

Blood and organ distribution of mono (2-ethylhexyl) phthalate (MEHP) in rats exposed to di (2-ethylhexyl) phthalate (DEHP) via diet

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Abstract

Di(2-ethylhexyl) phthalate (DEHP) undergoes hydrolysis in the gastrointestinal tract when orally administered to rats, and is mainly distributed in the body as mono(2-ethylhexyl) phthalate (MEHP). MEHP is thought to be involved in testicular and hepatotoxicity. Therefore, clarifying the distribution of MEHP in blood and organs after oral administration of DEHP is important.

In this study, male Sprague-Dawley rats were fed 1% and 2% DEHP diets for two weeks and then examined the distribution of MEHP and DEHP concentrations in blood and organs. The following results were obtained.

- MEHP concentrations in the 1% group were plasma: 16.7-40.8 µg/ml, testis: 0.9-3.2 µg/g, liver: 21.5-43.6 µg/g, kidney: 6.8-9.6 µg/g and MEHP concentrations in the 2 %group were plasma: 58.5-147.4 µg/ml, testis: 10.5-30.0 µg/g, liver: 44.2-97.1 µg/g, kidney: 18.6-56.7 µg/g.
- Mean MEHP concentrations in blood and organs were dose-dependent, but with considerable inter-individual variability.
- MEHP concentrations by organ were highest in liver, followed by kidney, and lowest in testis.
- DEHP was detected in plasma, testis, liver, and kidney in both 1% and 2% groups in the range of 1-20 µg/g (µg/ml).
- MEHP concentrations in testis, liver, and kidney were all strongly correlated with plasma MEHP concentrations.
- A strong negative correlation was observed between MEHP concentration in testis and testicular weight, and between plasma MEHP concentrations and testicular weight. This suggests a close relationship between MEHP and testicular toxicity.

Keywords: Di (2-ethylhexyl) Phthalate (DEHP); Mono (2-ethylhexyl) Phthalate (MEHP); Testicular atrophy

1. Introduction

The plasticizer di (2-ethylhexyl) phthalate (DEHP) is known to cause testicular atrophy and liver hypertrophy in animal studies [1-6]. In oral administration experiments to rats, DEHP is hydrolyzed in the gastrointestinal tract [7] and distributed in the body mainly as mono (2-ethylhexyl) phthalate (MEHP) [8]. It has been pointed out that MEHP is directly involved in toxicity in the testis and liver [5, 6, 11-15]. Therefore, it is important to clarify the distribution of MEHP in blood and organs when DEHP is orally administered.

The authors investigated MEHP concentrations in blood and organs of rats fed DEHP containing diet for 2 weeks.

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2. Material and methods

2.1. Chemicals and Animal Diet

DEHP was purchased from Wako pure chemical industries Ltd. (Osaka, Japan). The chemical purities of DEHP and were found to be >97%. CE-2 diets (Clea, Tokyo, Japan) containing DEHP by 1 or 2 w/w% were prepared by Oriental Yeast Company (Chiba, Japan). MEHP was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All other chemicals were the highest grade from commercial sources.

2.2. Animals and Ethics

Male Sprague-Dawley rats aged three-week-old purchased from Charles River (Kanagawa, Japan) were housed at the Laboratory Animal Center of Kagawa University. They were acclimated at 22–24 °C and 50–60% relative humidity with a 12-h light/dark cycle.

2.3. Experimental Design

Five-week-old rats weighing 166 ± 4 g were divided into control and treatment groups consisting of 6 animals. The treatment group consumed 1% (w / w) DEHP diet or 2% (w / w) DEHP diet for two weeks. At the end of the experiment, rats were sacrificed by ether anesthesia. The testes, liver and kidneys were removed and weighed. Cardiac blood samples were collected in heparinized tubes and plasma was separated from whole blood by centrifugation at 1500 g. Plasma and organs were frozen at -40 °C until MEHP measurement.

2.4. Plasma and organ MEHP Measurement

The concentrations of MEHP in plasma and testis were measured by high performance liquid chromatograph (HPLC).

