheduled termination, except for two animals found dead and two moribund sacrifice animals in the control group, one moribund sacrifice animal in the 0.5% dose group, one animal found dead and one humane sacrifice animal in the 2.5% dose group, and three moribund sacrifice animals and one humane sacrifice animal in the 5.0% dose group (Supplemental Tables 1 and 2). The cause of death was determined to be chronic progressive nephropathy in one control female and one 5.0% dose group male and lymphoma in one 5.0% dose group male. The two humane sacrifice animals sustained eye damage that was likely caused during a retroorbital blood collection procedure. A cause of death was not determined in the remainder of the animals.

#### 3.1.2. Clinical observations

Males and females had blue to black feces throughout treatment generally attributed to ingestion of gardenia blue at concentrations of 0.5% or greater. No other atypical clinical observations were present in treated or control rats.

#### 3.1.3. Body weight and food consumption

There were no statistically significant differences in initial body weight, final body weight or body weight gain of rats of either sex (Supplemental Tables 1 and 2). No significant differences in food consumption were noted between the animals fed control feed and those fed with gardenia blue. Exposure to 0.5%, 2.5%, or 5.0% gardenia blue in feed resulted in average doses of 251.7 and 336.5 mg gardenia blue/kg/day, 1257.5 and 1541.3 mg gardenia blue/kg/day, and 2478.7 and 3282.7 mg gardenia blue/kg/day in male and female rats, respectively.

#### 3.1.4. Ophthalmologic examinations

Mild bilateral corneal crystals were observed in seven rats prior to treatment and in two rats after 12 months of exposure to gardenia blue. Such corneal crystals are common in rats of this strain and age, and thus were not considered to be related to gardenia blue exposure.

#### 3.1.5. Neurotoxicity screening

All animals were examined for signs of neurotoxicity, using a screen consisting of a FOB and motor activity, after 6 months on study. No treatment-related effects on motor activity were observed for male or female rats administered gardenia blue in the diet. The only significant effect was fur discoloration from the test article, present at all dose levels of gardenia blue. There were no tremors, spasms, or seizures in any animal and no indication of alterations to sensory, motor, or autonomic function. Based on this neurotoxicity screening, administration of gardenia blue up to 5.0% in the diet was considered unlikely to present a neurotoxicity hazard, as outlined in the OECD rodent neurotoxicity guidelines.

#### 3.1.6. Clinical pathology

*3.1.6.1. 3 & 6-month timepoints.* There were no toxicologically or physiologically significant alterations in hematology, coagulation or clinical chemistry for male or female rats at the 3-month timepoint. Hematologic parameters were also within normal limits for both sexes at the 6-month sampling interval. Treatment related changes at the 6-month sampling interval included an 18% and 30% increase in activated partial thromboplastin time (APTT) in 2.5% and 5.0% gardenia blue exposure groups for males, alterations in blood urea nitrogen in 2.5% gardenia blue for males and 0.5% gardenia blue for females, and a scattering of other elevated clinical chemistry values in treated males or females versus controls (Supplemental Table 3). In all instances changes were minimal, within historical control values and not considered adverse.

*3.1.6.2. 12-Month timepoint.* While there were some statistically significant hematology alterations in the chronic toxicity group animals at the 12-month timepoint (Supplemental Table 4), all were well within normal ranges. Female rats showed no difference in clotting parameters, while male rats showed a dose dependent linear trend for decreasing APTT and statistically significant lower mean APTT levels in the 5.0% gardenia blue for males versus control. This reduced APTT was within normal ranges and was not considered toxicologically significant. Statistically significant alterations to clinical chemistry measurements were noted in female rats (Supplemental Table 5) but were generally within normal physiologic levels and not considered toxicologically relevant.

