A TWO-GENERATION REPRODUCTIVE TOXICITY STUDY OF n-BUTYLBENZENE IN RATS

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ABSTRACT — N-butylbenzene (n-BB), which is suspected of having endocrine disrupting effects, was administered orally by gavage at dose levels of 0, 30, 100, and 300 mg/kg/day to groups of Crj: CD (SD) IGS rats (24 males and 24 females per group) over 2 generations, and the effects on fertility of the parental animals and development/growth of the offspring were investigated. In the F0 and F1 parental animals, n-BB at the doses of 30 mg/kg/day and above increased the liver weights, and the doses of 100 and 300 mg/kg/day increased the kidney weights and caused histopathological changes in the liver and kidney. Moreover, the dose of 300 mg/kg/day also increase adrenal gland weights and there was a tendency for inhibition of body weight gain. With respect to effects on fertility, no significant findings were noted in the F0 parental males and females. Furthermore, it was concluded that n-BB did not induce serious reproductive toxicity in the F1 parental animals and no effects on the endocrine system were recognized. In the F1 and F2 offspring, n-BB at 300 mg/kg/day increased the thymus weights.

KEY WORDS: N-butylbenzene, Reproductive toxicity study, Endocrine system

INTRODUCTION

N-butylbenzene (n-BB) has been used as a synthetic intermediate product or raw material for manufacturing liquid crystals. It is reported to inhibit rabbit liver cytochrome P450 and induce rat hepatic cytochrome P450 (Backers *et al.*, 1993; Imaoka and Funae, 1991), but information on actions related to the drug metabolizing enzymes is insufficient and whether there is any endocrine disrupting action is unclear. While effects on binding of 17 β -estradiol to rainbow-trout hepatic estrogen receptors and proliferation of ZR-75, a human breast cancer cell have been studied (Jobling *et al.*, 1995), the data are equivocal. Furtherer, uterotrophic and Hershberger assays performed to investigate the estrogenic and androgenic effects of n-BB (Yamasaki *et al.*, 2002), did not indicate any endocrine disrupting properties.

The present study was conducted as part of the “Developmental Project of Evaluation and Testing Methods for Endocrine Disrupting Effects of Chemical Substances”, a study project of the New Energy and Industrial Technology Developmental Organization (NEDO). For this purpose, n-BB was administered

orally by gavage at dose levels of 0, 30, 100, and 300 mg/kg/day to groups of Crj: CD (SD) IGS rats (24 males and 24 females per group) over 2 generations, and the effects on reproductive capability of the parental animals and development/growth of the offspring were investigated.

MATERIALS AND METHODS

Chemical

N-butylbenzene (n-BB), purity 98% (Kanto Chemical; Lot No. 208G7508), was used as the test substance.

Laboratory animals and breeding conditions

Twenty-four male and 24 female SPF rats (Crj: CD (SD) IGS; Charles River Japan) per group were used.

Solid feed (NIH-07M; CLEA Japan) was given ad libitum. Well water supplemented with sodium hypochlorite (approx. 2 ppm) was made available ad libitum with an automatic water supply system or water supply bottles. Individual animals were placed in stainless cages with a wire netting floor (Toyo Riko) in a

breeding room with a barrier system set at a temperature of $24 \pm 2^\circ\text{C}$, and humidity of $55 \pm 10\%$ with 12-hr illumination (lights-on at 7 a.m. and light-off at 7 p.m.) and a ventilation frequency of 13-15 times/hr. During the period from gestation day 0 to weaning, individual animals and litters were placed in polycarbonate flat-floor cages (Toyo Riko) with bedding (White Flakes; Charles River Japan).

Administration of the test substance

The doses of the test substance were selected based on the results of a preliminary study of n-BB performed in our laboratory, in which the compound was administered orally at dose levels of 0, 30, 100, 300, and 1000 mg/kg/day to 6 males and 6 females per group of rats for 4 weeks before mating and during the gestation and lactation periods. The effects on the F0 parental animals included inhibition of body weight gain in both sexes receiving 1000 mg/kg/day and the elevated liver and kidney/adrenal weights or a tendency for elevation in males receiving 300 and 1000 mg/kg/day. No effects on the fertility of F0 parental animals were detected in any group. With the F1 offspring, viability on postnatal day 4 markedly decreased in the 1000 mg/kg group and body weight gain was inhibited during the lactation period. From these findings, the highest dose was set at 300 mg/kg/day, with which some toxic signs appear in the parental animals and sufficient F1 offspring for evaluation could be obtained. Then, the level was divided by a common ratio about 3, to give intermediate and low doses of 100 and 30 mg/kg/day, respectively.

Oral administration was done by gavage, using Teflon gastric tubes (CLEA Japan). N-BB was dissolved in olive oil (Wako Pure Chemical Industries) and the dose volume was adjusted to 5 mL/kg. The control group received an equal volume of olive oil alone. At the beginning, dosing of mixed feed was planned, since the volatility of n-BB is high. However, oral administration by gavage was eventually chosen, since it proved difficult to mix n-BB into basic feed, and it was found to be stable in olive oil solution.

The F0 male parental animals underwent dosing for approximately 16 weeks, including 10 weeks before mating from five weeks old and the mating (14 days at a maximum until completion of copulation) and later periods until one day prior to autopsy. The dosing period in the F0 female parental animals was about 14 - 16 weeks, including 10 weeks before mating from five weeks old, the mating period, the gestation period, and the period until day 21 after delivery. The

dosing period in the F1 male parental animals was about 18 weeks, including 10 weeks before mating from three weeks of age (at the time of weaning), through the mating period (14 days at a maximum until completion of copulation), and the period until one day prior to autopsy. The dosing period in the F1 female parental animals was about 19-21 weeks, including 10 weeks before mating from three weeks of age (at the time of weaning), the mating period, gestation period, and the period until day 21 after delivery. In F0 and F1 female parental animals, dosing was carried out after daily assessment of copulation during the mating period and males and females were returned to individual cages. The administration to test substance for the animals in the middle of delivery was conducted after completion of delivery.

Methods and study items

1. Clinical signs, mortality, body weight, and food consumption

Individual animals were observed twice a day before and after the administration for survival or death, external appearance, and behavior. The body weights and food consumption of the males were measured every week. The body weights of the females was measured once a week during the maturation period, on gestation days (GDs) 0, 7, 14, and 20 during the gestation period, and on postnatal days (PNDs) 0, 4, 7, 14, and 21 during the lactation period. The food consumption of the females was measured once a week during the maturation period, on GDs 1, 7, 14, and 20 during the gestation period and on PNDs 1, 4, 7, 14, and 21 during the lactation period.

2. Mating and confirmation of pregnancy

The females were moved to males' cages for mating in the evening and cohabited with males on a 1:1 basis when 15 weeks of age, 10 weeks from the dosing start in the F0 parental animals or at 13 - 15 weeks of age, 10 - 12 weeks from the dosing start at three weeks of age in the F1 parental animals. From the following day, they were examined on the next morning for the presence of vaginal plugs or sperm in vaginal smears. When either were observed, it was judged that copulation had taken place and the day was defined as GD 0. The animals were then moved to polycarbonate flat-floor cages and individually reared. The females in which mating had not been confirmed were put back into the original stainless cages with wire netting floor, and mating was repeated for 2 weeks according to the above described method or until copulation was con-

firmed.

Pregnancy was confirmed by investigating the presence of delivery and implantation scars at autopsy. In the F1 parent animals, one out of the males and one out of the females were selected from each litter of the F0 parent animals that experienced delivery. The remaining animals that were not selected were autopsied on PND 22.

3. Parameters of fertility

Based on observations during the reproductive period of the parental animals, the following parameters were calculated: the estrus count; estrous interval (estrous cycle); mating index = the number of the males or females in which copulation was confirmed / the number of the males or females used for mating; fertility index = the number of pregnant females / the number of the males or females in which copulation was confirmed; gestation rate = the number of females with viable offspring / the number of pregnant females; gestation length (the period from the day when the copulation was confirmed until completion of delivery); the number of implantations (implantation scars were counted at autopsy); and the litter size.

4. Sexual maturity

Using preputial separation in the males and vaginal opening in the females as parameters, individual animals were observed everyday until their completion from PND 35 or 28. The body weight was measured on the completion day.

