**2. 28-Day study in young rats (including the dose-finding study)**

In the 14-day dose-finding study at doses of 20, 60, 200 and 600 mg/kg, all animals died within 6 days after the first treatment in the highest group. They showed various toxic signs such as decrease in spontaneous movement, oligopnea and adoption of a prone/lateral position. Blood biochemical examination showed increase in total protein in males and in total cholesterol in females at 200 mg/kg. Increase in absolute and relative liver weights was observed in both sexes of the 60 and 200 mg/kg groups and relative liver weights in males of 10 mg/kg. In addition, increase was found in relative kidney weights in males and in absolute and relative kidney and heart weights in females at 200 mg/kg. There were no other dose-related changes evident. Based on the results, 250 mg/kg, at which it was predicted that clear toxic signs would appear, was selected as the top dose for the main study, and by one-fifth division 50 and 10 mg/kg were derived.

In the main study, salivation was observed from dosing Day 12 in 5 to 10 of each sex given 250 mg/kg. In males at this dose, body weights were significantly lowered by approx. 10% from dosing Day 18, in spite of no dose-related change in food consumption. On

**Table 1.** Histological findings for the liver after 18-day repeat dosing of 1,3-dibromopropane in newborn rats (main study).

| | Grade | Dose (mg/kg) 0 | 10 | 50 | 150 |
|---|---|---|---|---|---|
| Males | | | | | |
| No. of animals examined | | 6 | 6 | 6 | 6 |
| Liver | | | | | |
| – Single cell necrosis | ± | 0 | 0 | 0 | 3 |
| – Centrilobular hypertrophy of hepatocytes | ± | 0 | 0 | 0 | 3 |
| | + | 0 | 0 | 0 | 3 |
| | | | | | * |
| Females | | | | | |
| No. of animals examined | | 6 | 6 | 6 | 6 |
| Liver | | | | | |
| – Single cell necrosis | ± | 0 | 0 | 0 | 1 |
| – Centrilobular hypertrophy of hepatocytes | ± | 0 | 0 | 0 | 2 |
| | + | 0 | 0 | 0 | 4 |
| | | | | | * |

±: Slight, +: Mild, *: Significantly different from the control group ($p<0.01$).

**Table 2.** Summary of the results of the repeated dose studies of 1,3-dibromopropane in newborn rats.

| | Dose-finding Study (5 rats/sex/dose) | | | | Main Study (6 rats/sex/dose) | | |
|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 10 | 30 | 100 | 200 | 10 | 50 | 150 |
| Toxic Effects | | | | | | | |
| – Death (No. of dead animals) | 0 | 0 | 0 | 2M, 2F | 0 | 0 | 0 |
| – Body weight | – | – | – | 15-25%↓ | – | – | 10%↓ |
| – Blood biochemical parameters | – | – | – | F: Cho (↑) | – | – | M: GTP↑<br>F: TB↑ |
| – Relative liver weight | – | – | ↑ | ↑ | F: ↑ | ↑ | ↑ |
| – Histopathological changes ± | n.d. | n.d. | n.d. | n.d. | 0 | 0 | 3M, 2F |
| (No of animals with the findings*) + | n.d. | n.d. | n.d. | n.d. | 0 | 0 | 3M, 4F |

±: Slight change, +: Mild change, M: Males, F: Females, ↑: Increase, ↓: Decrease, (↑): Slight increase, –: No change, Cho: Total cholesterol, GTP: γ-GTP, TP: Total protein, n.d.: No available data, *Centrilobular hypertrophy of hepatocytes.

hematological examination at the scheduled sacrifice, slight anemic changes with decrease in Hb and Ht, and an increased reticulocyte ratio were observed in females receiving 250 mg/kg. At 250 mg/kg, many blood biochemical parameters, including total protein, albumin, total cholesterol, triglycerides, phospholipids and total bilirubin, were also increased with an upward trend of GOT and GPT. With 50 mg/kg, slight increase in total protein was only observed in males. Significant increases were found in absolute and relative liver weights of both sexes at 250 mg/kg and in relative liver weights of females at 50 mg/kg. There was also increase in relative heart weights and relative kidney weights in both sexes of the 250 mg/kg group. On histopathological examination, slight to mild centrilobular hypertrophy of hepatocytes was observed at 50 mg/kg and more (Table 3). Perilobular vacuolation of hepatocytes tended to decrease with the dose. Most of the above changes became less prevalent or disappeared during the recovery period. However, body weights remain lower throughout this period in males and the relative liver and heart weights continued to be

**Table 3.** Histological findings in the repeated dose study of 1,3-dibromopropane in young rats (main study).

| | | Scheduled-sacrifice group (mg/kg) | | | | Recovery group (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|
| | Grade | 0 | 10 | 50 | 250 | 0 | 50 | 250 |
| Males | | | | | | | | |
| No. of animals examined | | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Liver | | | | | | | | |
| – Centrilobular hypertrophy of hepatocytes | ± | 0 | 0 | 4 | 2 | 0 | – | 0 |
| | + | 0 | 0 | 0 | 4 | 0 | – | 0 |
| | | | * | | | | | |
| | | | ** | | | | | |
| – Perilobular vacuolation of hepatocytes | ± | 0 | 1 | 2 | 5 | 5 | – | 6 |
| | + | 6 | 5 | 4 | 1 | 1 | – | 0 |
| | | | ** | | | | | |
| Spleen | | | | | | | | |
| – Extramedullary hematopoiesis | ± | 5 | – | – | 5 | 6 | 3 | 0 |
| | + | 0 | – | – | 1 | 0 | 3 | 6 |
| | ++ | 1 | – | – | 0 | 0 | 0 | 0 |
| | | | | | | | ** | |
| – Deposits of brown pigment | ± | 6 | – | – | 6 | 6 | 6 | 1 |
| | + | 0 | – | – | 0 | 0 | 0 | 5 |
| | | | | | | | ** | |
| Females | | | | | | | | |
| No. of animals examined | | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Liver | | | | | | | | |
| – Centrilobular hypertrophy of hepatocytes | ± | 0 | 0 | 3 | 2 | 0 | – | 0 |
| | + | 0 | 0 | 0 | 4 | 0 | – | 0 |
| | | | | ** | | | | |
| – Perilobular vacuolation of hepatocytes | ± | 1 | 1 | 4 | 5 | 4 | – | 5 |
| | + | 5 | 5 | 2 | 1 | 2 | – | 1 |
| | | | | * | | | | |
| Spleen | | | | | | | | |
| – Extramedullary hematopoiesis | ± | 6 | – | – | 5 | 6 | 6 | 4 |
| | + | 0 | – | – | 1 | 0 | 0 | 2 |
| – Deposits of brown pigment | ± | 6 | – | – | 5 | 4 | 5 | 1 |
| | + | 0 | – | – | 1 | 2 | 1 | 5 |

±: Slight, +: Mild, ++: Moderate, *: Significantly different from the control group ($p<0.05$),
**: Significantly different from the control group ($p<0.01$).

high in females at 250 mg/kg. At the same time, decreases in RBC, Hb, Ht and increase in the reticulocyte ratio appeared in males given 250 mg/kg with an increased incidence of extramedullary hematopoiesis and deposits of brown pigment in the spleen (Table 3).

Summary of the results of the dose-finding and main study of DBP in young rats are shown in Table 4. The NOAEL was concluded to be 10 mg/kg/day from the main study, as the 20 mg/kg in dose-finding study was not appropriate because of the lack of histopathological examination. The unequivocally toxic level was concluded to be 250 mg/kg/day, at which increase of liver weight, mild centrilobular hypertrophy of hepatocytes, increase of many biochemical parameters with an upward trend of GOT and GPT, slight anemic effects and lowering body weight were observed in the main study.

**1,1,2,2-Tetrabromoethane (TBE)**

**1. 18-Day study in newborn rats (including the dose-finding study)**

In the dose-finding study, when newborn rats were given TBE at 12, 50 and 200 mg/kg, hypoactivity and bradypnea were observed during the dosing period in all animals of the high dose group, the body weights being lowered by 10-20% in both sexes at dosing Days 8 to 17. On blood biochemical examination for this group, slight increase in total bilirubin was found in both sexes. In addition, absolute and relative liver weights were increased in females receiving the 50 mg/kg and both sexes of the 200 mg/kg group, and relative liver weights in females of the 12 mg/kg and males of the 50 mg/kg groups. There were also increases in relative kidney weights of females and decreases in absolute spleen weights of both sexes and relative spleen weights of females at 200 mg/kg. No significant changes were observed on hematological and gross examination. Based on these results, it was predicted that some hepatotoxicity would be observed at 50 mg/kg, which was selected as the top dose in the main study, and 3 and 12 mg/kg were derived by approx. one-fourth divisions.

In the main study, no significant changes were noted in general behavior and body weight (Fig.2). There were also no definitive changes in the parameters for external and sexual development and reflex ontogeny at any dose. At scheduled sacrifice, blood biochemical examination in the 50 mg/kg group showed only a slight increase in total protein in males. There were also increases in absolute and relative liver weights in both sexes, relative kidney weights in males and relative heart weights in females of the 50 mg/kg group. After the recovery-maintenance period, no significant changes were observed in blood biochemical findings and in kidney and heart weights, but the relative liver weights still remained high in males at 50 mg/kg. There were no dose-related changes in food consumption, urinalysis, hematology and histopathology throughout the study, including the recovery-maintenance period.

