Table 4: Developmental findings in rats given DCBS by gavage.

Dose (mg/kg bw/day)	0 (control)	6	25	100	400
No. of litters	10	10	10	9	3
No. of corpora lutea[a]	19.7 ± 2.0	18.0 ± 2.3	18.5 ± 2.4	17.6 ± 1.3	16.3 ± 1.2*
No. of implantations[a]	18.8 ± 1.4	16.9 ± 2.3	17.4 ± 1.8	17.4 ± 1.4	15.3 ± 1.7*
Implantation index (%)[b]	95.9	94.0	94.8	99.3	93.7
No. of pups born[a]	17.0 ± 2.9	15.8 ± 1.9	15.5 ± 1.7	16.1 ± 1.9	12.0 ± 3.1*
No. of pups born alive[a]	16.8 ± 2.9	15.4 ± 1.6	14.5 ± 2.0	15.4 ± 1.9	4.0 ± 3.8*
No. of still born[a]	0.2 ± 0.4	0.4 ± 0.7	1.0 ± 2.0	0.7 ± 0.7	8.0 ± 4.0*
Delivery index (%)[c]	100	100	100	90	80
Live birth index (%)[d]	98.8	97.7	94.0	95.9	31.4*
Viability index on postnatal day 4 (%)[e]	97.1	99.3	93.6	88.9	0*
Sex ratio of pups born (males/females)	80/90	69/89	77/78	66/79	30/30
Body weight of male pups during lactation (g)[a]					
Day 0	6.4 ± 0.5	6.5 ± 0.5	6.7 ± 0.5	6.5 ± 0.2	5.6 ± 0.4
Day 4	10.2 ± 1.5	10.4 ± 1.2	10.9 ± 1.1	10.3 ± 0.6	
Body weight of female pups during lactation (g)[a]					
Day 0	6.0 ± 0.5	6.2 ± 0.4	6.4 ± 0.6	6.2 ± 0.2	5.3 ± 0.3
Day 4	9.5 ± 1.3	10.0 ± 1.0	10.6 ± 1.2	10.0 ± 0.8	
External examination of pups[f]					
Total no. of pups (litters) examined	170 (10)	158 (10)	154 (10)[h]	145 (9)	60 (5)
Total no. of pups (litters) with malformations	0	1	0	1	5 (1)
Cleft palate	0	0	0	0	5 (1)
Micromelia and ectrodactyly	0	1	0	0	0
Short tail	0	0	0	1	0
Internal examination of pups[g]					
Total no. of pups (litters) examined	169 (10)[h]	157 (10)[h]	151 (10)[i]	138 (9)[j]	57 (5)[k]
Total no. of pups (litters) with variations	1	6 (4)	2 (1)	4 (2)	1
Thymic remnants in neck	1	3 (3)	2 (1)	1	0
Left umbilical artery	0	3 (2)	0	1	0
Dilatated renal pelvis	0	0	9	2 (1)	1

[a]Values are expressed as Mean ± SD.
[b]Implantation index (%) = (no. of implantations /no. of corpora lutea) x 100.
[c]Delivery index (%) = (no. of pups born/no. of implantations) x 100.
[d]Live birth index (%) = (no. of pups born alive/no. of pups born) x 100.
[e]Viability index on postnatal day 4 (%) = (no. of live pups on postnatal day 4/no. of live pups on postnatal day 0) x 100.
[f]External examinations were performed in all pups born (live born and stillborn) on postnatal day 0.
[g]Internal examinations were performed in all pups (dead pups just after death and live pups on postnatal day 4).
[h]One pup was not examined because of cannibalism.
[i]Four pups were not examined because of cannibalism.
[j]Seven pups were not examined because of cannibalism.
[k]Three pups were not examined because of cannibalism.
*Significantly different from the control, $p < 0.05$.

male and female rats. The data show that DCBS exerts general toxicity and reproductive and developmental toxicity at relatively high doses.

DCBS was given to males during the premating and mating periods and to females during the premating, mating, and pregnancy periods and shortly after parturition. The dosage used in the current study was sufficiently high that it should be expected to induce general toxic effects. As expected, general toxicity, such as death, lowered body weight and food consumption, and toxicologic signs, was observed at 400 mg/kg bw per day. In males, lowered body weight during the whole period of administration and salivation was noted only in two of the 10 males at 400 mg/kg bw per day. In females, however, the lowered body weight was found only on day 20 of pregnancy, and deaths and significantly increased incidences of toxicologic signs, including decreased locomotor activity, soil of the lower abdominal fur, and reddish tears, were noted at 400 mg/kg bw per day. Some toxicologic signs during mid to late pregnancy and deaths during the periparturition period were noted in females. These findings may suggest that female rats have a higher susceptibility to the toxicity of DCBS than male rats. One possible explanation for the higher susceptibility to DCBS toxicity in females may be enhancement of the toxicity of DCBS by the stress of the pregnancy/parturition status in female rats. More precisely, DCBS may be more toxic in females during pregnancy and/or lactation.

Reduced platelet counts at 6 mg/kg bw per day, increased serum levels of total cholesterol at 25 and 100 mg/kg bw per day, and decreased serum levels of sodium at 6 and 400 mg/kg bw per day were found. However, changes in these parameters were thought to have no toxicologic meaning because these changes were relatively slight and were not dose-dependent. Several organ weights were affected by the administration of DCBS. Higher relative weight, but not absolute weight, of the testis and liver in females were observed at 400 mg/kg bw per day. Body weights of male and female rats on the day of scheduled sacrifice were lowered at 400 mg/kg bw per day. The higher relative weights of the testis and liver at the highest dose seem to be due to secondarily lowered body weight, but not due to the direct effects of DCBS on the organs. A decreased weight of the thymus was detected in both sexes at 400 mg/kg bw per day, and these changes were accompanied by atrophy revealed by histopathologic examinations. Atrophy of the spleen was also noted in females at the highest dose. These findings may suggest that one of the target systems of DCBS toxicity is the immune system. The increases in the absolute weights of the kidney at 6, 25, and 100 mg/kg bw per day in male rats are unlikely to be due to the toxic effects of DCBS, because the degree of changes in absolute weight was relatively small and no changes were noted in relative weight. Histopathologic examinations revealed the hyalin droplets in the proximal tubular epithelium in the kidney at 100 and 400 mg/kg bw per day in males. These histopathologic changes are thought to be due to the induction of α2u-globulin accumulation (Hamamura et al., 2006). In the current study,

this type of nephropathy was not detected in female rats. The nephropathy induced by α2u-globulin accumulation is male rat–specific and is unlikely to occur in humans (Hard et al., 1993). Consideration of these findings together suggests that the histopathologic changes in the male kidney are not relevant to human health although 100 mg/kg bw per day was an effect level in male rats.

Male reproductive parameters, including the histopathology of the reproductive organs, were not affected by the administration of DCBS even at the highest dose. These suggest that DCBS is not toxic to male reproduction in rats. In female rats, no adverse effects on the maternal reproductive parameters, including the mating index, fecundity index, and histopathology of the reproductive organs, were found. However, deaths and toxicologic signs during late pregnancy, a decrease in the gestation index, and total litter loss were noted in females at 400 mg/kg bw per day. These indicate that DCBS possesses toxic effects on female reproduction at 400 mg/kg bw per day.

As for the developmental parameters, decreases in the numbers of corpora lutea, implantations, total pups and live pups delivered, live birth index and viability on PND 4, and an increase in the number of stillborn were detected at 400 mg/kg bw per day. These findings indicate that DCBS is toxic to the survival and growth of offspring and exerts developmental toxicity at 400 mg/kg bw per day in rats.

In the current study, external malformations and variations of the internal organs were found in pups in the DCBS-treated groups. However, incidences of pups with malformations and variations were very low and not significantly different from those in the control group. No consistent tendency was found in the incidence of pups with these morphologic alterations. Furthermore, the external malformations and variations of the internal organs observed in the current study are of the types that occur spontaneously among control rat fetuses reported in the literature (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). Therefore, it seems unlikely that the morphologic changes in pups observed in the current study indicate a teratogenic response and that DCBS possessed teratogenic potential in rats. In the current study, external and internal examinations in the newborn rats were performed, but no skeletal examinations were carried out. To accurately evaluate the prenatal developmental toxicity, including teratogenicity, it is necessary to interrupt pregnancy 12–24 h before the expected term either by hysterectomy or the necropsy of maternal animals (Wilson, 1965, 1973).

The most deleterious effect of DCBS on reproduction and development is the marked decrease in the number of live pups. The most striking adverse effect noted in the current study is a total loss of pups until PND 4 at 400 mg/kg bw per day. The primary effects may be on the gestation index for dams and live birth index for pups, which appear to be affected at multiple points along the female reproductive process, as well as an viability of neonatal pups. The

current study was performed in compliance with the OECD guideline Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, and this screening test guideline does not provide complete information on all aspects of reproduction and development due to the relatively small numbers of animals in the dose groups and selectivity of the end points. In order to further evaluate the reproductive and developmental toxicity of DCSB in rats, a two-generation reproductive toxicity study is currently in progress.

