

Original Article

Developmental Toxicity Evaluation of Berberine in Rats and Mice

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BACKGROUND: Berberine, a plant alkaloid, is found in some herbal teas and health-related products. It is a component of goldenseal, an herbal supplement. Berberine chloride dihydrate (BCD) was evaluated for developmental toxicity in rats and mice. **METHODS:** Berberine chloride dihydrate was administered in the feed to timed-mated Sprague–Dawley (CD) rats (0, 3625, 7250, or 14,500 ppm; on gestational days [GD] 6–20), and Swiss Albino (CD-1) mice (0, 3500, 5250, or 7000 ppm; on GD 6–17). Ingested doses were 0, 282, 531, and 1313 mg/kg/day (rats) and 0, 569, 841, and 1155 mg/kg/day (mice). **RESULTS:** There were no maternal deaths. The rat maternal lowest observed adverse effect level (LOAEL), based on reduced maternal weight gain, was 7250 ppm. The rat developmental toxicity LOAEL, based on reduced fetal body weight per litter, was 14,500 ppm. In the mouse study, equivocal maternal and developmental toxicity LOAELs were 5250 ppm. Due to scattering of feed in the high dose groups, a gavage study at 1000 mg/kg/day was conducted in both species. **CONCLUSIONS:** In rats, maternal, but not fetal adverse effects were noted. The maternal toxicity LOAEL remained at 7250 ppm (531 mg/kg/day) based on the feed study and the developmental toxicity NOAEL was raised to 1000 mg/kg/day BCD based on the gavage study. In the mouse, 33% of the treated females died. Surviving animals had increased relative water intake, and average fetal body weight per litter decreased 5–6% with no change in live litter size. The maternal toxicity LOAEL remained at 5250 ppm (841 mg/kg/day) BCD, based on increased water consumption. The developmental toxicity LOAEL was raised to 1000 mg/kg/day BCD based on decreased fetal body weight. *Birth Defects Research (Part B) 77:195–206, 2006.* Published 2006 Wiley-Liss, Inc.†

Key words: berberine; development; birth defects; rats; mice; pregnancy; herbal remedy

INTRODUCTION

Berberine is a natural constituent of many plants, including plants of the Berberidaceae family and Oregon grape root (Bergner, 1997a; Merck, 1998). Berberine is found in tea, herbal dietary supplements, over-the-counter health products, and has antimicrobial properties (National Toxicology Program, 1998). It is the major alkaloid component of *Coptis chinensis* (huanglian) used in Chinese herbal medicine. Berberine is a component of goldenseal, *Hydrastis canadensis*, another dietary supplement (National Toxicology Program, 1998; O'Hara et al., 1998) and can be synthetically produced (The Merck Index, 1983).

Human exposure occurs most commonly by the oral route through the use of berberine salts, herbal remedies, or dietary supplements. In India and China, berberine is used as a remedy for diarrhea, respiratory infection, constipation, indigestion, and cardiac arrhythmia. It is also used in antipyretic preparations and as an anti-malarial and giardia treatment (Chopra et al., 1932; Choudry et al., 1972; Gupta, 1975; Sabir et al., 1976; Purohit et al., 1982; Ghosh et al., 1983; Sun et al., 1988; Huang, 1990; Vennerstrom et al., 1990; Newall et al., 1996; Schmeller et al., 1997; Birdsall and Kelly, 1998; Merck,

1998). High doses of berberine of approximately 5–60 mg/kg body weight are used in diarrhea treatment (Rabbani et al., 1987; Bergner, 1997b). A recommended treatment of children (1–10 years) for giardiasis is 5 mg berberine/kg/day, given orally in three divided doses for 6 days (Choudry et al., 1972). Berberine improved cardiac performance in heart patients when administered intravenously (i.v.) at the rate of 0.02–0.2 mg/kg/min (Marin-Neto et al., 1988). Recently, Kong et al. (2004) reported that berberine lowered total cholesterol by 29% and LDL-cholesterol by 25% in 91 hypercholesterolemic patients after 3 months treatment with 500 mg berberine given orally twice a day. The proposed mechanism of

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lowering circulating cholesterol is an increase in hepatic LDL receptors, increasing LDL-cholesterol hepatic uptake and catabolism. These findings have increased interest in berberine and berberine-like compounds as cholesterol-lowering drugs with a mechanism differing from statins.

Minimal information on the toxicity of berberine is available. Berberine displaces bilirubin from serum binding proteins, causing jaundice, kernicterus, and brain damage in infants (Bateman et al., 1998; Chan, 1993, 1994). Therefore, exposure of pregnant women to berberine is not recommended because it may lead to jaundice and kernicterus in the fetus (Chan, 1993). Absorption of berberine from the gastrointestinal tract has been demonstrated in rabbits and humans (Chopra et al., 1932; Bhide et al., 1969). No information was found on the toxicity of berberine given orally to rodents. LD₅₀ for intraperitoneal (i.p.) administration to mice and rats were reported to be 30 mg/kg and 205 mg/kg, allowing the mouse to be approximately six-fold more sensitive to toxicity from berberine (Registry of the Toxic Effects of Chemical Substances, 1998). No information describing developmental effects of berberine administered to laboratory animals during gestation was found in the scientific literature.

The National Toxicology Program selected berberine for study because of the potential of human exposure through herbal preparations and the absence of developmental toxicology data. Dose selection was based on the results of dose range-finding studies conducted in female Sprague-Dawley rats and Swiss Albino mice (National Toxicology Program, 2003a,c). Dosed feed was chosen to conform to the oral route used in herbal remedies. The present developmental toxicity studies were designed to assess the effects of berberine chloride dihydrate (BCD) on embryo/fetal growth, viability, and morphologic development, and to establish the NOAEL and LOAEL values for maternal and developmental toxicity in Sprague-Dawley (CD) rats and Swiss Albino (CD-1) mice. Because scattering of feed occurred in both the rat and mouse studies (possibly due to reduced palatability of dosed feed), an accurate determination of BCD intake was not possible for the high dose group in each study. Therefore, after the feed studies (National Toxicology Program, 2003b,d), a gavage study was completed in both species at the high dose (National Toxicology Program, 2002, 2003e).

MATERIALS AND METHODS

Chemical

Berberine chloride dihydrate (Fig. 1) (CAS No. 5956-60-5) was obtained from Sigma Chemical Company (St. Louis, MO; Lot No. 49H0532). The lot and purity of the BCD used in the rat and mouse definitive feed and gavage studies were the same (Lot No. 49H0532). The identity of the test article was confirmed by infrared (IR) spectroscopy as well as nuclear magnetic resonance (NMR) spectroscopy. The purity of the test article was determined to be approximately 87.7% expressed as berberine chloride. Each concentration of berberine was formulated independently in NIH-07 ground rodent chow for the feed studies or 0.5% aqueous methylcellulose for the gavage studies. The predosing and

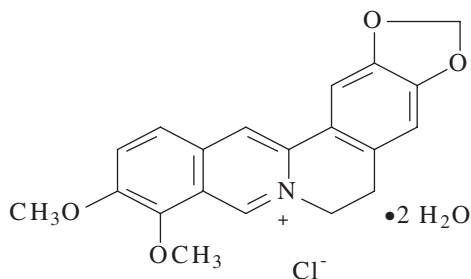


Fig. 1. Berberine chloride dihydrate.

postdosing concentrations of BCD were determined by HPLC (National Toxicology Program, 2002, 2003a-e). Formulations were administered within the period of proven stability.