As shown in summary of the results in Table 5, in the 50 mg/kg group, relative liver weights were increased in both dose-finding and main studies, and total protein was slightly increased only in males of the main study. These changes without histopathological alteration were not considered adverse effects. Therefore, the NOAEL was concluded to be 50 mg/kg/day. Unfortunately, no histopathological changes in the

**Table 4.** Summary of the results of the repeated dose studies of 1,3-dibromopropane in young rats.

| | Dose-finding Study (5 rats/sex/dose) | | | | Main Study(6 rats/sex/dose) | | |
|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 20 | 60 | 200 | 600 | 10 | 50 | 250 |
| Toxic Effects | | | | | | | |
| –Death (No. of dead animals) | 0 | 0 | 0 | 5M, 5F | 0 | 0 | 0 |
| –Body weight | – | – | – | n.d. | – | – | M: 10%↓ |
| –Blood biochemical parameters | – | – | M: TP↑<br>F: Cho↑ | n.d. | – | M: TP (↑) | Many↑ |
| –Relative liver weight | | M: ↑ | ↑ | n.d. | – | F: ↑ | ↑ |
| –Histopathological changes ± | n.d. | n.d. | n.d. | n.d. | 0 | 4M, 3F | 2M, 2F |
| (No of animals with the findings*) + | n.d. | n.d. | n.d. | n.d. | 0 | 0 | 4M, 4F |

±: Slight change, +: Mild change, M: Males, F: Females, ↑: Increase, ↓: Decrease, (↑): Slight increase, –: No change, Cho: Total cholesterol, TP: Total protein, Many: Many parameters including Cho, TP, albumin, triglycerides, phospholipids and total bilirubin, n.d.: No available data, * Centrilobular hypertrophy of hepatocytes.

liver were observed at the highest dose of 50 mg/kg in the main study, meaning that the dose setting was not appropriate. Therefore, an unequivocally toxic level could not be estimated. The dose of 200 mg/kg in the dose-finding study was clearly toxic because of effects on the central nervous system (hypoactivity and bradypnea) and lowering of body weight (10-20% reduction), although no histopathological examination was conducted.

**2. 28-Day study in young rats (including the dose-finding study)**

In the dose-finding study with 14-day exposure at 0, 10, 20, 50, 100 or 200 mg/kg, there were no significant changes in body weight, food consumption and urinalysis at any dose. Hematological examination showed increase in reticulocytes of both sexes at 200 mg/kg, and decrease in Hb in both sexes at 200 mg/kg and in males at 100 mg/kg, as well as Ht in males at 100 and 200 mg/kg and RBC in females at 200 mg/kg. On blood biochemical examination, increases in total

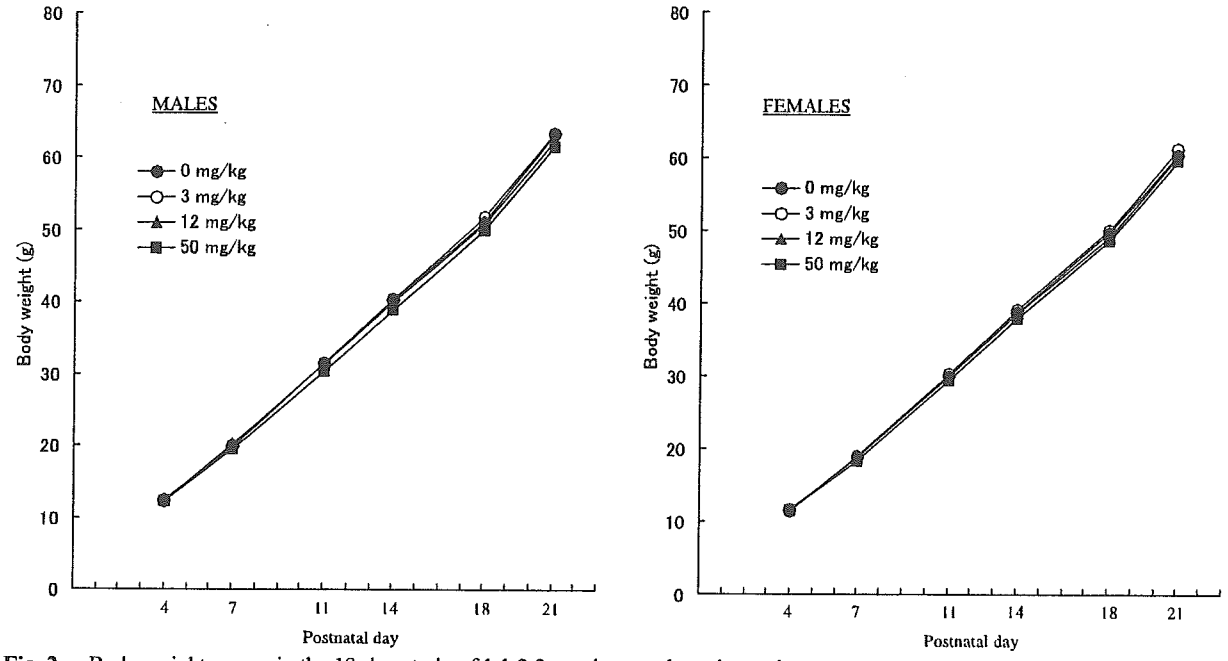


**Fig. 2.** Body weight curves in the 18-day study of 1,1,2,2-tetrabromoethane in newborn rats. Not significantly different from the controls.

**Table 5.** Summary of the results of the repeated dose studies of 1,1,2,2-tetrabromoethane in newborn rats.

| | Dose-finding Study (4 rats/sex/dose) | | | Main Study (6 rats/sex/dose) | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 12 | 50 | 200 | 3 | 12 | 50 |
| Toxic Effects | | | | | | |
| –Death (No. of dead animals) | 0 | 0 | 0* | 0 | 0 | 0 |
| –Body weight | – | – | 10-20%↓ | – | – | – |
| –Blood biochemical parameters | – | – | TB (↑) | – | – | M: TP (↑) |
| –Relative liver weight | F: ↑ | ↑ | ↑ | – | – | ↑ |
| –Histopathological changes | n.d. | n.d. | n.d. | 0 | 0 | 0 |
| (No of animals with the findings) | | | | | | |

M: Males, F: Females, ↑: Increase, ↓: Decrease, (↑): Slight increase, –: No change, TB: Total bilirubin, TP: Total protein, n.d.: No available data, *Although there were no deaths in this group, hypoactivity and bradypnea were observed in all animals.

cholesterol in both sexes, and total protein and triglycerides in females were noted at 200 mg/kg. In addition, increase in total cholesterol was found in females given 100 mg/kg. There were also increases in absolute liver weight in males at 100 and 200 mg/kg and in females at 200 mg/kg, relative liver weight in both sexes at 50 mg/kg and more, and kidney weights in females at 100 mg/kg and in both sexes at the highest dose. Because of the clear toxic effects, 200 mg/kg was selected as the top dose for the main study, and 60, 20 and 6 mg/kg were derived by one third division.

In the main study, there were no significant changes in body weight and food consumption. At scheduled sacrifice, hematological examination showed decrease in platelet counts in females of 200 mg/kg group. On blood biochemical examination, changes suggestive of effects on the liver, including increase in total protein, albumin, A/G, total cholesterol, were found in both sexes at the highest dose. There were also increases in total protein and albumin in females of the 20 and 60 mg/kg groups and increases in A/G in females of the 60 mg/kg groups. For organ weights, there were increases in absolute and relative liver weights of both sexes given 60 and 200 mg/kg and slight increase in relative liver weights in males given 20 mg/kg. In addition, relative kidney weights were higher in both sexes and absolute kidney weights in females of the 200 mg/kg group. On histopathological examination (Table 6), slight to mild centrilobular hypertrophy of hepatocytes was observed in both sexes given 20 mg/kg and more. In the thyroid, mild hypertrophy of follicular cells was found at 60 mg/kg and 200 mg/kg, and follicles were apt to be miniaturized and colloid to be decreased. At the end of the recovery period, changes observed in the scheduled-sacrifice group remained significant but with a tendency for recovery (total protein, total cholesterol, liver and thyroid weights, centrilobular hypertrophy of hepatocytes (Table 6)).

