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ORIGINAL ARTICLE

Reproductive toxicity evaluation of a new camptothecin anticancer agent, CKD-602, in pregnant/lactating female rats and their offspring

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Abstract CKD-602 is a camptothecin anticancer agent that was recently developed by the Chong Kun Dang Pharmaceutical Co. (Seoul, Korea). This study examined the potential adverse effects of CKD-602 on pregnancy, delivery, and lactation in female Sprague–Dawley rats as well as on the pre- and postnatal development of their offspring. One hundred pregnant females were divided into four groups: three treatment groups and a control group. CKD-602 was administered once daily by intravenous bolus injection to female rats at doses of 0, 5.7, 17, or 51 μg/kg/day from gestational day 6, through to parturition and throughout the period of lactation up to weaning [lactational day (LD) 21]. All the dams were sacrificed on LD 22 after weaning. The clinical signs, mortality, body weight change, food consumption, physical development, and behavioral function were evaluated in their progeny. When the exposed offspring reached maturity (postnatal day 70), their reproductive performance was assessed. In the high-dose group, suppressed body weight and a decrease in the amount of food consumption were observed in the dams during both the gestation and lactation periods. An increase in the incidence of thymic atrophy, decreased liver and ovary weight, and an increase in the weight of the spleen were also observed in the dams at the scheduled necropsy. In addition, an increase in the number of stillborn and postnatal mortality, a decrease in the live litter size, and a delay in physical development were observed in the F1 offspring. Teratological examinations showed an increase in the incidence of congenital anomalies in both the F1 offspring and F2 fetuses. In the medium dose group, only slight maternal toxicity including suppressed body weight and decreased food consumption was observed. There were no treatment-related effects on the maternal function and pre- and postnatal development in the low dose group. The no-observed-adverse-effect level (NOAEL) of CKD-602 for the dams are considered to be 5.7 μg/kg/day, however, the NOAEL for their offspring are estimated to be 17 μg/kg/day.

Keywords CKD-602 · Anticancer agent · Camptothecin · Pre- and postnatal developmental toxicity · Rats

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Introduction

Camptothecin (CPT) is a cytotoxic alkaloid extracted from the bark, fruit, and leaves of *Camptotheca acuminata*, which is a tree endemic to China. Although some antitumor activity has been observed, its development as an anticancer agent has been hindered by its poor solubility and unpredictable toxicity, which includes hemorrhagic cystitis, myelosuppression, and diarrhea [1–3]. There has been considerable effort made to develop structural analogues of CPT resulting in the discovery of several CPT analogues such as irinotecan (CPT-11), topotecan, and 9-aminocamptothecin [4–6].

These compounds have shown a high clinical efficacy in treating human neoplasms such as colorectal and ovarian cancers.

The mechanisms for the action of CPT derivatives lay in their ability to inhibit topoisomerase I, which is an important nuclear enzyme in various DNA functions including transcription and replication. However, there is one exception to this rule. Irinotecan, which is a prodrug, must first be converted to the active metabolite, SN-38, by a carboxyl esterase converting enzyme [7, 8]. CPTs all contain a terminal lactone ring that renders them unstable in aqueous solutions due to rapid, pH-dependent, non-enzymatic hydrolysis to form an open-ring hydroxy carboxylic acid [9], which significantly lowers its potency as a topoisomerase I inhibitor [10]. Because CPTs cause DNA damage, they are potentially mutagenic and can induce chromosomal aberrations including sister chromatid exchanges, gene deletions, and gene rearrangements [11]. Agents that can inhibit DNA synthesis and/or damage DNA have toxic side effects on multiple organ systems [12]. Therefore, there has been considerable research aimed at developing new CPTs with improved antitumor activity and safety profiles.

CKD-602 is a new CPT derivative and an antitumor agent with the formula (7-[2-(*N*-isopropylamino) ethyl]-(20S)-camptothecin) that was developed by the Chong Kun Dang Pharmaceutical Company in Seoul, Korea [13, 14]. Like other CPT derivatives, CKD-602 is a potent topoisomerase I inhibitor that successfully overcomes the poor water solubility and toxicity of the parent drug. Pre-clinical studies of CKD-602 have demonstrated it to have broad antitumor activity against various tumor cell lines that were equivalent or superior to those of topothecan, which is a clinically active antitumor drug [15, 16]. Genotoxicity studies have shown that it does not cause mutagenicity in *Salmonella typhimurium* TA 98, TA 100, TA 1535, and TA 1537 and does not cause any chromosome aberrations in Chinese Hamster lung cells in the presence of a metabolic activation system. In contrast, there was an increased incidence of micronucleated polychromatic erythrocytes in the bone marrow of ddY male mice [17]. The subacute toxicity study showed that a 4-week repeated intravenous dose of CKD-602 to rats at dose levels > 67 μg/kg/day caused various adverse effects on bone marrow, blood cells, spleen, liver, thymus, and heart in rats [18]. Recently we have reported that DW-116 is not only maternally toxic and embryotoxic, but also teratogenic in experimental animals. The rat developmental toxicity study showed that CKD-602 is embryotoxic and teratogenic to rats at a minimally maternotoxic dose of 80 μg/kg/day [19]. The rabbit developmental toxicity study also showed that the repeated dose of CKD-602 during pregnancy produces increased incidence of abortion and death, increased number of embryonic resorptions and fetal morphological alterations at 48 μg/kg/day [20]. The embryotoxic and teratogenic effects of CKD-602 observed in both rat and rabbit studies are consistent with the results of previous studies. Itabashi et al. reported that irinotecan caused decreased maternal body weight and food consumption, reduced fetal and placental weight, and increased number of fetal malformations, when it was given to rats intravenously at 6 mg/kg during organogenesis [21]. Because treatment of CPTs results in various reproductive and developmental toxicities in pregnant animals, they are not currently recommended for pregnant women. CKD-602 showed significant anticancer activity against pulmonary and ovarian cancer in humans and was approved by the Korea Food and Drug Administration on October 2003.

The aim of this study was to determine the potential adverse effects of CKD-602 on pregnancy, delivery, and lactation in dams, and on the pre- and postnatal development of F1 offspring in the Sprague–Dawley rats.