Treatment

Rats. Dose selection for the rat feed study was based on a previous screening study in rats (National Toxicology Program, 2003c). In that study, timed-mated rats were exposed from gestation day (GD) 6-20 to BCD in ground feed (NIH-07) at concentrations of 0, 2000, 5000, 8000, 11,000, or 14,000 ppm (145, 356, 571, 789, and 939 mg BCD/kg/day, respectively). Reductions in maternal weight gain were observed over the treatment period. However, gravid uterine weight was not affected. There was no evidence of a treatment-related effect on prenatal growth, survival, or external morphologic development. In the developmental toxicity study, female Sprague-Dawley (CD) rats (25/group) were dosed through ground feed with BCD at concentrations of 0, 3625, 7250, or 14,500 ppm (0, 282, 531, 1313 mg/kg/day) on GD 6-20. In the gavage study, timed-mated female Sprague-Dawley (CD) rats (25/group) were treated by gavage from GD 6-19 with either vehicle (0.5% methylcellulose) or 1000 mg/kg/day BCD in vehicle.

Mice. Dose selection for the mouse feed study was based on a previous screening study in mice (National Toxicology Program, 2003a). Female Swiss (CD-1) mice were dosed through ground feed (NIH-07) containing BCD (0, 1400, 2600, 3800, 5000, or 6200 ppm) on GD 6-17. The calculated chemical dose, based on daily feed intake, was 0, 237, 412, 623, 814, and 982 mg BCD/kg/day for the low- through high-dose groups, respectively. BCD had no adverse effect on maternal endpoints and did not adversely affect prenatal growth, viability, or external morphologic development. Based on the results of the screening study, time-mated female Swiss (CD-1) mice (25/group) in the developmental toxicity study were dosed through ground feed (NIH-07) containing BCD at concentrations of 0, 3500, 5250, or 7000 ppm (0, 569, 841, and 1155 mg BCD/kg of body weight/day) on GD 6-17. In the gavage study, timed-mated female Swiss (CD-1) mice (25/group) were treated by gavage from GD 6-16 with either vehicle (0.5% methylcellulose) or 1000 mg/kg/day BCD in vehicle.

Animals and Husbandry

Rats. Male and female Crl:CD BR VAF/Plus outbred albino rats (CD rats; Charles River Laboratories, Inc., Raleigh, NC) were maintained in quarantine for

a minimum of 10 days after arrival at RTI International. Animals were individually identified by ear tag. Individual breeding pairs were cohoused overnight. The morning on which sperm was found in the vaginal lavage (Hafez, 1970) was designated as GD 0. A total of 25 timed-mated females per group were assigned within each study. Confirmed-mated females were assigned to treatment groups by stratified randomization for body weight on GD 0, so that mean body weight on GD 0 did not differ among treatment groups. For the feed study, maternal body weights for confirmed pregnant females ranged from 216–273 g on GD 0. The study design included four consecutive breeding dates. In the gavage study maternal body weights, for confirmed pregnant females, ranged from 227–272 g on GD 0. This study design included three consecutive breeding dates.

Mice. Male and female CD-1 (ICR)BR VAF/Plus outbred albino mice (CD-1 mice, Charles River Laboratories, Inc.) were maintained in quarantine for 9–10 days after arrival at RTI International. Animals were individually identified by ear tag. Individual breeding pairs were cohoused overnight. The morning on which a vaginal plug was found (Hafez, 1970) was designated as GD 0. A total of 25 timed-mated females per group were assigned within each study. Confirmed-mated females were assigned to treatment groups by stratified randomization for body weight on GD 0, so that mean body weight on GD 0 did not differ among treatment groups. For the feed study, maternal body weights for confirmed pregnant females ranged from 25–32 g on GD 0. The study design included five consecutive breeding dates. In the gavage study, maternal body weights for confirmed pregnant females ranged from 21–28 g on GD 0. This study design included three consecutive breeding dates.

Rats and mice. Confirmed-mated females and 10 additional sentinel females per study were individually housed in solid-bottom polycarbonate cages with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ) and certified Sani-Chip hardwood cage litter (P.J. Murphy, Montville, NJ). Ground or pelleted rat/mouse diet NIH-07 (7022 CM) or Purina #5002 and tap water (City of Durham, NC) were available ad lib throughout the study. The rodent diets were certified by the vendor (Harlan Teklad, Madison, WI or PML, St. Louis, MO). Environmental conditions were monitored and controlled by a Siebe Barber-Colman Network 8000 System with SIGNAL software (Siebe Environmental Controls [SEC]/Siebe Barber-Colman Company, Loves Park, IL). Light cycles were maintained on a 12-hr light:12-hr dark cycle.

From the first GD 0 date until the final necropsy date for these studies, animal room temperature and relative humidity readings ranged from 67.2–78.2°F (rats), 69.2–73.9°F (mice) and 43.3–72.1% (rats), 35.2–67.1% (mice), respectively.

At termination of each study, blood samples were collected by cardiac puncture from ten sentinel females under terminal CO₂ anesthesia. Serum samples were submitted for evaluation of viral antibody titers. Mouse sera were tested for mouse adenovirus-Friend leukemia strain, ectromelia virus, epidemic diarrhea of infant mice, Theiler's mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, pneumonia virus of mice, reo viruses 1, 2 and 3, sendai virus, and

parvo virus. Rat sera were tested for pneumonia virus of mice, rat corona virus/sialodacryoadenitis, sendai virus, and parvo virus. All assays were negative.

Maternal Evaluations

Rats and mice. For confirmed-mated mice and rats in the feed studies, body weight was recorded on the mornings of GD 0, 6, 9, 12, 15. Mice were also weighed on GD 17 and immediately after termination on GD 17; rats were also weighed on GD 18, and 20, and immediately after termination on GD 20. In the gavage studies, confirmed-mated rats and mice were weighed on GD 0, daily during treatment, and on the day of termination (in-life and immediately after termination). Females were observed for clinical condition at least once a day on GD 0–5 (before dosing) and during treatment. On the termination date, females were observed for clinical condition at weighing and at scheduled termination. Feed and water consumption were monitored during each study, with measurements on the mornings of GD 0, 6, 9, 12, 15, 18, and 20 (rats) and GD 0, 6, 9, 12, 15, and 17 (mice). Feed weights were taken from the dosed feed jars. No attempt was made to retrieve ground feed from the bedding.

Timed-mated females were sacrificed by CO₂ asphyxiation. The body, liver, and gravid uterus of each timed-mated female were weighed. Thoracic and abdominal cavities were examined. No treatment-related gross pathology of the maternal organs was observed at necropsy. Ovarian corpora lutea were counted. Pregnancy status was confirmed by uterine examination. Uterine contents were examined to determine the number of implantation sites, resorptions, dead fetuses, and live fetuses. Dead fetuses were counted, weighed, and discarded. Uteri with no visible implantation sites were stained with ammonium sulfide (10%) to visualize any implantation sites that might have undergone very early resorption (Salewski, 1964).

