Table 2: Principal blood biochemical values in male and female rats given HDBB by gavage for 28 days.

	At the completion of the administration period					At the completion of the recovery period	
	0 mg kg^{-1} day^{-1}	0.5 mg kg^{-1} day^{-1}	2.5 mg kg^{-1} day^{-1}	12.5 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}	0 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}
Male							
No. of animals	5	5	5	5	5	5	5
Total protein (g/dL)	5.84 ± 0.34	5.52 ± 0.10	5.55 ± 0.24	5.72 ± 0.22	5.86 ± 0.40	6.02 ± 0.19	5.95 ± 0.49
Albumin (g/dL)	3.78 ± 0.22	3.90 ± 0.17	4.06 ± 0.20	4.43 ± 0.18**	4.40 ± 0.41**	3.75 ± 0.1	4.22 ± 0.45*
A/G ratio	1.85 ± 0.18	2.43 ± 0.23*	2.75 ± 0.29**	3.47 ± 0.25**	3.05 ± 0.55**	1.66 ± 0.11	2.46 ± 0.34*
Glucose (mg/dL)	122 ± 13	132 ± 15	170 ± 18**	170 ± 10**	156 ± 16**	166 ± 13	182 ± 22
Total cholesterol (mg/dL)	59 ± 11	46 ± 9	45 ± 4	49 ± 13	52 ± 20	62 ± 13	55 ± 19
Triglyceride (mg/dL)	25.5 ± 8.4	24.3 ± 4.5	34.5 ± 7.1	44.8 ± 20.9	45.8 ± 12.5	68.0 ± 52.0	47.5 ± 26.6
BUN (mg/dL)	13.0 ± 2.5	12.9 ± 0.5	15.5 ± 1.7	15.8 ± 1.3	17.2 ± 2.4**	14.5 ± 2.4	19.0 ± 1.9*
AST (U/L)	72 ± 7	71 ± 11	65 ± 5	83 ± 22	115 ± 16*	61 ± 7	68 ± 22
ALT (U/L)	30 ± 5	28 ± 4	32 ± 3	42 ± 5*	48 ± 10**	25 ± 5	49 ± 29**
ALP (U/L)	757 ± 175	992 ± 220	1089 ± 168	1569 ± 427**	1462 ± 250**	622 ± 123	906 ± 169*
Female							
No. of animals	5	5	5	5	5	5	5
Total protein (g/dL)	5.68 ± 0.14	5.61 ± 0.18	5.53 ± 0.19	5.93 ± 0.33	5.85 ± 0.19	5.91 ± 0.29	6.50 ± 0.30*
Albumin (g/dL)	3. 81 ± 0.23	3.67 ± 0.43	3.72 ± 0.12	4.12 ± 0.14	4.21 ± 0.18	3.85 ± 0.32	4.27 ± 0.10*
A/G ratio	2.04 ± 0.26	1.95 ± 0.44	2.09 ± 0.27	2.30 ± 0.25	2.59 ± 0.29*	1.89 ± 0.25	1.93 ± 0.18
Glucose (mg/dL)	110 ± 15	120 ± 20	114 ± 16	127 ± 22	151 ± 8**	117 ± 8	149 ± 16**
Total cholesterol (mg/dL)	49 ± 10	59 ± 5	50 ± 7	54 ± 6	84 ± 16**	63 ± 6	91 ± 14**
Triglyceride (mg/dL)	12.3 ± 5.6	12.1 ± 2.6	8.8 ± 3.7	12.2 ± 1.1	31.9 ± 4.8**	18.8 ± 7.6	37.7 ± 18.8
BUN (mg/dL)	16.1 ± 4.3	15.5 ± 1.5	16.6 ± 3.8	15.8 ± 2.4	16.9 ± 1.3	16.6 ± 1.2	16.8 ± 0.8
AST (U/L)	68 ± 5	69 ± 11	66 ± 7	68 ± 9	76 ± 12	66 ± 13	65 ± 19
ALT (U/L)	21 ± 2	22 ± 4	23 ± 3	27 ± 4	33 ± 6**	25 ± 4	36 ± 21
ALP (U/L)	490 ± 110	409 ± 86	414 ± 85	433 ± 83	633 ± 199	381 ± 138	247 ± 63

Values are expressed as the mean ± SD.
*Significantly different from the control, $p \leq 0.05$.
**Significantly different from the control, $p \leq 0.01$.

Table 3: Principal organ weights of male and female rats given HDBB by gavage for 28 days.

	At the completion of the administration period					At the completion of the recovery period	
	0 mg kg^{-1} day^{-1}	0.5 mg kg^{-1} day^{-1}	2.5 mg kg^{-1} day^{-1}	12.5 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}	0 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}
Male							
No. of animals	5	5	5	5	5	5	5
Brain (g)	2.02 ± 0.08	2.03 ± 0.07	2.12 ± 0.06	2.08 ± 0.05	2.06 ± 0.09	2.10 ± 0.10	2.07 ± 0.10
	(0.624 ± 0.009)[a]	(0.622 ± 0.038)	(0.633 ± 0.062)	(0.628 ± 0.044)	(0.630 ± 0.046)	(0.527 ± 0.046)	(0.580 ± 0.034)
Heart (g)	1.09 ± 0.09	1.10 ± 0.11	1.17 ± 0.14	1.18 ± 0.07	1.23 ± 0.19	1.20 ± 0.10	1.28 ± 0.16
	(0.337 ± 0.026)	(0.336 ± 0.028)	(0.346 ± 0.011)	(0.355 ± 0.017)	(0.374 ± 0.028)	(0.298 ± 0.008)	(0.356 ± 0.016**)
Liver (g)	9.40 ± 0.58	11.65 ± 1.90	17.11 ± 3.46*	21.64 ± 2.73*	24.47 ± 5.06*	11.8 ± 1.64	20.61 ± 3.36**
	(2.908 ± 0.139)	(3.533 ± 0.296*)	(5.045 ± 0.506*)	(6.507 ± 0.536*)	(7.413 ± 1.283*)	(2.930 ± 0.133)	(5.746 ± 0.527**)
Kidneys (g)	2.43 ± 0.22	2.54 ± 0.17	2.74 ± 0.29	2.88 ± 0.40	3.04 ± 0.45*	2.83 ± 0.23	2.91 ± 0.40
	(0.753 ± 0.075)	(0.775 ± 0.046)	(0.814 ± 0.053)	(0.865 ± 0.080)	(0.927 ± 0.119**)	(0.706 ± 0.046)	(0.814 ± 0.066*)
Testes (g)	2.90 ± 0.16	2.84 ± 0.12	2.88 ± 0.15	2.91 ± 0.15	2.92 ± 0.14	3.13 ± 0.11	3.07 ± 0.18
	(0.901 ± 0.080)	(0.871 ± 0.084)	(0.865 ± 0.121)	(0.879 ± 0.046)	(0.891 ± 0.068)	(0.787 ± 0.099)	(0.861 ± 0.043)
Female							
No. of animals	5	5	5	5	5	5	5
Brain (g)	1.94 ± 0.10	1.92 ± 0.08	1.95 ± 0.07	1.90 ± 0.12	1.90 ± 0.03	1.99 ± 0.02	1.94 ± 0.05
	(0.931 ± 0.053)	(0.884 ± 0.012)	(0.901 ± 0.052)	(0.857 ± 0.046)	(0.841 ± 0.058*)	(0.838 ± 0.086)	(0.802 ± 0.084)
Heart (g)	0.75 ± 0.07	0.77 ± 0.03	0.75 ± 0.02	0.78 ± 0.05	0.84 ± 0.06*	0.79 ± 0.04	0.87 ± 0.06
	(0.357 ± 0.019)	(0.356 ± 0.008)	(0.348 ± 0.007)	(0.351 ± 0.009)	(0.371 ± 0.024)	(0.333 ± 0.022)	(0.357 ± 0.028)
Liver (g)	6.39 ± 0.87	6.84 ± 0.63	6.73 ± 0.26	8.67 ± 1.16**	12.43 ± 0.89**	6.80 ± 0.86	8.85 ± 0.99**
	(3.053 ± 0.178)	(3.146 ± 0.197)	(3.112 ± 0.107)	(3.885 ± 0.324**)	(5.497 ± 0.172**)	(2.836 ± 0.076)	(3.626 ± 0.117**)
Kidneys (g)	1.70 ± 0.14	1.61 ± 0.08	1.71 ± 0.09	1.72 ± 0.11	1.87 ± 0.19	1.77 ± 0.18	1.86 ± 0.13
	(0.816 ± 0.057)	(0.742 ± 0.033*)	(0.789 ± 0.029)	(0.776 ± 0.040)	(0.827 ± 0.042)	(0.744 ± 0.075)	(0.766 ± 0.070)
Ovaries (mg)	87 ± 22	96 ± 18	82 ± 11	97 ± 9	89 ± 18	88 ± 12	101 ± 11
	(0.041 ± 0.007)	(0.044 ± 0.008)	(0.038 ± 0.005)	(0.044 ± 0.005)	(0.039 ± 0.008)	(0.037 ± 0.004)	(0.041 ± 0.003)

Values are expressed as the mean ± SD.
[a] Relative organ weight (organ weight per body weight) (%).
*Significantly different from the control, $p \leq 0.05$.
**Significantly different from the control, $p \leq 0.01$.