Analytical procedure and equipment are as follows, 200 μ l of 1M NaOH was mixed with 50 μ l of plasma, 850 μ l of acetonitrile was added, and the mixture was vigorously mixed, followed by ultrasonication for 10 minutes to extract DEHP and MEHP. Thereafter, 10 μ l of phosphoric acid was added for neutralization, and the supernatant after centrifugation was subjected to HPLC analysis. For the organs, 300-500 mg of the homogenate was added with 4 times the amount of 1M NaOH and mixed with a homogenizer.

The HPLC analysis conditions were as follows.

Column: TSKgel ODS (4.6mm ID x 150mm, TOSOH)

Mobile phase: Step gradient method; 1% phosphoric acid/acetonitrile 40/60 (13 min) - 1% phosphoric acid/acetonitrile 15/85 (30 min)

Flow rate: 0.8 ml/min, detection wavelength: UV 230 nm

2.5. Statistics

Results were expressed as means \pm standard deviations (SD). Statistical analyses were performed using one-way ANOVA procedure followed by Tukey HSD tests to detect difference between groups. Differences were regarded as significant at $P < 0.05$.

3. Results

3.1. HPLC analysis

DEHP and MEHP were added to rat plasma at 100 μ g/ml and to organs at 100 μ g/g, respectively, and the recovery rates were estimated to be 90-100%. The detection limit for MEHP was 0.1 μ g/g (or 0.1 μ g/ml). Chromatograms of HPLC analysis are shown in Figure 1. No interfering peaks were detected at the elution positions of MEHP and DEHP in the HPLC analysis of blood and organs.

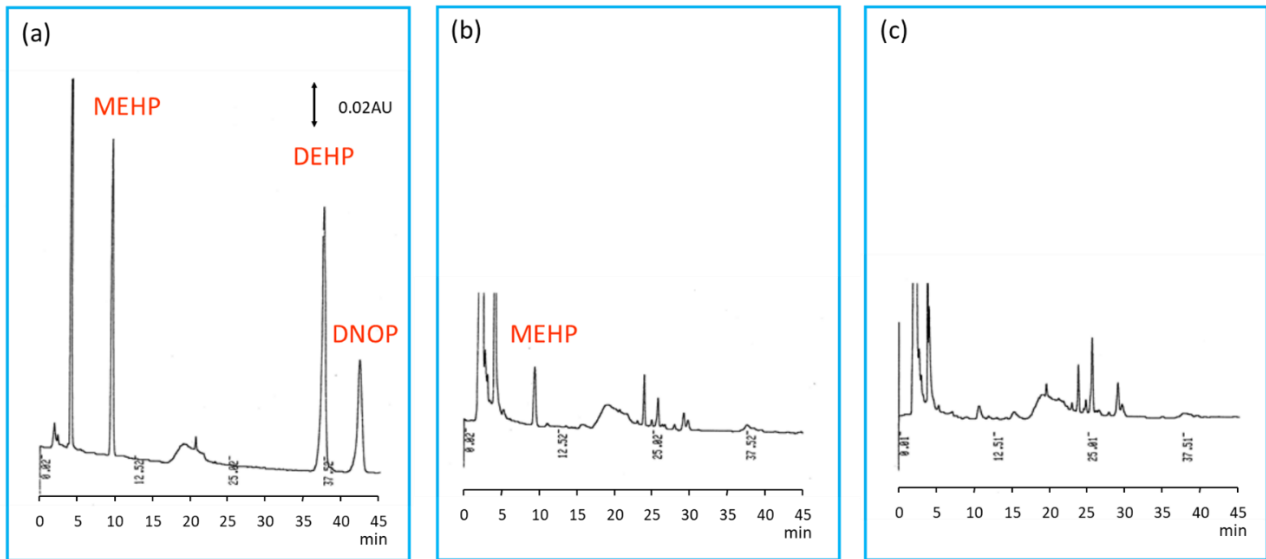


Figure 1 Chromatograms of HPLC. (a); Standard of phthalates, (b); Rat testis exposed to DEHP, (c); Control rat testis

3.2. Animal experiment

The DEHP dose estimated from food intake and average body weight during the dosing period was 0.9 g/kg/day in the 1% group and 1.6 g/kg/day in the 2% group.

Table 1 shows the body weight and organ weight of each group after the DEHP administration experiment. In the 1% group, significant liver hypertrophy was observed, but there was no difference in testis weight and body weight compared to the control. On the other hand, the 2% group showed marked reduction in testis weight, body weight suppression, and liver hypertrophy.