Embryo/Fetal Evaluations

Rats and mice. Live fetuses were dissected from the uterus and immediately placed on a moist paper towel over a tray of ice, to induce anesthesia by lowering the core body temperature below 25°C (Lumb and Jones, 1973; Danneman and Mandrell, 1997; Wixson and Smiler, 1997). All live fetuses were counted, weighed, sexed, and examined for external morphologic abnormalities, including cleft palate. Approximately one-half (50%) of the fetuses were terminated by decapitation and the remaining fetuses by evisceration under terminal anesthesia.

Approximately one-half (50%) of the fetal carcasses were sexed and examined for visceral morphologic abnormalities using a fresh tissue dissection method (Staples, 1974; Stuckhardt and Poppe, 1984). The same fetal carcasses were decapitated before dissection. Fetal heads were fixed and decalcified in Bouin's solution and subsequently examined using a free-hand sectioning technique (Wilson, 1965). All fetal carcasses were eviscerated (and gender determined for those not scheduled for a full visceral morphologic examination), and the skeletons macerated and stained with Alcian Blue/Alizarin Red S stain (Marr et al., 1988). Intact fetal skeletons (i.e., those fetuses that were not decapitated) were examined for skeletal morphologic abnormalities

(see final reports for specific descriptions of morphologic findings).

Statistics

The unit for statistical measurement was the pregnant female or the litter. All statistical procedures applied to selected measures from this study were based on SAS software (SAS Institute, Inc., 1989a,b, 1990a–c, 1992, 1996, 1997) available at RTI International. For the feed studies, quantitative continuous data (e.g., maternal body weights, fetal body weights, feed consumption, etc.) were compared among treatment groups by parametric statistical tests whenever Bartlett's test for homogeneity of variance was not significant. When Bartlett's test indicated a lack of homogeneity ($P < 0.001$), non-parametric statistical tests were applied (Winer, 1962). For the gavage studies, quantitative continuous data were compared among the single treatment group and the vehicle control group using the Student's *t*-test.

Parametric statistical procedures were applied to selected measures in these studies. General linear models (GLM) procedures were applied to the analyses of variance (ANOVA) and the tests for linear trend. Before GLM analysis, an arc sine-square root transformation was carried out on all litter-derived percentage data (Snedecor and Cochran, 1967). For litter-derived percentage data, the ANOVA was weighted according to litter size. For multiple treatment groups, when a significant ($P < 0.05$) main effect for dose occurred, Dunnett's multiple comparison test (Dunnett, 1955, 1964) was used to compare each treatment group to the control group for that measure. A one-tailed test (i.e., Dunnett's test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for maternal body and organ weight parameters, maternal feed and water consumption, fetal body weight, and percent males per litter.

For the feed studies, nonparametric tests applied to continuous variables included the Kruskal-Wallis one-way analysis of variance by ranks for among-group differences and, if significant ($P < 0.05$), the Mann-Whitney *U*-test for pairwise comparisons to the vehicle control group (Siegel, 1956). A one-tailed Mann-Whitney *U*-test was considered appropriate for all parameters, except that a two-tailed test was considered appropriate for maternal organ weights, maternal and fetal body weight parameters, maternal feed and water consumption, and percent male fetuses per litter. Jonckheere's test for *k* independent samples (Jonckheere, 1954) was used to identify significant dose-response trends.

Nominal scale measures were analyzed by χ^2 test for independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage test for linear trend on proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). If χ^2 showed significant ($P < 0.05$) differences among groups, then a one-tailed Fisher's exact probability test, with appropriate adjustments for multiple comparisons, was appropriate for pairwise comparisons between BCD-treated groups and the control group. For the gavage studies nominal scale measures were analyzed by χ^2 test for independence for differences among the single treatment group and vehicle control group (Snedecor and Cochran, 1967).

The α level for each statistical comparison was 0.05. The significance level for each trend test or pair-wise comparison (one- or two-tailed) was reported as $P < 0.05$ or $P < 0.01$.

RESULTS

Rats Maternal

Feed study. Twenty-five timed-mated rats were assigned to each treatment group by stratified randomization of body weight on GD 0. No females were removed from the study and all assigned females survived until scheduled termination on GD 20. Beginning on GD 7, yellow discoloration of the fur was noted at 14,500 ppm BCD (1–6 females/day), due to direct contact with feed containing the test chemical (yellow powder with an orange cast). No adverse clinical signs were noted. Pregnancy was confirmed at necropsy for 24–25 (96–100%) females per group.

Maternal body weight (Fig. 2) did not differ significantly among groups on GD 0 or 6 (i.e., before initiation of exposure). Exposure to BCD at 3625 or 7250 ppm was not associated with any significant changes in maternal body weight on GD 0, 6, 9, 12, 15, 18, or 20. Exposure to BCD at 14,500 ppm was associated with significant decreases (~4–10%) in maternal body weight on GD 9, 12, 15, 18, and 20.

Maternal body weight gain (Table 1) before treatment (GD 0–6) showed a spurious decreasing trend. At 3625 ppm BCD, there were no effects on maternal body weight gain during the exposure period. At 7250 ppm, maternal body weight gain was significantly reduced for the gestational period as a whole (GD 0–20), but corrected weight gain was not affected. At 14,500 ppm, significant reductions in maternal body weight gain were found for the following measurement periods: GD 6–9, 9–12, 12–15, 15–18, and 18–20. In addition, maternal body weight gain in the high-dose group was significantly reduced for the treatment period as a whole (GD 6–20), for the gestational period as a whole (GD 0–20), and for corrected weight gain (i.e., gestational weight gain minus gravid uterine weight).

Gravid uterine weight exhibited a decreasing trend, but differences between the control and individual treatment groups were not statistically significant (Table 1). Mean gravid uterine weight in the low-, mid- and high-dose groups was 95%, 92%, and 91% of the mean gravid uterine weight in the control group. Maternal liver weight (absolute and relative) showed decreasing trends and was significantly reduced at the high dose (Table 1).

Before the initiation of treatment (GD 0–6), maternal feed consumption (absolute or relative) was comparable among treatment groups. Exposure to 3625 ppm or 7250 ppm BCD was not associated with any significant changes in maternal feed consumption. Thus, calculated average intake of BCD for dams in the low- and mid-dose groups (282 and 531 mg/kg/day, respectively; $n = 23$ /group) was considered to provide an accurate measure of chemical consumption. However, erratic changes in relative maternal feed consumption were noted at 14,500 ppm BCD. Individual animal data for feed intake indicated an unusually high degree of variability within and between dams in the high-dose group. In addition,