5. Sperm examination

Using 10 animals of each group, sperm were collected from the caudal part of the right epididymis and examined in order of animal number. Counts of sperm and sperm motility were determined under a microscope using a hemocytometer. For motility, motile sperm (sperm which moved even slightly) and non-motile sperm (sperms which did not move at all) were discriminated in 100 sperm. Two examiners repeated the procedure twice, which resulted in observation and examination of 400 sperm, and the sperm motility ($[\frac{\text{total of motile sperm}}{400}] \times 100$) was calculated. For assessment of sperm morphology, stained smear specimens were made and 300 sperm for each individual were examined under a microscope to allow the rate for abnormal sperm to be determined.

6. Measurement of hormone level

In six males of each group, not used for sperm

examination, and 6 females in each group in descending order of animal number, plasma follicle stimulating hormone (FSH) and lutenizing hormone (LH) levels were measured by an RIA double antibody techniques. In addition, testosterone in males was measured by an RIA test-tube solid phase technique and estradiol in females was measured by an RIA double antibody technique.

7. Indices related to the offspring

Clinical signs were observed everyday during the lactation period. The number of offspring was adjusted randomly to eight for each litter on PND 4 (four males and four females where possible). The culled offspring were sacrificed under ether anesthesia, underwent autopsy and were then stored in 10% neutral buffered formalin. The body weights were measured on PNDs 0, 4, 7, 14, and 21. The anogenital distance was measured on PND 4 (only F1 offspring). Based on the observations during the lactation period of the offspring, the following parameters were calculated: sex ratio = the number of male offspring / the number of female offspring; birth rate (%) = $(\frac{\text{the number of offspring}}{\text{the number of implantations}}) \times 100$; viability on PND 4 (%) = $(\frac{\text{the number of live offspring on PND 4}}{\text{the number of offspring}}) \times 100$; and weaning rate (%) = $(\frac{\text{the number of the live offspring on PND 21}}{\text{the number of selected offspring on PND 4}}) \times 100$.

8. Physical development of the offspring

To detect effects on development and growth related to endocrine disruption, completion of the following items in all the F1 live offspring in each litter was recorded. The completion rates for littermates at each observation age were calculated for males and females.

- (1) Pinna detachment: from PND 4 to completion
- (2) Incisor eruption: from PND 10 to completion
- (3) Eyelid separation: from PND 15 to completion

9. Reflex response testing of the offspring

To detect effects on development and growth related to endocrine disruption, completion of the following items in all the F1 live offspring in each litter was recorded.

- (1) Righting reflex: from PND 5 to completion
- (2) Ipsilateral flexor reflex: from PND 5 to completion
- (3) Visual placing reflex: from PND 16 to completion
- (4) Preyer's reflex: from PND 28 to completion

10. Organ weights

The weights of the brain, pituitary gland, thyroid, thymus, liver, kidneys, spleen, adrenal glands, testes, epididymides, seminal vesicles (including coagulating glands), prostate, ovaries, and uterus in all male and female F0 and F1 parental animals were determined. The weights of the brain, thymus, spleen, testes, epididymides, ovaries, and uterus in the male and female F1 and F2 weanlings in all litters were also measured. The weights of bilateral organs were expressed on totals of the right and left organs.

11. Autopsy

All parental animals, offspring that died during the lactation period, offspring that were removed on PND 4, and weanlings which were not selected as parental animals for the next generation, were sacrificed for autopsy.

12. Histopathological examination

For all the F0 and F1 parental animals of the control and high dose groups, and other copulating non-fertile and non-copulating animals, the following were examined under a light microscope with paraffin sections prepared by routine methods and stained with hematoxylin and eosin (HE): the pituitary gland, thyroid (including parathyroid), liver, kidneys, adrenal glands, testes, epididymis, seminal vesicles, coagulating glands, prostate (ventral lobe and dorsal lobe), ovaries, oviduct, uterus, uterine cervical part, vagina and mammary glands and macroscopically abnormal sites. Because the 300 mg/kg group showed changes in the liver, kidneys, ovaries, oviduct, uterus, uterine cervical part, and vagina, those organs were also examined for the 30 and 100 mg/kg groups. Moreover, since the mating and fertility indices tended to be decreased, the non-copulating and copulating infertile animals of the 30 and 100 mg/kg groups were examined for their pituitary glands, testes, epididymides, prostate, seminal vesicles and coagulating glands, and stage analysis of the testes was conducted. In addition, the same analysis was also carried out for 5 animals of the control group in which fertility was observed.

Statistical analyses

For data for body weights, food consumption, the number of days needed for copulation, gestation length, organ weights, of estrus counts, estrous cycle length, anogenital distance, hormone levels, and the number of sperm, implantations, litter size and offspring born, the mean value and standard deviation for

each group were obtained and the homogeneity of variance was tested by Bartlett's method. Comparison with the control group was made using a Dunnett's multiple-comparison test when the variance was homogeneous among groups. When it was not homogeneous, the comparison was then conducted with Steel's multiple-comparison test. Comparisons between control and single dose groups were conducted as follows: for the gestation index, mating index, fertility index, and sex ratio, the chi-square test was used; for the still birth rate, birth rate, viability, weaning index, the results of physical development testing and reflex response testing, the rate for externally abnormal (by type) fetuses, sperm motility, and the rate for morphologically abnormal sperm, the Wilcoxon's rank-sum test was used; and for the results of histopathological examination, the Mann-Whitney's U test was adopted. Significance was concluded at the 1% and 5% level with all tests. For values gained from observation or measurement of offspring, the litter was treated as the unit.

RESULTS

The effects on the fertility in the parental animals

1. Mortality and clinical signs

One control F1 female parental animal and one F0 female parental animal receiving 300 mg/kg/day died. The causes of the deaths were technical error with oral gavage and spontaneous myelocytic leukemia, respectively.

As a clinical sign, salivation was found immediately after compound administration to the F0 and F1 male and female parental animals receiving 100 or 300 mg/kg/day (Table 1).

2. Body weights

Body weight changes in F0 and F1 parental animals are shown in Fig. 1 and 2, respectively. With 300 mg/kg/day, body weight gain tended to be inhibited during the nursing period, then persisting throughout the entire study period. In F1 female parental animals receiving 300 mg/kg/day, body weight gain on PNDs 0 - 21 was significantly high; however, this change was considered to be due to the low body weight on GD 0.

3. Food consumption

While significant variation was observed, this was considered to be accidental, since there was no correlation with the dose or period of exposure.

Reproductive toxicity study of n-butylbenzene.

4. Fertility

Regarding indices related to mating and fertility in F0 generation, the animals receiving 300 mg/kg/day showed a tendency for lower numbers of implantations and prolonged estrous interval (estrous cycle) (Table 2). The latter was also noted at 100 mg/kg/day.

In the F1 parental animals, a low reproductive performance was found in all the F1 animal groups including the control group (Table 2). These results were also lower than the background data from the study in which oral administration was conducted sim-

ilarly (data not shown).

Histopathological examination of the genital organs and stage analysis of the testes in controls, and in un-copulating as well as copulating infertile males was conducted additionally (Tables 3 and 4). The results of sperm examination and hormone measurement were also analyzed (Table 5) and demonstrated no differences between the normal cases and the non-copulating or copulating infertile cases, and no factors affecting the conception ability were found.

No changes associated with administration of the

Table 1. Clinical signs.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F0 males				
No abnormality	24 ^a	24	16	0
Salivation	0	0	8	24
F0 females				
No abnormality	24	24	23	2
Salivation	0	0	1	22
F1 males				
No abnormality	19	19	10	19
Salivation	0	0	11	19
F1 females				
No abnormality	19	19	18	0
Salivation	0	0	3	19

^a: The data shown are numbers of animals.

Fig. 1. Body weights of F0 parental males and females.

substance were found with regard to gestation period, gestation index, the sperm count, sperm motility, the rate of morphologically abnormal sperm, or sexual maturity (Table 6) of the F0 and/or F1 parental animals in any dose group.

5. Organ weights

Organ weights of the F0 and F1 parental animals are shown in Tables 7 and 8, respectively. There were significantly elevated relative and absolute weights of the liver and kidneys in both males and females. Higher liver weights were found in the females receiving 30 mg/kg/day or above and in the males receiving 100 mg/kg/day or above in the F0 parental animals, but only in the 300 mg/kg/day F1 male and female parental animals. Higher weights of the kidneys were observed in the F0 male and female parental animals receiving 300 mg/kg/day, in the F1 male parental animals receiving 100 mg/kg/day or above, and in the F1 females receiving 300 mg/kg/day. In addition, the absolute and relative weights of the adrenal glands in the F0 and F1 females were elevated or tended to be increased.