In conclusion, DCBS caused deaths in females, and decreased body weight and changes in urinalysis, blood chemistry, and/or histopathology in both sexes at 400 mg/kg bw per day. Adverse effects on reproductive and developmental parameters were noted at 400 mg/kg bw per day. At this dose, all dams lost their litters at delivery or by day 4 of lactation. The NOAEL for repeat dose toxicity is considered to be 100 mg kg^{-1} day^{-1} in male and female rats, and the NOAEL for reproductive/developmental toxicity is considered to be 100 mg kg^{-1} day^{-1}.

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ORIGINAL ARTICLE

Evaluation of reproductive and developmental toxicity of the rubber accelerator N,N-dicyclohexyl-2-benzothiazolesulfenamide in rats

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ABSTRACT **Male and female Crl:CD(SD) rats were fed a diet containing the rubber accelerator N,N-dicyclohexyl-2-benzothiazolesulfenamide (DCBS) at 0, 1500, 3000, 6000 or 10 000 p.p.m. (0, 83, 172, 343 or 551 mg/kg bw/day in males and 0, 126, 264, 476 or 707 mg/kg bw/day in females) for a total of 57 days beginning 16 days before mating in males, and a total of 61–65 days from 16 days before mating to day 21 of lactation in females. Body weight gains and food consumption were reduced in males at 6000 p.p.m. and higher and in females at 3000 p.p.m. and higher. The weights of the spleen at 6000 and 10 000 p.p.m. and of the thymus at 10 000 p.p.m. were decreased in females. No changes in estrous cyclicity, copulation index, fertility index, gestation index, delivery index, precoital interval or gestation length were observed at any dose of DCBS. Numbers of implantations at 6000 and 10 000 p.p.m. and pups delivered at 10 000 p.p.m. were reduced. There were no changes in the sex ratio or viability of pups. The body weights of male and female pups were lowered at 6000 p.p.m. and higher. Decreased weight of the spleen in weanlings was also observed in males at 1500 p.p.m. and higher and in females at 3000 p.p.m. and higher. The data indicate that DCBS possesses adverse effects on reproduction and development in rats.**

Key Words: developmental toxicity, N, N-dicyclohexyl-2-benzothiazolesulfenamide, rat, reproductive toxicity, rubber accelerator

INTRODUCTION

Sulfenamide accelerator compounds are widely used in the manufacture of automotive compartments and industrial rubber products such as tires, hoses, conveyer belts, bushings seals, gaskets and windshield wiper blades (EPA 2001). N,N-Dicyclohexyl-2-benzothiazolesulfenamide (DCBS, Fig. 1) is a sulfenamide accelerator. The annual production level of DCBS in Japan was approximately 1000 tons in 1990–1993 and 1900 tons in 2000–2003. Most of this amount was sold and handled domestically (OECD 2007). DCBS is used as an accelerator of vulcanization and is completely reacted in the vulcanizing process (OECD 2007). DCBS is regulated in Germany for use in articles that contact food, but is not regulated by the United States Food and Drug Administration for use in food contact applications (Flexsys 2000). Exposure of workers handling sulfenamide accelerator materials is likely to be highest in the area of materials packaging. During material packout at the manufacturing site, and to a lesser degree during weigh-up activities at the consumer site, there is a possibility of skin and inhalation exposure. Although consumer exposure should be minimal, the most likely route of consumer exposure is skin contact with rubber or latex articles (EPA 2001).

Only up to 6% biodegradation has been determined for DCBS in a ready biodegradability test, and a measured log Kow value of 4.8 suggests that DCBS may have a high bioaccumulation potential (OECD 2007). The possibility of such a chemical compound entering biological systems has aroused great concern regarding its toxicological potential. Generally, biological effects of chemicals should be studied in laboratory animals to investigate their possible influences on human health, and the results of animal tests of chemical toxicity relevant to humans (Clayson & Krewski 1990). However, very little information on the toxicity of DCBS has been published. The toxic effects of DCBS have been briefly summarized by the European Chemical Bureau (2000) and US EPA (2001). It was reported that the oral LD50 values were 1077–10 000 mg/kg bw in rats, the oral NOAEL for 44-day repeated dose toxicity was higher than 100 mg/kg bw/day in rats, and no effects on reproduction were observed at doses up to 400 mg/kg bw/day in rats (EPA 2001). The oral LD50 value was 8500 mg/kg bw in male mice, and repeated daily inhalation exposure of male rats for 15 days at 2 h/day and 350–400 mg/m^3 caused mucous membrane irritation (Vorobera 1969).

The Japanese Government (MHW 1998) conducted toxicity studies for DCBS, including acute toxicity, *in vitro* genotoxicity and repeat dose toxicity combined with reproductive/developmental toxicity as a part of the Safety Examination of Existing Chemical Substances and Chemical Safety Programmes. These toxicity studies are summarized in the IUCLID Data Sets (EPA 2006), OECD Screening Information Data Sets (OECD 2007) and the Hazard Assessment Sheet (CERI 2002). We previously reported the results of a screening test for repeat dose toxicity combined with a reproductive/developmental toxicity in rats, where DCBS at 400 mg/kg bw/day had a deleterious effect on reproduction and development and caused a marked decrease in the number of live pups as well as a total loss of pups by postnatal day (PND) 4 (Ema *et al.* 2007). The primary effects may be on the gestation index for dams and live birth index for pups, both of which appear to be affected at multiple points along the female reproductive process. The viability of neonatal pups may also be affected. To examine the adverse effect of dietary DCBS on survival and growth of pups, a reproductive and developmental toxicity study was performed in rats given DCBS during an extended administration period up to the weaning of pups.

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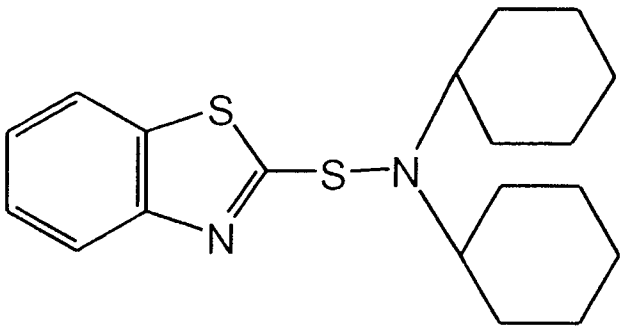

Fig. 1 Structural formula of N,N-dicyclohexyl-2-benzothiazolesulfenamide.

MATERIALS AND METHODS

This study was performed in 2005–2006 at the Safety Research Institute for Chemical Compounds (Sapporo, Japan) in compliance with *Law for the Humane Treatment and Management of Animals* (Law no. 105, October 1, 1973, revised December 22, 1999, Revised Law no. 221; revised June 22, 2005, Revised Law no. 68), *Standards Relating to the Care, Management and Refinement of Laboratory Animals* (Notification no. 88 of the Ministry of the Environment, Japan, April 28, 2006) and *Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in the Testing Facility under the Jurisdiction of the Ministry of Health, Labour and Welfare* (Notification no. 0601005 of the Health Sciences Division, Ministry of Health, Labour and Welfare, Japan, June 1, 2006).

Chemical and dosing

DCBS (CAS no. 4979-32-2) was obtained from Ouchishinko Chemical Industrial (Tokyo, Japan). DCBS in the form of off-white to tan granules is very slightly soluble in water and methanol but soluble in oil. Its melting point is 100–105°C, density is 1230 kg/m^3 and molecular weight is 347 (Flexsys 2000). DCBS (Lot no. 508001) used in this study was 99.7% pure and was kept in a sealed container under cool (1–8°C) and dark conditions. The purity and stability of the chemical were verified by analysis using high-performance liquid chromatography before and after the study. Rats were given dietary DCBS at a concentration of 0 (control), 1500, 3000, 6000 or 10 000 p.p.m. Males were fed a diet containing DCBS for a total of 57 days beginning 16 days before mating. Females were fed a diet containing DCBS for a total of 61–65 days from 16 days before mating to day 21 of lactation throughout the mating, gestation and lactation periods. Control rats were fed a basal diet only.

The dosage levels were determined based on the results of a previous study in rats that were given DCBS by gavage at 0, 6, 25, 100, or 400 mg/kg bw/day for a total of 44 days from 14 days before mating in males and a total of 40–51 days beginning 14 days before mating to day 3 of lactation throughout the mating and gestation periods in females (Ema *et al.* 2007). In that study, toxicologically significant changes were observed only at 400 mg/kg bw/day. Three of 10 females died during parturition. An increased incidence of females showing decreased locomotor activity, soil of the lower abdominal fur and reddish tears was observed. Decreased body weights were found in males and females. Decreased weight of the thymus in both sexes was noted. Decreases in the gestation index, numbers of corpora lutea, implantations, pups born and pups born alive, live birth index and viability index were detected.

