Original Article

Reproductive and Neurobehavioral Effects of Brilliant Blue FCF in Mice

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Brilliant blue FCF of food color was given in the diets of mice at levels of 0% (control), 0.08, 0.24, and 0.72% from 5 weeks of age in the F_0 generation and continuing to 11 weeks of age in the F_1 generation and selected reproductive and neurobehavioral parameters were measured. Mice were mated at 9 weeks of age and dams were delivered offspring at 12 weeks of age. Offspring were weaned at 4 weeks of age. Regarding exploratory behavior at 8 weeks of age in the F_0 generation, movement time (sec) displayed a significant tendency to be increased and the average time of rearing (sec) displayed a significant tendency to be decreased in females in the treatment groups in a trend test ($p = 0.019$ and 0.027, respectively). In the F_1 generation, the development of surface righting at postnatal day 4 was delayed significantly in the high-dose group (0.72%) in male and female offspring, and those effects were significantly related to dose in a trend test ($p < 0.01$ for both). Regarding exploratory behavior at 8 weeks of age in the F_1 generation, the number of horizontal activities exhibited a significant tendency to be decreased in females in the treatment groups in a trend test ($p = 0.015$). Regarding spontaneous behavior, average time of movement (sec) was significantly accelerated in females in the high-dose group. The dose levels of brilliant blue FCF used in the present study produced a few significant effects on neurobehavioral parameters in multiple generations in mice. *Birth Defects Res (Part B)* 95:395–409, 2012. © 2012 Wiley Periodicals, Inc.

Key words: ***behavioral development; brilliant blue FCF; exploratory behavior; mice; reproductive toxicity; spontaneous behavior***

INTRODUCTION

Brilliant blue FCF (also known as FD & C Blue No. 1, C.I. No. 42090, and Food Blue No. 1) is principally the disodium 2-[bis[4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl) amino]phenyl] methylio] benzenesulfonate. Brilliant blue FCF is a purplish red colored, water-soluble powder widely used in food products, drugs, cosmetics, and pharmaceuticals. The estimated amounts of brilliant blue FCF manufactured in 1996 were approximately 7.28 tons in Japan and 190.35 tons in the United States (Ishimitsu et al., 1998). The acceptable daily intake (ADI) of human has been set at 12.5 mg/kg body weight by the World Health Organization (WHO; JECFA, 1996).

In toxicological studies of brilliant blue FCF, Mannell et al. (1962) reported that brilliant blue FCF had no adverse effects on growth at diet levels of 0.03–3.0% in a chronic toxicity study (75 weeks) of rats. Mannell and Grice (1964) found that brilliant blue FCF produced no noticeable local and systemic effects in a subcutaneous chronic toxicity study of rats. Hansen et al. (1966) reported that brilliant blue FCF produced no effects on survival, hematology, or organ weight at diet levels of 0.5–5.0% in a chronic toxicity study (2 years) of rats. They also reported that brilliant blue FCF induced no clinical signs, gross lesions, or microscopic pathologies at diet levels of 1.0–2.0% in a chronic toxicity study (1 year) of dogs. Borzelleca et al. (1990) found that the no observed adverse effect levels (NOAELs) of brilliant blue FCF in rats were 2.0% for males and 1.0% for females (1072 and 631 mg/kg/day, respectively) in a multigeneration study that included chronic treatment of offspring (2 years). They also reported that NOAEL of brilliant blue FCF in mice was 5.0% (7354 and 8966 mg/kg/day for males and females, respectively) in a chronic toxicity study (104 weeks).

However, no published studies reported the reproductive and developmental toxicity of brilliant blue FCF. In addition, no published studies discussed the neurobehavioral toxicity of brilliant blue FCF. Therefore, the present study was designed to evaluate mating performance of F_0 animals and on the neurobehavioral functioning of the offspring of brilliant blue FCF in two generations of mice. In our institute, mice are typically used in reproductive and neurobehavioral toxicity studies to conserve resources (e.g., chemicals, diets, and space), and background data for mice are sufficient for evaluating

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neurobehavioral effects (Tanaka, 2004, 2010). The design of the present study was based on the guidelines issued by ICH (2005) and OECD (2007) adapted for mice.

MATERIALS AND METHODS

Materials

Brilliant blue FCF was obtained from Tokyo Kasei (lot no. ZSCBA, Tokyo, Japan). The purity of the chemical was >85.0% (w/w). The brilliant blue FCF products used in this study are used for food coloring as indicated by food sanitation law in Japan.

Animals and Maintenance

Male and female mice (Crlj:CD1, 4 weeks of age) were purchased from Charles River Japan (Kanagawa, Japan). They were housed individually in polycarbonate solid-floored cages with wood flakes and kept on a 12-hr light/12-hr dark cycle in a temperature-controlled room maintained at 25 ± 1°C with a relative humidity of 50 ± 5%. They were given control or experimental diets and water ad libitum.

Experimental Design

Brilliant blue FCF was administered in the diet to 80 mice (4 groups, 10/sex/group) at dietary levels of 0, 0.08, 0.24, and 0.72% from 5 weeks of age in the F_0 generation to 11 weeks of age in the F_1 generation. The control group was given the basal diets (Nihon Clea, CE-2) for the corresponding period. Mice were assigned to the groups by the stratified randomization method. The experimental diets were prepared bimonthly in our laboratory. After mixing brilliant blue FCF with the powdered diet, pellets were formed and fed to the mice. The homogeneity of the test compound was ensured by the preparation procedures in our laboratory. The individual food intake of mice was measured during five periods: preconception of the F_0 generation (from 5 weeks of age to mating), mating (5 days, males and females), gestation (14 days), lactation (from birth to weaning), and F_1 generation (4–11 weeks of age). Individual food intake (gm/kg bw/day) was calculated as follows: (the difference in feed weight between the previous and present weeks, excluding the weight of the fallen lumps in the cage)/(average body weight between the previous and present weeks)/days.

Reproductive Procedures

The animals from the F_0 generation were 5 weeks of age at the start of the study. The animals were weighed individually on experimental days 0, 3, 7, 14, 21, and 28 during preconception. At 9 weeks of age (day 30), each female was mated with 1 male from the same treatment group for a period of 5 days. The males were weighed individually on day 30 and 35, and females were individually weighed during mating (every day on weekdays). The females were examined for mating by appearance of the vaginal plug twice a day (morning and evening) on weekdays. The males were separated from the females after 5 days, and euthanized and autopsied after mating. The females were allowed to carry their litters to term, deliver, and rear all of their offspring. The dams were weighed during gestation (every day on weekdays) and lactation (once a week). The dams were examined for delivery at three times a day (morning, early afternoon and evening).

In the F_1 generation, the litter size, litter weight, and sex ratio (male/female) were measured on postnatal day (PND) 0 (at birth). The offspring were weighed individually on PNDs 0, 4, 7, 14, and 21 during lactation. The survival indices were calculated as (live offspring at each period)/(live and dead offspring at birth) × 100 (%), excluding litters from dead dams at each period. The offspring were weaned when they were 4 weeks of age, and one male and one female were randomly allocated to continue treatment from each litter. The rest of offspring were euthanized. The dams were euthanized and autopsied after weaning. The animals were weighed every week from 4 to 11 weeks of age after weaning. The animals were euthanized and autopsied at 12 weeks of age.

Neurobehavioral Procedures

The functional and behavioral developmental parameters were measured with stopwatch and scored by one investigator for all individual offspring during lactation in the F_1 generation (Tanaka et al., 1992) and analyzed as score frequencies (Tanaka, 1995). The variables measured were as follows:

(1) *Surface righting on PNDs 4 and 7* (Fox, 1965; Pantaleoni et al., 1988). Each offspring was placed on its back on a smooth surface and the time required to right itself to a position where all four limbs touched the surface was recorded. The scoring for successful righting was as follows: 0 = more than 2 sec; 1 = more than 1 sec but within 2 sec; and 2 = righting within 1 sec.
(2) *Inclined plane test on PNDs 4 and 7* (Fox, 1965; Altman and Sudarshan, 1975; Pantaleoni et al., 1988, Motz and Alberts, 2005). Each offspring was placed in a head-down position on a 30° inclined plane and the time required to reorient to a head-up position was recorded. The plane was made of plywood covered with sandpaper (fine grade). The following scoring was employed: 0 = no response within 60 sec; 1 = response within 60 sec; and 2 = response within 30 sec.
(3) *Cliff avoidance on PND 7* (Fox, 1965; Altman and Sudarshan, 1975; Pantaleoni et al., 1988). Each offspring was placed onto a platform elevated 10 cm above a tabletop. The forelimbs and snout of the animals were positioned so that the edge of the platform passed just behind an imaginary line drawn between the eye orbits. The following scoring was employed: 0 = no response within 20 sec; 1 = avoided backwards within 20 sec, and 2 = avoiding with turn.
(4) *Swimming behavior on PNDs 7 and 14* (Schapiro et al., 1970; Vorhees et al., 1979; Vorhees, 1986; Pantaleoni et al., 1988). Each offspring was placed into a tank of water maintained at 23 ± 1°C and swimming behavior was scored for direction (1 = floating; 2 = circling; and 3 = straight) and head angle (1 = unable to hold head up; 2 = nose and top of head out of water; 3 = ears half out of water; and 4 = ears out of water). Limb movement was rated as 1 (all four limbs used) or 2 (only hindlimbs used).
(5) *Olfactory orientation on PND 14* (Gregory and Pfaff, 1971; Altman and Sudarshan, 1975; Barlow et al.,

1978; Meyer and Hansen, 1980). Each offspring was placed in the arm of an apparatus consisting of two compartments connected by the arm. One compartment was covered with home wood flakes (from their cages), and the other was covered with fresh wood flakes. The time required to enter the compartment with the home wood flakes was recorded. Olfactory orientation was scored for time required (0 = no response within 90 sec; 1 = entered the home wood flakes compartment within 90 sec; 2 = entered within 60 sec; and 3 = entered within 30 sec) and for route (0 = not entered the home wood flakes compartment; 1 = entered the home wood flakes compartment *via* the fresh wood flakes compartment; and 2 = entered the home wood flakes compartment directly).