6. Plasma hormone measurement

In 6 animals in each sex of the F0 and F1 parental groups, LH and FSH were measured by RIA methods. Testosterone levels in males, and estradiol levels in females were also determined (Table 5). No changes associated with n-BB administration were found in any

groups. Of the 6 males and 6 females in the F0 groups, non-copulating or copulating infertile animals accounted for in 2 control males and one female receiving 300 mg/kg/day. In the F1 case, such animals were 1, 2, 1, and 1 male(s) in the control, 30, 100, and 300 mg/kg groups, respectively. However, no abnormal plasma hormone concentrations were evident that could have affected copulation or conception.

7. Autopsy and histopathological examination

At autopsy of the F0 and F1 parental animals, only spontaneous gross changes were seen and there were no lesions associated with n-BB administration.

Histopathological findings are shown in Table 9. In the liver, centrilobular hepatocytic hypertrophy was found in the F0 and F1 male parental animals receiving 300 mg/kg/day. Deposition of fat droplets in hepatocytes was found at a high rate in all the F0 male parental animals including the controls, and in the F1 male parental animals of the control and 30 mg/kg groups. The deposition was also observed in the control groups, and the incidence decreased with the dose.

In the kidneys, deposition of hyaline droplets in the proximal tubules was recognized in the F0 male parental animals receiving 100 mg/kg/day or above and the F1 male parental animals receiving 30 mg/kg/day or above. In the F0 male parental animals receiving 300 mg/kg/day or above and the F1 male parental animals receiving 100 mg/kg/day or above, the incidence

Fig. 2. Body weights of F1 parental males and females.

Reproductive toxicity study of n-butylbenzene.

was increased significantly in comparison with the control group and basophilic renal tubules were also observed.

In F0 and F1 female parental animals of all groups, including the controls, appearance of mucous cells in the vagina and cysts in the uterine cervical part was confirmed; however, the change was considered incidental, since there was no difference in the incidence or degree. In one F0 female parental animal receiving 300 mg/kg/day, myelocytic leukemia and ulceration of the glandular stomach were recognized. The myelocytic leukemia was considered sporadic, since its incidence was low and short-term administra-

tion of a test substance is unlikely to cause neoplastic change. The ulceration was considered due to stress that accompanied prostration of the animal.

Effects on the development and growth of offspring**1. Clinical signs and mortality**

No changes associated with n-BB administration were found in any groups of F1 and F2 offspring.

2. Body weights

Body weights of the F1 and F2 offspring are shown in Table 10. Significant inhibition of the body weight gain was found on PND 14 in the F2 offspring

Table 2. Fertility data.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F0 parents / F1 offspring				
Male animals				
Mating index ^a	22 / 24	23 / 24	24 / 24	24 / 24
Fertility index ^b	20 / 22	21 / 23	21 / 24	22 / 24
Female animals				
Estrus count	3.67 ± 0.48 ^g	3.58 ± 0.88	3.57 ± 0.68	3.83 ± 0.38
Estrous interval (estrous cycle; days)	4.09 ± 0.31	4.09 ± 0.20	4.40 ± 0.44*	4.48 ± 1.44
Mating index ^c	21 / 24	21 / 24	24 / 24	23 / 24
Fertility index ^d	19 / 21	19 / 21	21 / 24	21 / 23
Gestation ratio ^e	19 / 19	19 / 19	21 / 21	21 / 21
Gestation length (days) ^f	22.26 ± 0.45	22.37 ± 0.50	22.48 ± 0.51	22.57 ± 0.81
Number of implantations	14.26 ± 1.69	13.42 ± 2.81	14.24 ± 2.49	12.19 ± 3.91
The number of litter	13.26 ± 1.79	12.74 ± 2.62	13.48 ± 2.71	11.05 ± 3.97
F1 parents / F2 offspring				
Male animals				
Mating index ^a	15 / 18	15 / 19	17 / 21	15 / 18
Fertility index ^b	13 / 15	12 / 15	14 / 17	13 / 15
Female animals				
Estrus count	3.33 ± 0.77	3.63 ± 0.68	3.57 ± 0.75	3.68 ± 0.89
Estrous interval (estrous cycle; days)	4.42 ± 0.61	4.26 ± 0.42	4.25 ± 0.32	4.57 ± 0.74
Mating index ^c	15 / 18	15 / 19	17 / 21	15 / 18
Fertility index ^d	13 / 15	12 / 15	14 / 17	13 / 15
Gestation ratio ^e	13 / 13	11 / 12	13 / 14	13 / 13
Gestation length (days) ^f	22.31 ± 0.48	22.45 ± 0.52	22.31 ± 0.48	22.69 ± 0.48
Number of implantations	14.38 ± 2.66	13.45 ± 2.62	14.62 ± 2.40	13.54 ± 3.62
The number of litter	13.38 ± 2.47	12.18 ± 2.79	13.77 ± 1.96	12.31 ± 4.39

^a: Mating index = the number of males in which copulation was found / the number of males used for mating.

^b: Fertility index = the number of pregnant females / the number of males for which copulation was confirmed.

^c: Mating index = the number of the females for which copulation was confirmed / the number of females used for mating.

^d: Fertility index = the number of pregnant females / the number of females for which copulation was confirmed.

^e: Gestation ratio = the number of females with viable offspring / the number of pregnant females.

^f: Gestation length (days) = the period from the day when the copulation was confirmed until completion of delivery.

^g: Mean ± S.D.

: Significantly different from the control (, p<0.05; **, p<0.01).

receiving 100 mg/kg/day, but no other variation was evident.

3. Results for the number in a litter, sex ratio, viability of the offspring, anogenital distance, and reflex response test

Data for the number in a litter, sex ratio, and viability of offspring are shown in Table 11, and for the anogenital distance and reflex response tests are shown in Tables 12 and 13, respectively.

In the 300 mg/kg group of the F0 parental animals, the number in a litter and viability tended to be lower and the number of live offspring was significantly decreased. The low number of the live offspring was considered to be secondary to the low number of implantations in the parental animals. In the F1 parental animals, this was not observed. In addition, no changes associated with n-BB administration were found for the sex ratio, viability on PND 4 and weaning index in the F1 and F2 offspring, and the anogenital distance and the results of reflex response tests in the F1 offspring in any group.

4. Physical development

Completion days for pinna detachment, incisor eruption, and eyelid separation of the offspring are shown in Table 14. No effects of the administration of n-BB were observed.

5. Autopsy

No gross changes associated with n-BB administration were recognized at autopsy in any F1 or F2 offspring which died during the lactation period, the F1 or F2 offspring not selected on day 4 of lactation, the F1 weanlings not selected as F1 parental animals and the F2 weanlings.

6. Organ weights

Organ weights for the F1 and F2 offspring are shown in Table 15. In the F1 male and female offspring and F2 female offspring receiving 300 mg/kg/day, the absolute and relative weights of the thymus were significantly elevated or tended to be increased as compared with those of the control groups. Significantly elevated values were found for the relative ovary weights in the F1 female offspring receiving 100 mg/

Table 3. Stage analysis for the testes in control and non-copulating or copulating non-fertile F0 males.

n-butylbenzene (mg/kg/day)	Animal No.	Results of mating	Counts of stage (%) ^a													
			I	II,III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	
0 (Control)	801	pregnant	14	11	4	11	5	16	7	2	3	4	9	6	8	
	802	pregnant	20	9	4	8	7	15	10	3	6	2	8	4	4	
	803	pregnant	15	13	3	8	6	20	7	7	4	2	7	6	2	
	804	pregnant	11	13	9	10	8	16	10	6	1	0	8	4	4	
	805	pregnant	14	9	4	7	6	14	6	5	3	5	12	9	6	
0 (Control)	809	non-copulating	17	14	7	6	4	20	6	1	0	3	8	8	6	
	811	non-pregnant	14	14	5	10	7	18	5	2	4	3	8	5	5	
	815	non-copulating	11	11	4	7	7	21	8	3	2	2	10	7	7	
	821	non-pregnant	14	20	6	7	5	18	6	2	2	4	6	6	4	
30	830	non-copulating	16	18	7	6	7	17	6	2	2	1	6	7	5	
	835	non-pregnant	13	10	5	7	6	21	12	3	6	3	7	5	2	
	838	non-pregnant	11	15	6	8	7	18	8	1	2	1	8	6	9	
100	862	non-pregnant	11	15	5	7	5	22	4	4	1	4	6	10	6	
	864	non-pregnant	14	13	3	11	9	19	9	2	3	2	8	4	3	
	866	non-pregnant	15	13	5	7	8	18	9	4	3	2	6	5	5	
300	893	non-pregnant	15	14	8	6	7	12	6	2	1	3	12	9	5	
	896	non-pregnant	18	13	6	8	5	15	5	6	3	4	7	5	5	

^a: Values are percentages of seminiferous tubules examined.