Materials and methods

Animals

For mating, two females were placed into the cage of a single male overnight. Successful mating was determined by the presence of sperm in a vaginal smear, and the following 24 h was designated as gestational day (GD) 0. The mated females were housed individually in clear polycarbonate cages with stainless steel wire lids. The animals were fed standard irradiated rat and mouse pellets (Jeil Feed Co., Daejeon, Korea) and were given tap water sterilized by a ultra-violet sterilizer ad libitum. This experiment was carried out in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and the animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* [22].

Test substance

CKD-602 is a colorless white powder, which was provided by the Chong Kun Dang pharmaceuticals (Seoul, Korea). Figure 1 shows the chemical structure of CKD-602. The required amount of CKD-602 was dissolved in 1 ml of distilled water containing 50 mg D-mannitol,

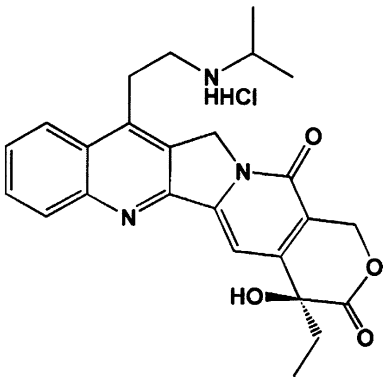

Fig. 1 Chemical structure of CKD-602

0.06 mg tartaric acid and adjusted to pH 3.50 with NaOH. A high-dose solution was prepared before treating the rats twice a week because the stability of the prepared test article over a 5-day period had already been confirmed. The dosing solutions for the lower dose groups were prepared by a stepwise dilution of the high-dose solution.

Experimental groups

Four experimental groups were constructed: three treatment groups receiving 5.7, 17, and 51 μg/kg/day CKD-602 and a vehicle control group. The vehicle used in this study was distilled water (pH 3.50) containing 50 mg D-mannitol, 0.06 mg tartaric acid in 1 ml. Each group contained 25 mated females.

Dose selection

Based on the results of our previous subacute and developmental toxicity studies [18–20], 51 μg/kg was selected for the high dose and two lower doses were determined using a scaling factor of 3. This dose was expected to be high enough to induce apparent toxic effects in pregnant/lactating dams and their offspring. The medium and low doses were expected to cause minimal or no adverse effects on both the dams and F1 offspring.

Drug treatment

The drug was administered intravenously in order to comply with a potential treatment route for humans. The formulated test chemical was administered once daily by intravenous bolus injection into the lateral tail vein of pregnant and lactating females from GD 6 to lactational day (LD) 21 at 2 ml/min through a 25G needle. The application volume (3 ml/kg) was calculated according to the most recent body weight.

Maternal examinations

All the F0 dams were observed daily for any clinical signs of toxicity, moribundity, and mortality throughout the duration of the study. Detailed clinical observations were recorded and printed. The effects of the test chemical on gestation, parturition, and nursing were also monitored. The maternal body weights were measured on GD 0, 6, 9, 12, 15, and 20 and on LD 0, 7, 14, and 21. The individual amount of food consumed was determined on GD 1, 7, 10, 13, 16, and 21 and on LD 1, 8, 15, and 22. At the scheduled termination (LD 22), all F0 females were euthanized by carbon dioxide asphyxiation and subjected to an external and internal macroscopic examination. The uteri were examined to determine the number of implantation sites. The absolute and relative (organ-to-body weight ratio) weights of the brain, heart, liver, kidneys, spleen, adrenal glands, and ovaries were measured for all survivors.

F1 offspring examinations

The morning on which parturition was complete was designated as postnatal day (PND) 0. All pups were examined as soon as possible on the day of birth in order to determine the number of live and stillborn pups in each litter. The number of live pups were counted, sexed, weighed, and examined externally. The cages were subsequently monitored daily for any dead or moribund pups. On PND 4, the size of each litter was adjusted by eliminating extra pups randomly to give four males and four females per litter whenever possible. The following four sub-groups within each litter were then constructed: growth, reproduction, behavior, and weaning necropsy groups (one male and one female each). The decision to cull was made in order to reduce the variability in the study data produced by different litter sizes between and within the treatment groups. Litters with eight or less pups were not culled. Approximately half of the surplus pups from each litter was examined viscerally using a free-hand razor sectioning technique [23] for the head and abdomen and a Nishimura's method [24] for the thorax. The other half was examined for skeletal malformations and variations after evisceration and staining with Alizarin Red S [25].

The body weights of the F1 offspring were measured once a week until 10 weeks of age. The physical signs of postnatal development (pinna detachment, incisor eruption, fur development, eyelid opening, testis descent, and vaginal opening), and behavioral tests [righting reflex (PND 5), negative geotaxis

(PND 10), grip-strength (PND 15), pupillary reflex (PND 22), acoustic startle response (PND 22), rotating rod for motor co-ordination (PND 23), open field for motor activity (PND 45), and swimming test for learning and memory (PND 60)] were evaluated. At weaning (PND 21), one male and one female per litter were sacrificed to evaluate structural abnormalities and pathological changes. On PND 70, one male and one female per litter were euthanized by carbon dioxide asphyxiation and subjected to an external and internal macroscopic examination. The weights of the brain, heart, liver, kidneys, spleen, adrenal glands, and testes or ovaries were measured. When the offspring reached maturity (PND 70), one male and one female from each litter were mated to evaluate their reproductive performance but with mating between siblings being avoided. The body weight of the F1 pregnant dams was measured on GD 0, 7, 14, and 20. At the caesarean section of the F1 dams on GD 20, the ovaries and uteri of each female were removed and examined for the number of corpora lutea and the status of all implantation sites, i.e., live and dead fetuses, early and late resorptions, and total implantations. Resorption was classified as 'early' when only the placental tissue was visible and 'late' when placental and embryonic tissues were visible at the caesarean section. All live fetuses were weighed, sexed, and evaluated for any external morphological abnormalities, including a cleft palate.