The results of the dose-finding and main study in young rats are summarized in Table 7. As slight hypertrophy of hepatocytes was observed at 20 mg/kg in the main study, the NOAEL was concluded to be 6 mg/kg/day. The unequivocally toxic level was considered to

**Table 6.** Histological findings in the repeated dose study of 1,1,2,2-tetrabromoethane in young rats (main study).

| | | Scheduled-sacrifice group | | | | | Recovery group | |
|---|---|---|---|---|---|---|---|---|
| | Grade | 0 | 6 | 20 | 60 | 200 | 0 | 200 |
| Males | | | | | | | | |
| No. of animals examined | | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Liver | | | | | | | | |
| – Centrilobular hepatocyte hypertrophy | ± | 0 | 0 | 3 | 4 | 0 | 0 | 3 |
| | + | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| | | | | | * | | | |
| | | | | | ** | | | |
| – Focal necrosis | ± | 2 | 1 | 3 | 1 | 5 | 1 | 0 |
| Thyroid | | | | | | | | |
| – Hypertrophy of follicular cells | ± | 0 | 0 | 0 | 1 | 4 | 0 | 0 |
| Females | | | | | | | | |
| No. of animals examined | | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Liver | | | | | | | | |
| – Centrilobular hepatocyte hypertrophy | ± | 0 | 0 | 3 | 5 | 1 | 0 | 2 |
| | + | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| | | | | | ** | | | |
| | | | | | ** | | | |
| – Focal necrosis | ± | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Thyroid | | | | | | | | |
| – Hypertrophy of follicular cells | ± | 0 | 0 | 0 | 2 | 5 | 0 | 0 |
| | | | | | | ** | | |

±: Slight, +: Mild, *: Significantly different from the control group ($p<0.05$), **: Significantly different from the control group ($p<0.01$).

be more than 200 mg/kg because of the lack of effects on body weights and parameters indicative of hepatotoxicity, such as GOT and GPT. Hypertrophy in the liver and thyroid, and increases in some biochemical parameters at this dose were not considered to be sufficient for a conclusion of toxicity.

## DISCUSSION

As with human neonates, the metabolic ability of the newborn rat is known to be extremely immature, with a low cytochrome P450 content (Rich and Boobis, 1997) and a low capacity for glucuronidation (Gow *et al.*, 2001). Therefore, it could be predicted that chemicals directly exerting adverse effects might show stronger toxicity in the newborn than in young/adult rats. As expected, our previous comparative studies demonstrated that the susceptibility to four chemicals (4-nitrophenol, 2,4-dinitorophenol, 3-aminophenol, 3-methylphenol), which may exert toxicity without metabolic activation, was 2 to 4 times greater in the newborn than in young rats (Koizumi *et al.*, 2001, 2002, 2003).

In the present study, DBP and TBE, which differ from the earlier chemicals in requiring biotransformation differently from previous chemicals, were therefore examined. Although hitherto there has been no information on the repeated dose toxicity of DBP, hepatotoxicity with slight centrilobular fatty degeneration or cytoplasmic vacuolization has been already reported for TBE (Hollingsworth *et al.*, 1963; NTP, 1996). The present study showed no effects of either chemical on early development in the newborn, but they caused hepatotoxicity, regardless of sex, in both newborn and young animals. The ratios for NOAELs and unequivocally toxic levels (young/newborn rats) for both chemicals are given in Table 8, the NOAELs for DBP and TBE being considerably higher in newborn than in young rats, so that the latter are clearly more susceptible. Unequivocally toxic levels could not be simply estimated for both chemicals because the hepatic influence observed was only hypertrophy of hepatocytes, usually without increase of GOT and GPT. Therefore, values were estimated on the basis of simultaneous changes of organ weights, histopathology, biochemical parameters and body weights. Based on our specified criteria, the unequivocally toxic level for DBP was in contrast lower in newborn than in young rats. Unfortunately an unequivocally toxic level of TBE could not be estimated for newborn or young rats. However, the dose of 200 mg/kg in the newborn dose-finding study was considered to be sufficiently toxic because of the 10 - 20% lowering of body weights observed, although no histopathology was conducted. The same dose in the young rat main study caused mild hypertrophy of hepatocytes but no change of body weights, was not considered a sufficient toxic level. These results suggest that the unequivocally toxic level of TBE in the newborn might be lower than that in young rats. The reasons for difference in susceptibility presumably lie with metabolic pathways and specific characteristics of newborn animals.

Three studies have demonstrated that DBP is conjugated with hepatic glutathione before or after oxidative biotransformation, leading to urinary excretion of cysteine or mercapturic acid derivatives and exhalation of $CO_2$ (James *et al.*, 1981, Jones and Wells, 1981, Onkenhout *et al.*, 1986). Activity of the conjugation

**Table 7.** Summary of the results of the repeated dose studies of 1,1,2,2-tetrabromoethane in young rats.

| | Dose-finding Study (4 rats/sex/dose) | | | | | Main Study (5 rats/sex/dose) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 10 | 20 | 50 | 100 | 200 | 6 | 20 | 60 | 200 |
| Toxic Effects | | | | | | | | | |
| – Death (No. of dead animals) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| – Body weight | – | – | – | – | – | – | – | – | – |
| – Blood biochemical parameters | – | – | – | F: TP↑ | M: Cho↑<br>F: Cho, TG, TP↑ | – | F: TP, Alb ↑ | F: TP, A/G, Alb ↑ | Many↑ |
| – Relative liver weight | – | – | ↑ | ↑ | ↑ | – | M: (↑) | ↑ | ↑ |
| – Histopathological changes ± | n.d. | n.d. | n.d. | n.d. | n.d. | 0 | 3M, 3F | 4M, 5F | 1F |
| (No of animals with the findings*) + | n.d. | n.d. | n.d. | n.d. | n.d. | 0 | 0 | 0 | 5M, 4F |

±: Slight, +: Mild, M: Males, F: Females, ↑: Increase, ↓: Decrease, (↑): Slight increase, –: No change, Alb: Albumin, Cho: Total cholesterol, TG: Triglycerides, TP: Total protein, Many: Many parameters including Alb, A/G, Cho and TP, n.d.: No available data, * Centrilobular hypertrophy of hepatocytes.

pathway is supported by a rapid drop in hepatic glutathione level after DBP administration (James *et al.*, 1981). Metabolism via conjugation with glutathione has in fact been indicated in common for dihaloalkanes or dihaloalkenes, such as 1,2-dibromopropane (Zoetemelk *et al.*, 1986), 1,2-dichloropropane (Trevisan *et al.*, 1989), 1,1-dichloroethylene (Jones and Hathway, 1978) and 1,3-dichloropropene (Climie *et al.*, 1979). In the case of 1,2-halogenated ethanes, it is considered that the oxidative metabolites might irreversibly bind to protein and that conjugate derivatives, episulphonium ions, might be responsible for the DNA adduct formation (Shih and Hill, 1981; Ozawa and Guengerich, 1983).

With TBE, Kennedy *et al.* (1993) identified various excretory metabolites after a single oral administration to rats, such as 1,2-dibromoethylene and tribromoethylene in exhaled air and dibromoacetic acid, glyoxylic acid, and oxalic acid in urine. They suggested that a number of metabolic intermediates produced by oxidative biotransformation may be involved in the mutagenicity, hepatotoxicity and nephrotoxicity of the compound. At least, dibromoacetic acid has unequivocal cytotoxicity and mutagenicity (Kargalioglu *et al.*, 2002).

Based on the available information, oxidative biotransformation mediated by cytochrome P450 might be a critical step for the initial hepatotoxic effects of both chemicals. The rate of production of active metabolites, including free radical intermediates, would be expected to be significantly less or negligible in newborn animals at least around 50 mg/kg, at which clearly hepatic changes were observed in young rats for both chemicals, because of their lower content of cytochrome P450 (Rich and Boobis, 1997). This metabolic character for both chemicals as well as the lower blood flow to the liver during the newborn period (Gow *et al.*, 2001) would make a major contribution to the much higher NOAEL in the newborn than in young rats. Similar results have already been demonstrated for aflatoxin B1 (Behroozikha *et al.*, 1992), acetaminophen, bromobenzene and carbon tetrachloride (Gergus and Klaassen, 1998). On the other hand, unequivocally toxic levels for both chemicals appeared to be only 3 to 4 times higher than the NOAELs in newborn rats, in contrast to 25 to >33 times higher in their young counterparts (Table 8). One possible explanation for these differences might be a low capacity for protection against deleterious oxidative stress in the newborn when the toxic chemical burden crosses a threshold in the liver. It has been reported that the content of glutathione and glutathione-*S*-transferase activity in rat liver drops in the early days after birth (Tee *et al.*, 1992).