Reproductive toxicity study of n-butylbenzene.

kg/day, the absolute ovary weights in the F1 female offspring receiving 300 mg/kg/day, the relative brain weights in the F2 males receiving 100mg/kg/day, and the relative thymus weights in the F2 females receiving 300mg/kg/day. Significantly low values for absolute uterus weights were found in the F2 female offspring receiving 300mg/kg/day. However, all the changes except for the increased thymus weights were considered sporadic and unrelated to n-BB administration,

since they were not consistently statistically significant.

DISCUSSION

With respect to effects of n-BB on parental animals, the following results were obtained. No mortality was caused and clinical signs were limited to salivation found in F0 and F1 males and females receiving 100

Table 4. Stage analysis for the testes in control and non-copulating or copulating non-fertile F1 males.

n-butylbenzene (mg/kg/day)	Animal No.	Results of mating	Counts of stage (%) ^a													
			I	II,III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	
0 (Control)	902-5	pregnant	17	13	5	6	7	18	5	4	2	2	9	8	4	
	904-5	pregnant	13	15	6	6	9	20	6	4	3	0	8	5	5	
	905-5	pregnant	15	9	6	5	9	16	7	5	3	3	7	6	9	
	906-5	pregnant	16	17	8	7	7	19	7	1	0	3	7	5	3	
	908-5	pregnant	14	14	6	10	8	15	10	3	2	2	6	6	4	
0 (Control)	901-5	non-copulating	15	15	3	6	7	14	10	2	4	3	10	5	6	
	903-5	non-pregnant	10	12	6	10	5	14	12	8	3	5	7	3	5	
	907-5	non-pregnant	13	15	5	7	7	19	8	5	2	4	7	5	3	
	910-5	non-copulating	17	13	6	7	5	15	6	0	1	1	10	9	10	
	918-5	non-copulating	15	14	3	8	7	16	9	3	3	3	9	5	5	
30	925-5	non-pregnant	11	14	4	8	8	18	11	1	1	2	14	2	6	
	929-5	non-pregnant	15	12	5	12	5	16	7	4	2	1	8	5	8	
	930-5	non-copulating	15	9	4	6	10	21	10	2	4	3	7	5	4	
	931-5	non-pregnant	16	13	6	6	6	16	7	1	5	1	12	6	5	
	939-6	non-copulating	16	14	3	5	8	12	13	4	0	3	9	8	5	
	942-5	non-copulating	19	15	4	7	5	19	10	1	3	2	8	4	3	
	948-5	non-copulating	13	10	4	8	10	17	7	4	2	2	9	7	7	
100	950-5	non-pregnant	14	14	5	7	8	16	7	6	2	2	10	5	4	
	952-5	non-copulating	13	13	6	9	11	13	7	2	3	4	8	6	5	
	955-5	non-copulating	14	16	4	7	8	16	7	4	2	1	10	6	5	
	957-4	non-pregnant	17	9	5	7	7	15	12	1	5	3	6	6	7	
	958-5	non-copulating	14	14	3	10	6	18	9	3	2	2	10	5	4	
	967-4	non-copulating	16	12	7	6	8	19	10	3	0	4	6	3	6	
	968-5	non-pregnant	14	13	7	5	5	19	9	7	3	2	7	5	4	
300	950-5	non-pregnant	14	14	5	7	8	16	7	6	2	2	10	5	4	
	952-5	non-copulating	13	13	6	9	11	13	7	2	3	4	8	6	5	
	955-5	non-copulating	14	16	4	7	8	16	7	4	2	1	10	6	5	
	957-4	non-pregnant	17	9	5	7	7	15	12	1	5	3	6	6	7	
	958-5	non-copulating	14	14	3	10	6	18	9	3	2	2	10	5	4	
	967-4	non-copulating	16	12	7	6	8	19	10	3	0	4	6	3	6	
	968-5	non-pregnant	14	13	7	5	5	19	9	7	3	2	7	5	4	

^a: Values are percentages of seminiferous tubules examined.

Table 5. Data for sperm and hormone levels.

n-butylbenzene (mg/kg/day)	0 (Control)		30		100		300	
F0 parent								
Sperm examination								
The number of males examined	10		10		10		10	
Sperm count (×10 ⁻⁶ g)	707.82 ± 137.79 ^a		676.81 ± 80.89		733.08 ± 123.25		769.06 ± 179.08	
Sperm motility (%)	98.53 ± 1.06		99.03 ± 0.92		98.00 ± 1.41		98.17 ± 1.34	
Morphologically abnormal sperm (%)	1.10 ± 0.52		1.30 ± 0.64		0.87 ± 0.57		1.57 ± 0.85	
Hormone levels								
The number of males examined	6		6		6		6	
LH (ng/mL)	1.44 ± 0.28		1.52 ± 0.42		1.56 ± 0.29		1.62 ± 0.37	
Testosterone (ng/mL)	3.21 ± 1.76		3.04 ± 1.82		2.68 ± 1.83		2.36 ± 1.61	
FSH (ng/mL)	12.00 ± 1.64		11.57 ± 1.75		11.56 ± 0.91		11.37 ± 0.99	
The number of females examined	6		6		6		6	
LH (ng/mL)	1.44 ± 0.24		1.38 ± 0.19		1.49 ± 0.28		1.42 ± 0.49	
FSH (ng/mL)	11.00 ± 3.72		9.83 ± 2.27		8.28 ± 0.88		7.99 ± 1.12	
Estradiol (pg/mL)	60.6 ± 8.6		38.9 ± 26.5		55.8 ± 26.6		51.0 ± 17.8	
F1 parent								
Sperm examination								
No. of males examined	10		10		10		10	
Sperm count (×10 ⁻⁶ g)	717.79 ± 91.89		696.08 ± 108.20		670.89 ± 159.93		664.35 ± 72.81	
Sperm motility (%)	97.16 ± 1.81		96.62 ± 2.26		96.57 ± 2.05		95.56 ± 2.40	
Morphologically abnormal sperm (%)	1.30 ± 0.74		1.57 ± 0.97		1.30 ± 1.09		1.13 ± 0.95	
Hormone levels								
The number of males examined	6		6		6		6	
LH (ng/mL)	1.80 ± 0.17		1.59 ± 0.38		1.39 ± 0.40		1.47 ± 0.42	
Testosterone (ng/mL)	3.47 ± 1.93		1.93 ± 0.90		3.21 ± 2.71		2.54 ± 1.09	
FSH (ng/mL)	13.83 ± 1.55		12.97 ± 2.20		13.42 ± 0.83		14.11 ± 1.52	
The number of females examined	6		6		6		6	
LH (ng/mL)	1.39 ± 0.43		1.55 ± 0.32		1.52 ± 0.30		1.36 ± 0.51	
FSH (ng/mL)	10.45 ± 2.81		11.56 ± 5.08		10.98 ± 3.90		10.94 ± 2.20	
Estradiol (pg/mL)	46.4 ± 21.3		40.6 ± 37.9		63.6 ± 50.2		68.5 ± 45.6	

^a: Mean $\pm$ S.D.**Table 6.** Sexual maturation.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F1 pups				
Preputial separation (Males)	19	19	21	19
At 35 day (%) ^a	40.63 ± 1.98 ^c	40.11 ± 1.45	40.86 ± 2.39	41.05 ± 1.47
Body Weight (g) ^b	245.7 ± 24.7	248.1 ± 21.8	245.0 ± 22.1	253.5 ± 33.9
Vaginal opening (Females)	18	19	21	19
At 28 day (%) ^a	31.17 ± 1.42	31.63 ± 1.83	31.24 ± 2.21	31.79 ± 1.87
Body Weight (g) ^b	111.4 ± 9.7	116.6 ± 13.3	113.3 ± 16.0	118.4 ± 11.8

^a: Percentage of completion animals at the time.^b: Body weight on completion day.^c: Mean $\pm$ S.D.

Reproductive toxicity study of n-butylbenzene.