#### 3.1.7. Urinalysis

There were statistical differences in urine color, white blood cell levels, and urine bilirubin levels for males exposed to gardenia blue at the 3-month timepoint (Supplemental Table 6). None of these changes was considered adverse or toxicologically significant. Urine bilirubin levels were elevated in male rats exposed to gardenia blue at the 6-month and 12-month timepoints and females at the 12-month timepoint, and these observations appeared to be dose related but were not considered adverse or toxicologically significant. There were no toxicologically or physiologically significant alterations in urinalysis parameters for female rats at any dose level. The urine was not blue at any stage of the study.

#### 3.1.8. Gross necropsy

The only gross pathology finding considered to be related to gardenia blue administration consisted of blue discoloration of the mesenteric lymph node, stomach, duodenum, jejunum, ileum, cecum, and colon, as well as dark discoloration of the kidneys (Supplemental Tables 1 and 2). At least one tissue was affected in one or more males and females in each of the gardenia blue-dosed groups. Other gross findings were scattered sporadically across groups and considered to be incidental with no association to gardenia blue

#### 3.1.9. Organ weights

There was a statistically significant decrease in the mean absolute brain weight in female rats exposed to 5.0% gardenia blue, with a dose-dependent linear trend, while relative brain weights (organ to body weight ratio) did not differ from controls. Statistically significant decreases in mean absolute and relative thyroid gland weights were present in males and females of the 0.5% and 2.5% exposure groups and the mean relative thyroid gland weight in females in the 5.0% exposure group, but there were no corresponding statistically significant dose-dependent linear trends nor correlating histopathological changes. Therefore, changes in organ weights and relative organ weights were

considered sporadic and not toxicologically relevant.

#### 3.1.10. Pathology

Changes to explain the blue or dark discoloration of several tissues observed grossly in some treated animals were not apparent microscopically. There were no remarkable findings observed during the enhanced histopathology review of the mandibular lymph node, mesenteric lymph node, GALT, and bone marrow. A small number of incidental background microscopic changes were observed, including but not limited to ectopic parathyroid gland; epithelial hyperplasia and decreased thymic lymphocyte cellularity; lymphoma in the spleen, thymus, and bone marrow; mandibular and mesenteric lymph node sinus erythrocytosis; and mesenteric lymph node erythrophagocytosis.

A variety of incidental microscopic changes occurring equally in control and high dose animals or sporadically across groups included the following: chronic progressive nephropathy, mixed cell infiltrates in the liver, cardiomyopathy, pulmonary alveolar histiocytosis, accessory capsule nodule and focal hypertrophy and/or hyperplasia in the cortex of the adrenal gland, C-cell hyperplasia in the thyroid gland, fibrous osteodystrophy in the femur and sternum, inflammation in various tissues, and cystic endometrial hyperplasia and endometrial squamous metaplasia in the uterus. A small number of incidental background neoplastic lesions were observed sporadically across groups, including lymphoma, benign pilomatricoma in the skin, pituitary gland pars distalis adenoma, thyroid gland C-cell adenoma, uterus endometrial stromal polyp, and mammary gland fibroadenoma, adenoma, and adenocarcinoma.

### 3.2. 24-Month carcinogenicity cohort

#### 3.2.1. Survival

Survival in males and females in the gardenia blue-exposed groups was similar to their respective concurrent control groups (Supplemental Tables 7 and 8). The terminal necropsy for male rats in the carcinogenicity cohort was performed 26 days before the originally scheduled termination due to early mortality in the control group after control group male survival fell below 25%. Terminal necropsy for the female animals in these groups occurred as planned following 24 months of exposure.

#### 3.2.2. Clinical observations

Males and females exposed to gardenia blue at test concentrations of 0.5% or greater had blue to black feces generally throughout the treatment period, similar to the changes observed at the 12-month timepoint. These changes in fecal color were attributed to the dark blue color of the test article and are not adverse. Other normally expected clinical observations for rats of this strain and age were noted across dose groups and sexes and were unrelated to gardenia blue exposure.