In our series of comparative studies, the results of the repeated dose toxicity study using newborn rats have been compared with those of routine repeated dose toxicity studies. The routine repeated dose studies have value in identifying target sites for toxicity and providing dose-response information that may be useful for human safety assessment, irrespective of life stage, but the developing period, which could be most vulnerablev to chemical toxicity during life, is not directly evaluated by the studies (Dourson *et al.*, 2002). To compensate for this period, reproductive/developmental toxicity studies that exposed the developing animals via placenta or maternal milk have been conducted. However, the direct exposure to chemicals dur-

**Table 8.** Comparison of NOAELs and unequivocally toxic levels in newborn and young rats.

| | Level (mg/kg/day) | | Ratio (young/newborn) |
|---|---|---|---|
| 1,3-Dibromopropane | | | |
| NOAEL (newborn) | 50 | × 3 | 0.2 |
| NOAEL (young) | 10 | | |
| Unequivocally toxic level (newborn) | 150 | × 25 | 1.67 |
| Unequivocally toxic level (young) | 250 | | |
| 1,1,2,2,-Tetrabromoethane | | | |
| NOAEL (newborn) | 50 | × 4* | 0.12 |
| NOAEL (young) | 6 | | |
| Unequivocally toxic level (newborn) | 200* | × >33* | >1.0* |
| Unequivocally toxic level (young) | > 200* | | |

*: Tentative levels or ratios, due to lack of histology alteration in the newborn and no change in body weight in young rats.

ing the newborn period is not included in these studies, despite the significant possibility that the newborn are exposed to chemicals directly via mouthing toys and household materials, or having chemical-contaminated milk and baby food, and so on. In the routine repeated dose toxicity study, rats at approximately 5-6 weeks of age have generally been used, and this start period is largely a matter of practical convenience and feasibility. Rats much younger than this age, especially newborn rats, are so difficult to handle such as grouping, direct dosing and other testing or observation. Economic issues and lack of the human resource with this technical difficulty make it impossible to subject the newborn rat study to the routine one. Our series of comparative studies are the first systematic study to look into the direct effects of chemicals in newborn animals, and the comparative analysis on the susceptibility of the newborn rats to the toxicity of chemicals with that of young rats would give important information for considering the effects by chemical exposure during the newborn period in risk assessment.

In conclusion, the target organ of DBP and TBE was here found to be the liver in both newborn and young rats, but the doses at which the toxic signs began to appear were higher in newborn rats. In contrast, the doses at which clear toxicity was observed appeared to be lower in the newborn case. However, no special concern with regard to newborn risk is necessary in cases of chemicals which induce toxicity after biotransformation via hepatic cytochrome P450, because the tolerable daily intake (TDI) used for regulation is generally derived from NOAEL in toxicity studies in young/adult animals.

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# Evaluation of developmental toxicity of 1-butanol given to rats in drinking water throughout pregnancy

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**Abstract**

The objective of this study was to evaluate the developmental toxicity of 1-butanol in rats. Pregnant rats were given drinking water containing 1-butanol at 0.2%, 1.0% or 5.0% (316, 1454 or 5654 mg/kg/day) on days 0–20 of pregnancy. A significant decrease in maternal body weight gain accompanied by reduced food and water consumption was found at 5.0%. No significant increase in the incidence of pre- and postimplantation embryonic loss was observed in any groups treated with 1-butanol. Fetal weight was significantly lowered at 5.0%. Although a significant increase in the incidence of fetuses with skeletal variations and decreased degree of ossification was found at 5.0%, no increase in the incidence of fetuses with external, skeletal and internal abnormalities was detected in any groups treated with 1-butanol. The data demonstrate that 1-butanol is developmental toxic only at maternal toxic doses. No evidence for teratogenicity of 1-butanol was noted in rats. Based on the significant decreases in maternal body weight gain and fetal weight, it is concluded that the no observed adverse effect levels (NOAELs) of 1-butanol for both dams and fetuses are 1.0% (1454 mg/kg/day) in rats.


*Keywords:* 1-Butanol; Developmental toxicity; Teratogenicity; Fetal abnormality; Rat

## 1. Introduction

1-Butanol (CAS no. 71-36-3, *n*-butanol; *n*-butyl alcohol), a flammable colorless liquid with a rancid sweet odor, is widely used as an organic solvent and intermediate in the manufacture of other organic chemicals (IPCS/WHO, 1987). Exposure of the general population is mainly through its natural occurrence in food and beverages and its use as a flavoring agent (IPCS/WHO, 1987).

Several reports on the developmental toxicity of 1-butanol are available. Nelson et al. (1989a) reported the results of a developmental toxicity study in which SD rats were exposed to 1-butanol by inhalation for 7 hr/day on days 1–19 of pregnancy at 3500, 6000 and 8000 ppm (equivalent to estimated daily absorbed doses of 350, 600 and 800 mg/kg). They observed maternal deaths at 8000 ppm, decreases in maternal food consumption and fetal weight at 6000 and 8000 ppm, and an increased incidence of rudimentary cervical ribs at 8000 ppm, and concluded that 1-butanol was not a selective developmental toxicant in rats. Nelson et al. (1989b) conducted a behavioral teratology study in which female SD rats were given 1-butanol by inhalation at 3000 or 6000 ppm for 7 hr/day throughout pregnancy (the maternal exposure group); male rats were

*Abbreviations:* NOAEL, no observed adverse effect level

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similarly exposed for 6 weeks and mated to unexposed females (the paternal exposure group), and offspring were behaviorally and neurochemically examined. The data from all tests in their study were within the range of control data in other research conducted by their laboratory. Sitarek et al. (1994) reported a significant increase in the incidence of fetuses with abnormalities after administration of 1-butanol at 0.24–4.0% (300–5000 mg/kg/day) in drinking water during the pre-mating period for 8 weeks and throughout the mating and pregnant period. No maternal toxicity was found at any dose of 1-butanol. The no observed adverse effect level (NOAEL) was not derived from the results of their study, because significant increases in the incidence of fetuses with dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain were found even at the lowest dose (0.24%). They have concluded that 1-butanol is a developmental toxicant and produces anomalies in the skeleton and central nervous system.

The present study was conducted to determine whether or not morphological abnormalities could be produced in fetuses of rats given 1-butanol prenatally and designed to replicate the observations of the study by Sitarek et al. (1994).

## 2. Materials and methods

This study was performed in compliance with regulatory guidelines (MHW, 1997a) and accordance with the principles for Good Laboratory Practice (MHW, 1997b) and "Guidance for Animal Care and Use" of Ina Research, Inc.

### 2.1. Animals

International Genetic Standard (Crj: CD (SD) IGS) rats were used throughout this study. This strain was chosen because it is most commonly used in reproductive and developmental toxicity studies and historical control data are available. Males at 10 weeks of age and females at 9 weeks of age were purchased from Tsukuba Breeding Center, Charles River Japan, Inc., (Yokohama, Japan). The rats were acclimated to the laboratory for 7 days prior to the start of the experiment. Male and female rats found to be in good health were selected for use. Animals were reared on a basal diet (NMF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum and maintained in an air-conditioned room at 21–25 °C, with a relative humidity of 40–70%, a 12-h light/dark cycle, and ventilation with 16 air charges/hour. Virgin female rats were mated overnight with male rats. The day when sperm were detected in the vaginal smear was considered to be day 0 of pregnancy. The pregnant rats, weighing 217–273 g and 10–11 weeks of age, were distributed using a computerized randomization procedure (TOXstaff 21 system) into 4 groups of 20 rats each and housed individually.

### 2.2. Chemicals and dosing

1-Butanol was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The 1-butanol used in this study was 99.9% pure and a special grade reagent (Lot no. CER5688), and it was kept in a dark place at room temperature under airtight conditions. The purity and stability of the chemical were verified by analysis before and after the study. Rats were given 1-butanol in their drinking water at a concentration of 0 (control), 0.2%, 1.0% or 5.0% on day 0 through day 20 of pregnancy. The dosage levels were determined based on the results of our range-finding study in which administration of 1-butanol in the drinking water on days 0–20 of pregnancy caused decreases in maternal body weight gain and food and water consumption and tended to reduce in fetal weight at 4% and 7% in rats. 1-Butanol was dissolved in distilled water (Otsuka Pharmaceutical Factory, Inc., Naruto, Japan). The control rats were given only water. The stability of formulations in a dark and cool place under airtight conditions has been confirmed for up to 3 days. During use, the formulations were maintained under such conditions for no more than 3 days and were 95.7–103.5% of the target concentration.

### 2.3. Observations

The maternal body weight and water consumption were recorded daily, and food consumption was recorded every 3 or 4 days. The pregnant rats were euthanized by exsanguinations under ether anesthesia on day 20 of pregnancy. The peritoneal cavity was opened, and the numbers of corpora lutea, implantation sites and live and dead fetuses and resorptions were counted. The live fetuses removed from the uterus were sexed, weighed, measured among their crown-rump length, and inspected for external malformations and malformations within the oral cavity. Approximately one-half of the live fetuses in each litter were randomly selected and fixed in alcohol, stained with alizarin red S (Dawson, 1926) and examined for skeletal anomalies. The remaining live fetuses in each litter were fixed in Bouin's solution. Their heads were subjected to a free-hand razor-blade sectioning (Wilson, 1973) and the thoracic areas were subjected to microdissecting (Nishimura, 1974) to reveal internal abnormalities. The placental weight was also measured.

### 2.4. Data analysis

The statistical analysis of fetuses was carried out using the litter as the experimental unit. The initial body

weight, body weight gain and food and water consumption of pregnant rats, numbers of corpora lutea, implantations and live fetuses per litter, fetal weight and crown-rump length and placental weight were analyzed with Bartlett's test (Snedecor and Cochran, 1980) for homogeneity of variance at the 5% level of significance. If it was homogeneous, the data were analyzed using Dunnett's multiple comparison test (Dunnett, 1955) to compare the mean of the control group with that of each dosage group, and if it was not homogeneous, the mean rank of the 1-butanol-treated groups was compared with that of the control group with the Dunnett type test. The Dunnett type test was used for the incidences of pre- and postimplantation embryonic loss and fetal anomalies and sex ratio of fetuses to compare the mean rank of groups treated with 1-butanol and that of the control group. The incidence of dams with anomalous fetuses was analyzed by Chi-square test or Fisher's exact test. The significance of differences from the control group was estimated at probability levels of 1% and 5%.