Table 7. Organ weights of F0 parent animals.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
Male				
The number of animals examined	24	24	24	24
Final body weight (g)	636.7 ± 72.9 ^c	627.6 ± 51.2	635.9 ± 70.2	623.4 ± 60.8
Brain (g) ^a	2.29 ± 0.10	2.27 ± 0.10	2.26 ± 0.12	2.29 ± 0.10
Brain (%) ^b	0.36 ± 0.04	0.36 ± 0.03	0.36 ± 0.04	0.37 ± 0.04
Pituitary (mg)	13.8 ± 1.7	13.6 ± 1.7	13.8 ± 1.4	13.5 ± 1.3
Pituitary (×10 ⁻³ %)	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.2
Thyroids (mg)	22.1 ± 3.8	21.5 ± 2.8	22.4 ± 4.2	22.1 ± 5.1
Thyroids (×10 ⁻³ %)	3.5 ± 0.7	3.4 ± 0.5	3.6 ± 0.8	3.6 ± 0.7
Thymus (g)	0.27 ± 0.08	0.26 ± 0.08	0.27 ± 0.09	0.25 ± 0.07
Thymus (%)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Liver (g)	21.35 ± 3.71	21.53 ± 2.54	22.78 ± 3.04	25.02 ± 3.42**
Liver (%)	3.34 ± 0.28	3.43 ± 0.21	3.58 ± 0.18**	4.00 ± 0.27**
Spleen (g)	0.83 ± 0.09	0.88 ± 0.12	0.90 ± 0.29	0.84 ± 0.10
Spleen (%)	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.05	0.14 ± 0.01
Kidneys (g)	3.51 ± 0.42	3.53 ± 0.26	3.70 ± 0.46	4.07 ± 0.50**
Kidneys (%)	0.55 ± 0.04	0.57 ± 0.05	0.58 ± 0.05	0.65 ± 0.05**
Adrenal glands (mg)	56.6 ± 7.1	57.9 ± 6.6	55.5 ± 10.4	59.2 ± 8.6
Adrenal glands (×10 ⁻³ %)	9.0 ± 1.2	9.3 ± 1.0	8.8 ± 1.6	9.6 ± 1.4
Seminal vesicle (g)	2.26 ± 0.35	2.36 ± 0.31	2.24 ± 0.21	2.35 ± 0.36
Seminal vesicle (%)	0.36 ± 0.07	0.38 ± 0.06	0.36 ± 0.05	0.38 ± 0.06
Prostate (g)	1.07 ± 0.20	1.10 ± 0.19	1.08 ± 0.16	1.12 ± 0.24
Prostate (%)	0.17 ± 0.04	0.18 ± 0.04	0.17 ± 0.03	0.18 ± 0.04
Epididymides (g)	1.30 ± 0.09	1.29 ± 0.13	1.33 ± 0.11	1.31 ± 0.10
Epididymides (%)	0.21 ± 0.02	0.21 ± 0.03	0.21 ± 0.02	0.21 ± 0.02
Testes (g)	3.43 ± 0.30	3.49 ± 0.27	3.51 ± 0.23	3.41 ± 0.30
Testes (%)	0.54 ± 0.06	0.56 ± 0.07	0.56 ± 0.07	0.55 ± 0.06
Female				
The number of animals examined	19	19	21	19
Final body weight (g)	322.8 ± 24.7	331.3 ± 23.7	328.4 ± 17.9	321.4 ± 22.6
Brain (g)	2.08 ± 0.09	2.10 ± 0.08	2.13 ± 0.07	2.12 ± 0.08
Brain (%)	0.64 ± 0.06	0.63 ± 0.04	0.65 ± 0.03	0.66 ± 0.04
Pituitary (mg)	15.6 ± 1.8	15.9 ± 1.6	16.2 ± 3.4	15.6 ± 2.9
Pituitary (×10 ⁻³ %)	4.9 ± 0.6	4.8 ± 0.5	4.9 ± 1.1	4.9 ± 0.8
Thyroids (mg)	16.8 ± 3.6	17.3 ± 4.2	16.5 ± 3.9	15.9 ± 4.7
Thyroids (×10 ⁻³ %)	5.2 ± 1.2	5.2 ± 1.1	5.1 ± 1.4	4.9 ± 1.4
Thymus (g)	0.21 ± 0.06	0.22 ± 0.05	0.21 ± 0.07	0.22 ± 0.07
Thymus (%)	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
Liver (g)	13.30 ± 1.62	15.09 ± 1.50**	15.11 ± 1.64**	15.20 ± 1.70**
Liver (%)	4.13 ± 0.47	4.56 ± 0.33*	4.61 ± 0.50**	4.74 ± 0.54**
Spleen (g)	0.64 ± 0.08	0.66 ± 0.07	0.64 ± 0.07	0.63 ± 0.10
Spleen (%)	0.19 ± 0.04	0.20 ± 0.02	0.19 ± 0.02	0.20 ± 0.03
Kidneys (g)	2.14 ± 0.22	2.19 ± 0.21	2.22 ± 0.18	2.29 ± 0.19
Kidneys (%)	0.66 ± 0.05	0.66 ± 0.05	0.68 ± 0.05	0.71 ± 0.05**
Adrenal glands (mg)	63.5 ± 11.2	63.6 ± 11.8	63.0 ± 13.8	72.4 ± 19.8
Adrenal glands (×10 ⁻³ %)	19.7 ± 3.5	19.3 ± 4.2	19.3 ± 5.0	22.6 ± 6.2
Ovaries (mg)	94.3 ± 16.6	101.6 ± 22.0	103.8 ± 15.3	97.9 ± 18.6
Ovaries (×10 ⁻³ %)	29.0 ± 6.1	30.7 ± 6.7	31.6 ± 4.5	30.5 ± 5.8
Uterus (g)	0.85 ± 0.26	0.88 ± 0.27	0.86 ± 0.32	0.92 ± 0.28
Uterus (%)	0.26 ± 0.08	0.27 ± 0.09	0.26 ± 0.09	0.29 ± 0.09

^a: Absolute organ weight.^b: Relative organ weight.^c: Mean ± S.D.

*: Significantly different from the controls; *, p<0.05; **, p<0.01.