Statistical analysis

The unit for statistical measurement was the pregnant/lactating female or the litter [26]. Quantitative continuous data such as the maternal body weight, food consumption, organ weight, and neonatal body weight were subjected to a one-way analysis of the variance (ANOVA), and a Scheffe's multiple comparison test was carried out when the differences were significant [27]. The number of corpora lutea, total implantations, live and dead fetuses, implantation loss rate, and fetal/neonatal malformations were evaluated statistically using the Kruskal–Wallis non-parametric ANOVA [28], followed by the Mann–Whitney *U*-test where appropriate. The clinical signs, necropsy findings, and histopathological findings are represented as frequencies, and were subjected to the Fisher's exact probability test [29] where necessary. The statistical analyses were performed by comparing the treatment groups with the vehicle control group using SAS software [30]. The significant probability values are represented as asterisks, $P < 0.05$ (*) or $P < 0.01$ (**).

Results

Effect on dams

Clinical signs and mortality

There were no treatment-related clinical signs or deaths in any of the groups during the gestation and lactation periods.

Body weight changes

As shown in Fig. 2, there was a statistically significant suppression in body weight observed on LD 0 and 7 at 17 μg/kg (5–8%) and on GD 12–20 and LD 0–21 at 51 μg/kg (6–9 and 9–16%, respectively).

Food consumption

As shown Fig. 3, there was a statistically significant decrease in food consumption observed on GD 16 at 17 μg/kg (13%) and on GD 13–21 and LD 1–15 at 51 μg/kg (19–28 and 26–35%, respectively).

Gross findings

At the necropsy of dams on LD 22, atrophy of the thymus was observed in 22 females in the 51 μg/kg group, but not in the 5.7 and 17 μg/kg groups (data not

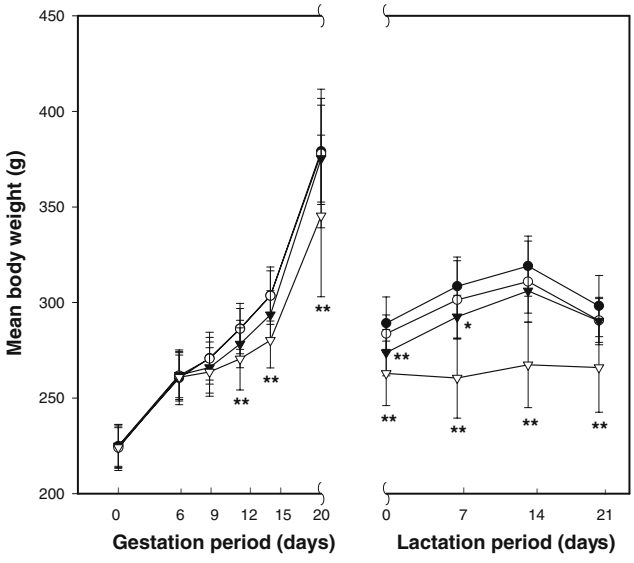

Fig. 2 Changes in the body weights of the dams treated with CKD-602 at doses of 0 (*filled circle*), 5.7 (*open circle*), 17 (*filled downward triangle*), and 51 (*open downward triangle*) μg/kg/day during the gestation and lactation periods. The values are presented as the mean ± SD. *Single asterisk* and *double asterisks* indicate a significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

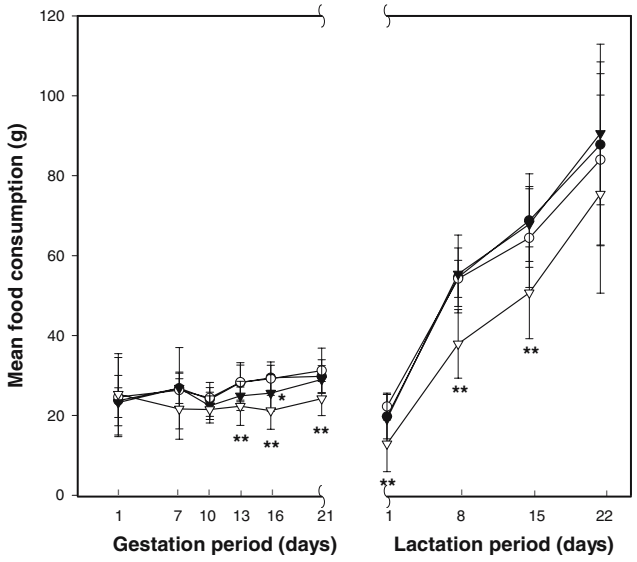

Fig. 3 Changes in the food consumption of the dams treated with CKD-602 at doses of 0 (*filled circle*), 5.7 (*open circle*), 17 (*filled downward triangle*), and 51 (*open downward triangle*) μg/kg/day during the gestation and lactation periods. The values are presented as the mean ± SD. *Single asterisk* and *double asterisks* indicate a significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

shown). There was a significantly higher incidence of thymic atrophy in the high dose group than in the control group.

Organ weights

The relative weight of the brain was significantly higher and the absolute weight of spleen was significantly lower in the 17 μg/kg group than in the control group (Table 1). The absolute and relative weights of the spleen and the relative weight of the brain, adrenal glands, kidneys, and heart were significantly higher in the 51 μg/kg group than the control group, while the absolute and relative weights of the liver and ovaries were significantly lower than the control group.

Effect on the pre- and postnatal development of F1 offspring

Delivery findings and the viability index

The number of implantations, the gender ratio of live newborns, pregnancy duration, delivery index, and weaning index were similar in all groups (Table 2). In contrast, there was a significantly higher number of stillbirths in the 51 μg/kg group than in the controls, while litter size was significantly lower. Although the difference was not statistically significant, the postnatal mortality to PND 4 in the 51 μg/kg group was higher than that in the control group.