## 3. Results

Table 1 shows the maternal findings in rats given 1-butanol during pregnancy. No death was found in female rats of any group. All females in all groups became pregnant. The body weight gains on days 0–7 of pregnancy were significantly reduced at 5.0%. The body weight gain during the whole period of pregnancy was also significantly decreased at 5.0%. No significant decrease in the body weight gain was noted at 0.2 or 1.0, except for a transient decrease on days 0–2 of pregnancy at 1.0%. The food consumption on days 0–7, days 7–14, days 14–20 and days 0–20 of pregnancy was significantly lower in the 1.0% and 5.0% groups than the control group. The water consumption on days 0–7 at 1.0 and 5.0% and on days 7–14, days 14–20 and days 0–20 at 5.0% was significantly decreased. The mean daily intakes of 1-butanol were 316 mg/kg for the 0.2% group, 1454 mg/kg for the 1.0% group and 5654 mg/kg for the 5.0% group.

Reproductive findings in rats given 1-butanol during pregnancy are presented in Table 2. No litters totally resorbed were found in any group. No effects of the administration of 1-butanol were observed on the numbers of corpora lutea, implantations, pre- or postimplantation loss, resorptions or dead or live fetuses or sex ratio of live fetuses. The body weights of male and female fetuses were significantly lower in the 5.0% group than in the control group. There was no significant difference in the crown-rump length of male and female fetuses or placental weight between the control and groups treated with 1-butanol.

A summary of morphological findings in live fetuses of rats given 1-butanol during pregnancy is shown in Table 3. One fetus with spina bifida in the control group and one fetus with thread-like tail and anal atresia in the 0.2% group were observed. Skeletal examination

Table 1
Maternal findings in rats given 1-butanol on days 0–20 of pregnancy

| Dose (%) | 0 (Control) | 0.2 | 1.0 | 5.0 |
|---|---|---|---|---|
| No. of rats | 20 | 20 | 20 | 20 |
| No. of pregnant rats | 20 | 20 | 20 | 20 |
| No. of dead rats | 0 | 0 | 0 | 0 |
| Initial body weight | 245 ± 14 | 247 ± 13 | 245 ± 11 | 244 ± 12 |
| *Body weight gain during pregnancy (g)*[a] | | | | |
| Days 0–7 | 44 ± 7 | 45 ± 7 | 40 ± 6 | 20 ± 28** |
| Days 7–14 | 40 ± 6 | 41 ± 5 | 41 ± 7 | 42 ± 10 |
| Days 14–20 | 78 ± 14 | 82 ± 8 | 84 ± 7 | 75 ± 11 |
| Days 0–20 | 162 ± 19 | 168 ± 16 | 165 ± 15 | 146 ± 16** |
| *Food consumption during pregnancy (g)*[a] | | | | |
| Days 0–7 | 179 ± 12 | 180 ± 16 | 164 ± 12* | 138 ± 21** |
| Days 7–14 | 193 ± 14 | 194 ± 17 | 177 ± 14** | 160 ± 11** |
| Days 14–20 | 176 ± 14 | 175 ± 15 | 161 ± 12** | 143 ± 11** |
| Days 0–20 | 548 ± 38 | 548 ± 46 | 503 ± 34** | 441 ± 34** |
| *Water consumption during pregnancy (ml)*[a] | | | | |
| Days 0–7 | 284 ± 28 | 305 ± 37 | 258 ± 29* | 175 ± 34** |
| Days 7–14 | 318 ± 35 | 337 ± 48 | 299 ± 40 | 239 ± 80** |
| Days 14–20 | 328 ± 47 | 342 ± 47 | 334 ± 46 | 256 ± 85** |
| Days 0–20 | 930 ± 105 | 983 ± 126 | 890 ± 106 | 669 ± 182** |
| Mean daily intakes of 1-butanol (mg/kg)[a] | 0 | 316 ± 30 | 1454 ± 186 | 5654 ± 1402 |

*,** Significantly different from the control, *$P < 0.05$ and **$P < 0.01$.
[a] Values are given as the mean ± SD.

Table 2
Reproductive findings in rats given 1-butanol on days 0–20 of pregnancy

| Dose (%) | 0 (Control) | 0.2 | 1.0 | 5.0 |
|---|---|---|---|---|
| No. of litters | 20 | 20 | 20 | 20 |
| No. of litters totally resorbed | 0 | 0 | 0 | 0 |
| No. of corpora lutea per litter[a] | 16.4 ± 3.6 | 16.7 ± 3.0[d] | 16.1 ± 2.1 | 16.3 ± 2.6 |
| No. of implantations per litter[a] | 14.3 ± 2.8 | 15.1 ± 1.7 | 15.2 ± 1.2 | 14.7 ± 2.5 |
| % Preimplantation loss per litter[b] | 9.0 | 9.0[d] | 4.4 | 9.2 |
| % Postimplantation loss per litter[c] | 6.0 | 5.4 | 3.7 | 8.0 |
| No. of live fetuses per litter[a] | 13.4 ± 2.6 | 14.3 ± 1.4 | 14.7 ± 1.5 | 13.5 ± 2.5 |
| Sex ratio of live fetuses (male/female) | 128/139 | 145/140 | 149/144 | 131/139 |
| *Body weight of live fetuses (g)*[a] | | | | |
| Male | 4.18 ± 0.27 | 4.00 ± 0.24 | 4.04 ± 0.25 | 3.83 ± 0.18** |
| Female | 3.97 ± 0.25 | 3.86 ± 0.20 | 3.83 ± 0.16 | 3.59 ± 0.17** |
| *Fetal crown-rump length (mm)*[a] | | | | |
| Male | 40.5 ± 1.2 | 40.3 ± 1.4 | 40.2 ± 1.2 | 39.7 ± 1.3 |
| Female | 39.4 ± 1.2 | 39.4 ± 1.2 | 39.3 ± 1.1 | 38.5 ± 1.4 |
| *Placental weight (g)* | | | | |
| Male | 0.50 ± 0.05 | 0.49 ± 0.05 | 0.48 ± 0.06 | 0.50 ± 0.06 |
| Female | 0.49 ± 0.05 | 0.48 ± 0.05 | 0.47 ± 0.05 | 0.49 ± 0.06 |

** Significantly different from the control, $P < 0.01$.
[a] Values are given as the mean ± SD.
[b] (No. of preimplantation embryonic loss/no. of corpora lutea) × 100.
[c] (No. of resorptions and dead fetuses/no. implantations) × 100.
[d] Value was obtained from 19 pregnant rats.

revealed one fetus with supernumerary thoracic vertebral bodies and malpositioned thoracic vertebrae at 1.0%. Although the total number of fetuses with skeletal variations was significantly increased at 5.0%, the number of fetuses with individual skeletal variations was not significantly increased, except for fetuses with short supernumerary ribs at 5.0%. A significantly lower number of forepaw proximal phalanges was observed at 5.0%. Membranous ventricular septum defect occurred in one fetus of the control and 0.2% groups and 3 fetuses in 3 dams of the 5.0% group. One fetus with a double aorta in the control group and one fetus with a left umbilical artery in the control and 2.0% groups were observed. Thymic remnants in the neck were found in 4–11 fetuses of the control and groups treated with 1-butanol. However, there was no significant difference in the incidence of fetuses with internal abnormalities between the control and groups treated with 1-butanol.

## 4. Discussion

The present study was conducted to determine the developmental toxicity of 1-butanol and designed to replicate the observations of the study by Sitarek et al. (1994). The data showed that prenatal administration of 1-butanol did not produce morphological anomalies in fetuses of rats. Thus, we have been unable to confirm the results of Sitarek's study in which prenatal exposure to 1-butanol produced fetal anomalies.

The doses of 1-butanol used in the present study expected to induce maternal and/or developmental toxicity, such as a decrease in maternal body weight gain and fetal weight, were given to pregnant rats during the whole period of pregnancy to characterize the effects of 1-butanol on embryonic/fetal development. Maternal toxicity, a significant decrease in body weight gain, was found at 5.0%. Maternal food and water consumptions were also reduced in this dose group. Although the only significant decrease in maternal body weight gain was observed on days 0–2 of pregnancy at 1.0%, this decrease was occasional and discontinuous and seems unlikely to be of toxicological significance. In this dose group, decreases in the maternal food consumption during the whole period of pregnancy and water consumption during the early period of pregnancy, which were unaccompanied by the continuous changes in body weight gain, were observed. No significant changes in maternal parameters were noted in the 0.2% group. These findings in maternal rats indicate that 1-butanol exerts maternal toxicity at 5.0% (equivalent to 5654 mg/kg/day) when administered during the entire period of pregnancy in rats.