Table 8. Organ weights of F1 parent animals.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
Male				
The number of animals examined	19	19	21	19
Final body weight (g)	680.4 ± 99.7 ^c	698.4 ± 45.1	668.0 ± 61.3	653.3 ± 75.5
Brain (g) ^a	2.26 ± 0.09	2.27 ± 0.09	2.27 ± 0.12	2.31 ± 0.09
Brain (%) ^b	0.34 ± 0.05	0.33 ± 0.02	0.34 ± 0.03	0.36 ± 0.04
Pituitary (mg)	14.1 ± 1.9	14.2 ± 1.7	14.4 ± 1.5	14.6 ± 1.9
Pituitary (×10 ⁻³ %)	2.1 ± 0.5	2.0 ± 0.3	2.2 ± 0.3	2.3 ± 0.3
Thyroids (mg)	22.4 ± 4.6	25.7 ± 5.3	22.2 ± 3.6	23.1 ± 4.0
Thyroids (×10 ⁻³ %)	3.3 ± 0.8	3.7 ± 0.8	3.4 ± 0.7	3.6 ± 0.7
Thymus (g)	0.29 ± 0.08	0.29 ± 0.07	0.30 ± 0.08	0.28 ± 0.12
Thymus (%)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
Liver (g)	24.05 ± 5.57	25.08 ± 2.95	24.15 ± 2.75	26.82 ± 4.14
Liver (%)	3.51 ± 0.34	3.58 ± 0.25	3.62 ± 0.24	4.09 ± 0.28**
Spleen (g)	0.83 ± 0.09	0.88 ± 0.12	0.90 ± 0.29	0.84 ± 0.10
Spleen (%)	0.13 ± 0.02	0.14 ± 0.02	0.12 ± 0.02	0.13 ± 0.02
Kidneys (g)	3.78 ± 0.46	4.12 ± 0.46	4.01 ± 0.54	4.45 ± 0.53**
Kidneys (%)	0.56 ± 0.04	0.59 ± 0.05	0.60 ± 0.06*	0.68 ± 0.07**
Adrenal glands (mg)	57.9 ± 9.6	56.1 ± 7.9	60.0 ± 7.2	58.8 ± 7.2
Adrenal glands (×10 ⁻³ %)	8.6 ± 1.5	8.1 ± 1.3	9.0 ± 1.0	9.1 ± 1.2
Seminal vesicle (g)	2.51 ± 0.40	2.44 ± 0.31	2.59 ± 0.24	2.62 ± 0.36
Seminal vesicle (%)	0.37 ± 0.07	0.35 ± 0.05	0.39 ± 0.05	0.40 ± 0.06
Prostate (g)	1.08 ± 0.18	1.06 ± 0.19	1.07 ± 0.23	1.05 ± 0.18
Prostate (%)	0.16 ± 0.03	0.15 ± 0.03	0.16 ± 0.03	0.16 ± 0.03
Epididymides (g)	1.38 ± 0.06	1.37 ± 0.11	1.34 ± 0.12	1.33 ± 0.07
Epididymides (%)	0.21 ± 0.03	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.03
Testes (g)	3.68 ± 0.25	3.78 ± 0.30	3.79 ± 0.32	3.69 ± 0.26
Testes (%)	0.55 ± 0.07	0.54 ± 0.06	0.57 ± 0.07	0.57 ± 0.08
Female				
The number of animals examined	12	11	12	13
Final body weight (g)	328.9 ± 21.9	329.2 ± 20.7	323.4 ± 33.6	325.4 ± 20.3
Brain (g)	2.08 ± 0.08	2.06 ± 0.09	2.11 ± 0.07	2.11 ± 0.07
Brain (%)	0.63 ± 0.05	0.63 ± 0.03	0.66 ± 0.05	0.65 ± 0.03
Pituitary (mg)	16.7 ± 2.0	16.1 ± 1.7	16.3 ± 1.8	16.3 ± 1.5
Pituitary (×10 ⁻³ %)	5.1 ± 0.9	4.9 ± 0.6	5.0 ± 0.4	5.1 ± 0.4
Thyroids (mg)	18.8 ± 3.8	18.6 ± 4.0	19.9 ± 4.5	20.3 ± 4.7
Thyroids (×10 ⁻³ %)	5.7 ± 1.0	5.7 ± 1.3	6.2 ± 1.4	6.3 ± 1.5
Thymus (g)	0.22 ± 0.07	0.22 ± 0.07	0.22 ± 0.07	0.21 ± 0.06
Thymus (%)	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.02
Liver (g)	15.15 ± 1.75	15.03 ± 1.44	14.90 ± 1.52	17.02 ± 2.26*
Liver (%)	4.62 ± 0.52	4.57 ± 0.37	4.62 ± 0.39	5.22 ± 0.48**
Spleen (g)	0.67 ± 0.07	0.68 ± 0.11	0.63 ± 0.11	0.67 ± 0.09
Spleen (%)	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.02	0.21 ± 0.02
Kidneys (g)	2.18 ± 0.17	2.39 ± 0.24	2.23 ± 0.22	2.45 ± 0.18**
Kidneys (%)	0.67 ± 0.05	0.72 ± 0.05	0.69 ± 0.05	0.75 ± 0.02**
Adrenal glands (mg)	60.5 ± 5.1	64.5 ± 8.0	60.9 ± 7.2	68.3 ± 6.3*
Adrenal glands (×10 ⁻³ %)	18.5 ± 1.9	19.6 ± 1.9	18.9 ± 2.4	21.0 ± 2.2*
Ovaries (mg)	104.9 ± 15.5	101.5 ± 16.1	111.7 ± 20.1	114.7 ± 20.4
Ovaries (×10 ⁻³ %)	32.1 ± 6.0	31.0 ± 5.3	34.6 ± 5.7	35.2 ± 5.8
Uterus (g)	0.88 ± 0.20	0.89 ± 0.23	0.84 ± 0.21	0.92 ± 0.24
Uterus (%)	0.27 ± 0.06	0.27 ± 0.06	0.26 ± 0.06	0.28 ± 0.06

^a: Absolute organ weight.^b: Relative organ weight.^c: Mean ± S.D.

*:Significantly different from the controls; *, p<0.05; **, p<0.01.

Reproductive toxicity study of n-butylbenzene.

and 300 mg/kg/day; furthermore, this was considered due to direct effects of the bitter taste stimulus of the test solution, and proved temporary. An increase in liver weight was observed in the animals receiving 30, 100, and 300 mg/kg/day and on histopathological examination, centrilobular hepatocytic hypertrophy was found in the F0 and F1 male parental animals receiving 300 mg/kg/day. Imaoka and Funae (1991) reported that this test substance induces rat liver cyto-

chrome P450 at a dose of 5 mmol/kg (equivalent to 670 mg/kg); therefore, this change was considered to be attributable to hyperplasia of the smooth endoplasmic reticulum accompanying induction of drug-metabolic enzymes. No histological change was observed in the liver of the female animals; however, it was considered that a similar change might have occurred in the females, since the liver weights were increased significantly. An increase in kidney weight was also observed

Table 9. Histopathological findings for F0/F1 parental animals.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F0 parent animals				
Males				
Liver				
Hypertrophy, hepatocytes	0 / 24 ^a	0 / 24	0 / 24	5 / 24*
Fatty degeneration, hepatocytes	8 / 24	6 / 24	2 / 24*	1 / 24
Kidney				
Hyaline droplets, proximal tubules	0 / 24	0 / 24	1 / 24	11 / 24**
Basophilic tubules	0 / 24	0 / 24	0 / 24	5 / 24*
Casts, proteinaceous	0 / 24	0 / 24	0 / 24	0 / 24
Females				
Glandular stomach				
Ulceration	0 / 24	0 / 24	0 / 24	1 / 24
Liver				
Leukemia, myeloid	0 / 24	0 / 24	0 / 24	1 / 24
Spleen				
Leukemia, myeloid	0 / 24	0 / 24	0 / 24	1 / 24
Adrenal				
Leukemia, myeloid	0 / 24	0 / 24	0 / 24	1 / 24
Vagina				
Appearance of mucosal cells	0 / 24	0 / 24	0 / 24	1 / 24
F1 parent animals				
Males				
Liver				
Hypertrophy, hepatocytes	0 / 19	0 / 19	0 / 21	6 / 19**
Fatty degeneration, hepatocytes	5 / 19	4 / 19	0 / 21*	0 / 19*
Kidney				
Hyaline droplets, proximal tubules	0 / 19	1 / 19	5 / 21*	12 / 19*
Basophilic tubules	0 / 19	0 / 19	1 / 21	5 / 19*
Casts, proteinaceous	1 / 19	4 / 19	3 / 21	2 / 19
Females				
Vagina				
Appearance of mucosal cells	1 / 19	2 / 19	1 / 21	1 / 19
Cervix				
Cysts	0 / 19	0 / 19	0 / 21	1 / 19

^a: The data shown are numbers of animals showing the finding / the number of animals examined.

*: Significantly different from the controls; *, p<0.05; **, p<0.01.

Table 10. Body weights of F1/F2 offspring.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F1 offspring				
Male (g)				
Postnatal day 0	6.8 ± 0.7 ^a	7.0 ± 0.7	7.0 ± 0.7	7.1 ± 0.7
Postnatal day 4	11.6 ± 1.5	12.1 ± 1.8	11.8 ± 1.6	12.0 ± 2.0
Postnatal day 7	18.9 ± 2.1	19.9 ± 2.3	19.0 ± 1.8	18.8 ± 2.6
Postnatal day 14	37.8 ± 3.2	38.9 ± 2.4	38.0 ± 3.0	38.1 ± 3.9
Postnatal day 21	57.4 ± 6.8	58.6 ± 4.9	55.5 ± 5.0	60.7 ± 7.3
Female (g)				
Postnatal day 0	6.6 ± 0.6	6.5 ± 0.7	6.5 ± 0.7	6.6 ± 0.7
Postnatal day 4	11.2 ± 1.4	11.5 ± 1.9	11.2 ± 1.7	11.3 ± 2.1
Postnatal day 7	18.2 ± 2.0	18.8 ± 2.4	18.3 ± 2.0	18.1 ± 2.6
Postnatal day 14	36.7 ± 3.3	37.1 ± 2.5	36.8 ± 3.2	36.7 ± 3.7
Postnatal day 21	55.7 ± 6.9	55.5 ± 4.8	53.7 ± 5.2	58.8 ± 6.3
F2 offspring				
Male (g)				
Postnatal day 0	6.8 ± 0.6	7.1 ± 0.6	6.4 ± 0.7	6.9 ± 0.6
Postnatal day 4	11.7 ± 1.4	12.5 ± 1.3	10.9 ± 1.5	12.3 ± 1.9
Postnatal day 7	19.6 ± 2.5	20.1 ± 1.5	17.8 ± 2.6	20.1 ± 2.0
Postnatal day 14	39.8 ± 4.0	39.5 ± 2.2	36.3 ± 4.1*	39.4 ± 3.2
Postnatal day 21	65.0 ± 5.6	65.9 ± 3.1	61.4 ± 5.8	65.3 ± 3.6
Female (g)				
Postnatal day 0	6.2 ± 0.5	6.7 ± 0.6	6.1 ± 0.6	6.6 ± 0.6
Postnatal day 4	11.0 ± 1.2	12.0 ± 1.1	10.4 ± 1.4	11.6 ± 1.6
Postnatal day 7	18.7 ± 2.1	19.3 ± 1.4	17.4 ± 2.3	18.8 ± 1.8
Postnatal day 14	37.9 ± 3.2	37.8 ± 2.9	35.9 ± 3.7	37.4 ± 3.2
Postnatal day 21	61.9 ± 5.0	62.9 ± 4.3	59.9 ± 5.9	62.0 ± 4.4

^a: Mean ± S.D.