On histopathology, test-substance-related changes were observed in the liver, heart, kidneys, thyroids, and spleen as shown in Table 4. In the liver, hypertrophy of hepatocytes in males at 0.5 mg/kg and more and in females at 12.5 and 62.5 mg/kg; bile duct proliferation and decreased incidence of hepatocellular fatty change in males at 0.5 mg/kg and more and in females at 62.5 mg/kg; vacuolar degeneration of hepatocytes in males at 2.5 mg/kg and more and in females at 62.5 mg/kg; focal necrosis in males at 2.5 mg/kg and more; increased mitosis of hepatocytes in males at 62.5 mg/kg and in females at 12.5 and 62.5 mg/kg; and hepatocellular pigmentation and/or cytoplasmic inclusion bodies in males at 62.5 mg/kg were observed. In the heart, cell infiltration at 2.5 mg/kg and more in males, and degeneration and/or hypertrophy of the myocardium at 12.5 and 62.5 mg/kg in both sexes were noted. Furthermore, hypertrophy of the tubular epithelium was observed in the kidneys of males at 12.5 and 62.5 mg/kg and of females at 62.5 mg/kg, and increased severity of basophilic tubules was found in males at 62.5 mg/kg. In the thyroids and spleen, diffuse follicular cell hyperplasia at 62.5 mg/kg in both sexes and extramedullary hematopoiesis at 2.5 mg/kg and more in males, respectively, were detected.

At the end of the recovery period, a significant decrease in red blood cell count, hematocrit, hemoglobin and MCHC, and increase in platelet count were still observed in males, and a significant decrease in MCH and increase in reticulocyte in males and increase in platelet count in females were additionally found (Table 1). A significant increase in serum levels of albumin, A/G ratio, BUN, ALT, and ALP in males, and in total protein, albumin, glucose and total cholesterol in females was also noted (Table 2). At necropsy, grossly enlarged liver was still observed, and the absolute and relative weight was significantly increased in both sexes (Table 3). In males, the liver was brown, and some were accompanied with a red or white patch/zone. A significant increase in the relative weight of the heart and kidneys was also noted in males (Table 3). Histopathologically, except for increased mitosis of hepatocytes, hepatic changes were observed with similar incidence as observed at the end of the administration period in males (Table 4). Degeneration of the myocardium and cell infiltration in the heart, diffuse follicular cell hyperplasia in the thyroid, and extramedullary hematopoiesis in the spleen were also detected in males. In females, hypertrophy of hepatocytes was found, but other histopathologic changes observed at the end of the administration period were not detected. In the liver, focal necrosis and hepatocellular pigmentation were also found in females.

DISCUSSION

The current study was conducted to obtain initial information on the possible repeated-dose toxicity of HDBB in rats. The dosage of HDBB used in this

Table 4: Histopathologic findings in the principal organs of male and female rats given HDBB by gavage for 28 days.

		At the completion of the administration period					At the completion of the recovery period	
	Grade	0 mg kg^{-1} day^{-1}	0.5 mg kg^{-1} day^{-1}	2.5 mg kg^{-1} day^{-1}	12.5 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}	0 mg kg^{-1} day^{-1}	62.5 mg kg^{-1} day^{-1}
Male								
No. of animals		5	5	5	5	5	5	5
Liver								
Hypertrophy of hepatocytes	+	0	3	5**	5**	5**	0	5**
Fatty change of hepatocytes	+	5	0**	0**	0**	0**	5	0**
Bile duct proliferation	+	0	1	1	4*	4*	0	4*
Vacuolar degeneration of hepatocytes	+	0	0	5**	5**	5**	0	4*
Focal necrosis	+	0	0	1	2	4*	0	3
Increased mitosis of hepatocytes	+	0	0	0	0	4*	0	0
Pigment deposit of hepatocytes	+	0	0	0	0	1	0	2
Cytoplasmic inclusion bodies	+	0	0	0	0	1	0	1
Heart								
Cell infiltration	+	0	1	5**	4*	4*	1	3
Degeneration of myocardium	+	0	0	0	5**	5**	0	4*
Hypertrophy of myocardium	+	0	0	0	3	4*	0	0
Kidney								
Hypertrophy of tubular epithelium	+	0	0	0	2	5**	0	0
Basophilic tubules	+	2	3	4	3	3	4	4
	++	0	0	0	0	2	0	0
Thyroid								
Diffuse follicular cell hyperplasia	+	0	0	0	0	2	0	3

Spleen								
Extramedullary hematopoiesis	+	0	0	3	2	2	0	3
Female								
No. of animals		5	5	5	5	5	5	5
Liver								
Hypertrophy of hepatocytes	+	0	0	0	5**	5**	0	3
Fatty change of hepatocytes	+	5	5	5	3	0**	5	4
Bile duct proliferation	+	0	0	0	0	1	0	0
Vacuolar degeneration of hepatocytes	+	0	0	0	0	2	0	0
Focal necrosis	+	0	0	0	0	0	0	2
Increased mitosis of hepatocytes	+	0	0	0	1	2	0	0
Pigment deposit of hepatocytes	+	0	0	0	0	0	0	1
Heart								
Cell infiltration	+	0	1	0	0	1	0	0
Degeneration of myocardium	+	0	0	0	3	5**	0	0
Hypertrophy of myocardium	+	0	0	0	1	3	0	0
Kidney								
Hypertrophy of tubular epithelium	+	0	0	0	0	2	0	0
Basophilic tubules	+	1	2	2	0	3	4	4
Thyroid								
Diffuse follicular cell hyperplasia	+	0	0	0	0	2	0	0
Spleen								
Extramedullary hematopoiesis	+	0	1	0	0	0	0	0

Values represent the number of animals with findings.
+: slight; ++: moderate.
*Significantly different from the control, $p \leq 0.05$.
**Significantly different from the control, $p \leq 0.01$.

study was sufficiently high to be expected to induce toxicity in the liver. As expected, histopathologic changes including vacuolar degeneration and hypertrophy of hepatocytes were observed in the liver. These findings showed that one of the toxicologically main targets of HDBB was the liver. Increased food consumption without body weight changes, increased blood glucose, total cholesterol and triglyceride, and decreased incidence of fatty changes of hepatocytes were noted after HDBB administration for 28 days. These changes indicate metabolic derangement and suggest possible adverse effects of HDBB in metabolic homeostasis. The current study showed that the heart was another toxicologic target organ for HDBB. Although degeneration and hypertrophy of the myocardium and cell infiltration were observed after HDBB administration, cardiac function was not evaluated in the current study. Further studies are required to clarify the adverse effects of HDBB on cardiac function, because functional parameters are considered to be more susceptible than histopathologic changes in the heart (Glaister, 1992). In our study, HDBB also caused anemic changes (decreased red blood cell count, hematocrit, hemoglobin and MCHC, and extramedullary hematopoiesis), and adverse effects on the kidneys (hypertrophy of tubular epithelium and increased severity of basophilic tubules with increased BUN) and the thyroids (diffuse follicular cell hyperplasia) at higher doses. Adverse effects on the liver and kidneys, and anemia, but not adverse effects on the heart and thyroid, were reported in the 90-day repeated feeding study on the structural analogue, 2-(2′-hydroxy-3′,5′-di-*tert*-amylphenyl)benzotriazole, in rats (U.S. EPA, 2001). Further studies are needed to clarify the differences in the toxicological profiles between the current study and study on the analogue.

