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Single and Two-Week Repeated Oral Dose Toxicity Study of DHP2, a Hydrophobic Drug Delivery Vehicle in Mice

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ABSTRACT. The present study was conducted to investigate the single and 2-week repeated dose toxicity of DHP2, a hydrophobic drug delivery vehicle, in ICR mice. The test article was administered orally to mice at the dose levels of 2.5, 12.5 and 37.5 g/kg for single dose toxicity study and at the dose levels of 0, 2.5, 5, and 10 g/kg for repeated dose toxicity study. In both studies, there were no treatment-related effects on mortality, clinical signs, food and water consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, necropsy findings and organ weights of all animals treated DHP2. Based on these results, it was concluded that the 2-week repeated oral dose of DHP2 may have no toxic effect in mice at a dose level of 10 g/kg. In the condition of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 10 g/kg/day for both sexes.

Keywords: DHP2, Single and 2-week repeated dose toxicity study, No-observed-adverse-effect level (NOAEL), Mice.

INTRODUCTION

In the present study we report the results of the single and 2-week repeated oral dose toxicity study in ICR mice performed as a part of the preclinical safety evaluation program for DHP2. DHP2 was developed as a peroral delivery system for hydrophobic drugs, such as paclitaxel and cyclosporin. DHP2 is composed of oils that could be dispersed in the intestine by peristalsis. Unlike many emulsion-type oral delivery systems, this formulation forms well-dispersed emulsion in aqueous environment. In water, DHP2 can be dispersed in water and forms suspension of oil droplets of a few micrometers in diameter. The solubilization process and the uptake mechanism are entirely different from those of conventional SEDDS (self emulsifying drug delivery system) or SMEDDS (self microemulsifying drug delivery system) formulations. The main difference comes from the mucoadhesiveness of DHP2 (Lee *et al.*, 2002). Since the dispersed particles of DHP2 are very mucoadhesive, they adhere and coat efficiently the mucosal epithelium where the drug can be taken up. DHP2 has been used successfully as a vehicle for peroral administration of paclitaxel (Lee *et al.*, 2002). Even though the components of DHP2 are known to be biocompatible, it is our aim to evaluate the toxicity of the formulation after oral administration in this paper. This study was conducted according to the test guidelines from the KFDA and OECD guidelines for the testing of chemicals under modern Good Laboratory Practice Regulation.

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List of abbreviations: AAALAC International, Association for Assessment and Accreditation of Laboratory Animal Care International; NOAEL, No-observed-adverse-effect level; NRC, National Research Council

MATERIALS AND METHODS

Preparation of DHP2

Distilled monoglyceride was purchased from Danisco Ingredients (Brabrand, Denmark). Triglyceride and tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO). DHP2 was prepared by mixing molten monoglyceride, triglyceride and tween 80 at the volume

ratio of 14 : 7 : 4 at 50°C. In order to mix the components completely, DHP2 was sonicated for 1 min in a bath-type sonicator at 45 ± 5°C.

Animal Husbandry and Maintenance

Thirty-six ICR mice of each sex were obtained from a specific pathogen free colony at Orient Inc. (Seoul, Korea) at 4 weeks of age and used after one week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23 ± 3°C and a relative humidity of 50 ± 10% with artificial lighting from 08 : 00 to 20 : 00 and with 13~18 air changes per hour. Only healthy animals were assigned to the study. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (PMI Nutritional International, 505 North 4th Street Richmond, IN, USA) *ad libitum*. This experiment was conducted in facilities approved by AAALAC International, and animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

Experimental Groups and Test Article Treatment

For single dose toxicity study, DHP2 (no vehicles or excipients were added, specific gravity 0.98 g/ml) was administered orally by gavage to mice at dose levels of 2.5, 12.5 and 37.5 g/kg based on the preliminary study (data not shown). The deaths or toxicological findings were not observed in the 37.5 g/kg group in the preliminary study, therefore we did not experiment on the vehicle control group for the single dose toxicity study. The oral administration was selected in the present study, because the oral route is the clinical route for human. Each group consisted of 3 mice of each sex.