Dosed diet preparations were formulated by mixing DCBS into an appropriate amount of a powdered basal diet (CRF-1; Oriental Yeast, Tokyo, Japan) for each dietary concentration. Chemical analysis showed that DCBS in the diet was stable for at least 21 days at room temperature and the formulations were maintained in a room temperature for no more than 21 days. Generally, the diet was replaced once a week.

Animals and housing conditions

Sprague–Dawley (Crl:CD[SD]) rats were used throughout this study. Rats of this strain were chosen because they are the most commonly used in reproductive and developmental toxicity studies and historical control data are available. Male and female rats at nine weeks of age were purchased from the Tsukuba Breeding Center (Charles River Laboratories Japan, Yokohama, Japan). The rats were acclimated to the laboratory for six days prior to the start of the experiment. Male and female rats found to be in good health were selected for use. Rats (F0) were randomly distributed into five groups of six males and six females each, and all animals were assigned a unique number and tattooed on the ear prior to the start of the experiment. Animals were housed individually in suspended aluminum/stainless steel cages except during the acclimation, mating and nursing periods. From day 17 of pregnancy to the day of weaning, individual dams and litters were reared using wood chips as bedding (White Flake; Charles River Laboratories Japan,).

Animals were reared on a basal diet or a diet containing DCBS and filtered tap water *ad libitum* and maintained in an air-conditioned room at 22 ± 3°C with a humidity of 50 ± 20% and a 12-h light (8:00–20:00)/dark (20:00–8:00) cycle. The room was ventilated 10–15 times/h.

Observations

All rats were observed twice a day for clinical signs of toxicity. The body weight was recorded once a week for males and once a week during the premating period, on days 0, 7, 14 and 20 of pregnancy, and on days 0, 4, 7, 14 and 21 of lactation for females. Food consumption was recorded once a week for males, and once a week during the premating period, on days 0, 7, 14 and 20 of pregnancy and on days 0, 7, 14 and 21 of lactation for females.

Rats were euthanized by exsanguination under ether anesthesia. Males were euthanized at 17 weeks and females at 18 weeks on day 21 of lactation. The external surfaces of the rats were examined for abnormalities. The abdomen and thoracic cavities were opened and gross internal examination was performed. In females, the number of implantation sites was recorded. The brain, pituitary, thymus, thyroid, liver, kidney, spleen, adrenal gland, testis, epididymis, seminal vesicle, ventral prostate, ovary and uterus were weighed. The thyroid and seminal vesicle were weighed after fixation with 10% neutral buffered formalin.

Daily vaginal lavage samples from each female were evaluated for estrous cyclicity for two weeks of the premating period. Females with repeated 4–6 day estrous cycles were judged to be normal. Each female rat was mated overnight with a single male rat of the same dosage group until copulation occurred. During the mating period, daily vaginal smears were examined for the presence of sperm. The presence of sperm in the vaginal smear and/or a vaginal plug was considered evidence of successful mating (day 0 of pregnancy). Copulated females were checked for signs of parturition three times a day on days 21–23 of pregnancy.

The females were allowed to deliver spontaneously and nurse their pups until PND 21. The day on which parturition was

completed by 13:00 was designated as PND 0. Total litter size and the numbers of live and dead pups were recorded. Live pups were counted, sexed, examined grossly and individually weighed on PND 0, 4, 7, 14 and 21. On PND 4, litters were randomly adjusted to eight pups comprised of four males and four females. No adjustment was made for litters with fewer than 8 pups. Selected pups were assigned a unique number and tattooed on a limb on PND 4. Unselected pups were necropsied on PND 4. Weanlings were necropsied on PND 21 and the brain, thymus, liver, spleen and uterus were weighed.

Statistical analysis

Statistical analysis of the offspring was carried out using the litter as the experimental unit.

Body weight, body weight gain, food consumption, length of estrous cycle, precoital interval, gestation length, number of implantations and pups delivered, delivery index, organ weight, organ/body weight ratio (relative organ weight) and the viability of pups were analyzed for statistical significance in the following way. Bartlett's test of homogeneity of variance was used to determine if the groups had equivalent variances. If the variances were equivalent, the groups were compared by one-way analysis of variance (ANOVA). If significant differences were found, Dunnett's multiple comparison test was performed. If the groups did not have equivalent variances, the Kruskal–Wallis test was used to assess the overall effects. Whenever significant differences were noted, pairwise comparisons were made by Mann–Whitney *U*-test. The incidence of females with normal estrous cycles, copulation index, fertility index, gestation index and neonatal sex ratio was analyzed by the χ^2 test or Fisher's exact test.

The 0.05 level of probability was used as the criterion for significance.

RESULTS

Clinical observations, body weight and food consumption (F0 males and females)

No deaths were found in F0 males and females. In males, there were no compound related clinical signs of toxicity at any doses. Hematuria and soil of perigenital fur were each observed at 10 000 p.p.m. in one female.

Table 1 shows body weight gain in F0 males and females during dosing. In males, body weight gain on days 0–7 of the dosing period at 6000 p.p.m. and higher was significantly lowered. In females,

Table 1 Body weight gains of F0 parental male and female rats given N,N-dicyclohexyl-2-benzothiazolesulfenamide

Dose (p.p.m.)	0 (Control)	1500	3000	6000	10 000
No. males	6	6	6	6	6
Initial body weight (g)†	367 ± 7	367 ± 6	366 ± 7	366 ± 8	366 ± 7
Body weight gain during dosing period (g)†					
Days 0–7	48.0 ± 10.4	36.8 ± 14.5	36.3 ± 4.8	26.7 ± 8.5**	25.2 ± 6.5**
Days 7–14	38.2 ± 9.2	33.7 ± 13.4	34.5 ± 8.7	35.2 ± 5.6	29.5 ± 5.5
Days 14–21	21.7 ± 9.2	27.3 ± 7.1	24.3 ± 5.2	23.0 ± 12.6	21.8 ± 3.1
Days 21–28	26.8 ± 10.2	25.7 ± 8.3	22.3 ± 10.5	23.5 ± 6.7	25.2 ± 5.0
Days 28–35	21.2 ± 7.7	20.8 ± 6.5	28.5 ± 12.6	24.0 ± 3.7	19.2 ± 4.1
Days 35–42	14.8 ± 6.9	15.3 ± 6.5	20.3 ± 6.9	17.3 ± 6.3	20.5 ± 5.2
Days 42–49	13.8 ± 8.3	19.5 ± 4.2	13.5 ± 2.7	19.8 ± 5.5	17.2 ± 2.9
Days 49–56	14.8 ± 7.6	19.5 ± 6.3	16.7 ± 5.0	20.5 ± 6.2	17.0 ± 3.5
No. females	6	6	6	6	6
Initial body weight (g)†	238 ± 6	239 ± 7	237 ± 5	238 ± 6	237 ± 7
Body weight gain during premating period (g)†					
Days 0–7	6.5 ± 7.7	8.8 ± 8.8	6.8 ± 4.6	−6.5 ± 9.7*	−19.3 ± 9.3**
Days 7–14	15.7 ± 8.5	16.3 ± 6.9	14.2 ± 5.9	12.2 ± 8.0	13.0 ± 8.7
Body weight gain during pregnancy (g)†					
Days 0–7	45.3 ± 6.5	42.5 ± 4.2	32.8 ± 5.4*	31.2 ± 8.6*	19.5 ± 12.0**
Days 7–14	38.3 ± 6.0	35.7 ± 5.4	36.8 ± 7.0	35.2 ± 6.2	31.2 ± 8.6
Days 14–20	76.7 ± 14.6	68.3 ± 4.3	75.8 ± 12.4	68.7 ± 7.7	62.5 ± 12.2
Body weight gain during lactation (g)†					
Days 0–4	28.0 ± 15.7	10.8 ± 24.3	28.0 ± 15.7	8.2 ± 8.7	−2.5 ± 14.6*
Days 4–7	6.5 ± 2.7	12.0 ± 9.0	10.0 ± 10.2	5.3 ± 7.3	0.5 ± 10.9
Days 7–14	1.3 ± 10.7	10.2 ± 7.3	4.2 ± 8.1	14.2 ± 11.1	6.0 ± 12.5‡
Days 14–21	−19.0 ± 14.7	−31.7 ± 9.9	−17.0 ± 8.3	−8.8 ± 9.8	2.6 ± 14.3‡†

*Significantly different from the control, $P < 0.05$; **significantly different from the control, $P < 0.01$.

†Values are given as mean ± SD; ‡data were obtained from five females because one female was excluded (total litter loss on day 9 of lactation).

body weight gains were decreased on days 0–7 of the premating period at 6000 p.p.m. and higher, on days 0–7 of pregnancy at 3000 p.p.m. and higher, and on days 0–4 of lactation at 10 000 p.p.m. Body weight gain on days 14–21 of lactation was significantly increased at 10 000 p.p.m.