The results of the current study clearly showed sex differences in the toxic susceptibility of rats to HDBB. In males, the development of anemia and histopathologic changes in the liver, heart, kidneys, thyroid, and spleen accompanied with related blood biochemical and organ weight changes were seen. Hypertrophy of hepatocytes, decreased incidence of fatty change of hepatocytes, bile duct proliferation, increase in relative liver weight and serum A/G ratio were noted even at 0.5 mg/kg. Most of the changes were not improved after a 14-day recovery period in the highest dose group. In females, however, no anemic effects of HDBB were observed, and other effects observed in males were noted only at 12.5 mg/kg and more in females. These changes in females mostly recovered after the recovery period. These findings suggest that male rats have a nearly 25 times higher susceptibility to HDBB toxicity than female rats.

Gender-related differences in toxic susceptibility have been documented for some other substances. For example, a recent subchronic toxicity study using F344 rats showed that fluoranthene, a polycyclic aromatic hydrocarbon, had greater effects on males than females (especially on the kidneys) (Knuckles et al., 2004). In contrast, it was reported that female rats exhibited a greater

susceptibility to hypothermic effects and inhibition of hypothalamic cholinesterase by a carbamate cholinesterase inhibitor, rivastigmine (Wang et al., 2001). Such gender-related variation is also reported in humans, mostly for drugs, such as more severe adverse effects with greater improvement in response to antipsychotic drugs such as chlorpromazine and fluspirilene in women (Fletcher et al., 1994; Harris et al., 1995). The various causes of these gender differences are indicated mainly for toxicokinetic determinants. It is well-known that hepatic metabolism differs between the sexes, with males generally having higher activity than females in rats (Gad, 2006). Furthermore, gender differences in membrane transport in various organs of the body including the kidneys, liver, intestine, and brain have emerged relatively recently (Morris et al., 2003). In the case of HDBB, it is difficult to discuss the cause of the gender differences because no other data are available on toxicity, including the toxicokinetics. However, because male rats showed higher susceptibility to various effects of HDBB (on the liver, heart, blood, etc.) consistently, such differences in metabolism or transports between the sexes might increase the blood concentration of causative substances (HDBB or its metabolites) in males.

For gender differences, it goes without saying that sexual hormones play an important role. In fact, Wang et al. (2001) reported that orchidectomy completely abolished the above-mentioned sex differences in hypothalamic cholinesterase inhibition induced by rivastigmine. Because testosterone decreased cholinesterase inhibition in gonadectomized males and females, it is apparent that testosterone interferes with the effects of rivastigmine. On the other hand, estrogen has been shown to act as a dopamine antagonist (Fletcher et al., 1994; Harris et al., 1995), which is considered to contribute at least in part to sex differences in response to antipsychotic drugs. It would be interesting to investigate the role of sex steroids in the mediation of sex differences in toxic susceptibility to HDBB, too. For the metabolic enzyme cytochrome P450, involved in the metabolism of many substances, gonadal hormones are known to play an important role in regulating the expression; however, gonadal hormones do not act directly on the liver to confer the sex-dependent pattern, but rather, indirectly via the hypothalamus, which regulates the pituitary and its secretion of the polypeptide hormone, growth hormone (Waxman and Chang, 2005).

Based on the findings of this study, the NOAEL for females was concluded to be 2.5 mg kg^{-1} day^{-1} based on the induction of hypertrophy and increased mitosis of hepatocytes and degeneration and hypertrophy of the myocardium at 12.5 mg/kg. On the other hand, the NOAEL for males could not be determined because hypertrophy and decreased incidence of fatty change of hepatocytes and bile duct proliferation were noted at the lowest dose of 0.5 mg/kg. Considering the toxic effects observed at a relatively low dose and the incomplete recovery, more severe damage by the longer exposure is a concern; therefore, we

are currently performing a 52-week repeated-dose toxicity study to clarify the potential toxic effects of this chemical.

CONCLUSION

The current results showed that the oral administration of HDBB for 28 days principally affected the liver and heart, and male rats were more susceptible to the toxic effects of this chemical than female rats. The NOAEL for repeated-dose toxicity was concluded to be less than 0.5 mg kg^{-1} day^{-1} in male rats and 2.5 mg kg^{-1} day^{-1} in female rats.

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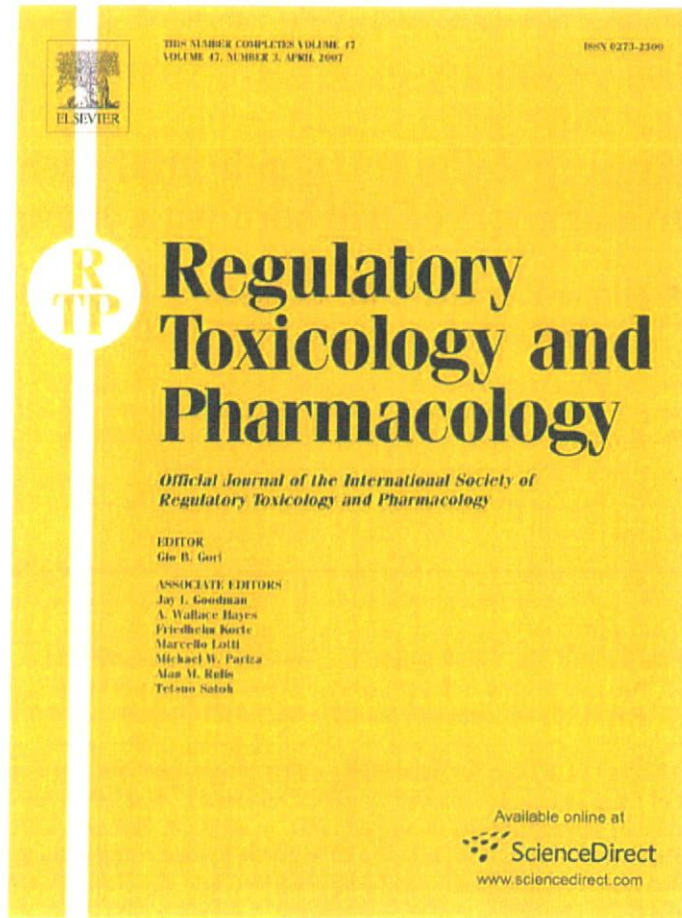

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Pediatric susceptibility to 18 industrial chemicals: A comparative analysis of newborn with young animals

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Abstract

We comprehensively re-analyzed the toxicity data for 18 industrial chemicals from repeated oral exposures in newborn and young rats, which were previously published. Two new toxicity endpoints specific to this comparative analysis were identified, the first, the presumed no observed adverse effect level (pNOAEL) was estimated based on results of both main and dose-finding studies, and the second, the presumed unequivocally toxic level (pUETL) was defined as a clear toxic dose giving similar severity in both newborn and young rats. Based on the analyses of both pNOAEL and pUETL ratios between the different ages, newborn rats demonstrated greater susceptibility (at most 8-fold) to nearly two thirds of these 18 chemicals (mostly phenolic substances), and less or nearly equal sensitivity to the other chemicals. Exceptionally one chemical only showed toxicity in newborn rats. In addition, Benchmark Dose Lower Bound (BMDL) estimates were calculated as an alternative endpoint. Most BMDLs were comparable to their corresponding pNOAELs and the overall correlation coefficient was 0.904. We discussed how our results can be incorporated into chemical risk assessment approaches to protect pediatric health from direct oral exposure to chemicals.

Keywords: Pediatric susceptibility; Industrial chemicals; Phenols; Newborn rats; Childhood exposure; Uncertainty factors; ADI; TDI; Benchmark dose

1. Introduction

Exposure of humans to environmental chemicals may occur via several routes such as the mouth, respiratory system, skin and eyes. As a result, regulatory/limit levels in food, water and air have been established to protect human health through risk assessment, which is usually based on toxicity data from animal studies (Hasegawa et al., 2004). However, the early postnatal period, especially the nursing phase, is not directly covered by current risk assessment approaches because of the inherent lack of toxicity information. Rather, two uncertainty factors are used to cover this data gap, one for human variability to toxic insult and the other for the lack of specific data to determine the critical effect (Dourson et al., 2002).

Repeated-dose oral rodent studies administer chemicals starting at approximately six weeks of age (OECD, 1995). In two-generation toxicity studies, chemicals are usually fed to rodents during the entire experimental period but newborn animals are only exposed to chemicals indirectly through maternal milk during nursing (up to 3 weeks old), or through small amounts of foods containing chemicals at about day 14 or older (OECD, 2001). Thus, there is generally no definitive toxicity information for chemical exposure in newborn animals.