(6) *Exploratory behavior*. The exploratory behavior of mice was measured using the animal movement analysis system SCANET CV-40 (Melquest, Toyama, Japan) in the distance (DT) mode at 8 weeks of age in the F_0 generation and at 3 and 8 weeks of age in the F_1 generation. The system consisted of a rectangular cage ($300 \times 202 \times 205$ mm^3) made of acrylate resins with two crossing sensor frames of 72 units of detectors of near-infrared photosensors for measuring spontaneous motor activity (Mikami et al., 2002). The behavioral parameters were recorded for 10 min for all mice at 8 weeks of age in the F_0 and F_1 generations and for 1 male and 1 female selected randomly from each litter at 3 weeks of age in the F_1 generation. The possibility that used the same animal at 3 and 8 week of age in the F_1 generation was approximately 5.6 and 3.9% in males and females, respectively. The measurement parameters in the DT mode were as follows: total distance (cm), number of horizontal activities, movement time (sec), number of rearing events, rearing time (sec), average time of movement (sec), average speed (cm/sec), average time of rearing (sec), and number of defecation.

(7) *Maze learning*. At the previous day of trials, each mouse performed premaze trials in a separate long straight swimming channel to acclimate animals to swimming and learning how to escape. Each mouse performed 1 trial per day for 3 days in a Biel-type multiple-T water maze adapted for mice at 7 weeks of age in the F_1 generation (Biel, 1940; Kitatani et al., 1988). The apparatus using the present study was an original type maze (Type A). The water temperature was maintained at $20 \pm 1^{\circ}$C. The time required and number of errors were measured from start to finish for a maximum of 120 sec. The errors were defined as mistake at choice points including moving backward of a course. If the time required was >120 sec, it was recorded as 120 sec (Kitatani et al., 1988), and the animal reached the 120 sec was guided out goal.

(8) *Spontaneous behavior*. The spontaneous behavior of mice was measured using SCANET CV-40 in the DT mode from 9 to 10 weeks of age in the F_1 generation. The behavioral parameters were measured for all mice for 120 min at an interval of 10 min after a 10-min latency period. The measurement parameters were the same as those for exploratory behavior, except for the number of defecation.

Statistical Analysis

Food intake, litter size, litter weight, and body weight were assessed using Bonferroni's multiple comparison test after an ANOVA. Sex ratio, survival, and behavioral developmental data were assessed using the χ^2 test or with Fisher's exact test of frequency analysis. Exploratory behavior data were assessed using Bonferroni's multiple comparison test after ANOVA. Regarding the spontaneous behavior data, the longitudinal pattern was assessed using profile analysis (test for equality of mean vectors, test for equality of covariance matrices and the parallelism hypothesis test; Fujikoshi et al., 2008; Fujikoshi, 2009), and variables at each time point were assessed using the Bonferroni's multiple comparison test within each treatment group. Multiple-T water maze performance data were assessed using the paired *t*-test for trials and assessed using Bonferroni's multiple comparison test within each treatment group after mixed ANOVA model for the longitudinal analysis. Dose–response effects were assessed using the Jonckheere test for ordered alternatives or the cumulative χ^2 test (multi) for frequency data.

Guidelines

The present study was conducted in accordance with the guidelines set by the National Research Council (2010) and the Science Council of Japan (2006). Animal experiments conformed with the following Japanese laws and relevant regulations: "Act on Welfare and Management of Animals" (Act No. 105 of October 1, 1973, revised on June 22, 2005); Notice No. 88 of April 28, 2006 of the Ministry of the Environment of Japan, "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain"; and Notification of June 1, 2006 of the Ministry of Health, Labour and Welfare of Japan, "Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Research Institutions under the jurisdiction of the Ministry of Health, Labour and Welfare."

RESULTS

Intake of Food and Chemicals

There was no significant difference ($p < 0.05$) related to the effect of brilliant blue FCF on the average food intake during any period, except for that in F_1 generation males (Table 1). In the F_1 generation, the average food intake of male mice decreased slightly in the intermediate-dose group. However, the intake of brilliant blue FCF was in accord with its concentrations in the diet during each period (Table 1).

F_0 Generation

Brilliant blue FCF treatment had no significant effects on the average body weight of male and female mice during preconception or mating, and the average body weight of dams displayed no significant effect during gestation or lactation except for the second week of lactation (Table 2). At second week of lactation, the average body weight of dams was significantly increased in the high-dose group. Regarding exploratory behavior at 8 weeks of age, number of horizontal activities was significantly

Table 1
Average Daily Food and Chemical Intake of Mice Administered Brilliant Blue FCF in a Two-Generation Toxicity Study

	Dose level (%)			
	0	0.08	0.24	0.72
Food intake (gm/kg/day)				
F_0 generation				
Preconception				
Male	159.5 ± 7.87	152.8 ± 9.64	151.9 ± 11.13	159.0 ± 8.50
Female	177.0 ± 20.33	169.7 ± 12.26	168.5 ± 22.84	172.4 ± 13.33
Mating	135.7 ± 12.23	139.0 ± 15.76	144.5 ± 11.37	143.4 ± 14.54
Gestation	160.9 ± 17.66	152.5 ± 17.02	146.3 ± 13.07	154.0 ± 7.70
Lactation	481.9 ± 117.41	508.6 ± 70.35	536.2 ± 66.84	535.5 ± 59.86
F_1 generation				
Male	162.9 ± 13.15	152.9 ± 5.62	148.7 ± 9.93*	161.8 ± 8.27
Female	175.9 ± 21.55	166.1 ± 13.30	178.0 ± 23.90	154.2 ± 9.31
Chemical intake (mg/kg/day)				
F_0 generation				
Preconception				
Male	–	122.3 ± 7.71	364.6 ± 26.70	1144.9 ± 61.21
Female	–	135.7 ± 9.80	404.4 ± 54.82	1241.2 ± 95.95
Mating	–	111.2 ± 12.60	346.7 ± 27.29	1032.1 ± 104.70
Gestation	–	122.0 ± 13.62	351.2 ± 31.36	1109.0 ± 55.46
Lactation	–	406.8 ± 56.28	1286.8 ± 160.41	3855.5 ± 430.98
F_1 generation				
Male	–	122.3 ± 4.50	356.9 ± 23.53	1110.2 ± 67.05
Female	–	132.9 ± 10.64	427.2 ± 57.36	1297.6 ± 191.22

Each value represents daily intake during each period (mean ± SD).
Significantly different from controls: $^{*}p < 0.05$.

Table 2
Summary of Average Body Weight (gm) of F_0 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
Day 0	26.04 ± 1.354	25.85 ± 1.468	26.05 ± 1.443	26.08 ± 1.668
Day 3	27.59 ± 1.318	27.41 ± 1.513	27.82 ± 1.479	27.74 ± 1.654
Day 7	29.87 ± 1.657	30.06 ± 1.559	30.26 ± 1.196	30.22 ± 1.924
Day 14	32.61 ± 2.374	33.25 ± 2.067	33.15 ± 1.257	33.29 ± 2.118
Day 21	34.38 ± 2.495	35.01 ± 2.170	34.69 ± 1.650	34.71 ± 2.200
Day 28	35.90 ± 2.545	35.88 ± 2.571	36.02 ± 1.808	36.06 ± 2.567
Day 30	35.53 ± 2.632	36.17 ± 2.678	36.10 ± 1.648	36.25 ± 2.482
Day 35	34.42 ± 2.232	34.85 ± 2.184	34.99 ± 1.519	35.00 ± 2.234
Female				
Day 0	23.84 ± 1.513	23.65 ± 1.167	23.71 ± 1.260	23.78 ± 1.514
Day 3	24.40 ± 1.471	24.33 ± 0.783	23.96 ± 1.266	24.45 ± 1.337
Day 7	25.99 ± 1.653	25.85 ± 0.982	25.58 ± 1.789	25.73 ± 0.950
Day 14	27.81 ± 1.746	27.43 ± 1.268	26.84 ± 1.434	27.12 ± 1.033
Day 21	29.10 ± 2.336	28.79 ± 1.701	27.77 ± 1.690	27.89 ± 1.379
Day 28	30.37 ± 2.411	29.15 ± 2.383	29.22 ± 1.827	29.05 ± 1.291
Day 30	30.22 ± 2.084	29.36 ± 2.199	28.99 ± 1.413	28.78 ± 1.044
Day 35	32.12 ± 2.280	31.08 ± 2.057	31.64 ± 2.102	30.68 ± 1.278
Dam				
Predelivery day	64.45 ± 6.143	63.61 ± 6.053	62.28 ± 3.551	62.78 ± 2.989
Postdelivery day	40.44 ± 1.798	40.04 ± 2.817	39.67 ± 1.837	39.80 ± 1.383
First week	47.07 ± 3.378	48.25 ± 2.548	48.59 ± 2.547	46.10 ± 4.767
Second week	48.47 ± 4.260	50.98 ± 2.579	50.10 ± 2.482	53.63 ± 2.619**
Third week	42.93 ± 2.260	41.62 ± 3.274	40.29 ± 2.348	41.64 ± 3.067

Each value represents the mean ± SD.
Significantly different from controls: $^{**}p < 0.01$.