No significant increase in the incidence of postimplantation loss was found at any dose of 1-butanol, and significantly decreased weights of male and female fetuses were found at 5.0%. No significant adverse effects on reproductive parameters were detected at 0.2% and 1.0%. These findings indicate that 1-butanol is not toxic to embryonic/fetal survival up to 5.0% or fetal growth up to 1.0% when administered during the whole period of pregnancy.

As for morphological examinations in the fetuses of exposed mothers, a few fetuses with external, skeletal

Table 3
Morphological examinations in fetuses of rats given 1-butanol on days 0–20 of pregnancy

| Dose (%) | 0 (Control) | 0.2 | 1.0 | 5.0 |
|---|---|---|---|---|
| *External examination* | | | | |
| Total no. of fetuses (litters) examined | 267 (20) | 285 (20) | 293 (20) | 270 (20) |
| Total no. of fetuses (litters) with abnormalities | 1 (1) | 1 (1) | 0 | 0 |
| Spina bifida | 1 (1) | 0 | 0 | 0 |
| Thread-like tail and anal atresia | 0 | 1 (1) | 0 | 0 |
| *Skeletal examination* | | | | |
| Total no. of fetuses (litters) examined | 139 (20) | 147 (20) | 152 (20) | 140 (20) |
| Total no. of fetuses (litters) with abnormalities | 0 | 0 | 1 (1) | 0 |
| Supernumerary of thoracic vertebral bodies and malpositioned thoracic vertebrae | 0 | 0 | 1 (1) | 0 |
| Total no. of fetuses (litters) with variations | 28 (11) | 23 (12) | 52 (17) | 69 (20)** |
| Bipartite ossification of thoracic centra | 1 (1) | 1 (1) | 1 (1) | 7 (5) |
| Dumbbell ossification of thoracic centra | 0 | 1 (1) | 2 (2) | 3 (3) |
| Bipartite ossification of lumbar centra | 0 | 0 | 0 | 2 (2) |
| Supernumerary lumbar vertebrae | 4 (1) | 1 (1) | 5 (3) | 5 (2) |
| Lumbarization | 0 | 0 | 1 (1) | 1 (1) |
| Bipartite ossification of sternebrae | 1 (1) | 1 (1) | 1 (1) | 1 (1) |
| Misaligned sternebrae | 0 | 0 | 0 | 1 (1) |
| Cervical ribs | 2 (2) | 3 (3) | 3 (3) | 7 (5) |
| Full supernumerary ribs | 5 (2) | 1 (1) | 10 (5) | 9 (5) |
| Short supernumerary ribs | 20 (10) | 18 (9) | 43 (16) | 55 (19)** |
| Wavy ribs | 0 | 0 | 0 | 1 (1) |
| Degree of ossification[a] | | | | |
| No. of sacral and caudal vertebrae | 8.4 ± 0.5 | 8.4 ± 0.4 | 8.3 ± 0.5 | 8.1 ± 0.3 |
| No. of sternebrae | 5.9 ± 0.2 | 5.8 ± 0.2 | 5.8 ± 0.2 | 5.8 ± 0.2 |
| No. of forepaw proximal phalanges | 1.6 ± 1.3 | 1.6 ± 0.9 | 1.2 ± 1.1 | 0.3 ± 0.4** |
| *Internal examination* | | | | |
| Total no. of fetuses (litters) examined | 128 (20) | 138 (20) | 141 (20) | 130 (20) |
| Total no. of fetuses (litters) with abnormalities | 7 (6) | 9 (6) | 11 (8) | 14 (9) |
| Membranous ventricular septum defect | 1 (1) | 1 (1) | 0 | 3 (3) |
| Double aorta | 1(1) | 0 | 0 | 0 |
| Left umbilical artery | 1 (1) | 0 | 1 (1) | 0 |
| Thymic remnant in neck | 4 (4) | 8 (5) | 10 (8) | 11 (8) |

** Significantly different from the control, $P < 0.01$.
[a] Values are given as the mean ± SD.

and/or internal abnormalities were found in all groups. The abnormalities observed in the present study are not thought to be due to the administration of 1-butanol, because they have occurred at a very low incidence and are of types that occur sporadically among control rat fetuses (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). Several types of skeletal variations were also found in the control and groups treated with 1-butanol. These skeletal variations are frequently observed in fetuses of rats at term (Kimmel and Wilson, 1973; Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Barnett et al., 2000). In the 5.0% group, a significant increase in the incidence of fetuses with skeletal variations and fetuses with short supernumerary ribs, but not full supernumerary ribs, and a significant decrease in the degree of ossification were accompanied by a significant decrease in the fetal weight. These findings show a correlation between these morphological alterations and growth retardation in fetuses. Although a skeletal variation, i.e., full supernumerary ribs, is a warning sign of possible teratogenicity, short supernumerary ribs, sternebral variations, and bilobed centra of the vertebral column are normal variations (Kimmel and Wilson, 1973). Chahoud et al. (1999) noted that variations are unlikely to adversely affect survival or health and this might result from a delay in growth or morphogenesis that has otherwise followed a normal pattern of development. Consideration of these findings together suggests that the morphological changes in fetuses observed in the present study do not indicate a teratogenic response and that 1-butanol possesses no teratogenic potential in rats.

In Sitarek's study (1994), significant increases in the incidences of wavy ribs at 300 mg/kg/day, dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain at 300 mg/kg/day and higher, dilation of the renal pelvis and external hydrocephaly at 1000 mg/kg/day, internal hydrocephaly at 1000 mg/kg/day and higher, and supernumerary ribs and delayed ossification at 5000 mg/kg/day were found. A significant decrease in fetal crown-rump length was

also observed at 5000 mg/kg/day. Based on these findings, Sitarek et al. (1994) concluded that 1-butanol had adverse effects on the morphological development of fetuses in rats. However, we did not confirm their findings. We have demonstrated here that prenatal 1-butanol has no adverse effect on the morphological development of rat offspring. There are some differences between Sitarek's study and the present study in experimental conditions, such as duration of administration and rat strain used in the experiments. Sitarek et al. (1994) administered 1-butanol to female rats for 8 weeks before mating and throughout the mating and pregnancy period and found fetal anomalies, such as hydrocephaly and dilation of the cerebral ventricles and the renal pelvis. On the other hand, we gave 1-butanol to female rats during the whole period of pregnancy and did not detect fetuses with these anomalies. Administration during the premating and mating period is thought to be excluded from the susceptible period for induction of morphological anomalies such as hydrocephaly/dilation of the cerebral ventricles and dilation of the renal pelvis, because rat fetuses are susceptible to induction of these anomalies during mid and late pregnancy (Wood and Hoar, 1972; Kameyama, 1985). The strain difference of rats used in the experiments may explain the discrepancy in the findings regarding fetal anomalies between the studies. In Sitarek's study (1994), ImP: DAK rats obtained from their own breeding colony were used. No detailed information on this strain of rats was available (Sitarek et al., 1994). In their study, dilation of the lateral ventricle and/or third ventricle of the brain was observed in 2% of fetuses (one of the 12 litters) in the control group. In their another study using Imp: DAK rats, extension of the lateral ventricle and/or third ventricle of the brain was observed in 11.7% of fetuses (8 of the 17 litters) in the control group (Sitarek et al., 1996). However, these anomalies were not found in the control group of their studies using Wistar rats (Baranski et al., 1982), Imp: Lodz rats (Sitarek, 1999, 2001) and Imp: WIST rats (Sitarek and Sapota, 2003). The incidences of dilation of the cerebral ventricles in Imp: DAK rats are thought to be higher than those in the background control data of other strains of rats. The fetal incidence of hydrocephaly/dilation of cerebral ventricles in the control rats of reproductive studies conducted between 1986 and 1993 in 63 research institutes is reported to be 0–0.09% and 0–0.26%, respectively (Nakatsuka et al., 1997). In Crj: CD (SD) IGS rats which were used in the present study, the incidence of dilation of the lateral ventricles of the brain in 19 studies conducted during 1998–2000 is reported to be 0–0.06% in fetuses and 0–0.44% in litters (Barnett et al., 2000). Thus, hydrocephaly/dilation of the cerebral ventricle is not commonly observed in fetuses of common strains of rats.

The difference in terminology used for classification of structural anomalies in fetuses may also explain the discrepancy in the findings regarding fetal anomalies between the studies. Sitarek et al. (1996) stated that minor abnormalities, such as enlarged lateral ventricle and/or third ventricle, are quite frequent in rat fetuses and without having the dose-dependent relationship should not be taken alone as evidence of tested chemical fetotoxicity. However, the Fourth Berlin Workshop on Terminology in Developmental Toxicity noted that changes affecting brain ventricles are more likely to be classified as malformations and classification should be based on the historical control incidences, the nature of the organ affected and the severity (Solecki et al., 2003). In Sitarek's study (1994), dilation of the subarachnoid space was observed in fetuses of rats given 1-butanol at 300 mg/kg/day and higher. This anomaly was also found in fetuses in Imp: DAK rats given *N*-cyclohexyl-2-benzothiazolesulfenamide (Sitarek et al., 1996) and Imp: Lodz rats given *N*-methylmorpholine (Sitarek, 1999). No information on the definition of this anomaly was available in their reports. We are unaware of this anomaly in other literature (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Horimoto et al., 1998; Barnett et al., 2000; Solecki et al., 2003).

