change in general behavior was observed in the others. In the 200 mg/kg group, body weights were also lower by 15-25% than the control values from dosing Day 4 in males and from dosing Day 8 in females. Blood biochemical examination showed a slight increase in total cholesterol in females given 200 mg/kg. For organ weight, increases in relative liver weights were demonstrated in both sexes at 100 mg/kg and more with absolute liver weights in males at 100 mg/kg. Decrease in absolute and relative testis weights were also observed in males of 200 mg/kg group. At autopsy, there were no gross abnormalities except hepatomegaly in all animals, including the dead rats at 200 mg/kg. Based on these results, 10, 50 and 150 mg/kg were selected as the doses for the main study in newborn rats.

In the main study, no change in general behavior was noted during the dosing period in any dose group. Body weights of both sexes given 150 mg/kg were lowered during the dosing period (Fig.1) and gain was also decreased by approx. 10%. No definitive changes in parameters for external and sexual development and reflex ontogeny were detected in any dose group. At the scheduled sacrifice, blood biochemical examination of the 150 mg/kg group showed increases in γ-GTP in males and total bilirubin in females. There were no dose-related changes in hematological parameters. Significant increase of absolute and relative liver weights was noted in males given 50 mg/kg and in both sexes given 150 mg/kg. The relative liver weights were also increased in females at 10 and 50 mg/kg. Absolute brain weights were lower in both sexes given 150 mg/kg, this being considered due to the lowered body weights. On histopathological examination, hypertrophy of centrilobular hepatocytes was noted in all animals given 150 mg/kg, being mild in 3/6 males and 4/6 females (Table 1). In four of each sex, the endoplasmic reticulum in hypertrophic hepatocytes showed a ground glass appearance. In addition, single cell necrosis was also noted in 3/6 males and 1/6 females at 150 mg/kg. During and at the end of the recovery-maintenance period, the changes observed in scheduled sacrificed group had disappeared.

The results of the dose-finding study and main study of DBP in newborn rats are summarized in Table 2. The NOAEL was concluded to be 50 mg/kg/day because increase in liver weight without biochemical and histopathological changes in this dose of the main study was not considered as an adverse effect. The unequivocally toxic level was concluded to be 150 mg/kg/day, based on increase of liver weight, mild centrilobular hypertrophy of hepatocytes, increase of γ-GTP and total bilirubin, and lowering of body weights at this dose in the main study, taking additional account of the 40% mortality rate at 200 mg/kg in the dose-finding study.

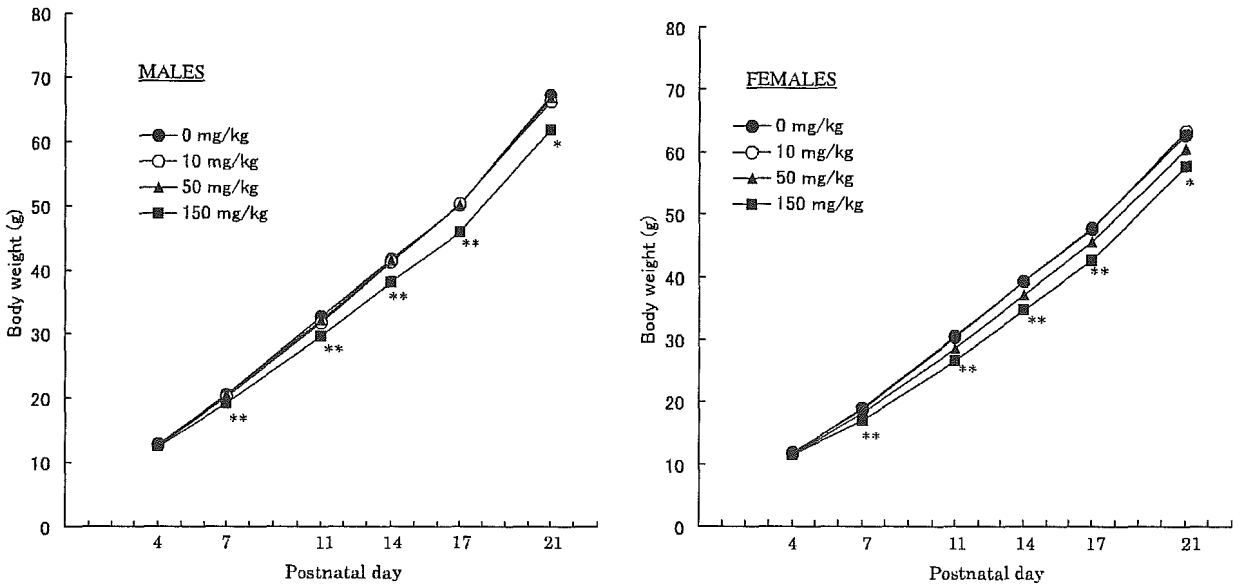

Fig. 1. Body weight curves for the 18-day study of 1,3-dibromopropane in newborn rats.
*: Significantly different from the controls ($p < 0.05$), **: Significantly different from the controls ($p < 0.01$).