Table 3
Summary of Exploratory Behavior at 8 Weeks of Age of F_0 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
Total distance (cm)	1436.8 ± 129.12	1517.0 ± 110.67	1394.2 ± 125.35	1332.9 ± 117.14
No. of horizontal activity	125.2 ± 3.33	137.3 ± 2.39*	132.8 ± 3.56	129.5 ± 2.02
Movement time (sec)	352.41 ± 14.152	379.58 ± 13.380	365.06 ± 15.044	350.29 ± 12.499
Average speed (cm/sec)	4.013 ± 0.2546	3.958 ± 0.1695	3.764 ± 0.2136	3.746 ± 2.2210
Average time of movement	2.817 ±0.0970	2.779 ± 0.1317	2.761 ± 0.1253	2.706 ± 0.0918
No. of rearing	83.4 ± 9.63	100.5 ± 10.42	89.4 ± 14.40	89.4 ± 7.21
Rearing time (sec)	180.71 ± 20.498	181.60 ± 14.204	179.45 ± 21.562	178.91 ± 13.713
Average time of rearing	2.200 ± 0.1969	1.899 ± 0.1621	2.1311 ± 0.1691	2.030 ± 0.1008
No. of defecation	4.6 ± 0.64	6.7 ± 1.20	6.5 ± 1.15	5.3 ± 1.03
Female				
Total distance (cm)	1256.9 ± 88.34	1296.3 ± 64.34	1435.5 ± 117.01	1532.4 ± 82.65
No. of horizontal activity	125.7 ± 5.48	124.4 ± 3.07	126.2 ± 2.85	135.4 ± 3.10
Movement time (sec)[a]	328.81 ± 16.618	323.42 ± 10.625	340.43 ± 13.580	367.97 ± 9.039
Average speed (cm/sec)	3.797 ± 0.1237	4.003 ± 0.1365	4.160 ± 0.2047	4.150 ± 0.1512
Average time of movement	2.612 ± 0.0587	2.601 ± 0.0652	2.699 ± 0.0975	2.730 ± 0.0891
No. of rearing	79.3 ± 7.32	83.4 ± 7.20	94.5 ± 9.97	102.6 ± 10.17
Rearing time (sec)	162.47 ± 19.874	196.09 ± 19.577	173.80 ± 17.980	182.78 ± 17.551
Average time of rearing[a]	2.032 ± 0.1615	2.343 ± 0.1282	1.869 ± 0.1037	1.799 ± 0.0814
No. of defecation	6.1 ± 0.92	7.3 ± 0.50	5.9 ± 0.98	5.8 ± 0.71

Each value represents the mean ± SE. Significantly different from controls: $^{*}p < 0.05$.
[a]Movement time and average time of rearing in females are significantly dependent on dose in a trend test ($p = 0.0019$ and 0.027, respectively).

increased in the low-dose group in males (Table 3). In females, movement time exhibited a significant tendency to be increased and the average time of rearing exhibited a significant tendency to be decreased in the treatment groups in a trend test (Jonckheere test: $p = 0.019$ and 0.027, respectively). Other measured variables displayed no significant effect of treatment in either sex.

Three females, one female each in the control, intermediate-dose, and high-dose groups, did not become pregnant (Table 4). Abortion (a litter consisting of resorption) was observed in one dam at first week of gestation in the control group (Table 4). One dam in the low-dose group died during the third week of lactation.

F_1 Generation

No significant effect of treatment was observed on litter size, litter weight, or sex ratio at birth (Table 4). In the low-dose group, the average body weight of male offspring was significantly lighter at birth (Table 5). The average body weight of male offspring increased significantly in a dose-dependent manner on PNDs 14 and 21 (Jonckheere test: $p = 0.037$ and 0.026, respectively; Table 5). The average body weight of female offspring increased significantly in a dose-dependent manner on PND 14 (Jonckheere test: $p = 0.018$; Table 5). There was no sex-related difference in average body weight during lactation. One dam killed its litter in the low-dose group during the first week of lactation. The survival indices

Table 4
Summary of Data of Litters at Birth in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose level (%)			
	0	0.08	0.24	0.72
No. of females examined	10	10	10	10
No. of pregnant females	9	10	9	9
No. of litters	8	10	9	9
No. of live offspring	97	131	116	119
No. of dead offspring	1	0	2	1
Average litter size	12.1 ± 3.56	13.1 ± 1.97	12.9 ± 1.96	13.2 ± 0.83
Average litter weight (gm)	19.40 ± 4.519	19.74 ± 2.608	19.94 ± 3.072	21.02 ± 2.336
Sex ratio (male/female)	0.94 (47/50)	1.18 (71/60)	1.42 (68/48)	1.38 (69/50)
Average sex ratio (male, %)	47.4 ± 16.82	53.5 ± 13.81	57.3 ± 13.99	57.7 ± 10.48

Each value represents the mean ± SD.

Table 5
Summary of Average Body Weight (gm) of Offspring during the Lactation Period in F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male offspring				
PND 0	1.65 ± 0.193	1.52 ± 0.147***	1.57 ± 0.133	1.63 ± 0.134
PND 4	3.01 ± 0.478	2.87 ± 0.339	2.93 ± 0.346	3.00 ± 0.268
PND 7	4.43 ± 0.680	4.27 ± 0.445	4.42 ± 0.500	4.42 ± 0.466
PND 14[a]	6.35 ± 1.335	6.38 ± 0.690	6.41 ± 0.615	6.56 ± 0.721
PND 21[a]	10.38 ± 3.752	9.80 ± 2.954	10.91 ± 1.927	10.98 ± 2.251
Female offspring				
PND 0	1.55 ± 0.168	1.49 ± 0.167	1.51 ± 0.141	1.54 ± 0.118
PND 4	2.84 ± 0.538	2.78 ± 0.397	2.83 ± 0.425	2.86 ± 0.255
PND 7	4.26 ± 0.880	4.15 ± 0.630	4.20 ± 0.616	4.27 ± 0.405
PND 14[a]	6.12 ± 1.756	6.21 ± 0.856	6.06 ± 0.784	6.43 ± 0.634*
PND 21	9.86 ± 3.732	10.26 ± 2.921	10.32 ± 2.133	10.59 ± 1.880

Each value represents the mean ± SD. Significantly different from controls: $^{*}p < 0.05$; $^{***}p < 0.001$.
[a]Significantly dependent on dose in a trend test at PND 14 and 21 in male offspring and PND 14 in female offspring ($p = 0.0369$, 0.0259, and 0.0176, respectively).

revealed no significant effect of exposure to brilliant blue FCF during lactation (Table 6).

Regarding the behavioral developmental parameters, surface righting testing revealed an increase in time required for high-dose males to right themselves on PND 4 (Table 7A), and those effects were significantly related to dose in a trend test (cumulative χ^2 test: $p < 0.01$). The time required for animals to reorient themselves in the inclined plane test assessment was significantly shorter in the low-dose males on PND 7 (Table 7A). Surface righting testing revealed an increase in time required for high-dose females to right themselves on PND 4 (Table 7B), and those effects were significantly related to dose in a trend test (cumulative χ^2 test: $p < 0.01$). The development of swimming direction was accelerated significantly in the intermediate-dose group in female offspring on PND 7 (Table 7B). All offspring reached the endpoints of the variables in the swimming behavior testing on PND 14 in both sexes. Other variables measured displayed no consistently significant effect of treatment on either sex during lactation. No significant effect was observed on exploratory behavior at 3 weeks of age in either sex (Table 8).

Table 6
Summary of Number of Offspring and Survival Index (%) during the Lactation Period in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose level (%)			
	0	0.08	0.24	0.72
Male offspring				
PND 0	47 (100.0)	71 (100.0)	68 (100.0)	69 (98.6)
PND 4	46 (97.9)	64 (90.1)	68 (100.0)	68 (98.6)
PND 7	46 (97.9)	64 (90.1)	68 (100.0)	68 (98.6)
PND 14	46 (97.9)	64 (90.1)	68 (100.0)	68 (98.6)
PND 21	44 (93.6)	59 (83.1)	68 (100.0)	68 (98.6)
Female offspring				
PND 0	50 (98.0)	60 (100.0)	48 (96.0)	50 (100.0)
PND 4	50 (100.0)	55 (91.7)	48 (100.0)	50 (100.0)
PND 7	50 (100.0)	54 (90.0)	48 (100.0)	50 (100.0)
PND 14	50 (100.0)	54 (90.0)	48 (100.0)	50 (100.0)
PND 21	47 (94.0)	49 (81.7)	47 (97.9)	50 (100.0)

Each value represents number of offspring: survival index (%) in parentheses. Survival indices are calculated except for litters from dead dams at each period.

The average body weight of male mice displayed no difference ($p < 0.05$) related to the treatment after weaning (Table 9). In females (Table 9), the average body weight exhibited a significant tendency to be decreased in a dose-dependent manner at 10 and 11 weeks of age (Jonckheere test: $p = 0.047$ and 0.023, respectively). Regarding multiple-T water maze performance, the time required showed no significant effects between groups but showed significant effects between trials (mixed ANOVA model: $p = 0.003$), while the interactions between groups and trials were not significant in males. The time required was significantly shortened through trials in the low-dose group of males (Table 10). In females, the time required showed no significant effects between groups but showed significant effects between trials (mixed ANOVA model: $p = 0.002$), and the interactions between groups and trials were significant (mixed ANOVA model: $p = 0.006$). The time required was significantly shortened through trials in the low- and high-dose groups of females (Table 10). The number of errors showed no significant effects between groups and trials in either sex. From the results, there was no significant effect caused by brilliant blue FCF treatment on multiple-T water maze performance in either sex at 7 weeks of age. Regarding exploratory behavior at 8 weeks of age, number of rearing was reduced significantly in the low-dose group ($p = 0.015$) in males (Table 11). In females, the number of horizontal activities displayed a significant tendency to be decreased in the treatment groups (Table 11) in a trend test (Jonckheere test: $p = 0.015$). Other variables measured exhibited no significant effect in either sex.