Human infants may ingest not only baby foods and liquids but also household materials, fluids, and soil. They have unique physiological characteristics with regard to their organ/body balance, and the immature structure

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and functions of various organs may lead to elevated susceptibility or sensitivity (Scheuplein et al., 2002; Polin et al., 2004). Even though newborn exposure studies cannot be conducted for all chemicals due to ethical limitations, limited human and economic resources, or handling difficulties, such studies are valuable for the assessment of pediatric health risk given the appropriate comparative attention now being drawn to infant and child health world wide (Landrigan et al., 2004; IFCS, 2005).

Therefore, we have established an 18 day repeated-dose newborn rat toxicity study protocol, and conducted newborn studies for 18 industrial chemicals using this protocol, although the selected chemicals were mostly limited to phenolic compounds due to financial support. In addition, we have compared the newborn results with the results of a 28 day repeated-dose study (young study) and published all of the detailed analysis in peer-reviewed journals (Koizumi et al., 2001, 2002, 2003; Fukuda et al., 2004; Takahashi et al., 2004, 2006; Hasegawa et al., 2005; Hirata-Koizumi et al., 2005a,b).

In this article, we compare the results of these published studies by first describing our comparative study conditions common to all chemicals, then providing a summary of the final re-analyzed data, and finally discussing how our results can be incorporated into chemical risk assessment approaches to protect pediatric health from direct oral exposure to chemicals.

2. Experimental conditions of newborn and young studies for comparison

To appropriately elucidate differences in chemical sensitivity, studies in newborn and young rat were conducted under the same experimental conditions as closely as possible. For example,

(1) Sprague-Dawley SPF rats [Crj:CD(SD)IGS] purchased from Charles River Japan Inc. (Yokohama, Japan) were used for all studies;
(2) the same Lot Number for each chemical was used for both newborn and young studies;
(3) test solutions were prepared by the same methods with the same vehicles for both studies and administered by gastric intubation;
(4) test solutions were prepared at least once a week and kept cool and in the dark until dosing; stability was confirmed to be at least 7 days under these conditions; and
(5) all other reagents used in this study were specific purity grade;
(6) all animal treatments were conducted in 5 Japanese contract laboratories according to their Animal Care Guidelines and Japanese GLP Guidelines inspected by the Government.

The only differences in conditions were the administration period of 18 days for newborn and 28 days for young rats, and the recovery (maintenance) period as described in Fig. 1. Since rearing conditions for newborn rats change abruptly from nursing by foster mothers to individual self-feeding at postnatal Day 21 it was considered to be the best termination time point for the newborn dosing (a dosing period of 18 days) rather than adopting the same dosing period for the young studies (28 days).

2.1. Young studies

All schedules and examinations were performed in compliance with the Test Guideline "28 day repeated-dose toxicity study using mammals" of the Japanese Chemical Control Act (Official Name: Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances). This guideline is equivalent to OECD Test Guideline 407.

A dose-finding study was conducted according to the results of a single oral toxicity study. The study had a shorter dosing period (14 days) when compared to the main

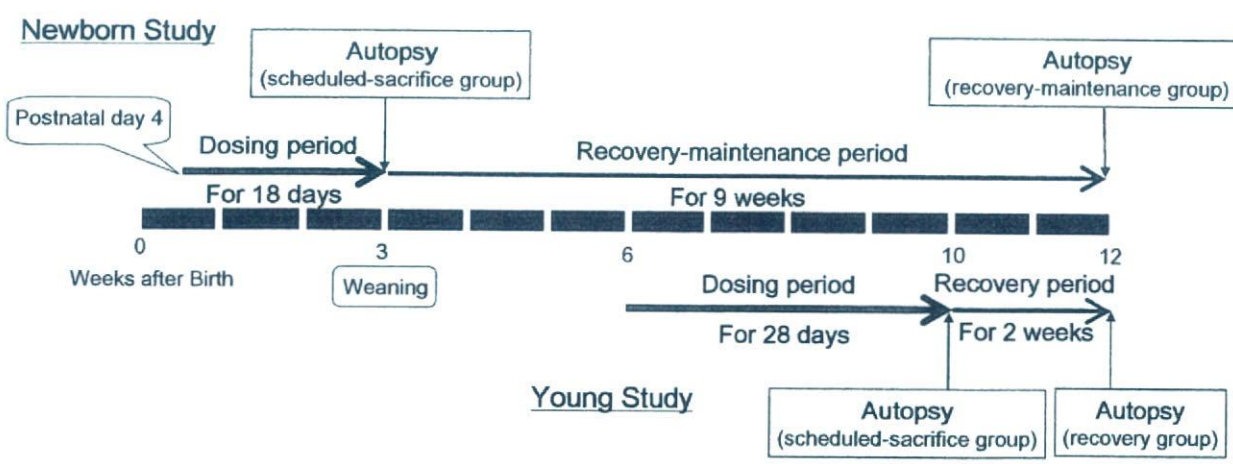

Fig. 1. Dynamic comparison of schedules for newborn and young studies.

study, and included most examination, but did not have examination of histopathology, urinalysis or recovery groups.

In the main study, at least 5 rats of both sexes were assigned to control, low, medium and high dose groups, and at least 5 rats of both sexes were assigned to control and high doses as recovery groups. Animals at 5–6 weeks of age were administered chemicals by gastric intubation daily for 28 days and sacrificed under ether anesthesia following the last treatment after overnight starvation (scheduled-sacrifice group). The recovery groups were maintained for 2 weeks without chemical treatment and sacrificed at 11 or 12 weeks of age. Observation of general behavior and estimation of body weight and food consumption were conducted during dosing and recovery periods. Macroscopic findings, blood chemistry (20 items), hematology (10 items), urinalysis (11 items), organ weights (15 organs) and histopathology (18 organs) were examined for the sacrificed animals.

2.2. Newborn studies

We established a newborn rat study protocol due to the lack of any standard test guideline for newborn animals. Fig. 1 shows the dosing and examination schedule for the newborn rat and young rat study. Pregnant rats (gestation day 14) were purchased and allowed to deliver spontaneously.

For a dose-finding study, all experimental conditions including the administration period were the same as the main study described below except that no examination of histopathology or urinalysis occurred, and no recovery-maintenance groups were maintained.

In the main study, dosing began on postnatal Day 4 with the administration of chemicals to 12 males and 12 female pups in each of 4 groups (control, low, medium and high doses). Each littermate consisted of 4 male and 4 female pups given different dose of chemical. Dosing to the pups continued up to weaning on postnatal Day 21 (18 days). On postnatal Day 22, half of the pups in each group were sacrificed under ether anesthesia (scheduled-sacrifice group), and remaining pups in all groups were maintained for 9 weeks without chemical treatment and subsequently sacrificed at 12 weeks of age (recovery-maintenance group). Observation of behavior and estimation of body weight and food consumption were conducted as with the young rat study protocol. The groups were examined for developmental parameters such as surface righting and visual placing reflexes for reflex ontogeny; fur appearance, incisor eruption and eye opening for external development during dosing period; and sexual development such as preputial separation, vaginal opening and estrous cycle during the recovery-maintenance period. The long recovery-maintenance period allowed for examination of sexual development after weaning and latent toxic effects in the early adulthood.

3. Unique approach to analysis of the susceptibility of newborn rats to chemicals

The no observed adverse effect level (NOAEL) is frequently used to determine safety or toxicity for environmental and industrial chemicals, with the NOAEL being the greatest dose at which no adverse effects are observed. However, the NOAEL is not always appropriate for an accurate comparison of toxicity levels between studies because the NOAEL is dependent on the dose setting. For example, in our early analysis of 2,4-dinitrophenol data, NOAELs for both newborn and young rat main studies were both 10 mg/kg/day because clinical signs of toxicity appeared at 20 mg/kg in newborn and 30 mg/kg in young rats. However, newborn rats seemed to be more sensitive to the chemical considering the intensity of lesions at higher doses. Further analysis of the data from the dose-finding young study showed no clinical toxicity signs at 20 mg/kg. Therefore, 20 mg/kg/day from the dose-finding young study was considered to be more appropriate as a NOAEL than the 10 mg/kg/day from the main young study. Including the dose-finding study in the determination of the NOAEL for a main study is not commonly done, thus, we decided to employ a new terminology in this document; the presumed NOAEL (pNOAEL) and defined it as the most likely no adverse effect dose for our specific purpose. The lack of information from dose-finding studies, such as histopathological examination in both newborn and young studies, and the shorter administration period in the young case was carefully considered in adopting the pNOAEL approach.