In conclusion, the administration of 1-butanol to pregnant rats throughout pregnancy had adverse effects on maternal rats and embryonic/fetal growth but had no adverse effects on fetal morphological development even at a maternally toxic dose. The data indicate that 1-butanol induces developmental toxicity only at maternally toxic doses in rats. Based on the significant decreases in maternal body weight gain and fetal weight at 5.0%, it is concluded that the NOAELs of 1-butanol for both dams and fetuses are 1454 mg/kg/day (1.0% in drinking water) in rats.

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医療の現場から

# 妊娠と薬相談外来

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1980年東京薬科大学薬学部卒，薬学博士．東京都病院薬剤師会副会長，日本病院薬剤師会常務理事・医薬情報委員長，日本薬剤師会薬価収載品目検討委員会委員長，厚生労働省保険医療専門審査員，日本医療薬学会評議員，日本医薬品情報学会幹事．

1960年代のサリドマイド事件が教訓となり，医療従事者はもとより国民全体に薬物の催奇形性に対する認識が浸透し，妊婦はむしろ過剰な不安を抱く傾向がある．当院では妊婦の服薬に対する不安を解消する目的で，1988年に産婦人科と薬剤部が共同で「妊娠と薬相談外来」を開設し，催奇形情報の提供やカウンセリングを行っている．

この外来で薬剤師は，薬物の催奇形情報を収集・評価するとともに妊婦への催奇形情報の提供，危険度有無の解説，服薬指導を行い，産婦人科医とともにカウンセリングを行っている．また，相談後の妊婦から出産結果の連絡を受け，妊婦服薬による胎児への影響に関して医療現場からの情報構築を行い，次世代妊婦への情報提供に活用している．

## 1.「妊娠と薬相談外来」の開設

当院「妊娠と薬相談外来」では，2005年9月末までに8,307人の妊婦の相談に応じており国内最大規模の相談実績となっている．胎児が薬物の影響を最も受けやすく催奇形性の観点から絶対過敏期と呼ばれる時期に，胎児に服薬による形態異常が生じているか否かを画像診断することは困難で，薬物自体に催奇形性があるのか網羅的な医薬品情報を調査し危険度を評価することが唯一の科学的な方法になる．

外来開設前の1987年当時は，外来中の産婦人科医から薬物の催奇形に関する問い合わせが薬剤部医薬情報科に毎週のように寄せられていた．医薬情報科の薬剤師は，動物の生殖試験から疫学調査まで丹念に調査し情報提供し，当時産婦人科の責任者であった佐藤孝道部長(現 聖路加国際病院女性総合診療部長)から，調査内容に対して高い評価を得た．1988年1月に産婦人科と薬剤部の共同で運営する外来開設に関する提案を受けて，現行の産婦人科医と薬剤師が同席する妊娠と薬相談外来がスタートした．

## 2.「妊娠と薬相談外来」の受診システム

当院の「妊娠と薬相談外来」を受診する妊婦は他施設で投薬を受けた方であり，他施設で出産を予定されている妊婦である．妊娠中の服薬による胎児の危険度に関する正しい情報を得る目的で来院される．妊婦の受診は2通りある．ひとつは産婦人科医師からの紹介で受診するもの，もうひとつはマスコミやインターネット等で存在を知り妊婦本人が直接受診申し込みする場合である．いずれにしても，薬剤部が窓口となり受診手順・予約管理から，催奇形性情報の調査・評価，産婦人科医との事前打ち合わせを行っている．薬剤部で行う薬物の催奇形性情報の網羅的調査は，本相談外来の薬剤師が担う重要な役割の第1点目といえる．

外来当日は，産婦人科の診察室で産婦人科医と薬剤師が同席してカウンセリングを行っている．薬剤師は，使用した薬剤が胎児に及ぼす影響に関する情報とその情報の持つ臨床的意味の評価に関する考え方を説明している．産婦人科医は，一人ひとりの妊娠の経過にあわせて服薬した時期の危険度，自然の奇形発生率に関する説明を行っている．この外来の目的は，妊娠中に服薬した薬物の胎児毒性(催奇形性)を心配し，無用な命の中断が発生することを防止することである．その意味で胎児に有害作用や催奇形性がないと考えられる根拠を，いかに平易で明快に情報提供し解説するかというリスクコミュニケーションは，本相談外来の薬剤師が担う重要な役割の2点目である．

また，本外来の特徴として，自由意思で協力が得られる妊婦から出産結果に関する情報を郵送形式で受け，妊婦の服薬による胎児への影響に関する独自

のデータベースを構築している．この出産結果情報を次回以降の相談妊婦に還元できるように，情報受理，整理，データ入力を薬剤師が行っており，本相談外来で担う重要な役割の3点目となっている．

## 3. 「妊娠と薬相談外来」の現状

1988年4月～2001年3月までの相談者の概要を紹介する．相談者の年齢は16～48歳まで幅広く分布し，28～30歳が最も多かった．服用した薬品数は，5種類以内が多いが最多は40種類であった．相談者が薬品を服用していた時期は絶対過敏期が61.4%と多いが，無影響期の服用薬も32.1%認められた．妊婦が服用していた薬剤の処方者は，内科，耳鼻咽喉科などの産婦人科以外の医師が最も多く，薬局で購入した薬剤を服用していた妊婦がこれに次いで多かった．薬局で医薬品を販売する際にも妊娠の有無を確認する等の注意が必要なことを示唆している．

相談の多い医薬品を薬効で分類すると，頭痛，発熱等で用いる解熱消炎鎮痛薬や感冒用薬，花粉症等に処方される抗ヒスタミン薬，抗アレルギー薬などが上位品目を占めていた．また，ストレス社会を反映してか精神神経用薬，SSRIやベンゾジアゼピン系の抗不安薬が上位品目となっていた(表1)．

表1 相談頻度の高い薬剤

| 順位 | 薬効名 | 使用数 | 順位 | 薬効名 | 使用数 |
|---|---|---|---|---|---|
| 1 | 解熱消炎鎮痛剤 | 3,392 | 11 | 酵素製剤 | 842 |
| 2 | 総合感冒剤 | 2,106 | 12 | 気管支拡張剤 | 802 |
| 3 | 消化性潰瘍用剤 | 1,906 | 13 | その他の消化器官用剤 | 796 |
| 4 | 抗生物質(CS，PC系) | 1,894 | 14 | ビタミン剤 | 770 |
| 5 | 催眠鎮静剤，抗不安剤 | 1,067 | 15 | 健胃消化剤 | 767 |
| 6 | 精神神経用剤 | 953 | 16 | ニューキノロン系抗菌薬 | 705 |
| 7 | その他のアレルギー用剤 | 938 | 17 | 止しゃ剤，整腸剤 | 675 |
| 8 | 漢方製剤 | 913 | 18 | 鎮咳去痰剤 | 630 |
| 9 | 鎮咳剤 | 873 | 19 | 去痰剤 | 580 |
| 9 | 抗ヒスタミン剤 | 873 | 20 | 副腎ホルモン剤 | 513 |

## 4. 妊娠と薬相談外来における危険度評価

### 1. 薬物自体の危険度評価

我が国では，妊娠期の薬物投与に関する危険度の公的評価として旧厚生省薬務局長通知(薬発第607号)に基づく医療用医薬品添付文書の使用上の注意，妊婦，産婦，授乳婦への投与の項の記載がある．一方，米国ではFDAによって胎児に対する薬剤の危険度を分類する基準が示されている．いずれの分類もリスクとベネフィットに基づく分類で，情報の危険度のみを標準化して位置づけるには構造が異なっていた．そこで当院の「妊娠と薬相談外来」では，薬剤の危険度を標準化して評価するために「薬剤催奇形危険度評価点」(表2)を定めて，疫学調査，症例報告，生殖試験の順で情報に重み付けした危険度評価をしている．

表2 薬物催奇形危険度評価点

| | |
|---|---|
| 5点 | 疫学調査で催奇形があると確定的に考えられている<br>または，生殖試験の結果，ヒトにも催奇形があると確定的に考えられている |
| 4点 | 疫学調査で催奇形を示唆する報告がある，または否定と肯定報告があり，どちらかといえば肯定的<br>または，疫学調査は行われていない，及び催奇形に関する信頼性の高い症例報告が複数ある |
| 3点 | 疫学調査で催奇形を示唆する報告と否定的報告があり，どちらかといえば否定的<br>または，疫学調査は行われていないが，催奇形の症例報告がある，または否定と肯定の報告があり優劣がつけがたい |
| 2点 | 疫学調査は行われていない，及びヒトでの催奇形を肯定する症例報告はない．しかし，生殖試験で催奇形の報告がある，または否定と肯定の報告があり優劣つけがたい |
| 1点 | 疫学調査は行われていない，及び催奇形を肯定する症例報告はない．及び生殖試験は行われていないか，または催奇形は認められていない<br>または，局所に使用するもの及び漢方薬 |
| 0点 | 疫学調査で催奇形の傾向はない，及び催奇形を肯定する症例報告はない．及び生殖試験は行われていないか，催奇形は認められていない．<br>または，食品としても使用されているもの |