Table 7A
Summary of Score Frequencies for Behavioral Development during Lactation in F_1 Generation Mice Administered Brilliant Blue FCF in the Diet

	Dose level (%)			
	0	0.08	0.24	0.72
Male offspring				
Surface righting on PND 4[a]				
0	32 (69.6)	52 (81.2)	56 (85.4)	63 (92.7)**
1	10 (21.7)	9 (14.1)	10 (14.7)	3 (4.4)
2	4 (8.7)	3 (4.7)	2 (2.9)	2 (2.9)
Surface righting on PND 7				
0	5 (10.9)	10 (15.6)	5 (7.4)	5 (7.4)
1	8 (17.4)	9 (14.1)	14 (20.6)	8 (11.8)
2	33 (71.7)	45 (70.3)	49 (72.0)	55 (80.8)
Inclined plane test on PND 4				
0	0 (0.0)	2 (3.1)	0 (0.0)	2 (2.9)
1	1 (2.2)	3 (4.7)	0 (0.0)	1 (1.5)
2	45 (97.8)	59 (92.2)	68 (100.0)	68 (95.6)
Inclined plane test on PND 7				
0	2 (4.3)	0 (0.0)**	0 (0.0)	0 (0.0)
1	4 (8.7)	0 (0.0)	1 (1.5)	2 (2.9)
2	40 (87.0)	64 (100.0)	67 (98.5)	66 (97.1)
Cliff avoidance on PND 7				
1	29 (63.0)	44 (68.7)	41 (60.3)	38 (55.9)
2	17 (37.0)	20 (31.3)	27 (39.7)	30 (44.1)
Swimming behavior on PND 7				
Direction				
2	18 (39.1)	27 (42.2)	28 (41.2)	26 (38.2)
3	28 (60.9)	37 (57.8)	40 (58.8)	42 (61.8)
Head angle				
2	20 (43.5)	29 (45.3)	25 (36.8)	27 (39.7)
3	26 (56.5)	35 (54.7)	43 (63.2)	41 (60.3)
Limb movement				
1	46 (100.0)	64 (100.0)	68 (100.0)	68 (100.0)
Olfactory orientation on PND 14				
Route				
0	1 (2.2)	5 (7.8)	2 (2.9)	1 (1.5)
1	4 (8.7)	3 (4.7)	8 (11.8)	10 (14.7)
2	41 (89.1)	56 (87.5)	58 (85.3)	57 (83.8)
Time required				
0	1 (2.2)	5 (7.8)	2 (2.9)	1 (1.5)
1	5 (10.9)	2 (3.1)	1 (1.5)	10 (14.7)
2	6 (13.0)	15 (23.4)	9 (13.2)	6 (8.8)
3	34 (73.9)	42 (65.7)	56 (82.4)	51 (75.0)

Each value represents number of offspring in each score: score frequency (%) in parentheses. Significantly different from control: **$p < 0.01$.
[a]Significantly dependent on dose in a trend test ($p < 0.01$).

Table 7B
Summary of Score Frequencies for Behavioral Development during Lactation in F_1 Generation Mice Administered Brilliant Blue FCF in the Diet

	Dose level (%)			
	0	0.08	0.24	0.72
Female offspring				
Surface righting on PND 4[a]				
0	40 (80.0)	49 (89.1)	41 (85.4)	49 (98.0)*
1	6 (12.0)	6 (10.9)	5 (10.4)	1 (2.0)
2	4 (8.0)	0 (0.0)	2 (4.2)	0 (0.0)
Surface righting on PND 7				
0	7 (14.0)	4 (7.4)	8 (16.7)	6 (12.0)
1	8 (16.0)	15 (27.8)	12 (25.0)	16 (32.0)
2	35 (70.0)	35 (64.8)	28 (58.3)	28 (56.0)
Inclined plane test on PND 4				
0	1 (2.0)	0 (0.0)	3 (6.3)	0 (0.0)
1	0 (0.0)	1 (1.8)	3 (6.3)	1 (2.0)
2	49 (98.0)	54 (98.2)	42 (87.4)	49 (98.0)
Inclined plane test on PND 7				
0	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)
1	3 (6.0)	0 (0.0)	2 (4.2)	0 (0.0)
2	47 (94.0)	53 (98.1)	46 (95.8)	50 (100.0)
Cliff avoidance on PND 7				
1	34 (68.0)	39 (72.2)	31 (64.6)	31 (62.0)
2	16 (32.0)	15 (27.8)	17 (35.4)	19 (38.0)
Swimming behavior on PND 7				
Direction				
2	26 (52.0)	22 (40.7)	14 (29.2)*	21 (42.0)
3	24 (48.0)	32 (59.3)	34 (70.8)	29 (58.0)
Head angle				
2	15 (30.0)	22 (40.7)	19 (39.6)	21 (42.0)
3	35 (70.0)	32 (59.3)	29 (60.4)	29 (58.0)
Limb movement				
1	50 (100.0)	54 (100.0)	48 (100.0)	50 (100.0)
Olfactory orientation on PND 14				
Route				
0	4 (8.0)	2 (3.7)	1 (2.1)	1 (2.0)
1	7 (14.0)	8 (14.8)	12 (25.0)	4 (8.0)
2	39 (78.0)	54 (81.5)	35 (72.9)	45 (90.0)
Time required				
0	4 (8.0)	2 (3.7)	1 (2.1)	1 (2.0)
1	1 (2.0)	2 (3.7)	1 (2.1)	2 (4.0)
2	8 (16.0)	7 (13.0)	5 (10.4)	6 (12.0)
3	37 (74.0)	43 (79.6)	41 (85.4)	41 (82.0)

Each value represents number of offspring in each score: score frequency (%) in parentheses. Significantly different from control: *$p < 0.05$.
[a]Significantly dependent on dose in a trend test ($p < 0.01$).

Regarding spontaneous behavior from 9 to 10 weeks of age in the F_1 generation, there was generally a similar decrease in activity levels over time in all groups in a longitudinal pattern. In males (Table 12A–C), the number of horizontal activities was significantly reduced at 30 min in the low-dose group (Table 12A), and the average time of rearing exceeded 10, 20, and 50 min in the intermediate-dose group (Table 12C). In females (Table 13A–C), the total distance (Table 13A), average speed and average time of movement (Table 13B) were significantly different between the control and high-dose groups (profile analysis: $p = 0.004$, 0.014, and 0.029, respectively). In the high-dose group, one female displayed hyperactive. The effects on spontaneous behavior in females were reevaluated except for the hyperactive female because the data from the hyperactive female prevented the statistical analysis. Excluding the hyperactive female (Table 13A–C), the average time of movement (Table 13B) was significantly different between the control and high-dose groups (profile test: $p = 0.049$).

DISCUSSION

In the present study, brilliant blue FCF exerted a few significant effects on reproductive and neurobehavioral parameters. In the F_1 generation, although the average body weights of male offspring in the low-dose group

Table 8
Summary of Exploratory Behavior at 3 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male offspring				
Total distance (cm)	1303.3 ± 159.04	1093.8 ± 52.91	1303.1 ± 113.22	1144.4 ± 125.52
No. of horizontal activity	109.6 ± 7.48	104.2 ± 6.38	111.1 ± 4.18	104.4 ± 4.20
Movement time (sec)	335.74 ± 20.635	297.10 ± 11.974	324.73 ± 15.963	323.48 ± 20.619
Average speed (cm/sec)	3.802 ± 0.3431	3.703 ± 0.1756	3.952 ± 0.1732	3.483 ± 0.1827
Average time of movement	3.174 ± 0.0970	2.946 ± 0.2396	2.957 ± 0.1987	3.097 ± 0.1567
No. of rearing	92.6 ± 17.44	75.3 ± 12.56	95.8 ± 11.90	89.2 ± 12.63
Rearing time (sec)	61.11 ± 18.449	47.38 ± 11.182	67.16 ± 11.912	49.23 ± 11.199
Average time of rearing	0.596 ± 0.1406	0.530 ± 0.0939	0.705 ± 0.0940	0.506 ± 0.0846
No. of defecation	4.9 ± 0.95	4.9 ± 0.81	4.4 ± 0.78	5.2 ± 0.55
Female offspring				
Total distance (cm)	1539.3 ± 243.18	1217.3 ± 65.89	1105.3 ± 158.11	1306.0 ± 128.70
No. of horizontal activity	107.3 ± 8.67	97.3 ± 5.02	106.6 ± 5.08	106.1 ± 3.37
Movement time (sec)	352.38 ± 27.518	296.59 ± 8.353	295.23 ± 24.596	332.32 ± 17.623
Average speed (cm/sec)	4.228 ± 0.3868	4.098 ± 0.1566	3.611 ± 0.2654	3.876 ± 0.2263
Average time of movement	3.584 ± 0.6505	3.117 ± 0.2098	2.752 ± 0.1697	3.148 ± 0.1853
No. of rearing	86.1 ± 18.81	67.4 ± 9.87	75.2 ± 8.53	81.8 ± 5.70
Rearing time (sec)	70.09 ± 24.13	37.41 ± 8.897	43.27 ± 7.628	38.59 ± 5.943
Av. Time of rearing	0.681 ± 0.2403	0.496 ± 0.0970	0.566 ± 0.0525	0.460 ± 0.0520
No. of defecation	4.1 ± 0.58	4.0 ± 0.42	4.6 ± 0.67	4.9 ± 0.61

Each value represents the mean ± SE.

was decreased significantly at birth, brilliant blue FCF treatment exerted slightly significant effects on the average body weight of male and female offspring throughout lactation. Litter size at birth was similar among the treatment groups. Therefore, it appears that brilliant blue FCF is unlikely to produce any significant effects on offspring weight during lactation. After weaning, it seems that the reduction in food intake of the intermediate-dose group in males was influenced by the reduction during first 2 weeks (4–6 weeks of age).