Tables 2 and 3 show the concentrations of MEHP and DEHP in the plasma, testis, liver and kidney of the 1% and 2% groups. The MEHP concentration in the 1% group was plasma: 16.7-40.8 µg/ml, testis: 0.9-3.2 µg/g, liver: 21.5-43.6 µg/g, kidney: 6.8-9.6 µg/g, and plasma in the 2% group. : 58.5-147.4µg/ml, testis: 10.5-30.0µg/g, liver: 44.2-97.1µg/g, kidney: 18.6-56.7µg/g. The average MEHP concentration in the 2% group was higher than that in the 1% group, and by organ, the liver was the highest, followed by the kidney, and the testis was the lowest. On the other hand, DEHP was detected in the range of 1-20 µg/g (µg/ml) in plasma, testis, liver and kidney in both the 1% and 2% groups, but most of them were 2 µg/g (µg/ml) or less, and the kidney showed a slightly higher value than the others.

Figure 2 shows the relationship between the MEHP plasma concentration and each organ concentration. Testis, liver, and kidney MEHP concentrations were all strongly correlated with the plasma MEHP concentration. In addition, the relationship between the MEHP concentration in the testis and the testicular weight, or the plasma MEHP concentration and the testicular weight, both showed a strong negative correlation (Figure 3).

Table 1 Body and organ weights of male rats (mean ± SD, n=6). The rats were fed 1% or 2% DEHP-containing diet for two weeks. *, p<0.05, **, p<0.01, ***, p<0.001, Significant differences as compared to control group

Group	Body weight (g)		Testes (g)			Liver (g)		Kidneys (g)				
	mean	SD	mean	SD		mean	SD	mean	SD			
Control	287.1	11.6	2.70	0.14		15.76	1.44	2.60	0.17			
1%DEHP diet	280.3	19.2	2.52	0.17		21.93	2.78	***	2.52	0.24		
2%DEHP diet	243.4	11.6	***	1.18	0.36	***	20.60	2.15	***	2.27	0.21	*

Table 2 MEHP concentrations in plasma and organs of the rats fed 1 or 2% DEHP-containing diet for two weeks

Group		Plasma		Testis		Liver		Kidney	
		MEHP (µg/ml)		MEHP (µg/g)		MEHP (µg/g)		MEHP (µg/g)	
1%DEHP diet	mean±SD	24.1	8.7	2.1	1.0	33.5	7.3	8.5	1.1
	min - max	16.7	40.8	0.9	3.2	21.5	43.6	6.8	9.6
2%DEHP diet	mean±SD	88.2	31.9	18.3	7.6	65.5	19.3	33.6	12.9
	min - max	58.5	147.4	10.4	30.0	44.2	97.1	18.6	56.7

Table 3 DEHP concentrations in plasma and organs of the rats fed 1 or 2% DEHP-containing diet for two weeks

Group		Plasma		Testis		Liver		Kidney	
		DEHP (µg/ml)		DEHP (µg/g)		DEHP (µg/g)		DEHP (µg/g)	
1%DEHP diet	mean±SD	6.0	2.7	1.7	1.1	2.3	0.6	10.3	5.6
	min - max	1.5	8.0	0.2	3.0	1.7	3.1	2.7	17.8
2%DEHP diet	mean±SD	0.9	1.1	3.0	1.7	4.5	2.8	11.6	4.8
	min - max	0.2	2.4	1.3	5.1	2.1	9.2	3.8	18.0