In addition, a Benchmark Dose (BMD) approach was applied to the same toxicity endpoint data that was used for the estimation of pNOAEL. Although clinical signs and histopathological changes are generally not appropriate for BMD analysis, since the frequency, duration and severity cannot usually be incorporated for the calculation, we attempted to employ incidences such as numbers of affected animals from both main and dose-finding studies where appropriate. Using the US EPA provided Benchmark Dose Software (Version 1.3.2), Benchmark Dose Lower Bound (BMDL) was estimated with 10% extra incidence at the 95% confidence level. In most cases, the incidence data were input to a Dichotomous model. For selection of the model, the lowest AIC (Akaike's Information Criterion) was used and the goodness-of-fit was confirmed visually with graphical displays.

At the first trial to evaluate the susceptibility of newborn rats to chemicals, we judged that the above endpoint comparison of pNOAEL/BMDL was not sufficient with respect of outcome reliability and the full toxicity data set should have been used. Alternatively, comparison of pNOAEL/BMDL might be sufficient for low dose responses but not with results at Lowest Observed Adverse Effect Level (LOAEL). In fact, it is reported that 17-day-old rats show higher susceptibility to chlorpyrifos at the maximum tolerated dose than adult rats (Moser and Padilla, 1998),

whereas no differential sensitivity was evident in NOAELs between the two groups (Pope and Liu, 1997)—a fact repeated with several chemicals in our series. Thus, we also considered the comparison of LOAELs among the newborn and young animal studies. Unfortunately, the traditional comparison of LOAELs has frequently suffered from a disparity in severities among studies, a situation that continues today with comparison of BMDLs.

This lead us again to employ a new terminology; the presumed unequivocally toxic level (pUETL) and defined it as the clear toxic dose giving similar severity for both newborn and young rats (at the same endpoints as far as possible). However, this was not simple to apply because the toxicity profile differed from chemical to chemical and also from newborn to young rats, the number per dose setting usually only being three in each group. Therefore, the most practical analytical strategy had to be a case by case approach. In most cases, the appropriate pUETL for either newborn or young rats was chosen first, thereafter the matching toxic dose or the range of doses was estimated giving similar severity for either group of rats, considering the whole data balance. Again, data from the dose-finding studies were also taken into account, especially considering the kinds of toxicity which appeared and the limits to be used. It should be noted that pUETL is not an absolute value, being different from pNOAEL/BMDL, but useful nevertheless to compare toxic responses between newborn and young rats at sufficient exposure.

This unique approach using two original definitions, with additional data from dose-finding studies concerning limitations, was fully supported by peer-reviewers of toxicology journals. On the other hand, the BMD approach for our whole data, including the dose-finding studies, was first conducted for this article.

4. Comparison of sensitivity of newborn and young rats to chemicals

4.1 Toxicity profiles

Critical toxicity data and the preliminary evaluation for 18 chemical studies have already been published in the literature. Table 1 shows a summary of the major findings for toxicities from the newborn and young studies. Fourteen chemicals commonly induced similar types of toxicities in both ages with the data considered in the pNOAEL or BMD approaches. With 3-ethylphenol and 1,1,2,2-tetrabromoethane, the toxicity profiles of both ages were not similar. In the case of 3-ethylphenol, the toxic similarity or difference between newborn and young rats cannot be predicted because of inadequate high dose setting in the newborn study. For 1,1,2,2-tetrabromoethane, hepatotoxicity in newborn rats can be speculated to appear at higher doses because a remarkable increase of relative liver weight was observed in the dose-finding study, although pathological examination was not conducted. In contrast, 2,4,6-trinitrophenol demonstrated a completely different profile of the major toxicities between the differently aged rats; also, tetrabromobisphenol A demonstrated unique toxicity in newborn rats.

4.1.1. Specific toxicity to reproductive organs in newborn rats

Although specific developmental parameters such as preputial separation and vaginal opening were carefully examined in newborn studies, no significant changes for any chemicals were observed.

In the case of *p*-(α,α-dimethylbenzyl) phenol, ovary weights were lowered at the end of the dosing as well as the recovery-maintenance periods and increased numbers

Table 1
Major types or symptoms of toxicities of 18 industrial chemicals in newborn and young studies

Chemical name	Newborn studies	Young studies	References
4-Nitrophenol	Convulsions	Hypoactivity, convulsions	Koizumi et al. (2001)
2,4-Dinitrophenol	Hypoactivity, convulsions	Hypoactivity, convulsions	Koizumi et al. (2001)
3-Aminophenol	Tremors, thyroid hypertrophy	Tremors, thyroid hypertrophy, anemia	Koizumi et al. (2002)
2-Chlorophenol	Tremors, renal toxicity	Tremors, hypoactivity	Hasegawa et al. (2005)
4-Chlorophenol	Tremors	Tremors, tachypnea	Hasegawa et al. (2005)
2-*tert*-Butylphenol	Hypoactivity, ataxia	Hypoactivity, ataxia	Hirata-Koizumi et al. (2005b)
2,4-Di-*tert*-butylphenol	Hepatic and renal toxicity	Hepatic and renal toxicity	Hirata-Koizumi et al. (2005b)
3-Methylphenol	Tremors, hyperactivity	Tremors	Koizumi et al. (2003)
3-Ethylphenol	Low BW	Ataxia, forestomach lesions	Takahashi et al. (2006)
4-Ethylphenol	Hypoactivity, delayed reflexes	Ataxia, forestomach lesions	Takahashi et al. (2006)
p-(α,α-Dimethylbenzyl) phenol	Renal toxicity, ovarian lesions	Renal toxicity, forestomach lesions	Hasegawa et al. (2005)
1,3,5-Trihydroxybenzene	Thyroid hypertrophy	Thyroid hypertrophy	Hasegawa et al. (2005)
2,4,6-Trinitrophenol	Low BW	Anemia, testicular atrophy	Takahashi et al. (2004)
(Hydroxyphenyl)methyl phenol	Low BW	Low BW, forestomach lesions	Hasegawa et al. (2005)
Trityl chloride	Low BW, hepatotoxicity	Low BW, hepatotoxicity	Hasegawa et al. (2005)
1,3-Dibromopropane	Low BW, hepatotoxicity	Low BW, hepatotoxicity, anemia	Hirata-Koizumi et al. (2005a)
1,1,2,2-Tetrabromoethane	Low BW	Hepatotoxicity	Hirata-Koizumi et al. (2005a)
Tetrabromobisphenol A	Renal toxicity	None	Fukuda et al. (2004)

BW: body weight.

of atretic follicles at the end of the dosing period. Most females continued to show various changes after the recovery-maintenance period, such as decreased numbers of corpa lutea in the ovaries, and hypertrophy of endometrial epithelium in the uteri. Therefore, further studies on this chemical should be conducted to elucidate the underlying mechanisms.

With (hydroxyphenyl)methyl phenol, some estrogenic effects were expected because it consists of bisphenol D, E and F isomers, and bisphenol F is reported to have estrogenic potential on the evidence of several *in vitro* and *in vivo* experiments (Hashimoto et al., 2001; Yamasaki et al., 2002; Stroheker et al., 2003). Some phenols such as nonylphenol, *p-tert*-octylphenol, bisphenol A and diethylstilbestrol have already been reported to induce morphological alteration of sex organs on early phase exposure after birth although the administration routes were either intraperitoneal or subcutaneous (Lee, 1998; Katsuda et al., 2000; Khan et al., 1998; Suzuki et al., 2002). The negative result in our study may be related to an insufficient component level of bisphenol F to induce such action.

4.1.2. Other specific toxicity in newborn rats

There was one exceptional case of toxicity limited to newborn rats. Tetrabromobisphenol A induced polycystic kidneys at 200 and 600 mg/kg in newborn rats but not in doses up to 1000 mg/kg in the main young study and 6000 mg/kg for 18 days exposure in an additional young study. Such specific renal toxicity in newborn rats has also been described for other chemicals such as chlorambucil (Kavlock et al., 1987), tetrachloro-1,4-dibenzodioxine (Couture-Haws et al., 1991) and difluoromethylornithine (Gray and Kavlock, 1991). Kidney nephrons of rats are formed in the period of the advanced stage of pregnancy until 2 weeks after birth (Chevalier, 1998), only 10% of nephrons are present at birth (Merlet-Benichou et al., 1994). It is possible that developing renal tubules in newborn rats may be sensitive to induction of hyperplasia of the tubular epithelium in response to cellular damage, leading to polycystic lesions. Although this toxicity is unusual—at least in newborn rats—it seems reasonable to consider similar unusual potential effects in newborn humans for some chemicals.