Regarding behavioral developmental parameters, brilliant blue FCF produced a few statistically significant effects on the measured variables in the treatment groups compared with the control group. In the F_1 generation,

Table 9
Summary of Average Body Weight (gm) after Weaning of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
4 weeks of age	17.96 ± 5.299	18.16 ± 4.291	19.95 ± 2.650	19.04 ± 2.300
5 weeks of age	27.18 ± 3.841	28.14 ± 3.353	28.86 ± 1.653	29.17 ± 1.774
6 weeks of age	32.32 ± 2.952	32.69 ± 2.252	32.61 ± 2.112	32.76 ± 2.436
7 weeks of age	35.00 ± 2.650	35.73 ± 2.635	34.69 ± 2.001	35.42 ± 2.458
8 weeks of age	37.51 ± 3.136	37.02 ± 3.084	35.79 ± 1.871	37.31 ± 3.712
9 weeks of age	39.02 ± 3.207	38.59 ± 3.328	37.04 ± 2.205	38.79 ± 3.466
10 weeks of age	40.21 ± 3.470	39.28 ± 3.458	38.07 ± 2.150	40.03 ± 3.677
11 weeks of age	41.86 ± 4.074	40.67 ± 3.540	39.01 ± 2.603	41.03 ± 3.800
Female				
4 weeks of age	15.67 ± 4.474	17.38 ± 3.682	16.63 ± 1.925	17.29 ± 2.050
5 weeks of age	22.96 ± 4.244	24.12 ± 2.211	23.22 ± 1.728	23.30 ± 1.632
6 weeks of age	25.14 ± 3.027	26.12 ± 1.523	24.53 ± 1.756	24.90 ± 1.274
7 weeks of age	27.14 ± 2.993	28.24 ± 1.602	26.46 ± 2.579	25.71 ± 1.409
8 weeks of age	28.16 ± 3.042	28.81 ± 0.997	27.47 ± 2.295	26.51 ± 1.672
9 weeks of age	29.60 ± 2.537	30.79 ± 1.090	28.54 ± 2.772	28.29 ± 1.757
10 weeks of age[a]	30.82 ± 2.828	30.92 ± 1.123	29.15 ± 3.246	28.54 ± 2.205
11 weeks of age[a]	31.29 ± 2.700	32.13 ± 1.881	30.20 ± 3.592	29.25 ± 1.973

Each value represents the mean ± SD.
[a]Significantly dependent on dose in a trend test at 10 and 11 weeks of age in females ($p = 0.047$ and 0.023, respectively).

Table 10
Summary of Multiple-T Water Maze Performance at 7 Weeks of Age in F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male offspring				
Time required (sec)				
First trial	53.9 ± 10.50	97.2 ± 11.37	74.6 ± 12.04	76.1 ± 14.62
Second trial	58.1 ± 10.47	62.9 ± 12.76**	73.3 ± 10.37	67.2 ± 15.38
Third trial	41.9 ± 7.56	61.0 ± 11.71*	48.4 ± 11.59	46.8 ± 9.90
Number of errors				
First trial	2.6 ± 0.57	3.6 ± 0.50	4.1 ± 0.96	2.9 ± 0.68
Second trial	3.8 ± 0.59	3.3 ± 0.62	4.7 ± 0.67	4.9 ± 1.46
Third trial	3.3 ± 0.62	2.6 ± 0.38	2.3 ± 0.50	3.4 ± 1.12
Female offspring				
Time required (sec)				
First trial	61.1 ± 14.08	88.1 ± 9.31	63.3 ± 10.03	82.9 ± 11.90
Second trial	75.5 ± 15.19	73.8 ± 11.88*	46.4 ± 10.53	51.4 ± 9.41*
Third trial	66.6 ± 12.27	34.0 ± 6.43**	65.9 ± 14.74	35.2 ± 6.11**
Number of errors				
First trial	3.4 ± 0.86	4.4 ± 0.78	4.8 ± 1.21	4.1 ± 0.56
Second trial	5.9 ± 1.59	4.4 ± 2.40	2.9 ± 0.59	2.6 ± 0.41*
Third trial	4.8 ± 1.06	4.9 ± 1.74	5.7 ± 2.20	3.4 ± 0.90

Each value represents the mean ± SE.
Significantly different from first trial in each group: *$p < 0.05$; **$p < 0.01$.

the time required of surface righting, indicative of coordinated movement, was prolonged significantly in the high-dose group in male and female offspring in the early lactation period (PND 4), and those effects were significantly related to dose ($p < 0.01$ for both). Thus, it appears that those effects on behavioral development were caused by brilliant blue FCF treatment because the average body weight, indicative of physical development, was similar among the treatment groups during lactation.

Concerning exploratory behavior, brilliant blue FCF treatment induced significant effects on several variables

Table 11
Summary of Exploratory Behavior at 8 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
Total distance (cm)	1707.0 ± 58.71	1313.4 ± 85.69	1610.1 ± 147.19	1587.6 ± 209.09
No. of horizontal activity	136.6 ± 1.96	131.9 ± 3.47	124.7 ± 2.83	130.0 ± 3.58
Movement time (sec)	379.40 ± 8.708	348.71 ± 13.033	352.02 ± 13.139	362.91 ± 19.020
Average speed (cm/sec)	4.498 ± 0.1108	3.748 ± 0.1786	4.520 ± 0.2861	4.262 ± 0.4050
Average time of movement	2.777 ± 0.0452	2.647 ± 0.0859	2.836 ± 0.1281	2.795 ± 0.1433
No. of rearing	127.4 ± 6.71	87.8 ± 5.40*	106.0 ± 5.83	114.8 ± 12.90
Rearing time (sec)	229.41 ± 13.607	194.22 ± 18.154	236.23 ± 12.823	207.23 ± 21.346
Average time of rearing	1.802 ± 0.0470	2.191 ± 0.1300	2.294 ± 0.1945	1.907 ± 0.1497
No. of defecation	7.1 ± 1.04	5.4 ± 0.50	6.1 ± 0.89	6.3 ± 0.80
Female				
Total distance (cm)	1476.3 ± 87.65	1404.9 ± 62.64	1566.2 ± 109.94	1560.6 ± 141.57
No. of horizontal activity[a]	128.0 ± 3.41	131.6 ± 2.63	124.0 ± 2.83	120.3 ± 2.65
Movement time (sec)	346.50 ± 9.022	338.24 ± 8.943	348.12 ± 9.763	338.82 ± 12.433
Average speed (cm/sec)	4.246 ± 0.1846	4.142 ± 0.1063	4.470 ± 0.1874	4.538 ± 0.2633
Average Time of movement	2.721 ± 0.1020	2.575 ± 0.0795	2.816 ± 0.0895	2.824 ± 0.1143
No. of rearing	103.0 ± 8.33	106.5 ± 6.20	106.3 ± 5.68	110.1 ± 11.59
Rearing time (sec)	207.96 ± 16.634	211.80 ± 15.763	197.32 ± 10.418	200.04 ± 22.049
Average time of rearing	2.045 ± 0.1123	1.985 ± 0.0999	1.869 ± 0.0850	1.836 ± 0.1479
No. of defecation	6.8 ± 0.75	5.9 ± 0.74	7.0 ± 1.01	6.0 ± 0.91

Each value represents the mean ± SE. Significantly different from controls: *$p < 0.05$.
[a]Significantly dependent on dose in a trend test ($p = 0.015$).

Table 12A
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
Total distance (cm)				
10 min	1206.8 ± 113.26	969.5 ± 105.16	1070.7 ± 56.40	1170.4 ± 142.71
20 min	1001.1 ± 124.67	878.5 ± 86.59	982.0 ± 80.92	1173.1 ± 150.48
30 min	1086.9 ± 101.55	746.5 ± 110.51	844.9 ± 75.56	1064.0 ± 154.12
40 min	836.3 ± 181.54	693.6 ± 89.86	841.4 ± 35.04	902.4 ± 137.35
50 min	854.1 ± 172.80	484.6 ± 70.05	627.6 ± 94.61	691.0 ± 105.43
60 min	681.0 ± 170.43	392.4 ± 104.54	483.3 ± 78.43	637.7 ± 79.92
70 min	632.3 ± 163.35	466.9 ± 94.97	396.8 ± 94.18	512.1 ± 118.36
80 min	530.6 ± 141.77	347.0 ± 92.05	377.3 ± 69.79	357.6 ± 86.70
90 min	607.8 ± 140.37	299.0 ± 100.86	290.6 ± 84.39	431.5 ± 87.11
100 min	505.8 ± 138.20	281.5 ± 100.70	241.5 ± 50.45	314.4 ± 101.20
110 min	522.5 ± 140.38	366.8 ± 86.33	285.9 ± 114.37	200.4 ± 61.13
120 min	448.3 ± 127.00	320.1 ± 92.10	138.9 ± 55.22	167.5 ± 50.02
No. of horizontal activity				
10 min	134.8 ± 4.55	119.8 ± 5.94	124.6 ± 2.66	124.7 ± 7.09
20 min	122.6 ± 6.05	117.4 ± 6.88	115.9 ± 2.76	118.2 ± 6.28
30 min	133.5 ± 4.82	102.6 ± 9.72*	111.0 ± 4.43	120.7 ± 5.77
40 min	108.0 ± 15.72	100.4 ± 9.21	106.9 ± 6.72	104.9 ± 8.82
50 min	110.4 ± 18.90	84.0 ± 11.49	92.2 ± 9.64	97.2 ± 9.58
60 min	90.0 ± 18.91	68.4 ± 15.91	76.2 ± 9.90	92.1 ± 7.47
70 min	86.6 ± 19.09	80.6 ± 14.13	66.7 ± 13.13	76.3 ± 11.47
80 min	78.6 ± 16.98	62.9 ± 14.20	64.4 ± 11.42	61.6 ± 12.41
90 min	87.5 ± 15.89	51.4 ± 14.97	58.0 ± 13.56	66.2 ± 9.57
100 min	73.4 ± 17.70	48.7 ± 15.88	47.0 ± 9.37	53.2 ± 12.22
110 min	74.8 ± 18.18	57.9 ± 14.22	37.6 ± 13.94	39.1 ± 10.44
120 min	76.8 ± 17.23	52.9 ± 13.98	26.1 ± 9.45	32.4 ± 9.47
Movement time (sec)				
10 min	321.15 ± 14.846	282.98 ± 21.853	296.07 ± 11.603	298.99 ± 16.505
20 min	277.71 ± 20.260	269.96 ± 21.812	282.57 ± 12.077	290.23 ± 19.150
30 min	304.40 ± 17.382	236.23 ± 28.875	257.83 ± 16.661	285.66 ± 22.947
40 min	241.69 ± 40.231	213.77 ± 19.919	251.66 ± 12.872	250.84 ± 26.043
50 min	244.96 ± 43.625	172.92 ± 24.076	203.87 ± 26.977	211.26 ± 24.428
60 min	201.54 ± 44.883	139.10 ± 34.289	159.89 ± 22.061	199.28 ± 17.071
70 min	192.31 ± 44.160	158.20 ± 29.259	144.84 ± 30.479	158.34 ± 26.209
80 min	166.20 ± 39.079	122.51 ± 28.42	131.38 ± 22.713	121.13 ± 25.574
90 min	187.68 ± 38.662	103.32 ± 31.742	114.02 ± 28.789	138.00 ± 22.489
100 min	154.89 ± 37.457	95.24 ± 31.560	91.76 ± 18.414	104.50 ± 25.706
110 min	156.14 ± 39.343	112.63 ± 26.449	76.83 ± 31.861	75.68 ± 21.25
120 min	148.28 ± 34.761	103.29 ± 27.670	52.29 ± 19.091	60.93 ± 17.697

