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Reproductive and neurobehavioural toxicity study of Ponceau 4R administered to mice in the diet

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Abstract

Ponceau 4R was given to mice in the diet at levels of 0 (control), 0.12%, 0.24%, and 0.48% from 5 weeks of age of the F_0 generation to 9 weeks of age of the F_1 generation, and selected reproductive and neurobehavioural parameters were measured. There was no adverse effect of Ponceau 4R on litter size, litter weight or sex ratio at birth. The average body weight of male and female offspring was increased significantly in the high-dose group at postnatal days (PNDs) 0, 4 and 21. In behavioural developmental parameters, surface righting at PND 4 was affected significantly in the high-dose group in male offspring. Other variables measured showed no consistently significant adverse effect on either sex in the lactation period. In multiple water T-maze performance in the F_1 generation, the time taken was significantly longer than the control in the middle-dose and high-dose groups in males, and those effects were significantly dose-related ($P < 0.01$). The dose level of Ponceau 4R in the present study produced no adverse effect on reproduction, and a few adverse effects on neurobehavioural parameters in mice. The non-observed adverse effect level (NOAEL) was presumed to be 0.12% in the diet (approximately 205 mg/kg per day) for maze learning by males in the F_1 generation. Nevertheless, the middle-dose and high-dose levels were in excess of the acceptable daily intake (ADI) of Ponceau 4R (0–4.0 mg/kg body weight), and the actual dietary intake of Ponceau 4R in humans is presumed to be much lower. It would appear, therefore, that the level of dietary intake of Ponceau 4R is unlikely to produce any adverse reproductive or neurobehavioural effect in humans.

Keywords: Behavioural development; Food dye; Maze learning; Mice; Movement activity; New coccine; Ponceau 4R; Reproductive toxicity

1. Introduction

Ponceau 4R (also known as: new coccine; cochineal red; C.I. no. 16255; E124; Food Red No. 102) is principally trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate. Ponceau 4R is a red, water-soluble powder widely used in food products, drugs, cosmetics and pharmaceuticals. The estimated amount of Ponceau 4R manufactured in Japan in 1996 was approximately 32.78 tonnes (Ishimitsu et al., 1998). The acceptable daily intake (ADI) for humans is 0–4.0 mg/kg body weight (JECFA, 1983, 1996).

In toxicological studies of Ponceau 4R, Gaunt et al. (1967) reported that the acute intraperitoneal LD_{50} in male and female rats was 0.6 g/kg and 2.6 g/kg, respectively, and the corresponding values in mice were 1.9 g/kg and 1.6 g/kg. The oral LD_{50} exceeded 8 g/kg in both species. Brantom et al. (1987a) reported that the no-observed-effect level (NOEL) of Ponceau 4R was 500 mg/kg per day in a long-term toxicity study (60 days in the F_0 generation, 114 weeks and 118 weeks in males and females, respectively, in the F_1 generation) *via utero*. Also, Brantom et al. (1987b) reported that the non-observed adverse effect level (NOAEL) of Ponceau 4R in rats was 1250 mg/kg per day in a three-generation reproduction study.

As regards reproductive and developmental toxicity studies, Meyer and Hansen (1975) found that Ponceau

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4R induced no embryotoxic effect in rats by gavage (1000–4000 mg/kg per day). Momma et al. (1981) reported that Ponceau 4R in the diets (0.05–0.7%) during pregnancy in mice produced no teratogenic or postnatal development effect.

To our knowledge, there are no reports of studies on neurobehavioural toxicity of Ponceau 4R. Therefore, the present study was designed to evaluate reproductive and neurobehavioural effects of Ponceau 4R in mice throughout two generations. In our institute, mice are usually used in reproductive and neurobehavioural toxicity studies to conserve resources (chemicals, diets, space, etc.) and background data for mice are sufficient for evaluation of neurobehavioural effects (Tanaka, 2004). The design of the present study was based on the guidelines issued by ICH (ICH, 1993) and OECD (OECD, 1999) adapted for mice.