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↑ 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↑ 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

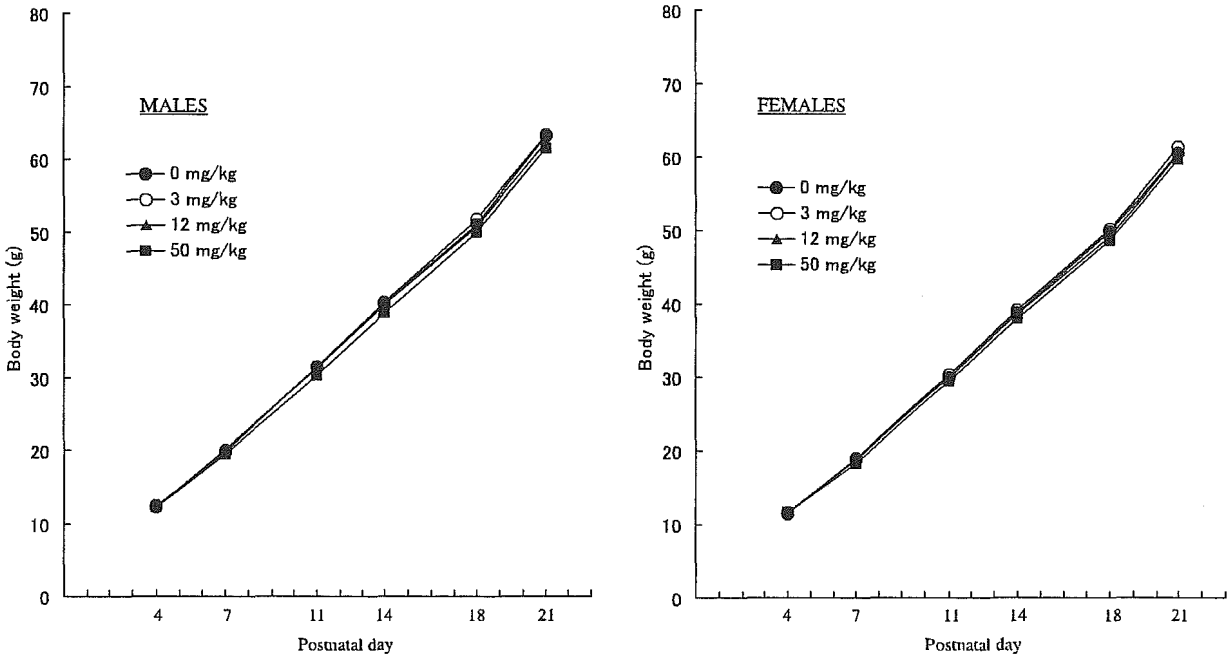

Fig. 2. Body weight curves in the 18-day study of 1,1,2,2-tetrabromoethane in newborn rats. Not significantly different from the controls.

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 (No of animals with the findings)	n.d.	n.d.	n.d.	0	0	0

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↑ 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.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of the Office of Chemical Safety, Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labor and Welfare, Japan.

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Food and Chemical Toxicology 43 (2005) 325–331
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Evaluation of developmental toxicity of 1-butanol given to rats in drinking water throughout pregnancy

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Received 21 September 2004; accepted 6 November 2004

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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doi:10.1016/j.fct.2004.11.003

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.

Acknowledgment

This study was performed in 2003 through 2004 at the Ina Research, Inc. (Ina, Japan) and supported by the Ministry of Health, Labour and Welfare, Japan.

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【報文】

OECD 化学物質対策の動向（第 6 報）

－第 14 回 OECD 高生産量化学物質初期評価会議（2002 年パリ）－

Progress on OECD Chemicals Programme (6): SIAM 14 in Paris, 2002.