Each value represents the mean ± SE. Significantly different from controls: $^{*}p < 0.05$.

in adult females in the F_0 generation. The results of exploratory behavior suggested that brilliant blue FCF treatment produced slightly active effects in adult females because movement time was slightly prolonged and average time of rearing was slightly shortened in the treatment groups of females. In addition, the number of horizontal activities displayed a significant tendency to be reduced in the treatment groups in adult females in the F_1 generation. The result of exploratory behavior suggested that brilliant blue FCF treatment produced slightly active effects in adult females because the number of horizontal activities was slightly reduced but total distance and movement time were similar in the treatment groups of females. Thus, it appears that brilliant blue FCF influenced exploratory behavior in adult female mice in the F_0 and F_1 generations. The results of this study suggest that brilliant blue FCF treatment may produce those effects on exploratory behavior in females throughout generations. Nevertheless, brilliant blue FCF treatment exerted inconsistent effects on exploratory behavior in adult males. Thus, brilliant blue FCF may have different effects on exploratory behavior in each sex.

Regarding spontaneous behavior, brilliant blue FCF treatment induced significant effects on several variables in the F_1 generation. Nevertheless, males exhibited inconsistent effects on spontaneous behavior, and the observed effects in females may have been caused by the hyperactive individual. The activity of the hyperactive female showed extremely higher (approximately 10-fold) than background data of control mice. Other females measured simultaneously with the hyperactive individual showed no significant effects on spontaneous behavior. The

Table 12B
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
Average speed (cm/sec)				
10 min	3.703 ± 0.2170	3.354 ± 0.1446	3.611 ± 0.1326	3.832 ± 0.2872
20 min	3.529 ± 0.2499	3.233 ± 0.1148	3.443 ± 0.1895	3.928 ± 0.2957
30 min	3.516 ± 0.2112	3.072 ± 0.1308	3.250 ± 0.1741	3.586 ± 0.2737
40 min	3.121 ± 0.3423	3.169 ± 0.1618	3.362 ± 0.0793	3.491 ± 0.2015
50 min	3.106 ± 0.3265	2.800 ± 0.0653	3.029 ± 0.0926	3.160 ± 0.1616
60 min	2.784 ± 0.3918	2.312 ± 0.3412	2.814 ± 0.2276	3.131 ± 0.1742
70 min	2.711 ± 0.3698	2.676 ± 0.2518	2.226 ± 0.3396	3.055 ± 0.3117
80 min	2.646 ± 0.3658	2.436 ± 0.2492	2.660 ± 0.2978	2.468 ± 0.3168
90 min	2.733 ± 0.3999	2.020 ± 0.3901	2.196 ± 0.3183	2.811 ± 0.3121
100 min	2.436 ± 0.5000	2.220 ± 0.3598	2.492 ± 0.2395	2.303 ± 0.4260
110 min	2.623 ± 0.4801	2.552 ± 0.4893	2.208 ± 0.4089	2.199 ± 0.2545
120 min	2.497 ± 0.3448	2.108 ± 0.4866	1.773 ± 0.4033	2.124 ± 0.3987
Average time of movement				
10 min	2.397 ± 0.1252	2.351 ± 0.1374	2.380 ± 0.0896	2.410 ± 0.0761
20 min	2.252 ± 0.0797	2.278 ± 0.0790	2.437 ± 0.0828	2.453 ± 0.1054
30 min	2.274 ± 0.0901	2.261 ± 0.1044	2.319 ± 0.1061	2.345 ± 0.1228
40 min	2.179 ± 0.0896	2.144 ± 0.0920	2.378 ± 0.0775	2.364 ± 0.1063
50 min	2.149 ± 0.0929	2.031 ± 0.0603	2.181 ± 0.0949	2.138 ± 0.0779
60 min	2.240 ± 0.1483	1.725 ± 0.2268	2.053 ± 0.0815	2.166 ± 0.0773
70 min	2.072 ± 0.1405	1.881 ± 0.0864	1.845 ± 0.2574	2.068 ± 0.1040
80 min	1.945 ± 0.1336	1.975 ± 0.0809	1.990 ± 0.1298	1.818 ± 0.1122
90 min	1.956 ± 0.1571	1.553 ± 0.2424	1.751 ± 0.2365	1.967 ± 0.1147
100 min	1.744 ± 0.2745	1.706 ± 0.1471	1.955 ± 0.1026	1.664 ± 0.2288
110 min	1.776 ± 0.2704	1.573 ± 0.3103	1.616 ± 0.2372	1.806 ± 0.1028
120 min	1.937 ± 0.1142	1.433 ± 0.2936	1.650 ± 0.2272	1.561 ± 0.2311

Each value represents the mean ± SE.

Table 12C
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72
Male				
No. of rearing				
10 min	88.8 ± 12.80	63.6 ± 7.85	64.7 ± 6.15	81.1 ± 11.01
20 min	81.0 ± 11.32	58.6 ± 6.73	56.8 ± 5.25	83.6 ± 8.81
30 min	88.0 ± 13.67	54.1 ± 8.72	52.3 ± 7.24	79.6 ± 11.54
40 min	69.9 ± 18.09	48.4 ± 7.79	55.4 ± 7.49	63.3 ± 10.29
50 min	70.3 ± 16.31	36.4 ± 6.45	50.7 ± 12.44	58.8 ± 9.18
60 min	60.5 ± 17.21	33.2 ± 9.99	39.3 ± 7.16	47.8 ± 7.54
70 min	54.8 ± 16.58	38.7 ± 8.20	28.0 ± 6.28	41.3 ± 8.83
80 min	46.6 ± 13.56	28.3 ± 8.63	28.4 ± 5.29	28.3 ± 6.89
90 min	56.4 ± 14.63	24.3 ± 8.65	19.7 ± 6.09	31.7 ± 5.89
100 min	52.9 ± 16.20	25.3 ± 9.61	17.0 ± 4.16	27.6 ± 8.04
110 min	49.4 ± 13.87	29.2 ± 8.08	14.3 ± 6.64	17.3 ± 6.62
120 min	45.1 ± 15.67	27.7 ± 7.58	11.9 ± 5.52	16.1 ± 5.09
Rearing time (sec)				
10 min	199.09 ± 24.664	160.49 ± 24.419	202.28 ± 14.861	194.69 ± 21.185
20 min	184.78 ± 23.595	172.17 ± 22.985	185.33 ± 12.245	228.13 ± 22.851
30 min	224.06 ± 28.927	154.14 ± 26.930	182.29 ± 20.029	209.37 ± 25.374
40 min	157.80 ± 36.642	133.94 ± 21.978	183.01 ± 20.937	171.13 ± 27.253
50 min	160.40 ± 35.125	116.61 ± 22.010	159.66 ± 26.636	159.80 ± 25.066
60 min	148.15 ± 40.600	111.98 ± 34.680	124.59 ± 21.131	146.36 ± 15.483
70 min	139.70 ± 41.423	128.78 ± 28.311	94.48 ± 21.191	127.11 ± 31.627
80 min	118.24 ± 33.730	86.80 ± 26.015	110.24 ± 4.878	87.71 ± 22.691

Table 12C
Continued

	Dose levels (%)			
	0	0.08	0.24	0.72
90 min	156.05 ± 39.026	71.71 ± 27.119	86.87 ± 29.055	97.48 ± 20.889
100 min	138.46 ± 42.503	74.40 ± 30.453	63.99 ± 16.863	76.92 ± 25.352
110 min	134.66 ± 36.764	97.76 ± 30.921	42.43 ± 18.311	60.81 ± 24.746
120 min	128.73 ± 43.413	85.79 ± 25.329	36.66 ± 18.570	46.61 ± 14.511
Av. Time of rearing				
10 min	2.306 ± 0.1681	2.452 ± 0.1560	3.217 ± 0.1703*	2.551 ± 0.2226
20 min	2.343 ± 0.1165	2.923 ± 0.1487	3.422 ± 0.3135*	2.892 ± 0.3028
30 min	2.654 ± 0.1320	2.763 ± 0.1484	3.795 ± 0.4051	3.070 ± 0.5198
40 min	2.611 ± 0.2703	2.782 ± 0.1504	3.465 ± 0.2944	2.879 ± 0.2395
50 min	2.120 ± 0.3268	3.180 ± 0.2632	3.686 ± 0.3705	2.764 ± 0.2488
60 min	1.902 ± 0.4271	2.790 ± 0.4608	3.303 ± 0.4563	3.672 ± 0.7016
70 min	2.150 ± 0.3379	2.863 ± 0.3801	2.940 ± 0.6663	2.857 ± 0.3335
80 min	2.490 ± 0.4089	2.994 ± 0.4871	3.419 ± 0.5682	2.416 ± 0.4920
90 min	2.121 ± 0.4874	1.734 ± 0.4904	4.398 ± 0.9809	3.179 ± 0.3510
100 min	1.971 ± 0.4309	2.021 ± 0.4489	3.683 ± 0.6423	2.081 ± 0.4802
110 min	2.114 ± 0.4695	2.394 ± 0.5084	2.374 ± 0.5176	2.148 ± 0.6963
120 min	2.266 ± 0.5360	2.001 ± 0.5437	1.508 ± 0.6456	2.284 ± 0.4980

Each value represents the mean ± SE. Significantly different from controls: $^{*}p < 0.05$.