Visceral examination of the F1 pups

Table 3 shows the types and incidences of visceral abnormalities observed in the F1 pups. Statistically higher incidences of viscerally malformed pups were found in the 51 μg/kg group than in the control group. Visceral malformations including anophthalmia, microphthalmia, and a dilated cerebral ventricle were observed in 5 of the 26 pups examined in the 51 μg/kg

Table 1 Organ weights in the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
No. of female rats	25	21	25	23
Body weight	296.6 ± 14.41	288.5 ± 15.91	285.4 ± 17.76	266.2 ± 24.78**
Brain (g)	1.82 ± 0.058	1.82 ± 0.074	1.84 ± 0.083	1.83 ± 0.093
per body weight (%)	0.62 ± 0.039	0.63 ± 0.039	0.65 ± 0.049*	0.69 ± 0.053**
Adrenal glands (g)	0.072 ± 0.009	0.071 ± 0.011	0.067 ± 0.007	0.071 ± 0.010
per body weight (%)	0.024 ± 0.003	0.024 ± 0.004	0.024 ± 0.003	0.027 ± 0.003*
Liver (g)	15.29 ± 1.307	14.78 ± 1.219	14.41 ± 1.717	12.68 ± 1.919**
per body weight (%)	5.15 ± 0.339	5.13 ± 0.417	5.06 ± 0.658	4.75 ± 0.380**
Spleen (g)	0.57 ± 0.105	0.52 ± 0.055	0.50 ± 0.056*	0.68 ± 0.162**
per body weight (%)	0.19 ± 0.031	0.18 ± 0.022	0.17 ± 0.026	0.26 ± 0.059**
Kidneys (g)	2.49 ± 0.202	2.47 ± 0.155	2.39 ± 0.206	2.49 ± 0.272
per body weight (%)	0.84 ± 0.067	0.86 ± 0.073	0.84 ± 0.073	0.94 ± 0.055**
Heart (g)	1.09 ± 0.079	1.06 ± 0.075	1.04 ± 0.084	1.13 ± 0.148
per body weight (%)	0.37 ± 0.024	0.37 ± 0.031	0.37 ± 0.034	0.43 ± 0.055**
Ovaries (g)	0.099 ± 0.016	0.097 ± 0.020	0.090 ± 0.016	0.070 ± 0.018**
per body weight (%)	0.033 ± 0.005	0.034 ± 0.007	0.032 ± 0.006	0.026 ± 0.006**

Values are presented as the mean ± SD

*, **Significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

Table 2 Reproductive and littering findings in the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
No. of dams	25	21	25	23
No. of implantations[a]	15.3 ± 3.17	16.0 ± 2.14	16.4 ± 1.78	15.1 ± 2.93
No. of stillbirth[a]	0.2 ± 0.41	0.1 ± 0.36	0.3 ± 0.63	0.7 ± 0.78*
Pre-implantation loss (%)[a,b]	1.0 ± 2.28	1.0 ± 2.73	1.4 ± 3.18	4.4 ± 5.09*
No. of live pups at birth				
Male/female	173/183	161/159	201/165	126/125
Sex ratio	0.95	1.01	1.22	1.01
Litter size[a]	14.2 ± 3.27	15.2 ± 1.92	14.6 ± 1.70	10.9 ± 2.97**
Post-implantation loss (%)[a,c]	9.1 ± 7.33	4.5 ± 4.85	10.3 ± 7.76	27.5 ± 16.74**
Pregnancy duration (day)[a]	21.8 ± 0.25	21.7 ± 0.30	21.8 ± 0.29	21.9 ± 0.27
Delivery index (%)[d]	100	100	100	100
Postnatal mortality to PND 4 (%)[a,e]	1.2 ± 2.49	1.9 ± 3.75	4.7 ± 15.73	8.1 ± 9.29
Weaning index (%)[a,f]	99.5 ± 2.50	100	99.5 ± 2.50	98.9 ± 3.60

*, **Significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

[a] Values are presented as the mean ± SD

[b] Pre-implantation loss (%) = [(no. of corpora lutea − no. of implantation sites)/no. of corpora lutea] × 100

[c] Post-implantation loss (%) = [(no. of implantation sites − no. of live fetuses)/no. of implantation sites] × 100

[d] Delivery index (%) = (no. of dams with live newborns/no. of pregnant females) × 100

[e] Postnatal mortality to PND 4 (%) = (no. of live pups at birth − no. of live pups on postnatal day 4/no. of live pups at birth) × 100

[f] Weaning index (%) = (no. of live pups on postnatal day 21/no. of live pups after culling on postnatal day 4) × 100

group. Visceral variations, such as a dilated renal pelvis, dilated ureter, and misshapen thymus, were observed in all groups and the incidences of visceral variations observed in the treatment groups were comparable to that in the control group. These findings are commonly observed in normal Sprague–Dawley rats [31–33].

Skeletal examination of the F1 pups

Table 3 shows the types and incidences of skeletal abnormalities observed in the F1 pups. The incidences of skeletal malformations and variations observed in the 51 μg/kg group were significantly higher than those of the control group, respectively. The incidence of litters with skeletal malformations was also significantly higher in the 51 μg/kg group. The malformations observed in the 51 μg/kg group were fused cervical arch, fused cervical centrum, fused thoracic arch, and fused sternebra. The variations observed were the cervical rib, extra sternebral ossification site, short/full supernumerary rib, misshapen sternebra, bipartite ossification of cervical/thoracic centrum, and dumbbell ossification of the cervical/thoracic centrum.

Clinical signs and mortality of the F1 offspring (growth group)

In the F1 male offspring (Table 4), dome-shaped head, absent eye bulge, small eye bulge, and death were observed in one, five, one, and two animals in the 51 μg/kg group, respectively. There was a significantly higher number of F1 males with clinical signs including death in the 51 μg/kg group than that in the control group. In the F1 females, emaciation and death was observed in one female of the control group. One female in the 17 μg/kg group died. An absent eye bulge, a small eye bulge, and dome-shaped head were observed in two, one, and one animals in the 51 μg/kg group, respectively. Although the incidence of clinical signs observed in the F1 females in the 51 μg/kg group was slightly higher than the controls, there was no a statistically significant difference between the groups.

Gross findings of the F1 offspring (weaning necropsy group)

No treatment-related gross findings were observed in any of the treatment groups at the necropsy of the F1 offspring on PND 21.