In F0 males, food consumption was significantly decreased during the first week at 6000 p.p.m. and higher and during the second week at 10 000 p.p.m. In F0 females, food consumption was significantly decreased throughout the premating, pregnancy and lactation periods at 6000 and 10 000 p.p.m., except on days 7–14 and 14–20 of pregnancy at 6000 p.p.m. A tendency towards decreased food consumption was observed on days 0–7 of pregnancy at 3000 p.p.m.

The mean daily intakes of DCBS were 83, 172, 343 and 551 mg/kg bw in F0 males, and 126, 264, 476 and 707 mg/kg bw in F0 females for 1500, 3000, 6000 and 10 000 p.p.m., respectively.

Estrous cyclicity (F0 females)

All F0 females showed normal estrous cycles in all groups, and the length of the estrous cycles was not significantly different between the control and DCBS-treated groups.

Reproductive and developmental effects (F0 parents/F1 offspring)

The reproductive and developmental parameters for F0 parents/F1 offspring are presented in Table 2. In F0 parent animals in all groups, all pairs copulated, all male and female rats were fertile and all females delivered live pups. All rats of all groups mated within four days. There were no significant differences between control and DCBS-treated groups in copulation index, fertility index, gestation index, precoital interval, gestation length, delivery index, sex ratio of F1 pups, or viability of F1 pups during lactation. Significantly lower numbers of implantations at 6000 and 10 000 p.p.m. and pups delivered at 10 000 p.p.m. were observed. Body weights of male pups were significantly lowered on PND 4, 7 and 21 at 6000 p.p.m. and on PND 7, 14 and 21 at 10 000 p.p.m. In female pups, significantly lower body weights were observed on PND 7, 14 and 21 at 6000 p.p.m. and higher. No malformed pups were detected in any groups.

Necropsy and organ weights (F0 males and females)

Atrophy of the thymus was found in two females at 10 000 p.p.m. No compound-related gross lesions of the reproductive organs were noted in F0 males and females. In males, significantly increased relative weights of the liver and kidney were observed at 10 000 p.p.m.

The organ weights of F0 females are shown in Table 3. The body weight at the scheduled terminal sacrifice was significantly lowered at 6000 and 10 000 p.p.m. The absolute weight of the ovary was significantly lowered at 10 000 p.p.m. Significantly increased relative weights were found for the pituitary at 3000 p.p.m., the liver at 6000 p.p.m., and the brain, kidney and adrenal gland at 10 000 p.p.m. The absolute and relative weights of the thymus at 10 000 p.p.m. and the spleen at 6000 p.p.m. and higher were significantly decreased.

Necropsy and organ weights (F1 weanlings)

No compound related gross lesions were observed in F1 weanlings.

The organ weights of F1 male weanlings are presented in Table 4. The body weight at the scheduled sacrifice was significantly reduced at 6000 and 10 000 p.p.m. The absolute weights of the brain at 6000 and 10 000 p.p.m. and the liver at 10 000 p.p.m. were also significantly reduced. The relative weights of the liver at 1500 and 6000 p.p.m. and of the brain at 10 000 p.p.m. were significantly increased. Significantly decreased absolute and relative weights of the spleen, except for the relative weight at 3000 p.p.m., were noted at 1500 p.p.m. and higher.

The organ weights of F1 female weanlings are presented in Table 5. The body weight at the scheduled sacrifice was significantly reduced at 6000 p.p.m. and higher. Significantly reduced absolute weights of the brain at 6000 and 10 000 p.p.m., the liver at 10 000 p.p.m., and the uterus at 3000 p.p.m. and 10 000 p.p.m. were also observed. The relative weight of the brain was significantly increased at 10 000 p.p.m. The absolute and relative weights of the spleen were significantly reduced at 3000 p.p.m. and higher.

DISCUSSION

This study was designed to assess the effects of DCBS on continuous parameters such as body weight and food consumption, as well as endpoints for reproductive and developmental toxicity.

Significant decreases in body weight gain and food consumption were observed at 6000 p.p.m. and higher in F0 males and females. In females at 3000 p.p.m., body weight gain was significantly decreased during early pregnancy. Food consumption also decreased, but not significantly. The data indicate that changes in body weight gain were associated with changes in food consumption and that DCBS adversely affects body weight gain and food consumption at 6000 p.p.m. in male rats and 3000 p.p.m. in female rats. The higher relative weights of the liver and kidney at the highest dose in F0 males seem to be due to secondary effects of lowered body weight rather than direct effects of DCBS on the organs. More pronounced effects on organ weights were noted in females. Lower absolute and relative weights of the thymus at 10 000 p.p.m. and spleen at 6000 p.p.m. and higher were detected. In our previous study, histopathological examination revealed atrophy of the thymus and spleen at 400 mg/kg bw/day (Ema *et al.* 2007). Other changes in female organ weight such as the relative weights of the brain, pituitary, liver, kidney and adrenal gland, as well as the absolute weight of the ovary are unlikely to be due to the toxic effects of DCBS because the degree of changes was relatively small, no dose-dependency was shown and no changes were noted in absolute or relative weight. These findings suggest that the immune system may be a target of DCBS toxicity, and that female rats have a higher susceptibility to the toxicity of DCBS than male rats. These findings are consistent with our previous study (Ema *et al.* 2007). The higher susceptibility to DCBS toxicity in females may be explained by the stress of pregnancy and lactation. DCBS is likely to be not reproductively toxic in male rats because DCBS caused neither pathological changes in male reproductive organs nor changes in male reproductive parameters.

In our previous study, DCBS given by gavage to rats at 400 mg/kg bw/day from 14 days before mating to day 3 of lactation caused significant decreases in the gestation index, number of corpora lutea, implantations, pups born and pups born alive, live birth index and viability index (Ema *et al.* 2007). This dose also caused severe maternal toxicity and a total loss of pups by PND 4. No maternal or reproductive/developmental toxicity was detected at 100 mg/kg bw/day in our previous study. In the present study, no serious reproductive difficulties were noted even at the highest dose of 10 000 p.p.m., and necropsy of the reproductive organs revealed no evidence of reproductive failure. Although decreased numbers of implantations and pups delivered were noted at the highest dose, the viability of pups until weaning was not significantly decreased. In the present feeding study, the mean daily intakes of DCBS at the

Table 2 Reproductive and developmental findings for F0 parents/F1 offspring of rats given N,N-dicyclohexyl-2-benzothiazolesulfenamide

Dose (p.p.m.)	Control	1500	3000	6000	10 000
No. pairs	6	6	6	6	6
Copulation index[b]					
Male/female (%)	100/100	100/100	100/100	100/100	100/100
Pre-coital interval (days)[a]	2.2 ± 0.8	2.3 ± 1.2	3.2 ± 0.8	3.0 ± 0.9	2.7 ± 1.2
Fertility index[c]					
Male/female (%)	100/100	100/100	100/100	100/100	100/100
Gestation index (%)[d]	100	100	100	100	100
Gestation length (days)[a]	22.2 ± 0.4	22.2 ± 0.4	22.2 ± 0.4	22.0 ± 0.0	22.2 ± 0.4
No. implantations[a]	16.0 ± 1.8	15.0 ± 0.9	16.3 ± 1.2	13.5 ± 2.0*	12.8 ± 1.2**
Delivery index (%)[a,e]	95.8 ± 8.0	96.7 ± 3.7	95.8 ± 5.3	95.6 ± 8.1	86.7 ± 21.1
No. pups delivered[a]	15.3 ± 2.2	14.5 ± 1.0	15.7 ± 1.8	13.0 ± 2.6	11.2 ± 3.1*
Sex ratio of F1 pups[f]	0.467	0.448	0.564	0.526	0.463
Viability index (%)[a]					
PND 0[g]	100 ± 0	100 ± 0	100 ± 0	100 ± 0	91.2 ± 12.9
PND 4[h]	99.1 ± 2.3	97.9 ± 3.3	95.9 ± 5.3	90.6 ± 12.2	72.1 ± 40.8
PND 21[i]	97.9 ± 5.1	97.9 ± 5.1	100.0 ± 0.0	89.6 ± 25.5	83.3 ± 40.8
Male pup body weight during lactation (g)[a]					
PND 0	6.8 ± 0.4	6.7 ± 0.7	6.3 ± 0.4	6.2 ± 0.6	6.5 ± 0.7
PND 4	10.6 ± 0.9	10.3 ± 0.8	9.6 ± 0.6	9.1 ± 0.7**	9.1 ± 2.2[j]
PND 7	18.7 ± 1.3	17.7 ± 1.3	17.6 ± 1.3	14.5 ± 2.2**	13.3 ± 3.7[j]**
PND 14	39.2 ± 3.0	36.2 ± 3.0	37.3 ± 2.9	33.0 ± 4.0	26.3 ± 7.2[j]**
PND 21	67.0 ± 4.6	61.1 ± 6.1	62.8 ± 3.2	55.7 ± 7.6*	44.1 ± 9.9[j]**
Female pup body weight during lactation (g)[a]					
PND 0	6.4 ± 0.4	6.4 ± 0.5	6.0 ± 0.3	5.8 ± 0.6	6.2 ± 0.5
PND 4	10.1 ± 1.1	9.9 ± 0.7	9.0 ± 0.6	8.7 ± 0.7	8.5 ± 1.9
PND 7	18.2 ± 2.0	17.4 ± 0.7	16.0 ± 1.2	13.8 ± 1.3**	11.7 ± 4.2*
PND 14	38.6 ± 3.5	36.1 ± 2.1	35.0 ± 2.4	31.5 ± 4.9*	25.3 ± 7.2[k]**
PND 21	65.1 ± 5.2	60.1 ± 3.7	58.2 ± 3.3	53.5 ± 9.0*	42.5 ± 9.9[k]**

*Significantly different from the control, $P < 0.05$; **significantly different from the control, $P < 0.01$.