Table 13A
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72 (e.g., hyperactive mouse)
Female				
Total distance (cm)[a]				
10 min	1029.5 ± 154.91	1002.3 ± 96.87	1103.7 ± 81.81	1757.2 ± 722.75 (1045.2 ± 141.19)
20 min	1013.8 ± 141.66	822.9 ± 88.18	1005.7 ± 66.39	1832.7 ± 726.49 (1110.8 ± 92.53)
30 min	883.5 ± 158.74	805.1 ± 81.90	856.5 ± 78.38	1721.0 ± 869.36 (861.6 ± 149.08)
40 min	797.9 ± 138.76	675.9 ± 93.44	807.3 ± 99.21	1713.2 ± 856.78 (860.9 ± 99.53)
50 min	728.7 ± 129.08	623.4 ± 95.45	587.5 ± 67.14	1704.1 ± 995.42 (716.2 ± 138.34)
60 min	698.3 ± 93.28	521.2 ± 68.87	643.9 ± 105.78	1690.1 ± 1006.08 (692.4 ± 146.85)
70 min	691.6 ± 108.98	509.2 ± 118.61	503.1 ± 103.59	1713.4 ± 1123.65 (591.7 ± 74.47)
80 min	720.9 ± 110.21	483.7 ± 120.19	445.1 ± 86.75	1823.7 ± 1307.78 (520.2 ± 120.62)
90 min	667.5 ± 125.12	401.3 ± 91.52	344.1 ± 99.97	1595.8 ± 1238.44 (360.3 ± 97.12)
100 min	482.7 ± 118.68	337.31 ± 89.77	305.6 ± 80.37	1563.8 ± 923.63 (649.4 ± 147.74)
110 min	517.1 ± 142.35	320.1 ± 109.37	218.9 ± 76.39	1448.8 ± 910.60 (541.24 ± 83.70)
120 min	574.5 ± 131.46	293.3 ± 141.73	284.5 ± 85.73	1304.7 ± 971.6 (336.5 ± 92.15)
No. of horizontal activity				
10 min	115.4 ± 8.26	114.5 ± 2.83	120.8 ± 3.08	101.0 ± 9.35 (107.6 ± 7.47)
20 min	111.8 ± 7.00	111.5 ± 6.24	107.7 ± 5.01	102.0 ± 8.53 (109.8 ± 4.03)
30 min	102.1 ± 14.68	109.5 ± 6.15	100.0 ± 4.71	89.4 ± 10.88 (97.4 ± 8.45)
40 min	104.4 ± 11.74	103.6 ± 6.86	99.9 ± 8.65	87.6 ± 10.48 (95.5 ± 7.74)
50 min	94.1 ± 13.23	91.1 ± 10.37	84.8 ± 8.00	75.4 ± 11.33 (82.0 ± 10.48)
60 min	95.3 ± 11.06	82.6 ± 8.03	85.4 ± 7.04	80.8 ± 10.34 (88.3 ± 8.10)
70 min	98.1 ± 11.20	81.9 ± 13.73	76.9 ± 12.29	71.1 ± 9.71 (78.6 ± 6.98)
80 min	95.4 ± 10.71	77.1 ± 14.26	65.6 ± 9.54	66.0 ± 12.56 (73.1 ± 11.73)
90 min	85.4 ± 13.56	64.9 ± 10.86	51.7 ± 13.44	48.3 ± 11.32 (52.1 ± 12.10)
100 min	68.9 ± 12.62	49.9 ± 13.12	52.1 ± 11.56	69.8 ± 13.69 (75.9 ± 13.90)
110 min	75.9 ± 17.35	53.8 ± 16.66	37.4 ± 12.92	69.6 ± 9.85 (74.0 ± 9.97)
120 min	78.0 ± 15.70	41.4 ± 16.75	41.4 ± 12.68	49.0 ± 10.27 (51.6 ± 11.26)
Movement time (sec)				
10 min	272.85 ± 27.663	266.41 ± 17.331	291.56 ± 10.163	297.77 ± 39.726 (263.93 ± 23.592)
20 min	270.93 ± 28.407	244.60 ± 20.150	262.91 ± 11.642	311.97 ± 34.234 (279.53 ± 12.394)
30 min	246.01 ± 38.007	235.85 ± 15.274	236.09 ± 12.591	268.61 ± 45.884 (229.55 ± 27.297)
40 min	231.41 ± 32.571	215.63 ± 19.233	223.83 ± 21.901	267.50 ± 42.581 (228.73 ± 19.952)
50 min	198.49 ± 29.809	190.48 ± 23.820	178.18 ± 17.723	237.00 ± 49.959 (193.44 ± 27.733)

Table 13A
Continued

	Dose levels (%)			
	0	0.08	0.24	0.72 (e.g., hyperactive mouse)
60 min	212.90 ± 25.568	170.20 ± 19.509	184.86 ± 20.596	242.87 ± 50.231 (200.31 ± 30.262)
70 min	206.50 ± 24.073	165.88 ± 32.396	155.73 ± 26.049	216.22 ± 48.400 (170.11 ± 16.683)
80 min	207.50 ± 24.728	153.19 ± 32.413	138.92 ± 21.127	204.06 ± 54.256 (155.91 ± 28.368)
90 min	190.60 ± 32.187	126.00 ± 22.904	107.87 ± 28.046	160.16 ± 57.863 (106.85 ± 25.522)
100 min	149.68 ± 31.307	105.60 ± 27.521	100.62 ± 22.274	220.79 ± 51.587 (180.45 ± 36.461)
110 min	153.04 ± 37.794	107.40 ± 34.169	66.54 ± 23.253	205.09 ± 48.060 (160.90 ± 21.426)
120 min	170.15 ± 35.948	84.16 ± 36.602	82.78 ± 24.182	156.14 ± 54.380 (106.36 ± 24.814)

Each value represents the mean ± SE.
[a]Significantly different between control and high-dose groups in longitudinal analysis ($p = 0.004$).

results suggest that those effects on spontaneous behavior in females were caused by brilliant blue FCF treatment. Excluding the hyperactive individual, females displayed significant effects on the average time of movement in the high-dose group. Therefore, it appears that brilliant blue FCF exerted slightly significant effects on spontaneous behavior only in female mice. It therefore seems that sensitivity for brilliant blue FCF is dependent on sex in mice.

According to the results, the dose levels of brilliant blue FCF used in the present study produced no significant effect on reproduction, but the high-dose level (0.72% in the diet) of brilliant blue FCF produced a few significant effects on behavioral development. Moreover, the dose levels of brilliant blue FCF used in the present study displayed a slight tendency to accelerate exploratory and spontaneous behavior in adult females. Park et al. (2009)

Table 13B
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72 (e.g., hyperactive mouse)
Female				
Average speed (cm/sec)[a]				
10 min	3.673 ± 0.2168	3.711 ± 0.1367	3.746 ± 0.1524	4.868 ± 1.0558 (3.838 ± 0.2622)
20 min	3.648 ± 0.1571	3.571 ± 0.0842	3.803 ± 0.1416	4.984 ± 1.0568 (3.943 ± 0.2069)
30 min	3.230 ± 0.3434	3.366 ± 0.1627	3.575 ± 0.1725	4.825 ± 1.2636 (3.579 ± 0.2390)
40 min	3.157 ± 0.3291	3.039 ± 0.1652	3.525 ± 0.1243	4.929 ± 1.2406 (3.699 ± 0.1844)
50 min	3.349 ± 0.4816	3.163 ± 0.1742	3.250 ± 0.1317	4.880 ± 1.4704 (3.438 ± 0.3310)
60 min	3.217 ± 0.1311	3.000 ± 0.0877	3.316 ± 0.2121	4.663 ± 1.5120 (3.173 ± 0.2931)
70 min	3.166 ± 0.2376	2.905 ± 0.1893	2.877 ± 0.3180	5.064 ± 1.6561 (3.413 ± 0.1571)
80 min	3.363 ± 0.1582	2.842 ± 0.2878	2.876 ± 0.3037	4.994 ± 1.9961 (3.019 ± 0.3287)
90 min	3.028 ± 0.4473	3.008 ± 0.2195	2.800 ± 0.3054	4.567 ± 1.9123 (2.692 ± 0.4243)
100 min	2.757 ± 0.3620	3.045 ± 0.2955	2.420 ± 0.3947	4.522 ± 1.5338 (3.045 ± 0.4698)
110 min	2.622 ± 0.4755	2.125 ± 0.4462	1.888 ± 0.3880	4.516 ± 1.4056 (3.131 ± 0.2746)
120 min	2.831 ± 0.4405	2.246 ± 0.4669	2.662 ± 0.5631	4.161 ± 1.5541 (2.640 ± 0.3642)
Average time of movement[a,b]				
10 min	2.333 ± 0.0910	2.318 ± 0.1117	2.417 ± 0.0724	3.473 ± 1.0503 (2.427 ± 0.1032)
20 min	2.383 ± 0.1311	2.173 ± 0.0851	2.452 ± 0.0780	3.858 ± 1.3067 (2.554 ± 0.1008)
30 min	2.309 ± 0.1241	2.155 ± 0.0705	2.362 ± 0.0683	4.535 ± 2.2291 (2.308 ± 0.1144)
40 min	2.116 ± 0.1341	2.061 ± 0.0617	2.224 ± 0.0844	4.802 ± 2.4095 (2.394 ± 0.0771)
50 min	1.994 ± 0.1317	2.060 ± 0.0482	2.098 ± 0.0730	4.886 ± 2.5730 (2.314 ± 0.1045)
60 min	2.244 ± 0.0778	2.029 ± 0.0602	2.124 ± 0.0970	5.021 ± 2.8483 (2.176 ± 0.1683)
70 min	2.104 ± 0.1007	1.942 ± 0.0904	2.062 ± 0.1197	7.830 ± 5.6706 (2.160 ± 0.0849)
80 min	2.163 ± 0.0762	1.915 ± 0.0992	2.012 ± 0.1405	9.086 ± 7.0484 (2.038 ± 0.1239)
90 min	1.946 ± 0.2882	1.928 ± 0.0680	2.202 ± 0.2589	5.238 ± 3.4217 (1.819 ± 0.1586)
100 min	1.997 ± 0.1643	2.062 ± 0.1510	1.689 ± 0.2189	4.698 ± 2.6622 (2.050 ± 0.3134)
110 min	1.692 ± 0.2577	1.591 ± 0.2548	1.472 ± 0.2026	3.741 ± 1.5874 (2.154 ± 0.0729)
120 min	1.924 ± 0.2905	1.638 ± 0.1674	1.620 ± 0.3306	4.109 ± 1.9700 (2.148 ± 0.2097)