Physical development of the F1 offspring (growth group)

A statistically significant delay in both eyelid opening of the males and the auricular detachment and eyelid opening of the females was observed in the 51 μg/kg group (Table 5). There were no significant

Table 3 Visceral and skeletal findings in the culled 4-day-old F1 pups of the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
Visceral anomalies				
Pups examined	74	67	73	26
Litters examined	23	20	24	16
Pups with malformations (%)	1 (1.4)	0	0	5** (19.2)[a]
Litters affected (%)	1(4.4)	0	0	5 (31.3)
Pups with variations (%)	23 (31.1)	18 (26.9)	19 (26.0)	10 (38.5)[b]
Litters affected (%)	14 (61.9)	12 (60.0)	13 (54.2)	8 (50.0)
Skeletal anomalies				
Pups examined	82	79	82	33
Litters examined	23	21	24	17
Pups with malformations (%)	0	2 (2.5)	1 (1.2)	10** (30.3)[c]
Litters affected (%)	0	2 (9.5)	1 (4.2)	8** (47.1)
Pups with variations (%)	22 (26.8)	32 (40.5)	25 (30.5)	26** (78.8)[d]
Litters affected (%)	15 (65.2)	16 (76.2)	13 (54.2)	15 (88.2)

**Significant difference at $P < 0.01$ compared with the control group

[a] Anophthalmia, microphthalmia, and dilated cerebral ventricle

[b] Dilated renal pelvis, dilated ureter, and misshapen thymus

[c] Fused cervical arch, fused cervical centrum, fused thoracic arch, fused sternebra, and misaligned sternebra

[d] Cervical rib, extra sternebral ossification site, short/full supernumerary rib, misshapen sternebra, bipartite ossification of cervical/thoracic centrum, and dumbbell ossification of cervical/thoracic centrum

Table 4 Clinical signs in the F1 pups from the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
Male				
No. of pups examined	25	21	25	23
Pups with clinical signs (%)	0	0	0	7* (30.4)
Dome-shaped head	0	0	0	1
Absent eye bulge	0	0	0	5
Small eye bulge	0	0	0	1
Death	0	0	0	2
Female				
No. of pups examined	25	21	25	23
Pups with clinical signs (%)	1 (4.0)	0	1 (4.0)	3 (13.0)
Emaciation	1	0	0	0
Dome-shaped head	0	0	0	2
Absent eye bulge	0	0	0	1
Small eye bulge	0	0	0	1
Death	1 (4.0)	0	1 (4.0)	0

*Significant difference at $P < 0.05$ compared with the control group

differences in hair growth, incisors eruption, testis descent, and vaginal opening in any of the treated groups.

Behavioral tests of the F1 offspring (behavior group)

The behavioral test results of the three treatment groups in terms of the following parameters examined were similar to those obtained from the control group: righting reflex, negative geotaxis, grip-strength, pupillary reflex, acoustic startle response, rotating rod, open field, and swimming test (data not shown).

Body weight of the F1 offspring (growth group)

In the F1 males (Fig. 4), there was a statistically significant suppression in body weight observed on PND 14 in the 17 μg/kg group and on PND 7–70 in the 51 μg/kg group. In the F1 females (Fig. 5), there was a statistically significant suppression in the body weight observed on PND 14 in the 5.7 and 17 μg/kg groups, and on PND 0–49 in the 51 μg/kg group. A statistically significant increase in body weight was observed in the 17 μg/kg group on PND 63.

Gross findings of the F1 offspring (growth group)

At the necropsy of the F1 males on PND 70, hydrocephalus and testicular atrophy were observed in one male in the control group (data not shown). A dilated renal pelvis and testicular atrophy were observed in one and one male in the 5.7 μg/kg group, respectively. A dilated renal pelvis was observed in one male in the 17 μg/kg group. Hydrocephalus, dilated renal pelvis, testicular atrophy, and indented eyeball were observed in three, two, one, and one males in the 51 μg/kg group, respectively. In the F1 females, renal pallor was observed in one female in the 17 μg/kg group. Hydrocephalus, a dilated renal pelvis and an indented eyeball were observed in two, one, and one females in the 51 μg/kg group, respectively.

Organ weights of the F1 offspring (growth group)

A statistically significant increase in the absolute and relative weights of the liver was observed in the males of the 5.7 μg/kg group (Table 6). There was a statistically significant increase in the relative weights of the brain in the males of the 51 μg/kg group. There was a statistically significant decrease in the absolute weight of the heart observed in both genders of the 51 μg/kg group (Table 7).

Table 5 Postnatal physical development in the F1 pups from the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
Male				
No. of pups examined	25	21	25	23
Auricular detachment	2.4 ± 0.51	2.5 ± 0.68	2.5 ± 0.51	2.9 ± 0.56
Hair growth	8.2 ± 0.37	8.2 ± 0.40	8.1 ± 0.33	8.2 ± 0.43
Incisors eruption	10.6 ± 0.51	10.7 ± 0.46	10.6 ± 0.50	10.7 ± 0.46
Eyelid opening	13.5 ± 0.51	13.3 ± 0.46	13.6 ± 0.51	14.1 ± 0.71**
Testis descent	23.2 ± 0.37	23.2 ± 0.40	23.1 ± 0.33	23.2 ± 0.54
Female				
No. of pups examined	25	21	25	23
Auricular detachment	2.4 ± 0.51	2.4 ± 0.60	2.5 ± 0.51	2.9 ± 0.67*
Hair growth	8.1 ± 0.28	8.1 ± 0.30	8.2 ± 0.37	8.2 ± 0.39
Incisors eruption	10.6 ± 0.50	10.4 ± 0.50	10.5 ± 0.51	10.7 ± 0.45
Eyelid opening	13.3 ± 0.48	13.4 ± 0.50	13.5 ± 0.51	14.0 ± 0.77**
Vaginal opening	31.2 ± 0.41	31.2 ± 0.54	31.3 ± 0.48	31.6 ± 0.94

Values are presented as the mean ± SD (day)

*, **Significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

Fertility data of the F1 offspring (reproduction group)

There was a similar the copulation index, fertility index, and pregnancy index in all groups.

Body weight changes in the F1 dams (reproduction group)

There was a statistically significant decrease in the body weight of the F1 dams observed on GD 0–20 in the 51 μg/kg group (Fig. 6).

Gross findings of the F1 dams (reproduction group)

There were no treatment-related gross findings in any of the groups at the necropsy on GD 20.