[a]Values are given as mean ± SD; [b]copulation index (%) (number of animals with successful copulation/number of animals paired) × 100; [c]fertility index (%) (number of animals that impregnated a female or were pregnant/number of animals with successful copulation) × 100; [d]gestation index (%) (number of females that delivered live pups/number of pregnant females) × 100; [e]delivery index (%) (number of pups delivered/number of implantations) × 100; [f]sex ratio (total number of male pups/total number of pups delivered); [g]viability index on PND 0 (number of live pups on PND 0/number of pups delivered) × 100; [h]viability index on PND 4 (number of live pups on PND 4/number of live pups on PND 0) × 100; [i]viability index on PND 21 (number of live pups on PND 21/number of live pups selected on PND 4) × 100; [j]data were obtained from five litters because one female experienced total male litter loss by day 1 of lactation; and [k]data were obtained from five litters because one female experienced total litter loss by day 9 of lactation.

PND, post natal day.

highest dose were 551 and 707 mg/kg bw in F0 males and females, respectively. One possible explanation for the discrepancy in the degree of reproductive and developmental toxicity between the present and previous studies may be the difference in administration method. Some studies have shown that gavage and feed administration result in different toxicokinetics for various chemicals (Yuan *et al.* 1994, 1995). Further studies are needed to clarify the difference in DCBS toxicokinetics between gavage and feed administrations.

Regarding the development of offspring, decreases in the numbers of implantations and pups delivered and lowered body weights of male and female pups were noted at 6000 p.p.m. and higher. These findings indicate that the dose level of 6000 p.p.m. used in this study was potent enough to adversely affect the survival and growth of pups. Reduced weight of the spleen was also observed in male and female weanlings. These findings also suggest that the immune system may be a target of DCBS toxicity. Other changes in the weights of organs, such as the brain and liver in male weanlings and the brain, liver and uterus in female weanlings are unlikely to be due to the toxic effects of DCBS because the degree of changes was relatively small, no dose-dependency was shown, no changes were noted in the absolute or relative weight, and also

Table 3 Absolute and relative organ weights of F0 female rats given N,N-dicyclohexyl-2-benzothiazolesulfenamide

Dose (p.p.m.)	Control	1500	3000	6000	10 000
No. females	6	6	6	6	5
Body weight (g)†	331 ± 18	316 ± 16	320 ± 11	306 ± 14*	274 ± 20**
Brain (g)†	2.10 ± 0.05‡	2.11 ± 0.08	2.10 ± 0.05	2.06 ± 0.10	2.06 ± 0.03
	0.63 ± 0.03§	0.67 ± 0.04	0.66 ± 0.02	0.67 ± 0.04	0.76 ± 0.05**
Pituitary (mg)†	13.3 ± 1.6‡	13.4 ± 2.4	15.4 ± 0.9	13.9 ± 1.9	12.9 ± 2.6
	4.03 ± 0.44§	4.24 ± 0.65	4.81 ± 0.32*	4.53 ± 0.46	4.70 ± 0.66
Thyroid (mg)†	18.3 ± 3.6‡	17.6 ± 3.5	17.7 ± 4.3	18.8 ± 2.7	17.5 ± 3.6
	5.52 ± 0.87§	5.55 ± 0.99	5.51 ± 1.18	6.15 ± 0.94	6.39 ± 1.02
Thymus (mg)†	255 ± 47‡	205 ± 63	237 ± 45	186 ± 89	116 ± 60**
	77.1 ± 14.4§	65.0 ± 19.6	74.2 ± 13.1	60.1 ± 26.5	41.7 ± 19.9*
Liver (g)†	13.03 ± 0.83‡	12.51 ± 0.71	13.42 ± 1.18	13.69 ± 0.68	12.18 ± 1.60
	3.94 ± 0.21§	3.97 ± 0.23	4.20 ± 0.27	4.48 ± 0.09**	4.46 ± 0.59
Kidney (g)†	2.34 ± 0.16‡	2.38 ± 0.13	2.35 ± 0.10	2.20 ± 0.12	2.51 ± 0.41
	0.71 ± 0.04§	0.75 ± 0.05	0.74 ± 0.04	0.72 ± 0.03	0.92 ± 0.18**
Spleen (mg)†	682 ± 74‡	589 ± 68	600 ± 89	493 ± 24**	459 ± 46**
	206 ± 20§	187 ± 19	188 ± 31	161 ± 5**	168 ± 15**
Adrenal (mg)†	75.5 ± 11.0‡	81.8 ± 12.9	77.0 ± 8.8	72.0 ± 8.8	88.2 ± 8.3
	22.9 ± 3.2§	26.0 ± 3.9	24.1 ± 2.7	23.5 ± 2.8	32.4 ± 3.8**
Ovary (mg)†	109 ± 18‡	113 ± 17	101 ± 5	101 ± 10	75 ± 23**
	32.9 ± 3.8§	36.1 ± 6.8	31.6 ± 2.4	32.9 ± 3.9	27.1 ± 6.4
Uterus (mg)†	513 ± 68‡	465 ± 73	489 ± 101	414 ± 71	369 ± 183
	156 ± 24§	148 ± 26	153 ± 32	135 ± 22	132 ± 56

*Significantly different from the control, $P < 0.05$; **significantly different from the control, $P < 0.01$.
†Values are given as the mean ± S.D; ‡absolute organ weight; §relative organ weight (organ weight [g or mg]/100 g body weight).

Table 4 Absolute and relative organ weights for F1 male weanlings of rats given N,N-dicyclohexyl-2-benzothiazolesulfenamide

Dose (p.p.m.)	Control	1500	3000	6000	10 000
No. males	6	6	6	6	5
Body weight (g)†	67.1 ± 6.7	62.5 ± 4.5	63.8 ± 4.2	55.3 ± 8.9*	43.8 ± 10.6**
Brain (g)†	1.70 ± 0.05‡	1.63 ± 0.12	1.59 ± 0.04	1.51 ± 0.05**	1.45 ± 0.11**
	2.55 ± 0.21§	2.61 ± 0.24	2.50 ± 0.15	2.78 ± 0.42	3.44 ± 0.74*
Thymus (mg)†	257 ± 44‡	219 ± 33	265 ± 45	246 ± 36	190 ± 65
	382 ± 50§	351 ± 57	415 ± 59	449 ± 60	424 ± 50
Liver (g)†	2.56 ± 0.35‡	2.65 ± 0.29	2.69 ± 0.38	2.37 ± 0.38	1.72 ± 0.49**
	3.80 ± 0.17§	4.22 ± 0.20*	4.20 ± 0.37	4.30 ± 0.33*	3.90 ± 0.22
Spleen (mg)†	372 ± 63‡	276 ± 53**	296 ± 32*	250 ± 45**	148 ± 36**
	556 ± 84§	442 ± 80*	466 ± 56	452 ± 32*	337 ± 31**

*Significantly different from the control, $P < 0.05$; **significantly different from the control, $P < 0.01$.
†Values are given as mean ± S.D; ‡absolute organ weight; §relative organ weight (organ weight [g or mg]/100 g body weight).

because the changes seem to be secondary effects of the lowered body weight. In the present study, external and internal morphological examinations of offspring were performed, but no skeletal examinations were conducted. To accurately evaluate prenatal developmental toxicity including teratogenicity, it is necessary to interrupt pregnancy 12–24 h before the expected term either by hysterectomy or the necropsy of maternal animals (Wilson 1965).