4.1.3. Specific toxicity in young rats

2,4,6-Trinitrophenol induced anemia and atrophy of seminiferous tubules of testes in young rats but only slight lowering of body weights in the main newborn study. Higher doses in the dose-finding newborn study induced severe suppression of body weight gain and death but not anemia or testicular toxicity. Sertoli cells in rats proliferate rapidly from day 19 of gestation to postnatal Day 15, then slow down and cease multiplying by approximately postnatal Day 20 (Orth, 1982, 1984; Toppari et al., 1996); 2,4,6-trinitrophenol seems unlikely to affect this stage rather affecting the maturation of spermatids. For anemia, the same pattern, of anemia only in young rats, was found for 3-aminophenol and 1,3-dibromopropane. Although methemoglobin levels were not determined in this study, it was reported that methemoglobin reductase levels in newborn rats are distinctly higher than in young animals (Gruener, 1976; Lo and Agar, 1986), which could be a reason for higher susceptibility in the latter. Another possible explanation is that major metabolites such as picramic acid may damage seminiferous tubules as well as induce hemolytic anemia but the metabolic rate may be very slow in newborn rats because of low P450 content (Rich and Boobis, 1997).

Hyperplasia of squamous cells in forestomach was observed for 3-ethylphenol, 4-ethylphenol, *p*-(α,α-dimethylbenzyl) phenol and (hydroxyphenyl)methyl phenol only at high toxic doses in young rats. Generally, phenols have similar toxicological effects due to their actions as extremely corrosive protoplasmic poisons (Manahan, 2003; Bloom and Brandt, 2001). The fact that the epithelium of the gastrointestinal tract of newborn rats may be more quickly renewed than that of young rats because of more active body metabolism in developing newborn rats, as well as a low capacity for gastric acid secretion, could explain any lower sensitivity in this regard.

4.2. Comparison of pNOAELs and pUETLs

pNOAELs for newborn and young rats with all chemicals were re-evaluated as shown in Table 2. Single pNOAELs for newborn and young rats were estimated for most chemicals on the basis of careful analyses of the results from the dose-finding and main studies. In two cases we judged that specification of a single value was not appropriate and therefore ranges were adopted. In case of 3-methylphenol for newborn rats, tremors only with contact stimuli were noted in three males on single days at the medium dose of 100 mg/kg in the main study. Thus the overt NOAEL became the low dose of 30 mg/kg, but the realistic NOAEL was considered to be slightly lower than 100 mg/kg, supported by overt NOAEL at 100 mg/kg in the dose-finding study. Therefore, the pNOAEL was established in the range of 60–80 mg/kg/day for more accurate comparison with data from the young study. The second case concerned the value for 2,4,6-trinitrophenol for newborn rats because they showed only a slight lowering of the body weight at 61.5 mg/kg and the low dose of 16.1 mg/kg was not considered appropriate as the pNOAEL; we adopted the range of 40–50 mg/kg/day instead. It should be noted that the pNOAEL of 1000 mg/kg/day of young rats for tetrabromobisphenol A is also not realistically appropriate because it was the highest limit dose indicated in the Test Guideline. As for estimation of pUETL, 8 values were given as ranges based on the definition of matching toxic dose ranges to induce clear toxicity at similar severity as described earlier. There were two chemicals without matches: 3-ethylphenol and tetrabromobisphenol A. For the former case, a dose in

Table 2
Summary of pNOAELs and pUETLs for 18 industrial chemicals in newborn and young rats

Chemical name	Newborn studies		Young studies		Young/Newborn	
	pNOAEL	pUETL	pNOAEL	pUETL	pNOAEL	pUETL
	(mg/kg/day)		(mg/kg/day)			
4-Nitrophenol	110	230	400	600–800	3.6	2.6–3.5
2,4-Dinitrophenol	10	30	20	80	2.0	2.7
3-Aminophenol	80	240	240	720	3.0	3.0
2-Chlorophenol	40	200–250	200	1000	5.0	4.0–5.0
4-Chlorophenol	100	300	100	500	1.0	1.7
2-*tert*-Butylphenol	20	100–150	100	500	5.0	3.3–5.0
2,4-Di-*tert*-butylphenol	5	100	20	500	4.0	5.0
3-Methylphenol	60–80	300	300	1000	4.0–5.0	3.3
3-Ethylphenol	100	—	300	—	3.0	—
4-Ethylphenol	30	200–250	100	1000	3.3	4.0–5.0
p-(α,α-Dimethylbenzyl) phenol	30	300	100	700–800	3.3	2.3-2.7
1,3,5-Trihydroxybenzene	100	500	300	1000	3.0	2.0
2,4,6-Trinitrophenol	40–50	65	20	100	0.4–0.5	1.5
(Hydroxyphenyl)methyl phenol	100	140–160	40	1000	0.4	6.3–7.1
Trityl chloride	60	400–500	12	300	0.2	0.6–0.8
1,3-Dibromopropane	50	150	10	250	0.2	1.7
1,1,2,2-Tetrabromoethane	50	200	6	300–400[a]	0.1	1.5–2.0
Tetrabromobisphenol A	40	—	1000[b]	—	25[b]	—

—: Appropriate values were not able to be given.
[a] These range values were estimated on the basis of all relevant toxicity data, including single dose toxicity data in young rats (the lowest mortality dose was 722 mg/kg for males and 852 for females) (MHLW, 2003).
[b] No accurate values for pNOAEL and pNOAEL ratio could be generated because 1000 mg/kg/day is the highest dose indicated in the Test Guideline.

newborn rats matching the toxic dose in young rats could not be predicted because the high dose in newborn rats did not induce any clear toxicity. The latter is that the high dose in young rats did not induce any toxicity.

The last column in Table 2 shows ratios for the young/newborn pNOAELs, and young/newborn pUETLs. Among the pNOAEL ratios for all 18 chemicals, newborn rats were less or nearly equal in sensitivity (less than 2-fold) to 6 chemicals (33%), clearly more sensitive (2–5-fold) to 11 chemicals (61%) and more than 25-fold for one exceptional case (6%). The mean ratio was 3.9 for all 18 chemicals or 2.5 for all but the exceptional case. Among the available pUETL ratios for 16 chemicals, 5 were less or nearly equal in newborn rats (less than 2-fold change) (31%) and 11 chemicals were clearly more toxic (2–8-fold) (69%). The mean ratio was 3.1 for the 16 chemicals.

Based on reliable calculated ratios for our two endpoints, approximately 94% of values (32 out of 34 ratios) demonstrated differences of 5-fold or less, one chemical had a 6–8-fold variation, and in the case of a 25-fold ratio of tetrabromobisphenol A, the nephrotoxicity in newborn rats is a specific toxicity rather than a higher susceptibility to the same toxic endpoint in young rats. These same ratios can be used to state that a higher susceptibility (more than 2-fold) in newborn rats was found for 62% of all tested chemicals in terms of pNOAELs and pUETLs, via oral repeated administration.

To appraise correlations between pUETL and pNOAEL ratios (young/newborn rats), available values were plotted on a logarithmic scale in a correlation diagram. As shown in Fig. 2, two separate groups became apparent, group 1 has the same or lower pNOAELs for newborn than young rats, and group 2 has higher pNOAELs for newborn than young rats. The mechanistic speculation for the differences is discussed next.

4.3. Speculation on differences in responses between low and high doses in newborn and young rats

Immature functions of organs (especially the liver and kidneys), in newborn rats may contribute to the difference of response. There were at least two types of dose response curve shifts between newborn and young rats, as illustrated in Fig. 3. The first was a parallel shift from right (young) to left (newborn) for 12 phenolic chemicals (group 1). The other 5 chemicals demonstrated a steeper shaped curve in newborn than young rats but young rats were clearly more sensitive around the pNOAEL doses (group 2).