Caesarean section data of the F1 dams (reproduction group)

There were no significant differences in the number of ovarian corpora lutea, uterine implantations, resorptions, dead fetuses and live fetuses, gender ratio, and

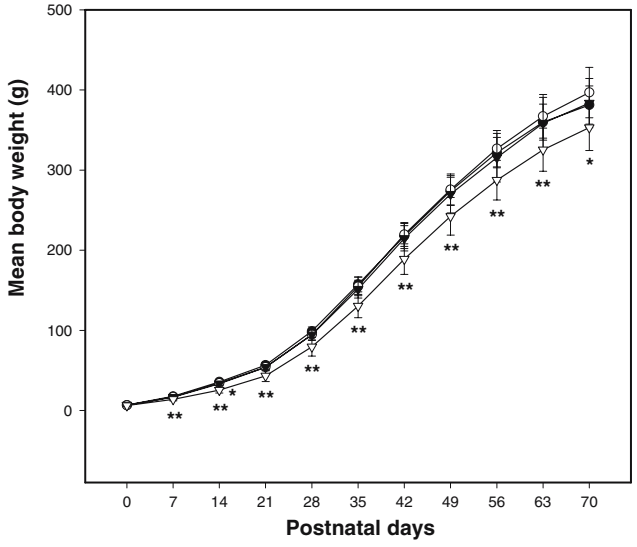

Fig. 4 Changes in the body weights of the F1 male rats from the dams treated with CKD-602 at doses of 0 (*filled circle*), 5.7 (*open circle*), 17 (*filled downward triangle*), and 51 (*open downward triangle*) μg/kg/day during gestation and lactation periods. The values are presented as the mean ± SD. *Single asterisk* and *double asterisks* indicate a significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

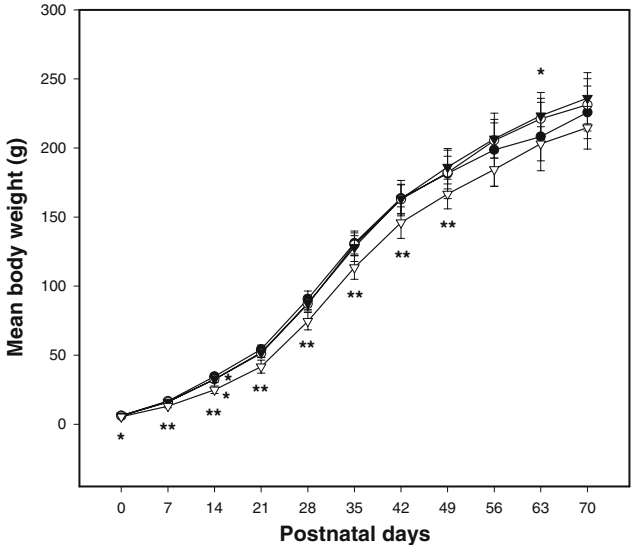

Fig. 5 Changes in the body weights of the F1 female rats from the dams treated with CKD-602 at doses of 0 (*filled circle*), 5.7 (*open circle*), 17 (*filled downward triangle*), and 51 (*open downward triangle*) μg/kg/day during the gestation and lactation periods. The values are presented as the mean ± SD. *Single asterisk* and *double asterisks* indicate a significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

Table 6 Organ weights in the F1 male rats from the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
No. of male rats	25	21	25	21
Body weight	381.3 ± 23.80	396.8 ± 31.44	383.6 ± 30.80	352.9 ± 28.57**
Brain (g)	1.91 ± 0.077	1.93 ± 0.057	1.90 ± 0.088	1.89 ± 0.085
per body weight (%)	0.50 ± 0.030	0.49 ± 0.038	0.50 ± 0.036	0.54 ± 0.034**
Adrenal glands (g)	0.052 ± 0.007	0.050 ± 0.006	0.052 ± 0.008	0.049 ± 0.008
per body weight (%)	0.014 ± 0.002	0.013 ± 0.002	0.014 ± 0.002	0.014 ± 0.002
Liver (g)	15.53 ± 1.621	17.22 ± 2.135**	15.80 ± 1.523	14.80 ± 1.715
per body weight (%)	4.06 ± 0.235	4.33 ± 0.291**	4.12 ± 0.209	4.19 ± 0.297
Spleen (g)	0.67 ± 0.100	0.69 ± 0.109	0.69 ± 0.092	0.63 ± 0.099
per body weight (%)	0.18 ± 0.024	0.17 ± 0.022	0.18 ± 0.025	0.18 ± 0.027
Kidneys (g)	2.85 ± 0.231	2.96 ± 0.282	2.83 ± 0.254	2.77 ± 0.358
per body weight (%)	0.75 ± 0.058	0.75 ± 0.050	0.74 ± 0.039	0.78 ± 0.071
Heart (g)	1.27 ± 0.099	1.29 ± 0.102	1.25 ± 0.101	1.15 ± 0.134**
per body weight (%)	0.33 ± 0.022	0.33 ± 0.025	0.33 ± 0.022	0.33 ± 0.022
Testes (g)	3.16 ± 0.450	3.13 ± 0.496	3.13 ± 0.472	2.95 ± 0.317
per body weight (%)	0.83 ± 0.121	0.79 ± 0.129	0.82 ± 0.128	0.84 ± 0.071

Values are presented as the mean ± SD

**Significant difference at $P < 0.05$ compared with the control group

Table 7 Organ weights in the F1 female rats from the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)			
	0	5.7	17	51
No. of female rats	24	21	24	23
Body weight	255.4 ± 19.22	220.8 ± 48.86	236.1 ± 18.57	213 ± 14.67
Brain (g)	1.81 ± 0.050	1.80 ± 0.056	1.78 ± 0.076	1.76 ± 0.069
per body weight (%)	0.81 ± 0.070	1.14 ± 1.606	0.76 ± 0.058	0.83 ± 0.061
Adrenal glands (g)	0.063 ± 0.011	0.064 ± 0.005	0.067 ± 0.008	0.066 ± 0.013
per body weight (%)	0.028 ± 0.006	0.041 ± 0.003	0.028 ± 0.003	0.031 ± 0.006
Liver (g)	8.78 ± 1.208	9.13 ± 1.246	9.30 ± 1.007	8.63 ± 0.794
per body weight (%)	3.88 ± 0.291	5.76 ± 0.392	3.94 ± 0.240	4.05 ± 0.216
Spleen (g)	0.46 ± 0.078	0.48 ± 0.062	0.49 ± 0.071	0.43 ± 0.065
per body weight (%)	0.21 ± 0.030	0.31 ± 0.494	0.21 ± 0.025	0.20 ± 0.027
Kidneys (g)	1.84 ± 0.173	1.83 ± 0.154	1.89 ± 0.192	1.75 ± 0.167
per body weight (%)	0.82 ± 0.065	1.12 ± 1.462	0.80 ± 0.055	0.82 ± 0.072
Heart (g)	0.86 ± 0.083	0.88 ± 0.062	0.89 ± 0.108	0.80 ± 0.064*
per body weight (%)	0.38 ± 0.029	0.55 ± 0.757	0.38 ± 0.034	0.38 ± 0.028
Ovaries (g)	0.093 ± 0.013	0.092 ± 0.014	0.094 ± 0.016	0.088 ± 0.018
per body weight (%)	0.042 ± 0.007	0.057 ± 0.078	0.040 ± 0.006	0.041 ± 0.008