The adverse effects of DCBS on reproduction and development noted in the present feeding study are almost consistent with the findings of our previous gavage study (Ema *et al.* 2007), which showed decreased numbers of implantations and pups delivered and decreased body weight of the pups at higher doses. These endpoints appear to be affected at multiple points of the female reproductive and developmental process. The decreased number of implantations

Table 5 Absolute and relative organ weights for F1 female weanlings of rats given N,N-dicyclohexyl-2-benzothiazolesulfenamide

Dose (p.p.m.)	Control	1500	3000	6000	10 000
No. females	6	6	6	6	5
Body weight (g)†	65.7 ± 7.2	61.1 ± 3.4	59.9 ± 4.6	54.0 ± 9.6*	42.8 ± 9.6**
Brain (g)†	1.60 ± 0.09‡	1.56 ± 0.07	1.53 ± 0.03	1.50 ± 0.05*	1.37 ± 0.08**
	2.46 ± 0.25§	2.56 ± 0.16	2.57 ± 0.18	2.84 ± 0.39	3.34 ± 0.78**
Thymus (mg)†	272 ± 46‡	253 ± 33	252 ± 27	243 ± 51	216 ± 82
	415 ± 56§	415 ± 57	422 ± 37	456 ± 101	491 ± 92
Liver (g)†	2.58 ± 0.31‡	2.47 ± 0.27	2.42 ± 0.42	2.27 ± 0.43	1.71 ± 0.49**
	3.93 ± 0.14§	4.03 ± 0.22	4.02 ± 0.41	4.19 ± 0.13	3.96 ± 0.29
Spleen (mg)†	360 ± 57‡	296 ± 16	267 ± 60*	247 ± 50**	163 ± 59**
	548 ± 66§	484 ± 9	442 ± 72*	456 ± 37*	371 ± 58**
Uterus (mg)†	44.7 ± 6.6‡	41.3 ± 6.1	35.7 ± 2.1*	42.0 ± 6.9	32.4 ± 4.8**
	68.9 ± 14.0 Temp.§	67.7 ± 9.8	60.0 ± 7.4	78.5 ± 10.8	77.3 ± 10.3

*Significantly different from the control, $P < 0.05$; ** significantly different from the control, $P < 0.01$.

†Values are given as the mean ± S.D; ‡absolute organ weight; §relative organ weight (organ weight [g or mg]/100 g body weight).

was the most striking effect in the present study. In our previous study, a decreased number of corpora lutea was noted in female rats given DCBS (Ema *et al.* 2007). Therefore, it is likely that the decreased number of implantations can be attributed to the decreased number of corpora lutea. The present study does not provide complete information on all aspects of reproduction and development due to the relatively small numbers of animals in the dose groups and selectivity of the endpoints. To further evaluate the reproductive and developmental toxicity of DCBS in rats, a two-generation reproductive toxicity study should be performed.

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妊娠とくすり

7．生殖発生毒性試験の役割

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Key Words/生殖発生毒性，催奇形性，動物実験

サリドマイド事件を契機として，各国における薬事制度の見直し，強化がはかられ，実験動物を用いた生殖発生毒性試験に関する資料の提出が新有効成分，新投与経路の承認申請時に必要となっている．本稿では医薬品によるヒトにおける生殖発生障害の例と動物実験との関わりについて述べ，実験動物を用いた生殖発生毒性試験の特徴および試験結果を評価する際の留意点について概説した．

生殖発生毒性試験

生殖（Reproduction）とは，種を存続させるための生物学的過程をいい，生殖毒性（reproductive toxicity）には，成熟動物の生殖能に対する有害作用と子孫における発生毒性（developmental toxicity）が含まれる．生殖能に対する有害影響とは，雌雄の生殖器や内分泌系の変化に起因する有害影響（春期発動，配偶子形成・輸送，生殖周期，性行動，繁殖，分娩，生殖系の統合性に依存するそのほかの機能に対する影響等）であり，発生毒性とは，親の妊娠前から児の性成熟までの曝露による正常な発生の障害（死亡，形態異常，成長の変化，機能障害）を指す[1]．すなわち，生殖毒性は親の世代を中心にとらえたときの環境要因による不妊や次世代の発生障害を指し，次世代を中心にとらえた発生毒性とほぼ同義である．生殖発生毒性試験の目的は，哺乳類の生殖発生に対するあらゆる影響を明らかにすることである[2]．薬物の即時的および遅発的影響を検出するために，親の世代の受精から次世代の受精までの完全な生殖周期（**図1**）に薬物を投与して，その間の観察を行う．新薬の申請時に必要とされている生殖発生毒性試験のうち，着床から硬口蓋閉鎖までの期間中の雌動物に投与を行う「胚・胎児発生に関する試験」，いわゆる催奇形性試験は，最も重要視されており，2種の動物を用いた試験が課せられている．動物の生殖発生の特定の段階に

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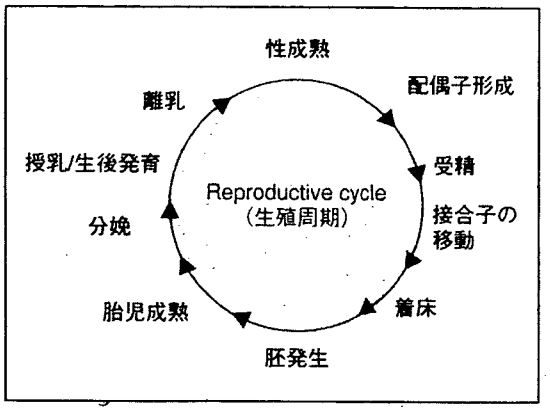

図1 生殖周期

生殖発生毒性試験においては，成熟動物および受精から性成熟までの発生の全過程にわたって薬物に曝露し，薬物の即時的および遅発的な影響を検出するため，完全な生殖周期，すなわち，一世代での受精から次の世代での受精までの観察を継続して行う．

被験物質を投与してその影響を観察することにより，どの生殖発生段階に障害を生じるかを明確にすることができる．このような試験方法は，大半の医薬品では亜急性的な服用が想定されることから，ヒトでの曝露状況をよく反映している．長期間曝露が想定される医薬品では，1世代または2世代試験が有用である．生殖発生毒性試験で得られた結果を，ヒトにおける生殖発生への危険性が他の毒性試験の結果から予見される危険性の程度と比較検討する．

生殖発生障害にかかわる出来事

生殖発生障害に関する主な出来事を**表1**に示

表1 生殖発生に関わる主な出来事

1744年	ヒドラの切断による多頭体
1870年	キニーネによるヒト児の難聴
1902年	妊娠モルモットへのブドウ球菌投与による児の白内障
1905年	妊娠ウサギへのX線処置による児の眼異常
1907年	妊娠ウサギへのコリン投与による実験（最初の化学物質の催奇形実験）
1911年	妊娠ウサギへのナフタリン投与による児の白内障等の眼異常
1913年	東北医専眼科教授小玉龍蔵，わが国最初の催奇形実験
1933年	ビタミンA欠乏食によるブタ児の無眼（近代実験奇形学のはじまり）
1941年	ヒト先天性風疹症候群
1950年	ストレプトマイシンによるヒト児の難聴
1952年	ヒト胎児性水俣病，アミノプテリンによるヒト胎児の髄膜脳瘤
1953年	男性ホルモンによるヒト女児の偽半陰陽
1956年	ベンデクチン発売開始
1957年	サリドマイド発売（鎮静薬「コンテルガン」西独グリュネンター社）
1961年	サリドマイド事件（米FDAフランシス・ケルシー承認与えず）
1963年	「胎児に及ぼす影響に関する動物試験法」厚生省薬務局長通知（わが国の最初の試験法ガイドライン）
1967年	「医薬品の製造承認等の基本方針」厚生省薬務局長通知（急性，亜急性，慢性，胎児及びその他の特殊毒性データの要求）
1968年	カネミ油症「コーラベビー」
1971年	ジエチルスチルベストロール（DES）服用の母親から生まれた女児の腟がん
1982年	「医薬品の安全性試験の実施に関する基準」（GLP）制定
1983年	ベンデクチン発売中止
1983年	イソトレチノイン（13-cis-retinoic acid）によるヒト児の小耳
1992年	ヒト精子の減少をスキャケベックが報告
1997年	薬審第316号「医薬品の生殖発生毒性に係わるガイドライン改定について」
2000年	薬審第1834号「医薬品の生殖発生毒性についてのガイドラインの改定について」：本ガイドラインの一部改訂

した．ヒトに関しては，1870年に小児の難聴とキニーネ（Quinine）の妊娠中の摂取との関係を疑った報告があり，その後，先天性風疹症候群，ストレプトマイシンによる難聴，胎児性水俣病，男性ホルモンによる女児の偽半陰陽，サリドマイド（Thalidomide）事件，カネミ油症，ジエチルスチルベストロール（DES:Diethystilbestrol）による女児の腟癌，イソトレチノイン（Isotretinoin）による小耳などが報告されているが，なかでも特筆すべきはサリドマイド事件である．1954年に西ドイツのグリュネンタール社においてサリドマイドが合成され，1957年10月にコンテルガン®という商品名で，睡眠薬，精神安定薬として発売され始め，世界の46カ国で発売された．睡眠薬としては即効性，持ち越し作用がなく，致死的作用もなく，当時の西ドイツおよび諸外国で大衆薬として広く使われた[3]．その後，サリドマイドを妊娠初期に服用した妊婦の出産児が先天異常を有することが報告され，1961年頃からサリドマイド禍として認識され始めた．このサリドマイド事件を契機として，医薬品の催奇形作用が問題視され，各国における薬事制度の見直し，強化がはかられた．わが国では1963年4月に「医薬品の胎児に及ぼす影響に関する動物試験法」が厚生省薬務局長から通知された．この通知はわが国で最初の具体的な毒性試験ガイドラインであり，2種類の動物を用いて行う器官形成期投与試験が示された．その後，何回かの改正を経て，現行の生殖発生毒性試験法が2000年12月に医薬審第1834号[2]として通知され，このガイドラインにしたがって新薬の承認申請のための生殖発生毒性試験が行われている．医薬品の承認申請には，医薬品の品質，有効性，安全性を評価するために，規格および試験方法，安定性，毒性，薬理作用，吸収・分布・代謝・排泄，臨床試験についての資料の提出が求められている．申請内容に応じて必要な資料が定められており，生殖発生毒性に関する資料の提出は新有効成分，新投与経路の承認申請時に必要とされている[4]．