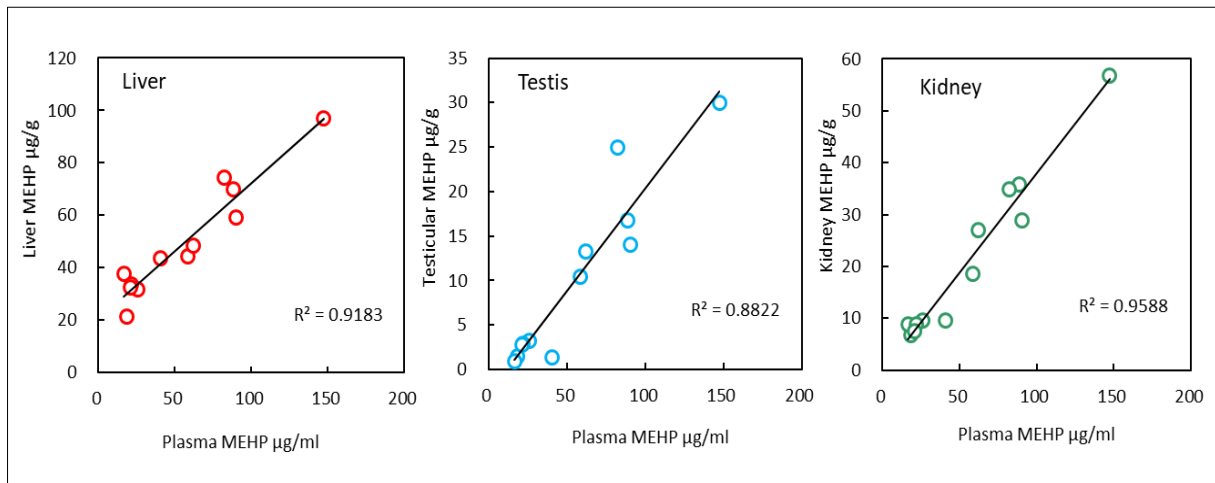


Figure 2 Relationship between plasma MEHP concentration and organ concentration

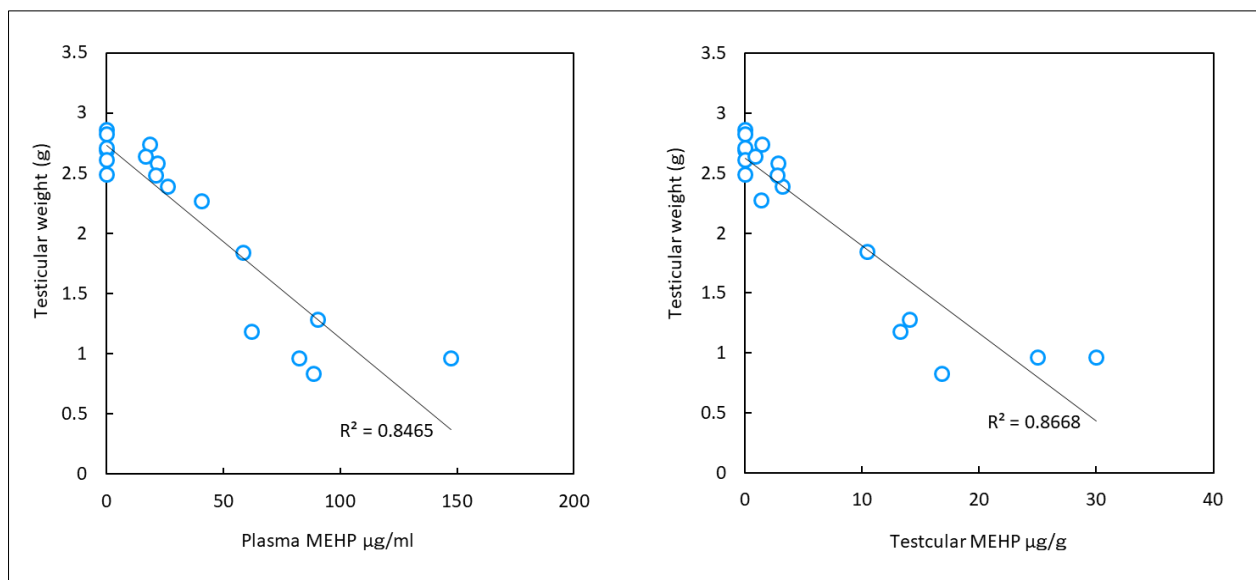


Figure 3 Relationship between plasma or testicular MEHP concentration and testicular weight

4. Discussion

Most of DEHP orally administered to rats is hydrolyzed by lipase in the gastrointestinal tract, and the produced monoester MEHP is rapidly absorbed into the body and distributed to organs [7, 8]. MEHP is mainly involved in toxicity such as testicular atrophy and liver hypertrophy [1-6]. MEHP is known as a peroxisome proliferator that induces rodent hepatocarcinogenesis [16-18]. MEHP has also been reported to be a reproductive toxicant that disrupts Sertoli cell function and causes germ cell apoptosis. Therefore, concentrations of MEHP in blood and organs is important in DEHP toxicity.