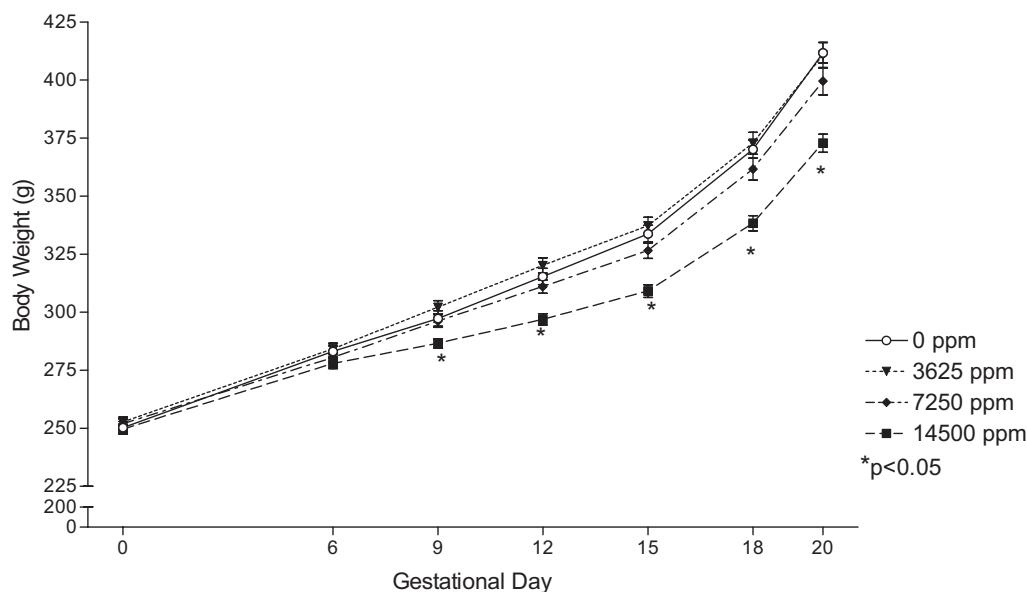


Fig. 2. Rat gestational body weights for BCD in feed.

seven dams in the high dose group had removed observable amounts of feed from their feed jars, presumably due to altered palatability or the novel taste of the chemical/feed mixture. Due to the nature of feed intake data at the high dose, calculated average intake of BCD (1313 mg/kg/day; $n = 16$) is an estimate of the chemical intake for that group. For the treatment period (GD 6–20) and the gestational period (GD 0–20) as a whole, maternal relative water consumption was not affected by BCD exposure.

Gavage study. Maternal body weight change corrected for gravid uterine weight and absolute and relative maternal liver weights were significantly decreased after exposure to BCD; gravid uterine weight was unaffected (Table 1). Maternal absolute feed consumption was significantly decreased in the BCD-dosed group. Relative maternal water consumption showed a treatment-related increase on GD 6–20.

Rats Developmental

Feed study. At termination on GD 20, there were 24–25 confirmed pregnancies per group and each pregnant female carried a live litter (Table 2). The numbers of corpora lutea per dam or implantation sites per litter were comparable among treatment groups. BCD exposure was not associated with changes in postimplantation mortality (i.e., there was no effect on the percent resorptions, late fetal deaths, non-live implants, or adversely affected implants per litter). Likewise, the number of litters with one or more resorptions, late fetal deaths, non-live implants, or adversely affected implants was unaffected. No adverse effects of treatment were observed for the number of live fetuses per litter or the percent male fetuses per litter. Significant decreasing trends were noted for average fetal body weight per litter (males, females or both genders), and fetal body weights were significantly reduced in the high-dose group. There was no effect on fetal body weight at 3625 or 7250 ppm, and a ~6% reduction at 14,500 ppm.

No external malformations or variations were observed in any dose group in this study (Table 2). Visceral malformations or variations occurred with an incidence of 0.4–2.6% (malformations) or 4.0–8.5% (variations). Skeletal malformations or variations occurred with an incidence of 0.5–1.1% (malformations) or 3.0–6.2% (variations). Incidences for visceral and skeletal anomalies did not occur with a dose-related incidence and were consistent with historic control data from RTI International.

Gavage study. There was comparable viability of the implants in treated and untreated groups. Average fetal body weight per litter was unaffected by treatment. No differences were observed in the incidences of fetal external, visceral, or skeletal malformations or variations (Table 2).

Mice Maternal

Feed study. Twenty-five timed-mated mice were assigned to each group by stratified randomization of body weight on GD 0. One control female was removed from the study because she delivered early (GD 15) and one high-dose female was removed due to inaccurate timing of chemical exposure. Otherwise, all assigned females survived until scheduled termination on GD 17. Beginning on GD 9, yellow discoloration of the fur was noted in all BCD-exposed groups, most likely the result of direct contact with feed containing the test chemical (yellow powder with an orange cast). No adverse clinical signs were noted throughout the treatment period. At scheduled necropsy (GD 17), pregnancy was confirmed for 21–23 females/group (Table 3).

There were no significant differences among groups for maternal body weight or weight gain during treatment and absolute maternal liver weight and gravid uterine weight (GD 17) were not affected by BCD exposure. Relative liver weight exhibited an increasing trend in the absence of a distinctive dose-response pattern.

Table 1
Maternal Toxicity in CD Rats Exposed to Berberine Chloride Dihydrate

mg/kg body wt/day (ppm)	Feed (GD 6–20)				Gavage (GD 6–19)	
	0	282 (3625)	531 (7250)	1313 (14,500)	0	1000
Maternal pregnancy status						
No. treated	25	25	25	25	25	25
No. removed ^a	0	0	0	0	0	1 [§]
No. dead or euthanized ^a	0	0	0	0	0	0
No. (%) pregnant at sacrifice	24 (96)	24 (96)	25 (100)	24 (96)	25 (100)	22 (91.7)
Maternal body weight changes (g)^{b,c}						
Pretreatment wt. gain (GD 0–6)	32.8±2.1 ^h	31.6±1.6	28.4±1.2	28.3±1.9	29.5±2.0	27.6±1.8
Gestation wt. gain (GD 0–20)	152.2±3.7 ⁱ	149.7±4.1	138.1±4.6 ^j	114.2±4.1 ^k	130.2±3.6	124.0±4.3
Treatment wt. gain (GD 6–20)	128.6±2.6 ⁱ	126.5±4.1	119.1±4.6	94.9±3.6 ^k	108.0±3.8	103.2±2.9
Corrected wt. gain ^d	57.3±2.9 ⁱ	59.4±2.3	50.5±2.4	27.6±2.9 ^k	55.9±2.7 ^o	42.9±3.84
Gravid uterine wt.	94.9±2.6 ^l	90.3±3.7	87.6±4.4	86.6±2.1	74.3±3.5	81.1±1.7
Maternal organ weights^b						
Liver						
Absolute (g)	18.06±0.28 ⁱ	18.13±0.22	17.54±0.31	15.46±0.23 ^k	17.34±0.25 ^o	15.9±0.37
Relative (% sacrifice wt.) ^e	4.48±0.05 ⁱ	4.52±0.06	4.49±0.06	4.25±0.04 ^k	4.58±0.07 ^o	4.28±0.06
Maternal feed consumption^{b,f}						
Pretreatment period (GD 0–6)						
Relative (g/kg/day)	85.8±1.4	87.3±1.6	86.3±1.3	85.2±1.9	87.1±1.4	84.1±2.2
Treatment period (GD 6–20)						
Relative (g/kg/day)	75.8±1.0	77.8±1.8	73.2±1.0	90.5±3.7 ^m	78.3±1.0 ^o	72.9±1.3
Maternal water consumption^{b,f}						
Pretreatment period (GD 0–6)						
Absolute (g/day)	33.5±1.3	33.3±0.7	34.3±0.8	31.2±0.9	34.6±0.9	34.4±1.3
Relative (g/kg/day)	126.0±5.1	124.3±2.9	129.1±2.8	118.3±3.6	131.1±3.4	131.7±4.8
Treatment period (GD 6–20)						
Absolute (g/day)	43.6±1.3	40.8±1.0	41.9±1.0	42.3±1.4	43.1±1.3	46.0±1.5
Relative (g/kg/day)	130.2±4.2	120.7±2.8	127.0±2.5	134.6±4.3	130.5±3.4 ⁿ	143.5±4.5

^aIn the feed study, no females died or were euthanized before scheduled termination on GD 20. GD, gestational day.