医薬品によるヒトにおける発生障害と動物実験との関わりの例

1．サリドマイド（Thalidomide）

サリドマイドに関しては，動物実験で催奇形性が証明される前に不幸にもヒトでの薬害が起こってしまった．サリドマイド禍当時の世界中のどこの国においても，医薬品や化学物質について発生中の生物に対する影響に関する試験は要求されておらず，食物中の重要な化学物質または生殖器官に選択的な反応を示すと推定される化学物質についてのみ生殖毒性試験が推奨されていたにすぎなかった．これらの生殖毒性試験では，数世代にわたって妊娠率，出生児数，児の成長などについて重点的に調べられていたが，胎児についての検査は十分に行われていなかった．したがって，当時の試験枠組みではサリドマイド禍は避けられなかったのかもしれない[5]．サリドマイドの催奇形性に対して，ラットおよびマウスはほとんど感受性を示さず（胚死亡は惹起されるとする報告はある），ハムスター，ブタ，ネコ，イヌ，フェレット，アルマジロおよびニワトリでは感受性を示すが，特異的な奇形は惹起されない[6][7]．ウサギおよび非ヒト霊長目では感受性を示し，特異的な奇形が惹起される．ウサギでは胎児致死作用が強く発現するような高用量でのみ四肢奇形およびその他の奇形が認められるが，奇形発現率は低く，系統間で感受性に差がある．9種中8種の非ヒト霊長目でヒトと同様の用量および感受期の投与により，特徴的な四肢奇形が観察されている[6]．非ヒト霊長目または最も鋭敏な系統のウサギを催奇形性スクリーニング試験に汎用することは現実的には困難である．Kalter（2003）[8]は，あ

る種の薬物が実験動物において先天奇形を惹起することはサリドマイド禍以前から知らされていたが，現在の生殖発生毒性試験の知識と技術をもってしても，サリドマイドのヒトにおける催奇形性をおそらく予見できなかっただろうと述べている．

2. ベンデクチン（Bendectin）

ベンデクチン（抗ヒスタミン剤のドキシラミン，抗痙攣剤のジサイクロミン，ビタミンB_6の合剤）は鎮吐薬として1956年から米国のメレル・ダウ社から発売開始され，米国の25%の妊婦が服用したと見積もられている[5]．本薬服用の女性が奇形児を出産したという訴えが起き，ベンデクチンの催奇形性作用についての大衆キャンペーンが行われた[3][6]．FDAでも，本薬が催奇形性の原因とはしなかったにもかかわらず，その後ベンデクチンの売り上げ収入よりも裁判費用が多くなったことから，1983年にメレル・ダウ社は販売を中止した[3]．ラット，ウサギおよび非ヒト霊長目を用いて，大量投与を行った実験でも，ヒトにおける催奇形性を支持する結果は得られていない[5][6]．

3. アンドロゲン（Androgens）

モルモット，ラット，マウス，ハムスター，ハリネズミ，フクロネズミ，モグラ，ウサギウシ，ヒツジおよび非ヒト霊長目において雌胎児に雄性化を引き起こすことが，1936～1950年にすでに報告されていた．その後の1953年，ヒトにおいて，乳癌の妊婦へのMethylandrostenediol投与による女児の外生殖器異常が報告された[5][8]．

4. プロゲステロン類

Ethisteroneについてはウサギの雌胎児の雄性化を引き起こすことが1942年に報告されていた．しかし，臨床家や発生学研究者の注意を引かず，切迫流産のために妊娠初期に黄体ホルモン剤を投与された女性から女児仮性半陰陽児が生まれた[5][8]．

5. 抗痙攣薬

抗痙攣薬については動物実験で奇形胎児の発現などの発生毒性試験結果が先に報告された．その後，ヒトにおける抗痙攣薬の発生障害に関する情報収集が行われた[8]．

6. ビタミンA類

ビタミンAの催奇形性については1953年にすでに報告されており，また，ビタミンA類似体のIsotretinoinやレチノイン酸類似体のEtretinateについては動物実験で催奇形性が認められていた．しかしながら，有用性のために医薬品として承認された後にヒトにおける発生障害の報告がなされた[5][8]．

ヒトにおいて発生毒性が報告されている医薬品

商業上および公衆衛生上重要であること，ヒトおよび動物における良質なデータが得られること，成長遅延，死亡，奇形，機能障害のいずれかの発生毒性を示すことを基準にSchardeinとMacina（2006）[7]が選定した50の化学物質のうちの42種の医薬品について，ヒトおよび実験動物で発生毒性が報告された年を**表2**に示した．ヒトにおいて，催奇形作用の報告があるものは40（95%），致死作用の報告があるものは24（57%），機能障害の報告があるものは21（50%），成長遅延の報告があるものは16（38%）であった．ヒトで成長遅延，死亡，奇形および機能障害のすべての発生障害の型を示すと報告されているのは，抗腫瘍薬（Aminopterin, Cyclophosphamide, Methotrexate），抗痙攣薬（Paramethadione），ACEインヒビター（Captopril），抗甲状腺剤（Methimazole），抗凝固剤（Warfarin），平滑筋収縮薬（Ergotamine）の8医薬品（19%）であり，これらは最も強い発生毒性物質と考えられる．ヒトで成長遅延，死亡，

表2 ヒトで発生毒性が報告されている医薬品のヒトおよび動物での発生障害の報告年

薬物名	ヒトにおける発生毒性（報告年）	動物における発生毒性（報告年）
Aminopterin	脳/口唇口蓋奇形，死亡（1952）	マウス胚死亡（1950），ラット奇形（1954）
Busulfan	口蓋/眼/生殖器/卵巣奇形，成長遅延，死亡（1960）	ラット卵巣性不妊（1964），マウス奇形（1966）
Chlorambucil	死亡（1962），腎臓/尿管奇形（1963）	マウス四肢/中枢神経系奇形，口蓋裂（1956）
Cyclophosphamide	指趾/口蓋/鼻奇形，皮膚異常（1964）	ラット奇形，成長遅延，胚致死（1962）
Methotrexate	頭蓋/指趾/耳/顔面/肋骨奇形（1968）	ラット四肢/指奇形，口蓋裂（1967）
Cytarabine	死亡（1978），骨/指趾/耳奇形（1980）	ラット四肢/指趾/尾奇形，口蓋裂，死亡（1968）
Mechlorethamine	死亡（1962），骨/指趾/脳/耳奇形（1974）	ラット奇形，成長遅延，胚致死（1948）
Vitamin A	尿管奇形（1965）	ラット頭顔面/脳奇形（1953）
Isotretinoin	耳奇形（1983）	ウサギ奇形（1982）
Etretinate	骨格/脳奇形（1984）	ウサギ催奇形性（1981）
Tretinoin	脳奇形（1991）	マウス顔面/四肢/神経系/心臓奇形（1967）
Acitretin	死亡（1994）	ウサギ/マウス/ラット四肢奇形（1985）
Phenytoin	奇形（1964）	マウス奇形（1966）
Phenobarbital	奇形（1964）	マウス口蓋裂（1977）
Paramethadione	口唇口蓋/脊椎/尿管/脳/心臓/血管系奇形，死亡（1970）	ラット胎児死亡，成長遅延，骨格変異（1976）
Primidone	顔面異常，成長遅延（1973）	マウス口蓋裂（1975）
Carbamazepine	死産児における奇形（1979）	マウス中枢神経系奇形（1977）
Valproic acid	顔面/脳/心臓/骨格奇形，成長遅延（1980）	マウス奇形（1971）
Ethisterone	女児雄性化（1955）	ウサギ雌胎児の雄性化（1942）
Methyltestosterone	女児雄性化（1957）	ウサギ雌胎児の雄性化（1947）
Norethindrone	女児雄性化（1958）	マウス雌胎児の雄性化（1972）
Medroxyprogesterone	女児雄性化（1963）	ラット雌胎児の雄性化（1960）
Danazol	死亡（1978），雄性化（1981）	ラット/ウサギで発生毒性（Physicians' Desk Reference, 2002）
Streptomycin	難聴（1950）	マウス顕微鏡的脳の変化（1963），マウス内耳障害（1985）
Tetracycline	歯/骨灰褐色化（1961）	ラット胎児骨石灰化，コラーゲン生合成（1968）
Trimethoprim	心血管系/口蓋/尿管奇形（2000）	ラット催奇形性（1969）
Penicillamine	消化管/血管系/骨奇形，皮膚異常，死亡（1971）	ラット奇形，成長遅延，胚致死（1972）
Methylen Blue	腸管異常（1990）	マウス奇形，胚致死（2000）
Quinine	耳障害（1933）	ウサギ耳神経障害（1938）
Propylthiouracil	甲状腺障害（1946）	モルモット甲状腺障害（1948）
Thalidomide	アザラシ肢症（1959）	ウサギ四肢奇形，胚致死（1963），サル四肢奇形（1964）
Disulfiram	四肢奇形，死亡（1965）	ラット胚致死（1974）
Warfarin	眼奇形，機能障害（1966）	マウス口蓋裂，出血，胎児死亡（1971）
Methimazole	四肢奇形，成長遅延（1966）	ラット生後行動変化（1982）
Diethylstilbestrol	腟腺がん（1970）	ラット間性（1940）
Ergotamine	心臓奇形，死亡（1971）	ラット/マウス低胎児体重，骨化遅延（1973）
Propranolol	子宮内成長遅延（1974）	ラット出生児数減少，出生児低成長（1985）
Captopril	腎臓/頭蓋/四肢奇形（1981）	ウサギ/ヒツジ死産（1980）
Misoprostol	頭蓋奇形（1991）	ラット着床阻害（妊娠0～7日に腟内投与）（1982）
Pseudoephedrine	腹壁破裂（1992）	ラット低胎児体重/骨化遅延（1989）
Fluconazole	頭蓋/口蓋/骨格奇形（1992）	ラット口蓋裂，頭顔面骨化異異常，胚致死，ウサギ流産(unpublished data)
Valsartan	頭蓋/顔面/腎臓/指趾奇形（2001）	ウサギ/マウス/ラット胚致死，成長遅延（unpublished data）