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要旨：第 14 回の OECD 高生産量化学物質初期評価会議が 2002 年 3 月にパリで開催された。日本が提出した 8 物質の初期評価文書については全ての評価結果の合意が得られた。本稿では、本会議で合意の得られた 8 物質の初期評価報告書の健康影響部分について、その要旨を紹介する。

Abstract: The 14th SIDS, the Screening Information Data Set, Initial Assessment Meeting (SIAM 14) was held at the Organisation for Economic Co-operation and Development (OECD) headquarters in Paris, France, hosted by the European Commission. The initial assessment documents of eight substances at SIAM 14 (CAS numbers: 88197, 126987, 839907, 2403885, 2867472, 3319311, 3452979, 16219753) were submitted by the Japanese Government with or without the International Council of Chemical Associations (ICCA) and all of them were agreed at the meeting. In this report, the human health effect parts in their eight substance documents are introduced.

Key words: OECD, HPV program, SIDS Initial Assessment Meeting

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連絡先：〒158-8501 東京都世田谷区上用賀 1-18-1　E-mail: ema@nihs.go.jp
受付日：2004 年 9 月 24 日　受理日：2004 年 10 月 13 日

1 はじめに

経済協力開発機構（Organisation for Economic Co-operation and Development : OECD）は加盟各国の経済の持続的な成長、多角的な貿易の拡大等を目的としているが、1960 年代から化学物質貿易の拡大に伴う環境汚染が深刻化して化学品対策がその重要な課題となり、化学品テストガイドラインや Good Laboratory Practice（GLP）の作成等の種々の活動が進められてきた（長谷川ら 1999a）。加盟各国における高生産量化学物質（High Production Volume Chemical : HPV）については、1992 年に始まった OECD 高生産量化学物質点検プログラム（HPV Program）により安全性の評価が行われている（長谷川ら 1999a）。点検プログラムにおいて加盟各国での生産量・既存の毒性データ量に基づき OECD HPV Chemicals List の作成及び評価の優先順位付けが行われ（長谷川ら 1999a）、現在は加盟各国と企業が、生産した化学物質に関する情報収集や試験を行って評価文書を完成させ、順次それらの文書が初期評価会議（SIAM : SIDS, the Screening Information Data Set, Initial Assessment Meeting）で討議されている。

日本政府は初回より評価文書を提出しており、第 6 回までに 27 物質の評価文書について合意を得た。第 7 回から第 13 回の初期評議会議において日本政府が担当し結論および勧告が合意された化学物質の評価文書のヒトの健康影響部分については既に紹介された（長谷川ら 1999b、2000、2001 ; 高橋ら 2004）。

評価文書は、物性、環境毒性、及びヒトの健康影響に関する記述から構成されているが、著者らがヒトの健康影響部分の担当であるため、本稿では SIAM14 で合意に至った化学物質名及び日本担当物質の評価文書のヒトの健康影響についての記述の概要を紹介する。

2 SIAM14 で合意された化学物質名と日本担当物質の初期評価内容

2002 年 3 月にパリ（フランス）で開催された SIAM14 において、33 化学物質の初期評価文書が検討され、表 1 に示す物質の初期評価結果および勧告が合意された。SIAM における合意は FW（Further Work）または LP（Low Priority）として示されている。FW は「今後も追加の調査研究作業が必要である」、LP は「現状の使用状況においては追加作業の必要はない」ことを示す。日本政府が担当した化学物質の初期評価報告書のヒトへの健康影響についての記述の概要を以下に示す。

（1）*o*-Toluenesulfonamide (88-19-7)（日本政府作成）

本化学物質はスルホンアミド類に属し、サッカリンの原料として使用される。

ラットへの経口投与では、ほとんどが速やかに尿中に排泄される。ヒトではラットより排泄は遅い。ラットとヒトにおける主要な代謝物質は 2-スルファモイル-ベンジルアルコール及びその硫酸塩、グルクロン酸塩である。さらに、ヒトでは代謝物質としてサッカリンが尿中で検知されている。

ラットにおける単回経口投与毒性試験（OECD TG 401）では、投与直後から鎮静、脱力、側臥位、刺激に対する無反応、促迫呼吸、呼吸数減少、体温低下、カタレプシー等が最低投与量の 700 mg/kg でもみられ、LD_{50} は雄で 2,000 mg/kg を上回り、雌で 1,000 ～ 2,000 mg/kg であった。ウサギの眼に対する刺激性が報告されているが、信頼性は不確実である。

反復投与毒性・生殖発生毒性併合試験（OECD TG 422）では、ラットの雌雄に交配前 2 週間

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受付日：2004 年 9 月 24 日　受理日：2004 年 10 月 13 日