^bIncludes all dams pregnant at sacrifice; mean±SEM.

^cBody weights were recorded in the morning of each designated GD.

^dWeight change during gestation minus gravid uterine weight.

^eCalculated using body weight at the time of sacrifice on GD 20.

^fBody weights were recorded in the morning of each designated GD. Relative feed and water consumption (g/kg/day) were calculated using these weights.

[§]One female was removed due to a dosing error (misdirected dose).

^h $P < 0.05$; test for linear trend.

ⁱ $P < 0.01$; test for linear trend.

^j $P < 0.05$; Dunnett's test.

^k $P < 0.01$; Dunnett's test.

^l $P < 0.05$; Jonckheere's test.

^m $P < 0.01$; Mann-Whitney *U*-test.

ⁿ $P < 0.05$; Student's *t*-test.

^o $P < 0.01$; Student's *t*-test.

During the pretreatment period, spurious differences among groups were noted for maternal relative feed consumption (g/kg/day), such that the mid- and high-dose groups consumed more than controls. These differences contributed to increased maternal relative feed consumption across the gestational period as a whole for the same groups. For individual measurement periods during treatment, there were no differences among groups for maternal relative feed consumption, although a significant increasing trend was noted for the treatment period as a whole.

Average daily intake of BCD was calculated from the concentration of BCD in the feed and relative maternal feed consumption during the treatment period (GD 6–17). Average daily maternal ingestion of BCD was approximately 0, 569, 841, and 1155 mg/kg/day for the

control through high-dose groups, respectively. Maternal relative water consumption (g/kg/day) was comparable among groups during the pretreatment period, but increases were noted in the mid- and high-dose groups over the treatment period.

Gavage study. One animal was removed from the control group due to a preexisting condition, and four animals were removed from the 1000 mg/kg/day dose group due to a misdirected dose (Table 3). Seven additional females in the 1000 mg/kg/day dose group were found moribund or dead. Recorded clinical observations indicated that six of seven animals effluxed dosing solution before death or moribundity. Observations at necropsy indicated that six of six necropsied animals had dosing solution in their stomachs; four of six animals had gas in the intestines Examination of the

Table 2
Developmental Toxicity in CD Rat Fetuses After Maternal Exposure to Berberine Chloride Dihydrate

mg/kg body wt/day (ppm)	Feed (GD 6–20)				Gavage (GD 6–19)	
	0	282 (3625)	531 (7250)	1313 (14,500)	0	1000
All litters ^a	24	24	25	24	25	22
No. corpora lutea/dam ^b	16.9±0.6	16.5±0.6	17.4±0.5	16.4±0.4	15.6±0.4	16.4±0.4
No. implantation sites/litter ^b	16.1±0.4	15.6±0.6	15.3±0.8	16.0±0.3	14.2±0.6	15.7±0.3
% preimplantation loss/litter ^b	4.4±1.3	7.1±2.3	14.4±4.3 ^e	3.7±1.3	9.8±3.0	5.3±1.2
% resorptions/litter ^b	1.8±0.6	3.9±1.3	1.8±0.6	3.5±1.2	5.7±2.2	3.1±1.1
% litters with resorptions	29	38	28	38	44.0	36.4
% late fetal deaths/litter ^b	0.3±0.3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
% litters with late fetal deaths	4	0±0	0±0	0±0	0.0	0.0
Live litters ^{b,c}	24	24	25	24	25	22
No. live fetuses/litter	15.8±0.4	15.0±0.6	15.0±0.8	15.5±0.4	13.4±0.7 ^g	15.2±0.3
Avg. male fetal body wt./litter (g)	3.91±0.06 ^d	3.89±0.04	3.82±0.06	3.66±0.05 ^f	3.54±0.04	3.45±0.04
Avg. female fetal body wt./litter(g)	3.71±0.05 ^d	3.72±0.05	3.61±0.05	3.50±0.05 ^e	3.39±0.04	3.30±0.05
% male fetuses/litter	46.4±2.6	52.7±2.0	48.9±3.0	48.4±2.5	50.1±2.6	53.3±2.7
% externally malformed fetuses/litter	0±0	0±0	0±0	0±0	0.00±0.00	0.32±0.32
% viscerally malformed fetuses/litter	1.8±1.1	0.5±0.5	0.4±0.4	2.6±1.3	1.90±1.43	0.65±0.65
% skeletally malformed fetuses/litter	1.0±0.7	0.9±0.9	1.1±0.8	0.5±0.5	1.00±0.69	0.65±0.65
% malformed fetuses/litter	1.4±0.7	0.7±0.5	0.8±0.5	1.5±0.8	1.37±0.70	1.00±0.72
% fetuses with external variations/litter	0±0	0±0	0±0	0±0	0±0	0±0
% fetuses with visceral variations/litter	5.3±2.0	4.0±1.6	8.5±2.4	5.2±1.9	7.05±3.45	6.26±1.79
% fetuses with skeletal variations/litter	6.2±3.2	3.8±1.4	3.0±1.3	5.2±2.1	17.27±3.98	16.32±3.88
% fetuses with variations/litter	5.7±1.6	3.9±0.9	5.7±1.4	5.3±1.2	12.36±2.41	11.05±2.03

^aIncludes all dams pregnant at sacrifice; litter size = no. implantation sites per dam. GD, gestational day.

^bReported as the mean ± SEM.

^cIncludes only dams with live fetuses; litter size = no. live fetuses per dam.

^d $P < 0.01$; test for linear trend.

^e $P < 0.05$; Dunnett's test.

^f $P < 0.01$; Dunnett's test.

^g $P < 0.05$; Student's *t*-test.

lungs of these animals did not provide any clear evidence of misdosing. No additional adverse clinical signs were noted. All remaining females survived until scheduled sacrifice on GD 17. Pregnancy was confirmed in 24 females in the control group (100%) and 14 in the BCD-treated group (100%) on GD 17 (Table 3).

Maternal body weight was comparable between groups on GD 0 and 6 (i.e., before the initiation of exposure), and throughout the study. Maternal body weight changes corrected for gravid uterine weight and absolute and relative maternal liver weight were also unaffected after exposure to BCD. Maternal absolute feed consumption was comparable in the control and treated groups. When calculated based on body weight, maternal feed consumption (mg/kg/day) was significantly increased in the treated group on GD 9–12, but was comparable at all other times. Absolute (g/day) and relative (mg/kg/day) maternal water consumption was increased in the BCD-treated group compared to the control group over the treatment period.