In this study, MEHP and DEHP concentrations in the blood and organs of male Sprague-Dawley rats were measured after 2 weeks of treatment with 1% and 2% DEHP diets. Mean MEHP concentrations in blood and organs corresponded to DEHP dose, but with considerable inter-individual variability. When looking at the distribution concentration in each organ, the highest was in the liver, followed by the kidney, and the lowest in the testis. Previous studies [8, 9] have shown that orally administered DEHP and its metabolites are distributed in large amounts in the liver and kidneys, with little distribution in the testis and brain, which is consistent with the present results. From these results, it was found that considerable concentration differences occur in the distribution of MEHP both by individual and by organ. On the other hand, DEHP was detected in the range of 1-20 µg/g (µg/ml) in plasma, testis, liver and kidney in both the 1% and 2% groups, but most of them were 2 µg/g (µg/ml) or less, and there was no particular relationship between the average DEHP concentrations in blood and organs and the dose of DEHP. Sjoberg et al. [3] detected 48-152 µg/ml of MEHP in the plasma of 25-, 40-, and 60-day-old rats orally administered 1.0 g/kg of DEHP per day for 14 days, but DEHP was not detected. In this experiment, 1% and 2% DEHP were administered for 2 weeks. /day. Our results are close to those of Sjoberg et al. [3], probably because the doses were almost the same. Either way. This experiment reconfirmed that orally administered DEHP is mainly distributed in the blood and organs as a monoester MEHP.

Next, it was clarified that MEHP concentration in testis, liver, and kidney showed a strong linear correlation with plasma MEHP concentration. From this, it seemed possible to estimate the amount of MEHP distribution in each organ by measuring the plasma MEHP concentration.

Furthermore, testicular toxicity of DEHP is thought to be related to its metabolite, MEHP [9]. A strong negative correlation was observed for both. These results suggest that the degree of testicular toxicity induced by oral administration of DEHP is dependent on the MEHP concentration in the plasma or the MEHP concentration in the target organ, the testis. After reaching the testis, MEHP damages the Sertoli cells in the seminiferous tubules by disrupting the vimentin filaments [12, 13], and as a result, the mechanism of germ cell apoptosis is induced [14, 15], which is thought to lead to testicular atrophy. Therefore, it is inferred that the relationship observed in this study reflects the dose-effect relationship between MEHP and germ cell apoptosis. Based on these findings, it seemed possible to some extent to evaluate testicular toxicity by measuring plasma MEHP concentrations.

5. Conclusion

Diets containing 1% and 2% DEHP were administered to male SD rats for 2 weeks, and the concentration distribution of MEHP and DEHP in blood and organs was investigated. The following results were obtained.

- MEHP concentrations in the 1% group were plasma: 16.7-40.8 µg/ml, testis: 0.9-3.2 µg/g, liver: 21.5-43.6 µg/g, kidney: 6.8-9.6 µg/g and MEHP concentrations in the 2 %group were plasma: 58.5-147.4 µg/ml, testis: 10.5-30.0 µg/g, liver: 44.2-97.1 µg/g, kidney: 18.6-56.7 µg/g.
- Mean MEHP concentrations in blood and organs were dose-dependent, but with considerable inter-individual variability.
- MEHP concentrations by organ were highest in liver, followed by kidney, and lowest in testis.
- DEHP was detected in plasma, testis, liver, and kidney in both 1% and 2% groups in the range of 1-20 µg/g (µg/ml).
- MEHP concentrations in testis, liver, and kidney were all strongly correlated with plasma MEHP concentrations.
- A strong negative correlation was observed between MEHP concentrations in testis and testicular weight, and between plasma MEHP concentrations and testicular weight. This suggests a close relationship between MEHP and testicular toxicity.

Compliance with ethical standards

Acknowledgments

This study was conducted at Kagawa University. The author appreciates the help of colleagues in the laboratory.

Disclosure of conflict of interest

There is no conflict of interest in this work.

Statement of ethical approval

The experiment protocols had the approval by the Kagawa University Animal Committee.

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