Group 1 chemicals may primarily have direct actions on their target organs such as the central nervous system, kidneys or thyroid. They may be detoxified by the formation of conjugates, for example, glucuronidation of 4-nitrophenol (Robinson et al., 1951) and 3-methylphenol (Bray et al., 1950). UDP-glucuronyltransferase activity at birth in the rat liver is known to be comparable to that in adults but nearly 50% lower during nursing (Watkins and Klaassen, 1985; Rachmel and Hazelton, 1986). Therefore, a low capacity for glucuronidation may be one of the major causes of higher susceptibility of newborn rats to these phenols. This may also occur in human infants since immature hepatic glucuronidation and low activity of bilirubin glucuronidation at birth have been shown in human infants

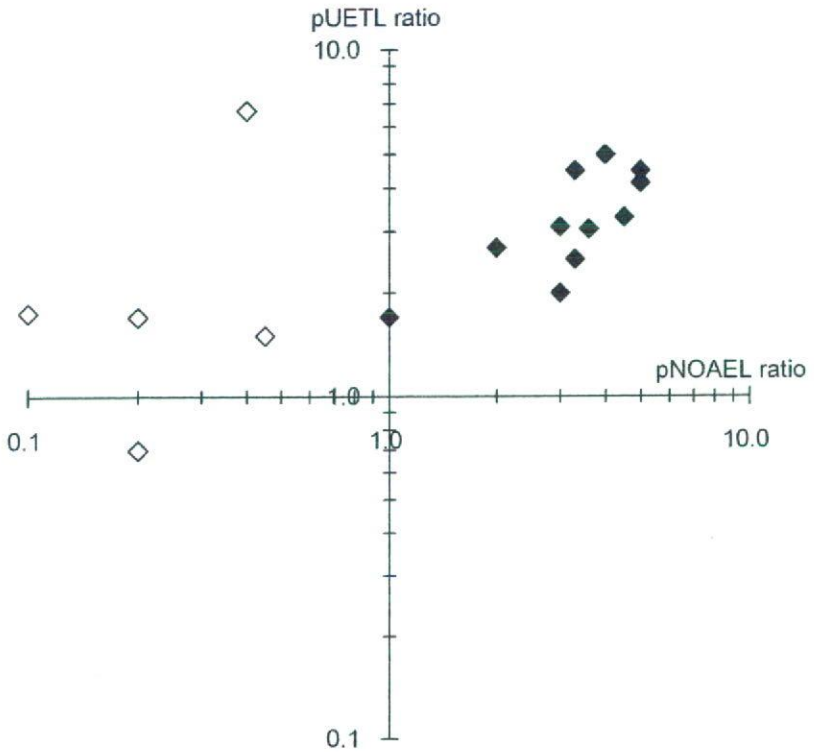

Fig. 2. Correlations of pUETL and pNOAEL ratios (young/newborn). Each point is plotted on a logarithmic scale from the ratios for young/newborn pUETLs and pNOAELs. Closed and open diamonds indicate group 1 and 2 chemicals, respectively.

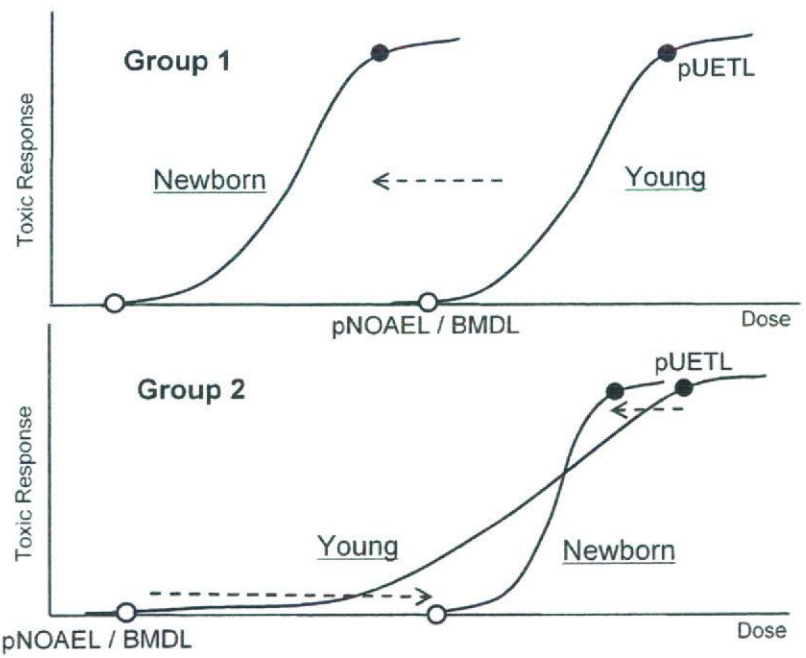

Fig. 3. Illustration of two patterns of shift of dose response curves from young to newborn rats.

(Gow et al., 2001; Kawade and Onishi, 1981). In addition, there is a possibility that high susceptibility may be due to a low capacity for hepatic cytochrome P450 (Rich and Boobis, 1997) and renal excretion (Horster, 1977), and increased permeability of the blood-brain barrier (Cremer et al., 1979).

Group 2 chemicals did not demonstrate as many effects at the low dose but nearly the same or higher

number of effects at the high dose in newborn compared to young rats. These chemicals may need metabolic activation to exert toxic effects. Newborn rats have been shown to have a low content of hepatic cytochrome P450 (Rich and Boobis, 1997) and a drop of glutathione-S-transferase activity in the early days after birth (Tee et al., 1992). Therefore, production of active metabolites may be significantly lower in newborn rats. In fact, it has been suggested that 1,1,2,2-tetrabromoethane requires an oxidative biotransformation to produce active intermediates (Kennedy et al., 1993) and 1,3-dibromopropane is conjugated with glutathione before or after oxidative biotransformation (James et al., 1981) as is common for dihaloalkanes or dihaloalkenes (Zoetemelk et al., 1986; Trevisan et al., 1989). However, pUETLs for newborn rats for 4–5 chemicals were in approximately the same ranges as in young rats. Although major reasons for variation in susceptibility are unclear, one possible explanation 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, which has a low activity of catalase and glutathione peroxidase during the nursing period (Yoshida et al., 1982).

4.4. Application trial of the BMD approach

We attempted to derive BMDLs as sensitive and appropriate endpoints in each study in addition to pNOAELs whenever possible. These calculated values are shown in Table 3. Most BMDLs seem to be relatively close to the corresponding pNOAELs but there are some cases in which BMDLs were lower than the probable values from toxicity profiles. One major reason may be the nature of the toxicity data used for the BMDL calculations. For example, no changes were observed with histopathological data in the young study for trityl chloride at 12 mg/kg, only slight changes in 3 of 6 animals at 60 mg/kg, and 4 mild and 2 moderate levels of change in 6 animals at 300 mg/kg. For the BMDL estimation from these data we input an incidence of 3 animals in 6 at 60 mg/kg and 6 animals in 6 animals at 300 mg/kg, even though the severities of these changes were different. So an actual dose response curve was obviously steeper than the input data curve, leading to a lower BMDL of 6.8 mg/kg/day, compared to the pNOAEL of 12 mg/kg/day. Nonetheless, Fig. 4 shows a good relationship between pNOAEL and BMDL since the correlation efficient was 0.904 (calculated without logarithmic conversion). The BMDL ratios in Table 3 are slightly lower than pNOAEL ratios in Table 2, with 9 chemicals (53%) demonstrating less or nearly equal sensitivity in newborn rats (less than 2-fold) and 8 chemicals (47%) demonstrating more sensitivity (2–8-fold) in newborn rats. However, a correlation diagram of the pUETL ratios versus the BMDL ratios also showed the same profile as Fig. 2 (not shown here). Therefore, the BMD approach can be considered very useful for the present purposes and somewhat easier than our pNOAEL estimation because extensive experience in toxicology is necessary for the latter estimations.

Table 3
Summary of BMDL values and ratios for 18 industrial chemicals in newborn and young rats

Chemical name	Newborn studies (mg/kg/day)	Young studies (mg/kg/day)	Young/newborn
4-Nitrophenol	141	392	2.8
2,4-Dinitrophenol	11	14	1.3
3-Aminophenol	54	254	4.7
2-Chlorophenol	31	126	4.1
4-Chlorophenol	79	63	0.8
2-*tert*-Butylphenol	43	130	3.0
2,4-Di-*tert*-butylphenol	7.5	48	5.1
3-Methylphenol	50	397	7.9
3-Ethylphenol	276	376	1.4
4-Ethylphenol	53	173	3.3
p-(α,α-Dimethylbenzyl) phenol	28	42	1.5
1,3,5-Trihydroxybenzene	63	206	3.3
2,4,6-Trinitrophenol	41	15	0.4
(Hydroxyphenyl)methyl phenol	108	42	0.4
Trityl chloride	34	6.8	0.2
1,3-Dibromopropane	32	6.1	0.2
1,1,2,2-Tetrabromoethane	82	3.1	0.04
Tetrabromobisphenol A	45	—	—

—, Appropriate values could not be generated because no toxicity was apparent in the young study.