Mice Developmental

Feed study. At scheduled necropsy, pregnancy was confirmed in 21–23 females per group (88–96% per group), and each pregnant female carried a live litter (Table 4). The number of corpora lutea per dam, number of implantation sites per litter, and percent preimplantation loss were each comparable among treatment groups. Prenatal mortality (resorptions or late fetal deaths),

average live litter size, and percent male fetuses also did not differ among groups. Average fetal body weight per litter (males or both genders) was not affected by BCD exposure. A decreasing trend was noted for average female fetal body weight per litter, but the 4% reduction at the high dose was not significant.

There were no statistically significant effects on the incidences of malformations or variations grouped according to their primary classifications (external, visceral, and skeletal) (Table 4). Percent fetuses with malformations per litter showed an increasing trend, and group means followed a clearly dose-related pattern. Specifically, the average percent malformed fetuses per litter were 1.21, 1.23, 3.29, and 5.13% for the control through high-dose groups, respectively. Two individual malformations (discontinuous rib and cleft palate) occurred with a somewhat higher incidence in BCD-exposed groups, although the incidences were not significantly different from controls. Cleft palate, a malformation to which this species and strain are predisposed, occurred with an incidence of 0/291 (0%), 1/294 (0.3%), 2/287 (0.7%), and 6/261 (2.3%) (data not shown). The number (%) of litters with cleft palate was 0/23 (0%), 1/22 (5%), 1/23 (4%), and 2/21 (10%) for the control through high-dose groups, respectively (data not shown).

The percent fetuses with variations and percent litters with variations were increased at the mid dose (both genders or males, but not females), but were not increased in the high dose group, indicating the lack of

Table 3
Maternal Toxicity in Swiss (CD-1) Mice Exposed to Berberine Chloride Dihydrate

mg/kg body wt/day (ppm)	Feed (GD 6–17)				Gavage (GD 6–16)	
	0	569 (3500)	841 (5250)	1155 (7000)	0	1000
Maternal pregnancy status						
No. treated	25	25	25	25	25	25
No. removed	1 ^a	0	0	1 ^a	1 ^g	4 ^h
No. dead or euthanized ^a	0	0	0	0	0	7 ⁱ
No. (%) pregnant at sacrifice	23 (96)	22 (88)	23 (92)	21 (88)	24 (100)	14 (100)
Maternal body weight changes (g)^{b,c}						
Pretreatment wt. gain (GD 0–6)	2.65±0.21	3.05±0.26	2.41±0.21	2.32±0.18	3.22±0.19	3.15±0.16
Gestation wt. gain (GD 0–17)	25.46±0.86	26.85±0.63	25.11±0.57	24.71±1.15	24.09±0.96	23.14±0.82
Treatment wt. gain (GD 6–17)	24.62±0.84	26.06±0.54	24.54±0.58	24.18±1.09	21.95±0.91	20.78±0.80
Corrected wt. gain ^d	5.77±0.46	6.90±0.36	6.37±0.38	6.48±0.72	6.68±0.27	6.18±0.46
Gravid uterine wt.	19.69±0.74	19.94±0.51	18.74±0.48	18.23±0.82	17.41±0.87	16.96±0.66
Maternal organ weights^b						
Liver						
Absolute (g)	3.10±0.08	3.32±0.07	3.16±0.07	3.18±0.08	2.79±0.05	2.69±0.07
Relative (% sacrifice wt.) ^e	5.72±0.10 ^j	6.07±0.12	5.96±0.10	6.08±0.15	5.79±0.11	5.65±0.11
Maternal feed consumption^{b,f}						
Pretreatment period (GD 0–6)						
Relative (g/kg/day)	158.5±10.3 ⁿ	162.5±8.8	190.1±6.2 ^o	185.2±3.2 ^o	219.5±4.9	222.7±3.9
Treatment period (GD 6–17)						
Relative (g/kg/day)	158.1±1.8 ^j	162.4±2.0	160.2±1.9	165.1±2.0	175.8±2.5	181.7±4.0
Maternal water consumption^{b,f}						
Pretreatment period (GD 0–6)						
Absolute (g/day)	6.8±0.2	6.8±0.2	6.9±0.2	7.3±0.6	6.8±0.2	6.8±0.2
Relative (g/kg/day)	230.1±5.5	232.5±4.9	237.2±8.1	252.0±19.9	230.1±5.5	232.5±4.9
Treatment period (GD 6–17)						
Absolute (g/day)	8.5±0.2 ^k	8.9±0.2	9.3±0.3 ^l	9.6±0.2 ^m	9.5±0.3 ^p	10.6±0.4
Relative (g/kg/day)	205.7±4.2 ^k	214.4±5.6	228.9±6.3 ^l	236.8±7.0 ^m	245.6±7.8 ^p	278.1±11.8

^aOne control female delivered early (GD 15) and one female in the high-dose group was removed from the study because dosing was started on the wrong GD. GD, gestational day.

^bIncludes all dams pregnant at sacrifice; mean±SEM.

^cBody weights were recorded in the morning of each designated GD.

^dWeight change during gestation minus gravid uterine weight.

^eCalculated using body weight at the time of sacrifice on GD 17.

^fBody weights were recorded in the morning of each designated GD. Relative feed and water consumption (g/kg/day) were calculated using these weights.

^gOne control female was removed due to a preexisting condition (anophthalmia).

^hFour females were removed due to misdirected dosing.

ⁱSeven females were found dead or moribund; cause of death or moribundity was unknown, but was not due to technician error.

^j $P < 0.05$; test for linear trend.

^k $P < 0.01$; test for linear trend.

^l $P < 0.05$; Dunnett's test.

^m $P < 0.01$; Dunnett's test.

ⁿ $P < 0.01$; Jonckheere's test.

^o $P < 0.01$; Mann-Whitney *U*-test.

^p $P < 0.05$; Student's *t*-test.

a dose-response trend. The increase was due to the collective incidences of several variations commonly found in this species and strain, and these specifically included enlarged lateral ventricles, short rib, wavy rib, or rib on lumbar I.

Gavage study. At termination on GD 17, the number of ovarian corpora lutea per dam, number of implantation sites per litter, and percent preimplantation loss per litter were comparable between groups (Table 4). These data indicate comparable reproductive status between treatment groups at the time dosing was initiated on GD 6. Prenatal (postimplantation) mortality (number or percent resorptions, late fetal deaths, or non-

live implants per litter), average live litter size, and percent male fetuses per litter were not adversely affected by treatment, suggesting comparable viability of the implants across treatment groups.

Average fetal body weight per litter (genders combined, males or females) exhibited a 5–6% decrease in the BCD-treated group compared to the control group, which was statistically significant for the males only.

No differences were observed in the incidences of fetal malformations or variations. Specifically, BCD treatment at 1000 mg/kg/day on GD 6–16 did not affect the incidence of external, visceral, or skeletal malformations or variations.