奇形，機能障害のうちの3つの型の発生障害を惹起すると報告されているのは13（31%），2つの型の発生障害を惹起すると報告されているのは9（21%），1つの型の発生障害を惹起すると報告されているのは12（29%）であった．ヒトでは成長遅延の報告はあまり多くはないが，胎児の成長遅延は実験動物においては最も鋭敏で，最も検出しやすい発生毒性指標であり，母体および胎児の両者に対する毒性影響によって起こりうる．ヒトにおける子宮内成長遅延は3～10%の頻度で起こり，これらの児の死亡率は正常児の3倍高く，自然流産の20%程度が重篤な成長遅延を有しているとの報告もある[5]．また，周産期死亡，先天奇形，神経学的機能障害との

関連も明らかになっている[7].

動物実験の特徴と評価の際の留意点

ヒトで発生毒性を現す化学物質は，いずれかの動物種で発生毒性を現し，いかなる化学物質も適切な量を適切な時期ある動物種に与えたときに発生毒性を現しうる（Karnofskyの法則）．これらのことはある動物種で発生毒性が惹起されれば，ヒトでも発生毒性が惹起される可能性があることを示している．Schardein（2000）[5]は，調査した4,153の化学物質のうち，動物で催奇形性が報告されているものは約1/3であり，そのうちの291の化学物質では2種以上の動物において催奇形性の報告があるが，2,760の化学物質については催奇形性は示されていないと述べている．さらに，70,000以上の化学物質が環境中に存在し，そのうち70物質がヒトでの発生毒性物質であると述べている[7]．また，実験動物で催奇形性を示した1,200の化学物質のうち40物質がヒトにおける催奇形性物質であったとの記述もある[9]．動物を用いた生殖発生毒性試験では，その動物における試験の科学的真実性を考察し，その動物種における生殖発生毒性の機序と薬物動態を検索し，曝露量を考慮し，ヒトへの外挿を行う[2]．ヒトの生殖発生障害の情報がない場合には，動物を用いた実験結果からヒトへの外挿を行わなければならない．

生殖発生毒性の発現には，因子特異性，時期特異性，投与量と投与経路，母児の遺伝子，母体の生理及び病態等が複雑に係わっているので，これらの要因を十分に考慮して実験データを考察する必要がある．特定の型の発生障害には特定の感受期が存在し，観察の時期によって検出しうる発生障害の型が限定される．したがって，同じ化学物質が投与条件によって異なった発生障害を惹起し，同じ発生障害でも異なった機序や発生過程から惹起されうる．このように，発生障害発現には多くの要因が関与しているので，実験結果の再現性に問題がある場合がある．動物の生殖発生毒性試験では，生殖周期のあらゆる時期に化学物質が投与されるので，あらゆる型の生殖発生異常が惹起される可能性がある．投与量が高いと，胚/胎児死亡が惹起され，投与量が低くなれば，奇形，成長遅延，次いで，機能障害，さらに低くなれば作用はみられなくなるのが一般的である．胚/胎児致死作用が強くみられたときには，催奇形性が隠されていないかを吟味する必要がある．また，実験動物とヒトでは系統発生学的な差，生殖生理学的な差があり，実験動物とヒトとの比較が困難な場合がある．子宮内発生にも差があり，発生毒性試験によく用いられるラットやマウスなどのげっ歯類，ウサギでは主要な奇形の感受期である器官形成期は1～2週間と短いが，ヒトを含めて高等霊長類では4～6週間と長く，高等霊長類では催奇形因子の侵襲に対する修復過程の時間がある．また，出生時の成熟の程度がヒトと実験動物では異なっており，ラットやマウスでは，ヒトに比べて未熟な状態で出生するので，周産期の発生障害の評価には注意を要する．さらに，代謝の種差，薬物動態の差異も考慮する必要がある．生殖発生毒性試験に用いられる用語および異常の分類等については，文献ごとに違いがある場合があり，毒性評価の際に注意を要するが，これらについては統一化の試みが行われている[11]～[13]．また，公表されている生殖発生毒性試験の報告には質的な差があり，公表された催奇形性についての試験のうち10%しか適切に実施されていない[9]ともいわれており，ヒトへの外挿を難しくしている要因となっている．

ヒトにおける生殖発生毒性を検出するための理想的な動物種はなく，動物1種における毒性だけでヒトにおける作用を予測することは不可

能であるので，複数の動物種を用いて毒性試験が実施される必要がある．毒性発現に著しい動物種差が認められるときには，薬物動態や胚/胎児の組織の感受性に差がある可能性を考察する必要がある．薬物代謝が類似している多くの動物種において，母体毒性量よりも低用量で同じ型の発生毒性が発現するときには，ヒトでの毒性が発現する可能性が高くなる．動物実験では通常健常な動物を使って行われることがヒトの場合とは異なっている．ヒトでは，例えば，抗痙攣薬の場合，治療に複数の医薬品が使われ，また，痙攣などの母体の要因が介在するために，これらの要因による作用の増強または軽減の影響を考慮しなければならない．また，当然のことながら医薬品は比較的大量を意図的に与えるものであり，処方する者が医薬品についての情報を熟知している必要がある．

これまで述べたような動物実験の特徴を十分に理解し，動物実験のデータを適切に評価して，ヒトへの外挿が行われることが望まれる．

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妊娠とくすり

1．わが国における妊娠とくすりの問題点

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Key Words/薬剤添付文書，妊婦・授乳婦・禁忌

要旨

薬剤添付文書は，以前に比べればだいぶその記載がよくなってはいるが，特に妊婦への使用については訂正するべきものが多い．記載が不明瞭で参考にならないものが多いが，なかには誤った記載，さらに，禁忌にすべきものがそうなっていないこともある．また，抗菌薬など一群の薬剤で，リスクの高いもの，低いものがまったく区別できていないことも改善すべき課題である．また，厳しすぎる記載により不要な妊娠中絶が行われることも問題である．

はじめに

すべての薬剤には，本来の目的の薬効のほかに好ましくない副作用がある．特に高齢者，小児および妊婦・授乳婦は通常の成人とは異なった注意が必要とされており，薬剤の添付文書にもこれらの場合については特別に注意点が記載されている．しかし，多くの薬剤では「妊娠中の投与に関する安全性については確立していないので，治療上の有益性が危険性を上回ると判断される場合にだけ投与する」という趣旨の記載が大部分であり，実際の臨床の場ではあまり参考にならないことが多い．また，いくつかの薬剤では明らかに誤った評価もなされている．このため，妊婦に薬剤を投与する場合，この薬剤添付文書は無視されることも多いのではないかと思われる．しかし，この薬剤添付文書も日々更新する作業が行われており，特に新しい副作用についてはかなりのスピードで改定の作業が行われており，これを無視するのは誤りである．本稿では，わが国の薬剤添付文書を中心に妊娠中の薬剤使用の問題について筆者の考えを述べることにする．

薬剤添付文書を無視してはいけない理由

薬剤を使用し副作用が認められた場合，医師

0386-9792/07/¥100/頁/JCLS