5. Discussion of pediatric susceptibility

Major uncertainty exists in the derivation of human safety doses from animal experimental data. This uncertainty consists primarily of toxicokinetic and toxicodynamic differences between experimental animals and humans and among humans, and is addressed through the use of two factors, inter-species differences and human variability (intra-species differences). For either factor, a value of 10-fold has generally been applied for most assessments.

The aim of risk assessment is to derive the estimated no adverse toxic response level in sensitive humans. Thus, NOAELs or BMDLs are used as the starting point values, and not higher doses exhibiting toxicity, although descriptions of such toxicity provide critical information on risk assessment. Human variability implies appreciable differences of NOAELs or BMDLs between average populations and sensitive subpopulations as indicated by Dourson et al. (2002). Since the general human population or a more uniform experimental animal population is typically the focus group for toxicity evaluation, risk assessment needs to include sensitive subpopulations, such as infants, children, the elderly, and specific subgroups with minor diseases or relevant genetic polymorphisms. However, some hypersusceptible individuals might be excluded, for example, patients with severe hepatic or renal dysfunction should

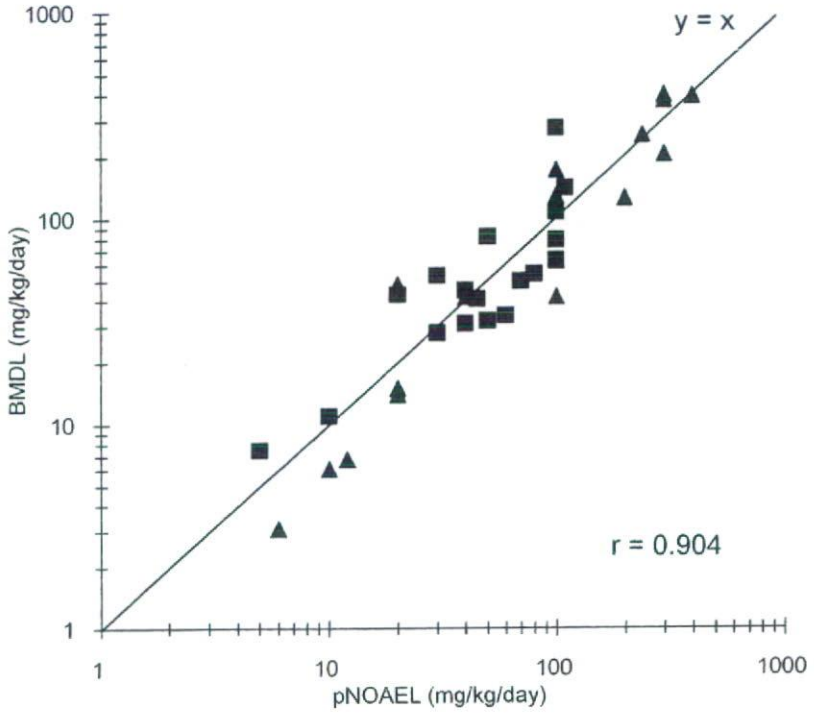

Fig. 4. Correlation between pNOAELs and BMDLs from both newborn (closed squares) and young (triangles) studies.

be excluded because they may be extremely sensitive to chemicals due to their impaired capacity for detoxification or excretion. Pregnant women and fetuses are also sensitive subpopulations, but the toxicity to these groups is routinely tested by reproductive and developmental toxicity studies.

Recently, Dourson et al. (2002) summarized considerations on adequacy of uncertainty factors of human variability for children. With human data, Glaubiger et al. (1981) reported that overall differences in sensitivity between children and adults are quite small on the basis of comparing maximum tolerated doses for 17 anticancer drugs. Using animal data analyses, Charnley and Putzrath (2001) demonstrated that younger animals appear to be less susceptible to 47% and more susceptible to 40% of the tested chemicals in carcinogenesis studies. Sheehan and Gaylor (1990) showed newborn mammals to be more sensitive than adults (86% within 10-fold) by comparing LD_{50} ratios for 238 chemicals. Calabrese (2001) also reported that younger animals have a greater sensitivity than older animals in 54% of cases (more than 10-fold in 14%) with LD_{50} ratio analyses for 313 chemicals. All these reports suggest the degree of variation in sensitivity of infants/younger animals as compared to adults for most chemicals may be within 10-fold, so that a 10-fold uncertainty factor may be sufficient to cover the variation (Dourson et al., 2002). However, only Sheehan and Gaylor (1990) targeted newborn rather than young animals and the report was a meeting abstract.

Concerning the methodology for risk assessment with repeated exposure, NOAELs or BMDLs from repeated-dose toxicity studies are starting values to derive risk values such as acceptable daily intake (ADI) or tolerable daily intake (TDI). These studies might be as short as 28 days or as long as 2 years, but invariably dosing generally starts around 6 weeks of age for rodents. These animals are referred to as "young" in this article rather than adult because their growth is still vigorous. Therefore, toxicity responses of young animals, equivalent to late childhood in humans, may already be covered by the general repeated-dose toxicity studies (see also Table 2 of Dourson et al., 2002 which summarized work by Scheuplein et al., 2002, on this point).

However, only limited data exist for animals from birth to 5 or 6 weeks of age. During these initial few weeks after birth, susceptibility to toxic insult might be expected to be greater than at later periods because organ growth rates are higher. Moreover, metabolism and elimination pathways are not yet mature (see for example the discussion of kinetic comparisons of newborn, infants and children as compared with adults by Rane, 1992 and Renwick, 1998 in Dourson et al., 2002). Although for some chemicals this lack of maturation in metabolism and elimination might serve to protect the newborn, it is clearly very important to clarify newborn sensitivity versus young animal sensitivity. Thus we have designed our newborn rat study protocol (18 day newborn study) to follow the conditions of the 28 day repeated-dose toxicity study (onset of administration at 5–6 weeks old) as closely as possible using 18, mostly phenolic, compounds. In addition to the unique design of the 18 day newborn study, new clarifying terminology has been developed, pNOAELs and pUETLs, in order to more appropriately determine ratios between newborn and young studies.

Our analyses of 18 such pNOAEL ratios or 17 BMDL ratios revealed less or nearly equal sensitivity in newborn animals (less than 2-fold) in 33–53%, clearly greater sensitivity (2–8-fold) in 47–61% and one exceptional case of more than 25-fold sensitivity in the newborn. In the case of 16 pUETL ratios, 31% of chemicals showed less or nearly equal toxicity in newborns (less than 2-fold) and 69% more toxicity (2–8-fold) in newborns. This distribution and the extent of newborn susceptibility in toxicity are in line with the conclusions of several investigators summarized previously by Dourson et al. (2002), but evidence presented here is more direct because of careful design of the comparative studies and comprehensive toxicological analyses and judgments. In addition, two kinetic analyses showing newborns to be more sensitive than adults, with a 3.5 arithmetic average difference in elimination half life (Rane, 1992) or a 4-fold longer average half life (Ginsberg et al., 2002), support relatively similar degrees of average susceptibility as we have found.

Collectively, all of this work suggests that studying the early life stage sensitivity to toxic insult is important. When such studies determine the critical effect, then ADIs or TDIs should be based on their findings. When such studies do not determine the critical effect, then the ADI or TDI is appropriately based on a critical effect found in a different study and the newborn is protected. It is when such studies have not been conducted that uncertainty factors must be invoked to protect the newborn, and other potential sensitive subpopulations, and several investigators have looked at the adequacy of such factors (e.g., Burin and Saunders, 1999; Dourson et al., 2002). Based on our results and those of other investigators, we suggest that an uncertainty factor of 10-fold for human variability and an uncertainty factor of between 3- and 10-fold for database completeness can be considered appropriate for risk assessment unless knowledge of particular toxicity in newborn or infants is present, or if not present is discountable due to other credible information on the chemical.

In conclusion, newborn rats are clearly more susceptible than young animals (at most 8-fold) to two thirds of the present series of 18 chemicals, mostly phenolic substances, and less or nearly equal sensitive to the others for oral repeated exposure. However, it should be noted that there was one exceptional case in which the toxicity appeared only in newborn rats. These repeated oral exposure newborn studies are unique for this limited group of chemicals, and perhaps for other chemicals as well.

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