Table 4
Developmental Toxicity in Swiss (CD-1) Mouse Fetuses After Maternal Exposure to Berberine Chloride Dihydrate

mg/kg body wt/day (ppm)	Feed (GD 6–17)				Gavage (GD 6–16)	
	0	569 (3500)	841 (5250)	1155 (7000)	0	1000
All litters^{a,b}	23	22	23	21	24	14
No. corpora lutea/dam	14.30±0.47	14.24±0.44	13.23±0.40	13.86±0.65	12.00±0.48	12.54±0.46
No. implantation sites/litter	13.65±0.37	13.77±0.38	12.83±0.40	12.95±0.68	11.75±0.56	12.36±0.49
% preimplantation loss/litter	5.63±1.73	4.96±1.58	5.26±2.27	9.63±3.21	7.03±2.98	6.63±3.45
% resorptions/litter	6.71±2.61	2.82±1.24	1.94±0.98	3.63±1.37	4.35±1.71	6.41±1.93
% litters with resorptions	39	27	17	29	33.33	50.00
% late fetal deaths/litter	0.57±0.39	0.00±0.00	0.69±0.49	0.37±0.37	0.00±0.00	0.51±0.51
% litters with late fetal deaths	9	0	9	5	0.00	7.14
Live litters^{b,c}						
No. live fetuses/litter	12.65±0.49	13.36±0.38	12.48±0.39	12.43±0.70	11.25±0.59	11.50±0.50
Avg. male fetal body wt./litter (g)	1.096±0.013	1.058±0.017	1.049±0.017	1.067±0.025	1.075±0.023 ^f	1.020±0.024
Avg. female fetal body wt./litter (g)	1.050±0.013 ^d	1.016±0.015	1.014±0.016	1.006±0.021	1.032±0.017	0.965±0.021
% male fetuses/litter	48.8±3.0	46.5±2.7	47.3±2.3	54.0±3.3	51.08±3.53	50.15±4.52
% externally malformed fetuses/litter	0.00±0.00	0.32±0.32	0.54±0.54	1.79±1.50	0.35±0.35	1.02±1.02
% viscerally malformed fetuses/litter	0.62±0.62	0.65±0.65	1.27±0.89	1.87±1.34	0.00±0.00	0.00±0.00
% skeletally malformed fetuses/litter	1.89±1.05	1.22±0.84	4.79±2.17	4.92±2.01	14.19±4.48	11.26±5.32
% malformed fetuses/litter	1.21±0.56 ^d	1.23±0.57	3.29±1.39	5.13±1.90	7.47±2.24	6.58±2.68
% fetuses with external variations/litter	0.00±0.00	0.00±0.00	0.00±0.00	0.37±0.37	0.00±0.00	0.55±0.55
% fetuses with visceral variations/litter	0.00±0.00	2.16±1.19	4.55±1.95	1.98±1.10	36.17±6.41	45.27±10.59
% fetuses with skeletal variations/litter	14.87±4.67	9.90±2.76	23.95±4.95	19.38±4.52	28.94±6.45	34.06±8.05
% fetuses with variations/litter	7.37±2.32 ^d	6.05±1.57	14.38±2.42 ^e	10.81±2.36	32.47±5.18	40.00±6.34

^aIncludes all dams pregnant at sacrifice; litter size = no. implantation sites per dam. GD, gestational day.

^bReported as the mean ± SEM.

^cIncludes only dams with live fetuses; litter size = no. live fetuses per dam.

^d $P < 0.05$; test for linear trend.

^e $P < 0.05$; Dunnett's test

^f $P < 0.05$; Student's *t*-test.

DISCUSSION

Before these studies, no information describing the developmental effects of berberine compounds administered to laboratory animals during gestation was found in the literature.

In the definitive rat feed study, there was no clinical or gross evidence of maternal toxicity at the lowest concentration of BCD in the feed (3625 ppm). Sporadic decreases in maternal relative water intake were noted in the low-dose group (GD 12–15 and 18–20). However, these changes were not associated with any consistent pattern of effects on water intake across the range of BCD exposures. Furthermore, there were no other statistically significant differences between the low-dose group and the control group for any measure of maternal status in this study. Thus, the no-observed-adverse-effect level (NOAEL) for maternal toxicity was considered to be 3625 ppm (equivalent to an average daily intake of 282 mg BCD/kg of body weight/day or 223 mg berberine chloride/kg/day). At 7250 ppm, maternal gestational body weight gain was reduced by ~9% relative to weight gain in the control group, and this decrease was statistically significant. In addition, there was a robust decreasing dose-response trend associated with this endpoint. Thus, the lowest-observed-adverse-effect level (LOAEL) for maternal toxicity was considered to be 7250 ppm (equivalent to 531 mg BCD/kg/day or 420 mg berberine chloride/kg/day). Maternal toxicity at the highest concentration of BCD in feed (14,500 ppm) included reduced maternal body weight and reduced

weight gain, and reduced liver weight (absolute and relative). Acknowledging the unusual variability for maternal feed intake data at the high dose (indicative of decreased palatability), the best available estimate for average daily ingestion was 1313 mg/kg/day as BCD or 1040 mg/kg/day as berberine chloride. This value likely represents an overestimation of actual chemical intake for that group. Due to uncertainty about the accuracy of maternal feed intake data at 14500 ppm, the possibility existed that altered feed intake contributed to the reduction in maternal or fetal body weight at the high dose.

There was no evidence of developmental toxicity at the low (3625 ppm) or mid (7250 ppm) concentrations of BCD in the diet. At the highest concentration (14,500 ppm), indices of prenatal mortality and morphologic development were comparable to controls, but there was a significant decrease (~6%) in average fetal body weight per litter accompanied by a decreasing dose-response trend. Thus, the NOAEL for developmental toxicity was considered to be 7250 ppm (531 mg BCD/kg/day or 420 mg berberine chloride/kg/day). The LOAEL for developmental toxicity was considered to be 14,500 ppm (1313 mg BCD/kg/day or 1040 mg berberine chloride/kg/day).

The extreme variability of the maternal feed consumption data at the high dose in the rat and mouse developmental toxicity feed studies precluded an accurate calculation of berberine exposure, and thus an accurate evaluation of the maternal and developmental toxicity NOAEL and LOAEL. To more clearly define the maternal and developmental toxicity of BCD, pregnant

Sprague–Dawley (CD) rats were given 1000 mg berberine chloride dihydrate/kg/day in 0.5% aqueous methylcellulose by gavage on GD 6–19. Formulation analysis indicated that the actual exposure to BCD in this study was 1125–1155 mg berberine chloride dihydrate/kg/day (987–1013 berberine chloride/kg/day, or 779–800 mg berberine/kg/day). Maternal toxicity was observed as decreased maternal body weight and corrected maternal weight gain. Yellow feces, not observed in the treated animals from previous studies, were noted and presumed to be from the presence of yellow-orange berberine compounds in the gastrointestinal system. No developmental toxicity was observed. The results of this study indicate that the maternal toxicity LOAEL observed in the feed study is supported by more severe maternal toxicity when a higher dose is administered by gavage, but that the developmental effects (reduced fetal body weight) observed at the high dose in the feed study are most likely secondary to a reduction in maternal corrected weight gain. The gavage study suggests that the developmental toxicity NOAEL is ~1000 mg berberine chloride dihydrate/kg body weight/day, and that the developmental toxicity LOAEL has not been determined (Table 5).