Values are presented as the mean ± SD

*Significant difference at $P < 0.05$ when compared with the control group

fetal weight between the treatment and control groups. However, an external examination of F2 fetuses revealed external malformations in 2 of the 311 fetuses within 2 of the 21 litters in the 17 μg/kg group, and 3 of the 239 fetuses within 2 of the 17 litters in the 51 μg/kg group (Table 8). Although the incidences of fetal malformations observed in the medium- and high-dose groups were not significantly different from the controls, they showed an increasing tendency. The fetal malformations observed were umbilical hernia, absent eye bulge, agnathia, anal atresia, edema, lordosis, short snout, and a thread-like tail.

Discussion

This study investigated the potential adverse effects of CKD-602 on the maternal function and pre- and postnatal development of Sprague–Dawley rats when administered at doses of 0, 5.7, 17, and 51 μg/kg/day via

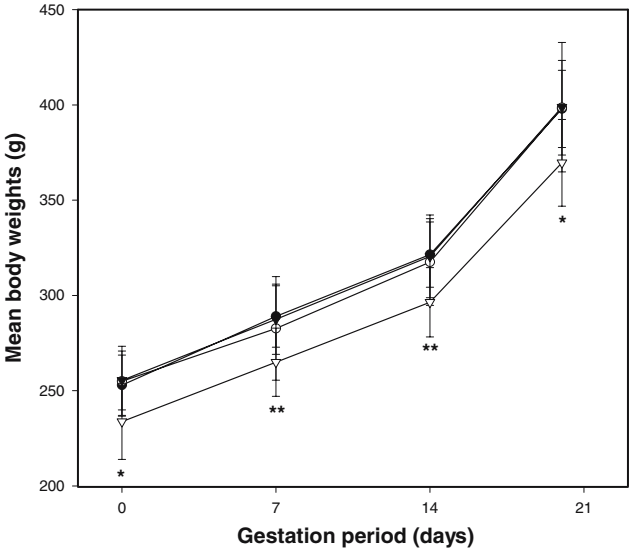

Fig. 6 Changes in the body weights of the F1 pregnant females from the dams treated with CKD-602 at doses of 0 (*filled circle*), 5.7 (*open circle*), 17 (*filled downward triangle*), and 51 (*open downward triangle*) μg/kg/day during the gestation and lactation periods. The values are presented as the mean ± SD. Single *asterisk* and *double asterisks* indicate a significant difference at $P < 0.05$ and $P < 0.01$ compared with the control group, respectively

Table 8 External findings in the F2 fetuses from the dams treated with CKD-602 during the gestation and lactation periods

Parameter	Dose (μg/kg/day)				Historical control range[a]
	0	5.7	17	51	
Fetuses examined	256	270	311	239	4,164
Litters examined	17	19	21	17	305
Fetuses with external abnormalities (%)[b]	0	0	2 (0.6)	3 (1.3)	0–1.4
Litters affected (%)[c]	0	0	2 (9.5)	2 (11.8)	0–16.0
Umbilical hernia (%)	0	0	2 (0.6)	0	0–1.0
Absent eye bulge (%)	0	0	0	2 (0.8)	0
Agnathia (%)	0	0	0	1 (0.4)	0
Anal atresia (%)	0	0	0	1 (0.4)	0
Edema (%)	0	0	0	2 (0.8)	0
Lordosis (%)	0	0	0	1 (0.4)	0
Short snout (%)	0	0	0	1 (0.4)	0
Tread-like tail (%)	0	0	0	1 (0.4)	0–0.5

[a] Kim et al. [31]

[b] A single fetus may be represented more than once in listing the individual defects

[c] Includes litters with one more affected fetuses

an intravenous injection from GD 6 through to LD 21. The results showed that the daily intravenous injection of 51 μg/kg CKD-602 during the gestation and lactation period had several reproductive and developmental effects on the pregnant/lactating dams and their offspring.

Significant maternal toxicity of CKD-602, as evidenced by the suppression in body weight and the reduction in food consumption, was observed in the medium- and high-dose groups. The dose-dependent suppression in the maternal body weight during the gestation and lactation periods was attributed to the administration of the test chemical, which is consistent with the decreased food consumption observed in those groups during the treatment period. This is a clear indication of the general toxicity induced by the CKD-602 treatment, which suggests that this chemical causes anorexia followed by the suppression of body weight in rats. These findings are consistent with those reported in previous toxicity studies [18, 19].

In general toxicity studies, it is well known that the body weight and organ weight are sensitive indicators of potentially toxic chemicals [34, 35]. As described above, the administration of CKD-602 to pregnant/lactating rats caused a significant suppression in body weight in the 17 and 51 μg/kg groups, indicating that the doses of more than 17 μg/kg are maternally toxic to rats. The suppressed body weight in the groups affected the relative weights of some organs such as the brain in the medium dose group, and the brain, adrenal glands, kidneys, and heart in the high-dose group. However, the weight changes in the above organs are of uncertain toxicological significance because they are considered to be the result of a body weight reduction. The significant increase in the absolute spleen weight observed in the medium dose group is believed to be an incidental finding because it did not show a dose–response relationship and a similar change was not found in the high-dose group. In contrast, the decreased absolute and relative weights of the liver and ovaries as well as the increased absolute and relative weights of the spleen observed in the high-dose group suggest a close relationship with the administration of CKD-602. This is because these changes were remarkable and/or showed a dose–response relationship.