Each value represents the mean ± SE.
[a]Average speed and average time of movement are significantly different between control and high-dose groups in longitudinal analysis ($p = 0.014$ and 0.029, respectively).
[b]Significantly different between control and high-dose groups in longitudinal analysis ($p = 0.049$) excluding hyperactive mouse in high-dose group.

Table 13C
Summary of Spontaneous Behavior at 9–10 Weeks of Age of F_1 Generation Mice in a Two-Generation Toxicity Study of Brilliant Blue FCF Administered to Mice

	Dose levels (%)			
	0	0.08	0.24	0.72 (e.g., hyperactive mouse)
Female				
No. of rearing				
10 min	68.9 ± 9.09	73.4 ± 12.09	74.1 ± 7.35	119.2 ± 44.32 (76.0 ± 11.12)
20 min	77.1 ± 10.57	70.0 ± 10.68	67.0 ± 7.87	106.8 ± 39.13 (68.0 ± 5.97)
30 min	71.3 ± 13.88	63.5 ± 8.86	65.3 ± 10.58	109.4 ± 47.07 (63.4 ± 10.96)
40 min	68.6 ± 11.45	50.1 ± 11.08	64.0 ± 7.82	109.6 ± 45.33 (65.4 ± 11.51)
50 min	64.1 ± 11.42	52.4 ± 11.38	47.1 ± 8.16	100.4 ± 46.38 (55.1 ± 11.16)
60 min	69.4 ± 8.98	43.8 ± 9.26	52.3 ± 8.77	105.6 ± 49.77 (56.9 ± 11.77)
70 min	70.4 ± 14.07	43.5 ± 12.54	45.3 ± 10.37	95.3 ± 45.85 (50.1 ± 8.70)
80 min	64.4 ± 9.98	48.3 ± 14.68	39.2 ± 9.54	86.4 ± 42.92 (44.4 ± 9.63)
90 min	66.0 ± 11.64	38.0 ± 11.94	34.4 ± 12.00	76.6 ± 42.92 (34.8 ± 11.03)
100 min	46.6 ± 11.42	28.8 ± 9.60	28.4 ± 9.16	96.0 ± 43.11 (54.3 ± 12.17)
110 min	53.9 ± 14.22	32.4 ± 12.25	16.7 ± 8.21	82.1 ± 36.30 (46.4 ± 7.25)
120 min	56.3 ± 13.07	32.3 ± 16.50	24.4 ± 8.53	63.8 ± 38.04 (26.6 ± 9.24)
Rearing time (sec)				
10 min	190.19 ± 23.140	187.39 ± 24.276	168.32 ± 18.768	215.97 ± 25.477 (208.60 ± 27.654)
20 min	214.00 ± 26.981	194.79 ± 28.340	177.89 ± 17.950	222.07 ± 14.130 (217.63 ± 15.210)
30 min	203.99 ± 37.759	208.03 ± 23.939	199.41 ± 32.933	213.43 ± 25.724 (209.60 ± 28.842)
40 min	202.25 ± 31.004	171.94 ± 25.717	188.79 ± 28.629	233.08 ± 24.317 (232.94 ± 27.572)
50 min	196.63 ± 29.619	200.96 ± 38.180	169.47 ± 34.356	208.54 ± 36.623 (210.40 ± 41.473)
60 min	243.23 ± 35.440	162.40 ± 27.386	183.24 ± 33.392	196.52 ± 28.007 (196.18 ± 31.755)
70 min	228.45 ± 36.729	167.28 ± 42.546	164.27 ± 40.821	198.62 ± 31.429 (202.75 ± 35.328)
80 min	225.36 ± 28.114	155.93 ± 38.627	155.54 ± 37.826	168.31 ± 28.291 (169.96 ± 32.024)
90 min	227.40 ± 39.473	140.14 ± 35.144	129.02 ± 42.382	179.49 ± 51.758 (181.10 ± 58.660)
100 min	174.99 ± 36.472	114.09 ± 33.568	116.56 ± 38.146	221.91 ± 45.394 (225.63 ± 51.299)
110 min	172.60 ± 38.580	134.12 ± 48.946	84.77 ± 46.425	184.99 ± 28.316 (172.60 ± 38.580)
120 min	196.68 ± 48.933	102.05 ± 49.567	92.33 ± 36.294	152.69 ± 48.549 (147.94 ± 54.785)
Average time of rearing				
10 min	2.999 ± 0.3514	2.765 ± 0.2536	2.269 ± 0.0955	2.661 ± 0.3236 (2.920 ± 0.2205)
20 min	2.955 ± 0.2955	2.820 ± 0.1315	2.712 ± 0.1101	3.013 ± 0.3974 (3.313 ± 0.2962)
30 min	2.645 ± 0.5202	3.398 ± 0.2439	3.089 ± 0.1737	3.355 ± 0.5936 (3.710 ± 0.5390)
40 min	3.303 ± 0.3562	3.784 ± 0.4081	2.904 ± 0.1970	3.518 ± 0.5131 (3.894 ± 0.3952)
50 min	2.868 ± 0.5329	4.271 ± 0.5795	3.465 ± 0.1720	3.643 ± 0.7093 (4.046 ± 0.6617)
60 min	3.413 ± 0.1979	3.885 ± 0.3401	3.434 ± 0.2383	3.297 ± 0.6026 (3.659 ± 0.5464)
70 min	3.672 ± 0.5605	4.154 ± 0.6768	3.051 ± 0.4759	4.005 ± 0.8381 (4.461 ± 0.7977)
80 min	3.863 ± 0.4690	4.289 ± 0.8673	3.517 ± 0.4995	3.277 ± 0.7343 (3.641 ± 0.7232)
90 min	3.107 ± 0.5666	4.297 ± 0.6413	3.328 ± 0.5317	3.800 ± 1.2115 (4.224 ± 1.2867)
100 min	3.765 ± 0.7909	3.913 ± 0.7475	3.408 ± 0.5976	4.778 ± 2.2773 (5.320 ± 2.5082)
110 min	2.756 ± 0.7737	2.676 ± 0.9394	2.347 ± 0.8023	3.747 ± 0.7657 (4.154 ± 0.7356)
120 min	2.868 ± 0.5089	2.093 ± 0.6646	2.731 ± 0.5880	4.281 ± 1.6106 (4.750 ± 1.7470)

Each value represents the mean ± SE.

found that the combination of tartrazine and brilliant blue FCF at 1000-fold higher than the average daily intake in Korea significantly decreased the numbers of newly generated cells in the adult mouse hippocampus, indicating adverse actions on hippocampal neurogenesis. The report suggests that brilliant blue FCF influences the development of central nervous system (CNS). Thus, the significant effects of brilliant blue FCF on CNS development may have resulted in the observed effects on behavior.

The high-dose level (1032–3856 mg/kg/day) was based on the current ADI of brilliant blue FCF set by WHO (12.5 mg/kg bw). The results of behavioral development tests suggest that the high-dose level of brilliant blue FCF inhibits the development of surface righting in the early lactation period. Therefore, these results suggest the need to reappraise the ADI of brilliant blue FCF. The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) (2010) proposed a new ADI of brilliant blue FCF (6 mg/kg bw) that was calculated from a chronic toxicity study in rats (Borzelleca et al., 1990). The intermediate-dose level (347–1287 mg/kg/day) of brilliant blue FCF induced any significant effects in the present study. The results of the present study support the value of a new ADI set by EFSA ANS (2010). Brown et al. (1980) reported that brilliant blue FCF was poorly absorbed from the gastrointestinal tract and that it underwent subsequent rapid and complete biliary excretion in rats. Phillips et al. (1980) reported that the fetuses of pregnant rats administered ^{14}C-labeled brilliant blue FCF showed no radioactivity. Thus, it appears that the actual level of brilliant blue FCF in the body is much lower than that anticipated. The actual dietary intake of

brilliant blue FCF in Japan (1987–1997) is presumed to be approximately 0.28–0.56 μg/kg/day, which is much lower than that expected (Japan Food Additive Society, 2001). It would therefore appear that the actual dietary levels of brilliant blue FCF are unlikely to produce any significant effects in humans.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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