2. Materials and methods

2.1. Materials

Ponceau 4R was obtained from Tokyo Kasei Co., Ltd., Tokyo, Japan (lot no. GL 01). The purity of the chemical was more than 85.0% (w/w). The products of Ponceau 4R used in this study are standardized for food additives. The impurities of Ponceau 4R products are: matter loss on drying at 135 °C, together with chloride and sulfate calculated as sodium salts, is not more than 20% (w/w); water insoluble matter is not more than 0.2% (w/w); subsidiary coloring matter is not more than 1% (w/w); organic compounds other than coloring matter are not more than 0.5% (w/w) of the sum of: 4-amino-1-naphthalenesulfonic acid, 7-hydroxy-1,3-naphthalenedisulfonic acid, 3-hydroxy-2,7-naphthalenesulfonic acid, 6-hydroxy-2-naphthalenesulfonic acid, and 7-hydroxy-1,3,6-naphthalenetrisulfonic acid; unsulfonated primary aromatic amines calculated as aniline are not more than 0.01% (w/w); and ether-extractable matter is not more than 0.2% (w/w).

2.2. Animals and maintenance

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

2.3. Experimental design

Ponceau 4R was administered in the diet to 60 mice (six groups, each of 10, same-sex mice) at dietary levels of 0.12%, 0.24%, and 0.48%, from 5 weeks of age of the F_0 generation to 9 weeks of age of the F_1 generation. The control group (two groups, each of 10 same-sex mice) were given the basal diets (Nihon Clea, CE-2) for the corresponding period of time. Since all offspring were examined for behavioural development during the lactation period, number of mice was 10 per sex in each group. Mice were assigned to the groups by the stratified randomization method. The experimental diets were prepared bimonthly (twice) in our laboratory. After mixing Ponceau 4R with the powdered diet, pellets were formed and fed to mice. Ponceau 4R was stable in the pellets during the experimental period when previously measured by HPLC. The homogeneity of the test compound was ensured by the preparation procedures in our laboratory. The concentration of the test compound in the diet was not tested during the experimental period. Individual food intake of mice was measured during five periods: preconception (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 (4–9 weeks of age).

2.4. Reproductive procedure

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, 2, 4, 7, 14, 21, 28, and 30 during the preconception period. At 9 weeks of age, each female was paired with one male from the same treatment group, for a period of 5 days. The males were removed from females after 5 days, and the females were allowed to carry their litters to term, deliver, and rear all of their offspring.

In the F_1 generation, 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 the lactation period. The survival indices were calculated as (live offspring at each period)/(live and dead offspring at birth) × 100 (%). The offspring were weaned when they were 4 weeks of age, and one male and one female were selected at random from each litter to continue treatment. The animals were weighed individually at 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8weeks, and 9 weeks of age after weaning.

2.5. Neurobehavioural procedure

The functional and behavioural developmental parameters were measured and scored for all individual offspring during the lactation period in the F_1 generation (Tanaka et al., 1992), and were 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). The 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 rate for successful righting was: 2 = righting within 1 s; 1 = more than 1 s but within 2 s; 0 = more than 2 s.
2. *Negative geotaxis* on PNDs 4 and 7 (Fox, 1965; Altman and Sudarshan, 1975; Pantaleoni et al., 1988). The 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 rate was employed: 0 = no response within 60 s; 1 = response within 60 s; 2 = response within 30 s.
3. *Cliff avoidance* on PND 7 (Fox, 1965; Altman and Sudarshan, 1975; Pantaleoni et al., 1988). The offspring was placed onto a platform elevated 10 cm above a table top. 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 rate was employed: 0 = no response within 20 s; 1 = avoided backwards within 20 s, 2 = avoiding with turn.
4. *Swimming behaviour* on PNDs 4 and 14 (Fox, 1965; Pantaleoni et al., 1988). The offspring was placed into a tank of water maintained at 23 ± 1 °C and swimming behaviour was scored for direction (straight = 3; circling = 2; floating = 1) and head angle (ears out of water = 4; ears half out of water = 3; nose and top of head out of water = 2; and unable to hold head up = 1). Limb movement was rated as either 1 = all four limbs used, or 2 = only hindlimbs used.
5. *Olfactory orientation* on PND 14 (Altman and Sudarshan, 1975; Barlow et al., 1978; Meyer and Hansen, 1980). The offspring was placed into the arm of an apparatus consisting of two compartment 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. The following scoring rate was employed: 0 = no response within 90 s; 1 = entered the home wood flakes compartment *via* the fresh wood flakes compartment; 2 = entered the home wood flakes compartment directly.