At the scheduled necropsy, the significant increase in thymic atrophy observed in the high-dose group is believed to be a treatment-related effect because this finding showed a dramatic increase in both the incidence and severity in comparison with the control group. It is in good agreement with the result of a previous subacute toxicity study [18]. Therefore, it is believed that the thymus, which is an immune organ, is one of the major target organs of CKD-602 in rats.

The pre- and postnatal developmental toxicity of CKD-602 included an increase in the number of stillbirths, a decrease in the litter size, an increase in the postnatal mortality and number of congenital abnormalities, as well as a delay in postnatal development.

The dose-dependent increase in developmental toxicity indicates that this is caused by CKD-602. The increase in the number of stillbirths observed in the high-dose group is closely related to the administration of CKD-602 because there was a clear dose–response relationship, which is in good agreement with the increased postnatal mortality to PND 4. The increased number of stillbirths and postnatal mortality was probably due to an increase in the major congenital abnormalities, as shown in Tables 3 and 5. It should be noted that a congenital anomaly is a major cause of fetal and neonatal death in both humans and experimental animals [36–38]. The main signs of congenital anomalies observed in the high-dose group were detected in the face, brain, vertebra, rib, and sternum. Characteristic anomalies included a dome-shaped head, absent/small eye bulge, ano-/microphthalmia, dilated cerebral ventricle, fused vertebra, fused/misaligned/misshapen sternebra, cervical/lumbar rib, extra sternebral ossification site, bipartite/dumbbell ossification of the vertebral centrum, etc.

The dose-dependent decrease in the litter size at birth observed in the high-dose group resulted from the increase in post-implantation loss, indicating that this dose is lethal to embryo–fetuses in rats. It was previously reported that an intravenous injection of CKD-602 into rats at a dose level of 80 μg/kg/day during the major organogenetic period caused an increase in the number of fetal deaths, a decrease in the litter size, and an increase in the number of fetal malformations [19]. These results as well as our previous findings [19] suggest that CKD-602 adversely affects the viability of F1 generation not only during the embryo–fetal developmental period but also during the postnatal developmental period. These results also indicate that the embryo–fetuses and neonates are more susceptible to the adverse effects of CKD-602 treatment than their dams.

The significant delay in auricular detachment and eyelid opening observed in the F1 offspring of the high-dose group is believed to be treatment-related because there was a clear dose–response relationship, which is consistent with the significant suppression of body weight during the postnatal period. The delayed auricular detachment and eyelid opening along with the suppressed body weight of the F1 offspring observed in the high-dose group are believed to be indications of the growth retardation effects induced by the CKD-602 treatment. The significant suppression in body weight observed in the F1 males in the 17 μg/kg group and the F1 females in the 5.7 and 17 μg/kg groups on PND 14 is also considered to be a treatment-related effect. However, it was of no toxicological significance because it was very slight and there were no accompanying pathological findings. The significant increase in body weight observed in the 17 μg/kg group on PND 63 is believed to be an accidental finding because it was a transient change and there was no dose–response relationship.

At the scheduled necropsy of the F1 offspring on PND 70, there were some abnormal gross findings found in the medium and high-dose groups at a low frequency. However, these findings were not believed to be treatment-related because they occurred infrequently and the incidences were similar to the controls. On the other hand, the slight increase in the incidence of hydrocephalus and indented eyeballs observed in the high-dose group of both genders was considered to be treatment-related because they were uncommon in Sprague–Dawley rats and consistent with the increased incidence of external malformations. The organ weight changes observed in the F1 offspring of the treatment groups are not believed to be treatment-related because they were slight and there was no dose–response relationship. In addition, the behavioral and mating trials showed that the gestational and lactational administration of CKD-602 did not significantly affect the behavioral function and mating performance of the F1 offspring at the doses tested.

The suppressed body weight of the F1 dams observed in the 51 μg/kg group during pregnancy may largely be due to the growth retardation effects of CKD-602 on the growing F1 offspring, which suggests that the gestational and lactational administration of CKD-602 did not have an adverse effect on the maternal body weight of the F1 dams. Although the slight increase in the incidence of malformed F2 fetuses observed in the high-dose group was not statistically significant in comparison with the controls, it showed a clear-cut dose–response relationship and most of the findings were classified as major congenital malformations that are uncommon in normal Sprague–Dawley rats [31–33]. Therefore, we considered that the gestational and lactational administration of CKD-602 to F0 dams causes a slight increase in the incidence of external malformations in their F2 fetuses. The fact that the administration of CKD-602 to the F0 dams induced teratogenicity in not only the F1 offspring but also in the F2 fetuses is quite interesting, and will require further study. Meanwhile, the umbilical hernia observed in the medium dose group was not considered to be treatment-related, because the incidence was within the limits of normal biological variations and the finding is common in the normal Sprague–Dawley rat [31–33].

Camptothecins are cancer chemotherapeutic agents that suppress the topoisomerase I enzyme, which is present at high levels in solid tumors [10, 39]. Because

of the unique mechanism of action, they have various adverse effects on not only multiple organs such as the bone marrow, gastrointestinal tract, reproductive organs, mucosal membrane, and hair follicles but also an embryo–fetuses, which have a very high cell proliferation rate. Although there are few reports of the potential effects of CPTs on reproduction and development in animals [19, 21, 40, 41], the results in this study as well as those reported in the literature clearly show that the CPT anticancer agents have adverse effects on reproduction and development in experimental animals. These results are expected to provide some information relevant to the safety assessment of test chemical exposure during pregnancy and lactation, with particular focus on in utero growth, viability, pre- and postnatal development, as well as on the behavioral and reproductive function.

In conclusion, the intravenous administration of CKD-602 at 51 μg/kg/day to rats during gestation and lactation periods caused significant maternal toxicity including a suppression in body weight, a reduction in food consumption, an increase in the incidence of thymic atrophy, a decrease in the weights of the liver and ovaries, and an increase in the weight of the spleen in dams. It also caused significant developmental toxicity including an increase in the number of stillbirths and postnatal mortality, a decrease in the litter size, a delay in physical development, and an increase in the incidence of congenital anomalies in their offspring. A dose of 17 μg/kg/day caused only slight maternal toxicity including a suppression in body weight and a reduction in food consumption. Under the present experimental conditions, the no-observed-adverse-effect levels of CKD-602 for dams and their offspring are believed to be 5.7 and 17 μg/kg/day, respectively.

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