及び交配期間 2 週間、さらに、雄では交配期間終了後 2 週間、雌では妊娠期間及び分娩後哺育 3 日まで、0、20、100 及び 500 mg/kg/day が強制経口投与された。雄の 100 mg/kg/day 以上の群で自発運動低下、腹臥姿勢、体重増加抑制、摂餌量減少、流涎が観察された。剖検時の血液学検査では、赤血球血色素濃度が 100 mg/kg/day 以上の群で、また、血小板数が 500 mg/kg/day 群で増加した。血液生化学検査では、100 mg/kg/day 以上の群で総コレストロール濃度が増加した。また、500 mg/kg/day 群では総蛋白濃度が増加し、A/G 比、ブドウ糖濃度及びトリグリセライド濃度が低下した。100 mg/kg/day 以上の群で肝臓及び腎臓に暗色化あるいは腫大が認められ、病理組織学検査において、肝臓では小葉中心性肝細胞肥大が認められ、腎臓では好酸性小体が被験物質投与群で頻度及び程度ともに増強された。雌の 500 mg/kg/day 群において自発運動低下、腹臥姿勢、鎮静、紅涙、体温低下、触発反応の喪失、流涙、呼吸困難、褐色尿などを示した後に 13 例中 3 例が死亡し、2 例が瀕死剖検された。雌の生存例では、100 mg/kg/day 以上の群で雄と同様の一般状態の変化が観察され、500 mg/kg/day 群ではさらに四肢の伸展及びよろめき歩行が認められた。哺育 4 日の剖検では、100 mg/kg/day 以上の群の肝臓に腫大あるいは暗色化、胸腺には小型化が観察された。100 mg/kg/day 以上の群の肝臓、500 mg/kg/day 群の腎臓、脳及び副腎の重量あるいは比体重が増加し、病理組織学検査では、100 mg/kg/day 以上の群で小葉中心性肝細胞肥大が、500 mg/kg/day 群では心外膜の線維化及び細胞浸潤、胸腺の萎縮ならびに被膜の線維化および細胞浸潤が認められた。

以上の臨床症状と肝臓の変化に基づいて反復経口投与の無毒性量（NOAEL: No Observed Adverse Effect Level）は 20 mg/kg/day と判断された。

上述の OECD の反復投与毒性・生殖発生毒性併合試験において、交尾及び受胎能、分娩及び哺育状態の異常は認められなかった。出生児に被験物質投与に起因した形態異常は観察されなかったが、500 mg/kg/day 群における哺育 0 及び 4 日における雌雄生存児体重に低下が認められた。また、0、2.5、25 及び 250 mg/kg/day を混餌投与したラットの二世代生涯試験では、250 mg/kg/day 群で児数及び体重の減少が認められた。これらの試験結果を考慮して、生殖発生毒性の NOAEL は 100 mg/kg/day と判断された。

細菌を用いた復帰突然変異試験では S9 mix 存在および非存在下で陰性であり、チャイニーズ・ハムスター培養細胞を用いた染色体異常試験でも陰性であった。*In vivo* の 2 種類の小核試験では陰性であったが、マウスの Spot 試験では結果が曖昧であった。これら *in vivo* の試験は実験条件が十分に記載されていないことも考慮して、*in vivo* での遺伝毒性の可能性については結論できないとされた。

上述の二世代生涯試験では、いずれの投与群でも両世代に腫瘍発生率上昇は認められず、２年間経口投与試験でも本物質に発がん性がないことが示された。一世代生涯試験において膀胱の腫瘍発生率の低下が示されているが、この研究の報告は記述が不十分であるため、信頼性は不確実である。ほ乳類の培養細胞を用いた細胞トランスフォーメーション活性は陰性であった。これらのデータの総合判断から本化学物質に発がん性はないと判断された。

（2）Methyl acrylonitrile (126-98-7)（日本政府作成）

本化学物質はメタクリル酸誘導体やポリマーの中間体として使用される。

消化管で速やかに吸収されるが生体内蓄積の可能性は低い。主として呼気中に二酸化炭素として排泄される。投与に用いた溶媒の違いや試験に用いた動物の種差／系統差が本化学物質の代謝と排泄に影響を及ぼしている。

急性毒性に明確な種差があり、経口 LD_{50} はラットで 64～240 mg/kg、マウスで 17 mg/kg、ウサギで 16 mg/kg、スナネズミで 3.8～4.9 mg/kg であった。自発運動低下、腹臥姿勢や側

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