#### 3.2.3. Body weight and food consumption

There were no statistically significant differences in body weight gain or final body weight of rats of either sex nor differences in food consumption for treated versus control groups (Supplemental Tables 7 and 8). For animals that survived to the scheduled final necropsy, exposure to 0.5%, 2.5%, or 5.0% gardenia blue in feed resulted in average doses of 262.7 and 357.6 mg gardenia blue/kg/day, 1185.2 and 1564.1 mg gardenia blue/kg/day, and 2175.3 and 3075.4 mg gardenia blue/kg/day in male and female rats, respectively.

#### 3.2.4. Clinical pathology

Aside from statistically significant decreasing trends in unstained leukocytes in treated females, mean hematologic measurements as well as coagulation parameters were all within normal ranges. Statistically significant clinical chemistry alterations in treated females were limited to a decreasing linear trend with dose for mean alkaline phosphatase without any significant pairwise differences relative to the control. Clinical pathology measurements were all within normal physiologic levels.

#### 3.2.5. Urinalysis

There were no toxicologically or physiologically significant alterations in urinalysis parameters for either sex at any dose level. The urine was not blue at any stage of the study.

#### 3.2.6. Gross necropsy

Gross pathology findings related to gardenia blue administration were similar to the ones observed at the 12-month timepoint, and consisted of blue discoloration of the stomach, duodenum, jejunum, ileum, cecum, and colon, as well as dark or blue discoloration of the kidneys and mesenteric lymph nodes (Supplemental Tables 7 and 8). At least one tissue was affected in one or more males and females in each of the 0.5%, 2.5%, and 5.0% carcinogenicity dose groups. Other gross findings were scattered sporadically across groups and considered to be background findings with no association to the test article.

#### 3.2.7. Organ weights

There were statistically significant dose-dependent linear trends in higher mean absolute and relative heart weights in the male rats but without statistically significant pairwise differences in dose groups versus control. There was a significant dose-dependent linear increase in mean absolute kidney weight in females with a statistically significant increase in the 5.0% group. In the absence of histopathological changes, these heart and kidney weight effects were considered sporadic and not toxicologically relevant.

#### 3.2.8. Pathology

No microscopic findings, including enhanced histopathology of the lymphoid system, associated with gardenia blue exposure versus controls were present. The blue or dark discoloration of several tissues observed grossly was not apparent microscopically in any of the tissue, probably because of loss of the pigment during pathology tissue processing. Spontaneous, age-associated neoplastic and non-neoplastic background lesions occurred equally in treated and control groups. The histopathological findings are summarized in Supplemental Table 9.

Chronic progressive nephropathy (CPN) was recorded in kidneys of almost all males and females in the control and three dose groups. CPN severity ranged from minimal to marked and was notably increased in males versus females. The CPN in a large percentage of the males in this study was severe enough to have been considered end-stage kidney disease and was likely responsible for the higher number of early deaths in males versus females.

## 4. Discussion

Here we present the results of a GLP-compliant 2-year carcinogenicity study with a 12-month toxicity interim necropsy in SD rats. The animals were exposed to gardenia blue at dose levels of 0.0% (control), 0.5%, 2.5%, or 5.0% (corresponding to 0.0, 262.7, 1185.2, and 2175.3 mg gardenia blue/kg/day in males and 0.0, 357.6, 1564.1, and 3075.4 mg gardenia blue/kg/day in females, respectively). Overall, no signs of toxicity or carcinogenicity were observed in animals exposed to gardenia blue after treatment for 12- or 24-months.

Our results are consistent with two previous studies; a two-year carcinogenicity study performed in Fischer 344 (F344) rats at dietary doses of 0, 2.5% and 5% gardenia blue (Imazawa et al., 2000) and a 12-month toxicity study in SD rats at 0, 0.1%, 0.5%, 1.5%, 3.0% and 5% gardenia blue commencing with *in utero* exposure (Streicker, 2020). In the former study, no adverse changes were observed in clinical signs, mortality, hematology parameters, or histopathology. In the later study there was good survival with no consistent treatment-related changes in parental animals, including histopathology. There were no adverse findings in F1 offspring for all study parameters (hematology, clinical

chemistry, enhanced evaluation of the lymphoid system, and T-cell antibody response) including tissue histopathology at the 3- or 6-month interim necropsy or 12-month terminal necropsy. Treatment-related blue discoloration of the intestinal contents, mesenteric lymph nodes, and darkening of the kidneys was present at necropsy in the Streicker (2020) study as well as in the present 2-year study without associated histopathology in either study, and this tissue coloration was considered to be non-adverse. Prior reports of liver toxicity following exposure to gardenia blue (Cui et al., 2017; Li et al., 2019a, 2019b; Xu et al., 2018) were not observed in the Imazawa et al. (2000) study or the present study.