### 2. 服薬時期の危険度評価

妊娠期間中の服薬の影響は，前述の薬物の危険度とともに服薬時期が大きな要因となる．催奇形の観点から最も危険な時期は，胎児の中枢神経，心臓，消化器，四肢などの臓器や器官が発生，分化する時期にあたる妊娠4～7週目の期間と評価している．当院では，この期間を，催奇形性に関して薬剤に最も敏感な「絶対過敏期」として服用時期の危険度を5点とし，以下「服薬時期の催奇形危険度評価点」(表3)に従って，相対過敏期3点から無影響期0点と5段階に分類している．

### 3. 催奇形危険度の総合評価

妊娠中に使用した薬物が胎児に及ぼす影響は，薬

表3　服薬時期の催奇形危険度評価点

| 最終月経開始日からの日数 | | 評価点 |
|---|---|---|
| 0 ～ 27日 | 無影響日 | 0点 |
| 28 ～ 50日 | 絶対過敏期 | 5点 |
| 51 ～ 84日 | 相対過敏期 | 3点 |
| 85 ～112日 | 比較過敏期 | 2点 |
| 113 ～ 出産日まで | 潜在過敏期 | 1点 |

表4　総合得点判定と患者への説明

| 総合得点 | 判定 | 患者への説明 |
|---|---|---|
| 0～6 | 無影響 | 薬剤による胎児への催奇形性は，全く考えられない．胎児に奇形が起こる確率は薬剤を服用しなかった人と全く同じである |
| 7～11 | 注意 | 薬剤による胎児への催奇形性は，皆無とはいえない．しかし，胎児に奇形がある確率は薬剤を服用しなかった人と同じか，それとほとんど差はない．薬剤が市販後間もない新薬であったり，ヒトでは否定的であるが一部の動物実験で催奇形作用が報告されているために安全といい切れないだけで，まず安全と考えられる． |
| 12～19 | 警戒 | 胎児への催奇形性の可能性はあるが危険性は低い．薬剤を服用していない場合に胎児に奇形がある確率を1%とすると，この危険性が2～3%程度になるかもしれない．専門家は人工妊娠中絶を考慮する対象になるとは考えない |
| 20～25 | 危険 | 薬剤の服用によって胎児に奇形がある可能性は，服用しなかった場合と比較して明らかに増加する．これを理由に人工妊娠中絶が行われたとしても，一部の専門家はその判断が根拠のないものとは考えない． |

物自体の危険度と服薬時期の危険度によって左右される．当院では，下記の式によって薬物の危険度点数と服薬時期の危険度点数を乗じた点数を算出し，それを胎児危険度点数として0～6点は「影響なし」，7～11点は「注意」，12～19点は「警戒」，20～25点は「危険」の4段階に分け評価している（表4）．

胎児危険度算出式

胎児危険度点数（総合得点）　＝

薬物危険度評価点　×　服薬時期の危険度評価点

## 5. 妊婦服薬カウンセリングの留意点

妊娠と薬相談外来では，薬物の催奇形情報に関する情報提供を行った上で，危険度の評価を共有できるようにカウンセリングを行っている．多くの統計では，健常妊婦の出生児の2～3%になんらかの先天的な異常が生じている．したがって，妊婦が自然の奇形発生率を正しく理解した上で服薬していないと，薬物や医療機関への不信感を生じるおそれがある．このため，妊婦に胎児への危険度を説明する際には，まず自然の奇形発生率について理解できるよう指導し，この奇形発生率を薬物が増加させるか否かという観点から，客観的な指導を行っている．

## 6. 相談事例の解析

当院の妊娠と薬相談外来では出産結果を郵送で確認する方式をとっており，8,000例を超える妊婦服薬例とその出産結果に関する情報を蓄積している．相談外来を受診する妊婦へ情報として提供し還元することはもとより，医学・薬学情報としても適正使用に必要な情報を社会にフィードバックする必要があると考えている．

国内で繁用されている解熱消炎鎮痛剤のロキソプロフェンは，妊婦使用に関する薬剤疫学データは極めて少ない．ベンゾジアゼピン系の抗不安薬エチゾラムも国内で繁用されており，妊娠と気づかずに服用する妊婦が少なくないが，国際的な調査を行っても妊婦服薬例の胎児への影響に関する情報は極めて少ない．当外来で，絶対過敏期にロキソプロフェンナトリウムを服用し，出産結果を確認できた相談事例226名中奇形の認められた新生児は6名であった．また絶対過敏期にエチゾラムを服用し，出産結果の確認できた相談事例130名中奇形の認められた新生児は2名であった．いずれの薬物も，我が国における一般的な自然奇形発生率と大きな違いは認められなかった．

## 7. おわりに

本年10月に成育医療センターに妊娠と薬情報センターが開設された．欧米では催奇形性情報の提供と出産結果の解析を多施設共同で行う，OTISやENTISなどの組織がある．我が国においても拠点病院の連携による妊婦服薬相談体制と出産結果収集システムの構築が必要であり，こうした時代の要請に当院の妊娠と薬相談外来としても取り組んでいく必要があると考えている．

# 妊婦に対する抗菌薬投与の注意点

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## 妊婦に対する抗菌薬投与の特殊性 ●

### 1. 妊娠中の薬物療法の特殊性

妊娠中の抗菌薬による治療では，母体治療上の必要性とともに感染症が妊娠経過と胎児に及ぼす影響，妊娠期の薬物体内動態の変化(**表1**)などを考慮した上で，胎児に悪影響を及ぼさない薬物を選択する必要がある．一方，胎児への影響を懸念するために，細菌性肺炎，クラミジア感染症，肺結核などの治療に必要な薬物の処方が控えられることによる母児の不利益は避けなければならない．そのためには，薬物の胎児毒性に関する情報を適正に評価し，治療上の必要性を満たし催奇形の危険度が低い薬物を使用する必要がある．本稿では，公的な妊婦リスクカテゴリーに加えて，虎の門病院の「妊娠と薬相談外来」におけるリスク評価についても紹介する．

また，サリドマイドによる催奇形の問題が教訓となり，一般の妊婦は妊娠中の薬物療法に過剰な不安を抱く傾向がある．妊婦自身が薬物療法の必要性と安全性を理解できるように服薬指導し，積極的に治療に参加できる環境を整える必要がある．

### 2. 妊娠中の薬物療法の原則

妊婦を対象とした臨床試験は倫理的問題から行われないため，新薬に関する母児への安全性情報は限られている．一方，使用歴が長く国際的に使用されている薬剤の中には，市販後調査，後向きケース・コントロール研究，前向きコホート研究などにより，母親の薬剤使用と催奇形の関連は認められなかったと判断し得る根拠情報が存在する薬剤がある．日常診療で使用される抗生物質のうち，こうした薬剤の代表例としてペニシリン系抗生物質のアモキシシリン，セフェム系抗生物質のセファレキシン，マクロライド系抗生物質のエリスロマイシンなどの抗生物質があげられる．

表1　妊娠期の薬物体内動態の変化[1)]

| | 半減期 ($t_{1/2}\beta$) | 分布容積 ($l$) | クリアランス (m$l$/分) |
|---|---|---|---|
| アンピシリン | | | |
| [妊娠時] | 52.4±3.9 | 32.8±2.5 | 450±31 |
| [非妊娠] | 69.6±6.1 | 34.5±2.7 | 370±30 |
| ピペラシリン | | | |
| [妊娠時] | 46.5±10 | 67.6±11.8 | 1,538±362 |
| [非妊娠] | 53.7±4.6 | 41.9±6.2 | 540±75 |
| セフロキシム | | | |
| [妊娠時] | 44±5 | 17.8±1.9 | 282±34 |
| [非妊娠] | 58±8 | 16.3±2.1 | 198±27 |
| イミペネム | | | |
| [妊娠時] | 36±8 | 47.1±14.8 | 973±47 |
| [非妊娠] | 41±16 | 18.9±5.8 | 338±85 |

抗生物質，抗菌薬，抗結核薬など，妊娠中であっても投薬の必然性(必要性と安全性)がある薬剤は，必要量を必要な期間投薬することが原則となる．妊婦であっても治療上の必要性があって投薬する原点に立ち返れば，投与量，投与期間を制限することにより有効性が得られなければ，投薬自体が意味を失うからである．

### 3. 妊娠中の薬物療法の公的リスクカテゴリー

わが国では，医療用医薬品添付文書の使用上の注意「妊婦，産婦，授乳婦への投与」の項に必要な注意が記載されており国内唯一の公的リスクカテゴリーとなっている．記載は，旧厚生省薬務局長通知(薬発第607号)に基づき，以下のように記載するよう定められている．

① 特に妊婦，産婦，授乳婦に注意する必要がある場合や，適正使用に関する情報がある場合に，必要な注意を記載し投与してはならない場合は禁忌の項にも記載する．

② 動物実験，臨床使用経験，疫学的調査などで得られている情報に基づき，必要な事項を記載す

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