*: Significantly different from the controls; *, p<0.05; **, p<0.01.

Table 11. Results for numbers in a litter, the sex ratio, and viability of the offspring.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F1 offspring				
Number in a litter	13.26 ± 1.79 ^e	12.74 ± 2.62	13.48 ± 2.71	11.05 ± 3.97
Sex ratio ^a	134 / 118	125 / 114	138 / 143	115 / 113
Birth rate (%) ^b	92.99	93.73	93.98	89.06
Viability on post natal day 4 (%) ^c	98.81	99.58	99.64	98.25
Weaning rate (%) ^d	100	99.33	100	97.37
F2 offspring				
Number in a litter	13.38 ± 2.47	12.18 ± 2.79	13.77 ± 1.96	12.31 ± 4.39
Sex ratio	88 / 85	68 / 66	82 / 85	66 / 88
Birth rate (%)	92.51	90.54	87.89	87.50
Viability on post natal day 4 (%)	96.53	99.25	92.22	98.70
Weaning rate (%)	100	100	100	100

^a: Sex ratio = the number of the male offspring born / the number of the female offspring born.^b: Birth ratio (%) = (the number of offspring born / the number of implantations) × 100.^c: Viability on postnatal day (PND) 4 (%) = (the number of live offspring on PND 4 / the number of offspring born) × 100.^d: Weaning rate (%) = (the number of the live offspring on PND 21 / the number of the selected offspring on PND 4) × 100.^e: Mean ± S.D.

Reproductive toxicity study of n-butylbenzene.

in the F0 and F1 parental animals receiving 100 and 300 mg/kg/day. On histopathological examination, deposition of hyaline droplets in the proximal tubules was observed in the F0 male parental animals receiving

100 mg/kg/day or above and the F1 male parental animals receiving 30 mg/kg/day or above. In the F0 male parental animals receiving 300 mg/kg/day or above and the F1 male parental animals receiving 100 mg/kg/

Table 12. Anogenital distance.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F1 offspring ^a				
No. of males examined	76	76	84	75
AGD (mm) ^b	4.95 ± 0.37 ^c	5.09 ± 0.54	4.98 ± 0.34	5.09 ± 0.48
No. of females examined	76	74	82	77
AGD (mm)	2.38 ± 0.17	2.40 ± 0.21	2.47 ± 0.17	2.42 ± 0.22

^a: AGD was measured on postnatal day 4.

^b: Distance between the anus and genital node (mm).

^c: Mean ± S.D.

Table 13. Results of reflex response tests of F1 offspring.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
Male animals				
Righting reflex ^a				
Postnatal day 5	71 / 76 ^e	73 / 76	81 / 84	65 / 75
Postnatal day 6	76 / 76	76 / 76	84 / 84	74 / 75
Postnatal day 7	Not tested	Not tested	Not tested	75 / 75
Ipsilateral flexor reflex ^b				
Postnatal day 5	76 / 76	76 / 76	84 / 84	75 / 75
Visual placing ^c				
Postnatal day 16	76 / 76	76 / 76	84 / 84	71 / 71
Preyer's reflex ^d , 500 Hz				
Postnatal day 28	19 / 19	19 / 19	21 / 21	19 / 19
Preyer's reflex, 2000 Hz				
Postnatal day 28	19 / 19	19 / 19	21 / 21	19 / 19
Female animals				
Righting reflex				
Postnatal day 5	67 / 76	63 / 74	73 / 82	65 / 74
Postnatal day 6	75 / 76	73 / 74	82 / 82	72 / 74
Postnatal day 7	76 / 76	74 / 74	Not tested	73 / 74
Ipsilateral flexor reflex				
Postnatal day 5	76 / 76	74 / 74	82 / 82	74 / 74
Visual placing				
Postnatal day 16	76 / 76	73 / 73	82 / 82	69 / 69
Preyer's reflex, 500 Hz	19 / 19	19 / 19	21 / 21	19 / 19
Preyer's reflex, 2000 Hz				
Postnatal day 28	19 / 19	19 / 19	21 / 21	19 / 19

^a: Animals were examined for the righting reflex from PND 5 while its observation.

^b: Animals were examined for the ipsilateral flexor reflex from PND 5 while its observation.

^c: Animals were examined for the visual placing was observed from PND 16 while its observation.

^d: Animals were examined for the preyer's reflex from PND 28 while its observation.

^e: The data shown are numbers of animals showing the item / the number of animals examined.

day or above, basophilic renal tubules were also observed. These changes were not observed in the females, and the toxicological significance can be considered limited, since the deposition of hyaline droplets was inferred to be due to α_2 -globulin, a protein specific to male rats. The basophilic renal tubules were considered to indicate secondary tubular damage following on deposition of hyaline droplets; however, the possibility of change occurring by a mechanism independent of hyaline droplets, and α_2 -globulin could not be precluded because: the degree of deposition appeared too mild to induce the tubular damage (Greaves, 1990); kidney weights were increased in the F0 and F1 female parental animals receiving 300 mg/kg/day with no histological renal change; and there have been reports of renal tubular damage without deposition of hyaline droplets with other benzene chemical substances (Kamada, 1994a and 1994b; Hagita, 1996). In addition, the absolute and relative weights of the adrenal glands in the F0 and F1 females were elevated or showed a tendency for increase. The toxicological significance of this is unknown, and the change was slight with no associated histopathological alteration.

Of the indices related to fertility in the parental animals, the F0 parental animals receiving 300 mg/kg/day showed a tendency for lowering of the number of implantations; however, this was considered to be an incidental change, since there were 2 litters with implantation numbers of 2 or 3 in this group, and background data for the same strain collected at this laboratory showed the occurrence of such animals with low implantations at low frequency (0.86%). Similar change was not found in the F1 parental animals or in the 1000 mg/kg group of the preliminary study. The estrous interval (estrous cycle) was prolonged significantly in the F0 female parental animals receiving 100 mg/kg/day and a tendency for prolongation was observed in the F0 female parental animals receiving 300 mg/kg/day; however, this was considered to be incidental, since the prolongation was within the normal estrous interval range (4 or 5 days) of rats of this strain, and no other related changes were not found. Furthermore abnormal estrous cycling was not found in the 1000 mg/kg group of the preliminary study. In the F1 parental animals, a low reproductive performance was found in all F1 animal groups, including the controls. The results of histopathological examina-

Table 14. Physical development of F1 offspring.

n-butylbenzene (mg/kg/day)		0 (Control)	30	100	300
Male animals					
Pinna detachment ^a					
	Postnatal day 4	76 / 76 ^d	76 / 76	84 / 84	75 / 75
Incisor eruption ^b					
	Postnatal day 10	53 / 76	54 / 76	67 / 84	55 / 75
	Postnatal day 11	72 / 76	75 / 76	82 / 84	73 / 75
	Postnatal day 12	75 / 76	75 / 76	84 / 84	75 / 75
	Postnatal day 13	76 / 76	76 / 76	Not tested	Not tested
Eyelid separation ^c					
	Postnatal day 15	76 / 76	76 / 76	84 / 84	71 / 71
Female animals					
Pinna detachment					
	Postnatal day 4	76 / 76	74 / 74	82 / 82	77 / 77
Incisor eruption					
	Postnatal day 10	51 / 76	47 / 74	57 / 82	51 / 73
	Postnatal day 11	70 / 76	72 / 74	80 / 82	70 / 73
	Postnatal day 12	76 / 76	74 / 74	82 / 82	73 / 73
Eyelid separation					
	Postnatal day 15	76 / 76	73 / 73	82 / 82	69 / 69

^a: Animals were examined for the pinna detachment from PND 4 while its observation.