In the definitive mouse feed study, there was no maternal mortality and no adverse clinical signs. During the pretreatment period, spurious differences among groups were noted for maternal relative feed consumption. These differences contributed to increased maternal relative feed consumption across the gestational period as a whole. For individual measurement periods during treatment, there were no differences among groups for maternal relative feed consumption, although a significant increasing trend was noted for the treatment period as a whole. Maternal relative water consumption was comparable among groups during the pretreatment period, but increased at 5230 and 7000 ppm from GD 6–9, 9–12, 12–15, 6–17, and 0–17, but not from GD 15–17. Based on increased water consumption with berberine dose, 5250 ppm (841 mg/kg/day) BCD was considered a conservative estimate of maternal toxicity LOAEL. At scheduled necropsy, pregnancy was confirmed in 21–23 females/group (88–96%). Prenatal mortality (resorptions or late fetal deaths), average live litter size and percent male fetuses did not differ among groups. Average male fetal body weight/litter was not affected by BCD exposure. A decreasing trend was noted for average

female fetal body weight/litter (but not for males or for both genders combined), and the 4% reduction for females at the high dose was not significant. There were no significant effects on incidences of malformations or variations grouped by primary classification (external, visceral, skeletal). Percent fetuses with malformations/litter showed an increasing trend (both genders combined), but no significant effects for either gender alone. Two individual malformations (discontinuous rib and cleft palate) occurred with higher incidences in BCD-exposed groups, but were not significantly different from controls. Percent fetuses with variations/litter and percent litters with variations was increased at the mid dose (both genders or males, but not females) due to the collective incidences of common variations, including enlarged lateral ventricles, short rib, wavy rib, or rib on lumbar I.

The incidence of cleft palate failed to reach statistical significance in this study, but it nevertheless exhibited an apparent dose-response relationship. Specifically, cleft palate was found in 0/291 fetuses (0/23 litters) in the control group, 1/294 fetuses (1/22 litters) in the low-dose group, 2/287 fetuses (1/23 litters) in the mid-dose group, and 6/261 fetuses (2/21 litters) in the high-dose group. It has long been recognized that cleft palate occurs spontaneously with a low incidence in the CD-1 mouse (Palmer, 1972; Perraud, 1976; Fritz et al., 1978). Historic control data from RTI International were examined (i.e., developmental toxicity screen or definitive studies in which the CD-1 mouse was administered the vehicle control treatment by the oral route). The incidences of cleft palate were 0/292 fetuses (0/23 litters) and 1/297 fetuses (1/24 litters) for two studies in which control treatment was administered by gavage on GD 6–15. Additional control data from studies not yet added to the historic control database were also examined. In a gavage study (GD 6–15), the incidence of cleft palate was 1/271 fetuses (1/22 litters). No cases of cleft palate were found among 873 fetuses (69 litters) in control groups from four studies conducted during 1999 in which the test article had been administered to CD-1 mice in the feed from GD 6–17. Based on this background incidence of cleft palate, the occurrence of one case in the low-dose group is not considered evidence of a treatment-related effect, occurrence of two cases in the mid dose group provides equivocal evidence of a treatment-related effect, and the occurrence of six cases in the high dose group (albeit from only two litters) is clearly higher than expected. The observed incidence of cleft palate was used to set an equivocal LOAEL for developmental toxicity at 5250 ppm BCD in the diet.

In light of the equivocal results obtained from the developmental toxicity study conducted previously in mice and an observed palatability problem with BCD in the diet, an evaluation of the high dose, administered by gavage, was warranted. Pregnant Swiss (CD-1) mice were given 1000 mg BCD/kg/day (877 mg berberine chloride/kg/day or 693 mg berberine/kg/day) in 0.5% aqueous methylcellulose by gavage on GD 6–16. Formulation analysis indicated that the actual exposure to BCD in this study was 1068–1071 mg BCD/kg/day (937–938 berberine chloride/kg/day, or 740–742 mg berberine/kg/day).

Maternal mortality/moribundity was observed in 7/21 (33%) of the mice exposed to 1000 mg/kg/day BCD by

Table 5
Developmental Toxicity Endpoints for Berberine Chloride Dihydrate^a

Toxicity endpoint	LOAEL (mg/kg/day)		NOAEL (mg/kg/day)	
	BC ^b	BCD	BC	BCD
Rat maternal	420	531	223	282
Rat developmental	>792	>1000	792	1000
Mouse maternal	666	841	450	569
Mouse developmental	792	1000	666	841

^aNOAEL/LOAEL values based on the collective results from studies using two routes of administration.

^bBerberine chloride.

gavage. Animals died or were moribund after 1–8 doses with no additional clinical signs and no distinctive gross pathology. Of six moribund animals necropsied, all had dosing solution in their stomach and there was no evidence of tissue tears or misdirected dose. In four of six animals, there was gas noted in the intestines. These results suggest that a response of severe toxicity, not observed in the feed study, may have occurred via the gavage route. In the absence of gross organ pathology, maternal organs were not fixed for histopathology, and the study design did not include clinical pathology endpoints. Further study of this phenomenon seems to be warranted. Surviving animals exhibited increased (absolute and relative) maternal water consumption, but no effect on maternal body weight. The results of this study indicate that the equivocal maternal toxicity LOAEL observed in the feed study is supported by more severe maternal toxicity when a higher dose is administered by gavage.

The gavage study in mice resulted in a 5–6% decrease in fetal body weight that was statistically significant for the males only. Examination of the historic control data from RTI International for CD-1 mouse fetuses indicates a variation of only 1.5–2.7% in fetal body weight among control fetuses. The 5–6% decrease in fetal body weight in the present study seems to be biologically relevant. These results support the developmental toxicity NOAEL 841 mg BCD/kg/day or 666 mg berberine chloride, based on the absence of cleft palate at 1000 mg/kg/day berberine chloride dihydrate, and a developmental LOAEL of 1000 mg BCD/kg/day (Table 5).

From the evidence of severe toxicity observed in the gavage study, it can be concluded that pregnant mice are more sensitive to gavage administration of BCD than pregnant rats. These data are consistent with studies in non-pregnant mice and rats with berberine administered by the intraperitoneal injection (Registry of the Toxic Effects of Chemical Substances, 1998). LD₅₀ for i.p. administration to mice and rats were reported to be 30 mg/kg and 205 mg/kg, indicating that the mouse is approximately six-fold more sensitive to toxicity from berberine (Registry of the Toxic Effects of Chemical Substances, 1998). In the feed studies, mouse maternal toxicity was manifested as increased water consumption at doses of 841 mg/kg/d and 1155 mg/kg/d BCD. Although there was evidence of feed scattering in the high dose group, there was no observed effect on maternal body weight (i.e., due to decreased feed consumption) or other qualitative signs of maternal toxicity. Therefore, evaluation of the kinetics of BCD after gavage and feed administration would be required to clarify the differences in maternal toxicity at nominal concentrations of 1000 mg/kg/day BCD.

The developmental toxicity NOAELs in both the rat and mouse studies are ~500 times higher than the amount of berberine taken for dietary supplements (e.g., goldenseal) by humans and ~60–100 times higher than a pharmacologic dose.

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