Against the background of expected age-associated histopathological changes in the present study, there were no remarkable or consistent clinical or histopathological changes attributed to gardenia blue exposure based on sampling at multiple timepoints. The grossly evident blue discoloration of intestinal contents and feces is attributed to ingested gardenia blue in the formulated diet. With an average molecular weight of 15,600 Da, absorption of gardenia blue polymer would not be expected. However, blue/dark discoloration of mesenteric lymph nodes and kidneys in the present study indicates that some lower molecular weight component(s) of gardenia blue entered the systemic circulation via the lymphatics with excretion via the kidneys. This is supported by a recently completed ADME study in which 0.25% and 0.35% radioactivity was recovered in urine of male and female rats, respectively, after gavage administration of 1000 mg/kg of radiolabeled gardenia blue (Fennell, 2021). 89–97% of the administered dose was eliminated in the feces and 0.25% in bile. Chromatography of feces suggests gardenia blue remains unchanged. In the ADME study the highest measured tissue recovery was in the kidneys accounting for 0.01–0.03% of the administered dose. Mesenteric lymph nodes were too small to permit accurate analysis. Thus, it is apparent that a small amount of low molecular weight gardenia blue is systemically absorbed at the high doses used in the present study but without any associated histopathological tissue changes, including mesenteric lymph nodes and kidneys.

To evaluate the reversibility of grossly observed blue/dark discoloration of the kidneys, we performed an additional study using the same lot of gardenia blue and using the same diet with the study carried out in the same laboratory. Male SD rats were provided gardenia blue diet at dose levels of 0.0%, 0.5% or 5.0% for 45 days at which time kidneys were grossly darkened in gardenia blue treated rats. Subsequent gross evaluation following administration of control diet confirmed return to normal kidney color in the 0.5% treated rats at day 103 and decreased kidney darkening from marked to minimal in the 5% treated rats at day 166 (Creamer-Hente, 2020). Based on this study we believe that kidney discoloration is reversible following discontinuation of exposure to gardenia blue. The reversibility of renal color change along with the absence of any related histopathology support our conclusion that this discoloration is not adverse. It should be noted that gardenia blue has been on the market in Japan and other Asian countries for several years without evidence of blue feces or urine.

In conclusion, gardenia blue was not associated with any adverse effect when administered to SD rats for up to 2 years with the NOAEL for dietary gardenia blue exposure was 5.0% (2175.3 mg/kg/day in male rats and 3075.4 mg/kg/day in female rats).

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## CRediT authorship contribution statement

**Robert Maronpot:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Yuval Ramot:** Writing – original draft. **Abraham Nyska:** Conceptualization, Methodology, Writing – review & editing, Formal peer review. **Christopher Sproul:** Formal analysis, Methodology, Writing – review & editing. **Rebecca Moore:** Formal analysis, Methodology, Writing – review & editing. **Mihoko Koyanagi:** Writing – review & editing, Funding acquisition. **Shuichi Chiba:** Writing – review & editing, Funding acquisition. **Masayuki Nishino:** Writing – review & editing, Funding acquisition. **Shim-mo Hayashi:** Conceptualization, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mihoko Koyanagi, Shuichi Chiba, and Masayuki Nishino are employees of San-Ei Gen F.F.I., Inc., Osaka, Japan, and Robert Maronpot and Abraham Nyska are consultants for San-Ei Gen F.F.I., Inc., Osaka, Japan.

## Data availability

The authors are unable or have chosen not to specify which data has been used.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.113734.

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