^b: Animals were examined for the incisor eruption from PND 10 while its observation.

^c: Animals were examined for the eyelid separation from PND 15 while its observation.

^d: The data shown are the numbers of animals showing the item / the number of animals examined.

Reproductive toxicity study of n-butylbenzene.

Table 15. Organ weights of F1/F2 offspring.

n-butylbenzene (mg/kg/day)	0 (Control)	30	100	300
F1 offspring				
The number of F1 males examined	19	19	21	17
Final body weight (g)	61.4 ± 6.6 ^c	61.5 ± 6.0	59.1 ± 6.1	65.3 ± 7.6
Brain (g) ^a	1.58 ± 0.05	1.56 ± 0.07	1.57 ± 0.06	1.62 ± 0.07
Brain (%) ^b	2.59 ± 0.26	2.55 ± 0.26	2.68 ± 0.27	2.50 ± 0.23
Thymus (g)	0.22 ± 0.07	0.24 ± 0.04	0.21 ± 0.04	0.28 ± 0.04*
Thymus (%)	0.36 ± 0.07	0.39 ± 0.05	0.35 ± 0.05	0.43 ± 0.05**
Spleen (g)	0.30 ± 0.07	0.29 ± 0.08	0.27 ± 0.06	0.30 ± 0.09
Spleen (%)	0.49 ± 0.08	0.48 ± 0.10	0.45 ± 0.10	0.46 ± 0.10
Epididymides (mg)	45.4 ± 7.3	46.8 ± 6.5	45.2 ± 6.9	48.3 ± 5.6
Epididymides (10 ⁻³ %)	74.1 ± 9.4	76.4 ± 10.3	76.5 ± 8.5	74.4 ± 8.9
Testes (mg)	312.1 ± 41.2	321.9 ± 40.3	319.1 ± 48.2	304.6 ± 58.3
Testes (10 ⁻³ %)	509.8 ± 54.4	524.1 ± 51.3	538.7 ± 47.6	468.7 ± 82.2
The number of F1 females examined	19	19	21	17
Final body weight (g)	57.6 ± 10.8	59.9 ± 5.0	57.3 ± 5.9	64.0 ± 6.0
Brain (g)	1.50 ± 0.12	1.53 ± 0.06	1.50 ± 0.08	1.57 ± 0.05
Brain (%)	2.71 ± 0.56	2.58 ± 0.21	2.65 ± 0.25	2.47 ± 0.20
Thymus (g)	0.23 ± 0.07	0.24 ± 0.04	0.21 ± 0.05	0.28 ± 0.06*
Thymus (%)	0.40 ± 0.08	0.40 ± 0.06	0.37 ± 0.07	0.44 ± 0.08
Spleen (g)	0.27 ± 0.08	0.28 ± 0.05	0.27 ± 0.06	0.31 ± 0.05
Spleen (%)	0.46 ± 0.10	0.47 ± 0.08	0.47 ± 0.09	0.48 ± 0.06
Ovaries (mg)	15.7 ± 3.6	18.4 ± 4.2	17.9 ± 2.6	18.2 ± 1.6*
Ovaries (10 ⁻³ %)	27.4 ± 4.5	30.5 ± 6.0	31.3 ± 4.1*	28.7 ± 3.0
Uterus (mg)	37.4 ± 8.3	37.1 ± 5.7	35.9 ± 6.3	40.4 ± 5.8
Uterus (10 ⁻³ %)	66.3 ± 13.5	62.0 ± 8.7	62.6 ± 9.0	63.4 ± 9.4
F2 offspring				
The number of F1 males examined	12	11	12	13
Final body weight (g)	71.4 ± 6.2	71.9 ± 3.3	66.6 ± 5.5	71.8 ± 4.1
Brain (g) ^a	1.61 ± 0.05	1.62 ± 0.05	1.60 ± 0.07	1.62 ± 0.07
Brain (%) ^b	2.26 ± 0.17	2.26 ± 0.10	2.41 ± 0.11*	2.26 ± 0.13
Thymus (g)	0.29 ± 0.06	0.30 ± 0.05	0.26 ± 0.06	0.29 ± 0.03
Thymus (%)	0.40 ± 0.06	0.41 ± 0.07	0.38 ± 0.08	0.41 ± 0.05
Spleen (g)	0.39 ± 0.05	0.41 ± 0.07	0.37 ± 0.06	0.38 ± 0.08
Spleen (%)	0.54 ± 0.07	0.57 ± 0.09	0.55 ± 0.06	0.53 ± 0.09
Epididymides (mg)	50.8 ± 7.6	54.6 ± 7.2	49.9 ± 4.6	54.4 ± 5.7
Epididymides (10 ⁻³ %)	71.2 ± 9.6	75.9 ± 9.7	75.1 ± 6.0	75.6 ± 5.7
Testes (mg)	330.1 ± 43.2	344.4 ± 42.3	322.9 ± 44.6	348.3 ± 38.4
Testes (10 ⁻³ %)	463.6 ± 54.6	479.0 ± 53.9	484.4 ± 40.0	483.9 ± 34.5
The number of F1 females examined	12	11	12	13
Final body weight (g)	68.3 ± 5.8	66.1 ± 5.5	64.9 ± 7.1	67.2 ± 6.3
Brain (g)	1.60 ± 0.04	1.56 ± 0.07	1.59 ± 0.06	1.56 ± 0.05
Brain (%)	2.36 ± 0.17	2.38 ± 0.21	2.46 ± 0.22	2.34 ± 0.19
Thymus (g)	0.25 ± 0.05	0.28 ± 0.05	0.26 ± 0.06	0.29 ± 0.03
Thymus (%)	0.37 ± 0.07	0.41 ± 0.07	0.40 ± 0.06	0.43 ± 0.05*
Spleen (g)	0.34 ± 0.08	0.38 ± 0.07	0.34 ± 0.08	0.36 ± 0.08
Spleen (%)	0.49 ± 0.08	0.57 ± 0.07	0.52 ± 0.10	0.53 ± 0.09
Ovaries (mg)	21.5 ± 3.7	19.5 ± 2.5	18.8 ± 2.8	19.4 ± 3.2
Ovaries (10 ⁻³ %)	31.6 ± 5.6	29.7 ± 3.9	29.0 ± 3.4	28.8 ± 4.2
Uterus (mg)	46.9 ± 8.4	45.7 ± 6.1	41.7 ± 5.8	40.3 ± 4.1*
Uterus (10 ⁻³ %)	68.7 ± 10.9	69.5 ± 11.2	64.5 ± 7.7	60.2 ± 7.0

^a: Absolute organ weight.^b: Relative organ weight.^c: Mean ± S.D.

*: Significantly different from the controls; *, p<0.05; **, p<0.01.

tions of the genital organs and stage analysis of the testes for the non-copulating and copulating infertile animals, sperm examination, and hormone measurement revealed no differences between normal cases and non-copulating or copulating infertility cases in any of the groups including the controls, and no factors affecting conception ability were found.

Thus, n-BB at doses up to 300 mg/kg/day did not show any effects on endpoints regarding fertility. In the F1 parental animals, however, a possibility of adverse influence was noted. As for effects on the endocrine system, n-BB administration did not alter plasma FSH, LH, testosterone, or estradiol concentrations, the sex ratio, anogenital distance, and sexual maturation.

In the F1 and F2 offspring, the dose of 300 mg/kg/day was considered to increase the weight of the thymus, since both absolute and relative values were elevated.

Therefore, from the findings when n-BB was administered over two successive generations to rats, the NOEL for parental animals is concluded to be less than 30 mg/kg/day, since the liver weights were increased at doses of 30 mg/kg/day and above. The NOAEL in the parental animals was estimated to be 30 mg/kg/day, for of the following reasons: the liver weight gain observed in the 30 mg/kg group can be considered an adaptive change accompany induction of drug metabolizing enzymes and was judged to have no toxicological significance; increase in weight and histopathological changes of the kidneys were recognized in the groups at doses of 100 mg/kg/day or above. On the other hand, the NOEL and NOAEL for fertility in F0 parental animals were both estimated to be 300 mg/kg/day for males and females. Judging from the results for effects in the F1 parental animals, it is concluded that n-BB is unlikely to cause serious reproductive toxicity. The NOEL and NOAEL for development and growth of offspring are both estimated to be 100 mg/kg/day, since increase in the thymus weights was observed at 300 mg/kg/day in both F1 and F2 offspring.

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