For 2-week repeated dose toxicity study, DHP2 (no vehicles or excipients were added) was administered once a day by gavage to mice for 2 weeks at dose levels of 0, 2.5, 5 and 10 g/kg. In the single dose toxicity study, treatment-related toxicological effect was not observed. Therefore a dose of 10 g/kg, estimated maximum dosage, was selected for the highest dose in this study. Doses of 5 and 2.5 g/kg were selected as middle and low doses, respectively, using a common ratio of 2. Each group consisted of 5 mice of each sex. The vehicle control mice received distilled water.

Experimentals

Single dose toxicity study.

(1) Clinical observation and mortality: Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality.

(2) Body weights: Body weight of each mouse was measured shortly before administration and on days 1, 3 and 7 after administration.

(3) Gross findings: On day 7 after the treatment, all surviving animals were euthanized by carbon dioxide overdose and the necropsy was performed with special attention to all vital organs and tissues.

2-Week repeated oral dose toxicity study.

(1) Clinical observation and mortality: Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality. Detailed clinical observations were recorded and printed by Path/Tox System (ver 4.2.2, Xybion Medical Systems Corporation, USA), respectively.

(2) Body weights: Body weight of each mouse was measured at the initiation of treatment, once a week thereafter, and on the day of scheduled autopsy.

(3) Food consumption: Food consumption were measured per cage at the start of treatment and at weekly intervals thereafter. The measured amounts of food were supplied to each cage and their remnants were measured on the next day to calculate the difference which was regarded as the daily food consumption (g/mouse/day).

(4) Ophthalmoscopy: External eye examination of all males and females per group was conducted shortly before the initiation of treatment and the scheduled termination, respectively. The ocular fundus of all males and females per group was examined shortly before the scheduled termination using a indirect binocular ophthalmoscope (IO-H, Neitz Instruments Co., Japan). Conjunctiva, sclera, cornea, lens and iris of each eye were also examined.

(5) Urinalysis: During the last week of treatment, urinalysis of all animals was conducted with fresh urine for the items of specific gravity, pH, protein, glucose, ketone body, occult blood, bilirubin, urobilinogen, and nitrite by using a CliniTek-100 urine chemistry analyzer (Ames Division, Miles Laboratory, USA).

(6) Hematology: Blood samples were drawn from the posterior vena cava by using a syringe with a 24 gauge needle under ether anesthesia. The animals had been fasted overnight prior to necropsy and blood collecting. The blood samples were collected into CBC bottles containing EDTA-2K (Green Cross Medical Industry, Korea). Red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and white blood cell count (WBC) were measured by a hematological autoanalyzer (ADVIA120, Bayer, USA).

(7) Serum biochemistry: To get the sera for blood biochemistry, blood samples on a separation tube were centrifuged at 3,000 rpm for 10 minutes on the day of

necropsy. The sera were stored in the -80°C freezer before they were analyzed. Serum biochemistry parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, albumin/globulin ratio (A/G ratio), total protein (TP), albumin, creatine phosphokinase (CPK), triglyceride, phospholipid and total bilirubin were evaluated by an autoanalyzer (Shimadzu CL-7200, Shimadazu Co., Japan).

(8) Gross findings: At scheduled termination, all animals were anesthetized by ether inhalation, blood samples taken and then terminated by exsanguinating the abdominal aorta. Complete gross postmortem examinations were performed on all terminated animals.

(9) Organ weights: The absolute organ weights were measured and their relative organ weights (organ-to-body weight ratios) were calculated from the termainal body weight for the following organs of all survivors when they were sacrificed: brain, hypophysis (pituitary gland), liver, spleen, heart, prostates, lung, thymus, kidneys, testes, adrenal glands, epdidymides, ovaries, and uterus.

Statistical Analysis

Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Bartlet Test. If the Bartlet Test indicated no significant deviations from variance homogeneity, the ANOVA multiple comparison test (Dunett Test) was conducted to determine which pairs of group comparison were significantly different. In case that significant deviations from variance homogeneity were observed, a non-parametric comparison test (Kruskal-Walliss(H) Test) was conducted. When a significant difference is observed in the Kruskal-Walliss(H) Test, the Dunn's Rank Test was conducted to determine the specific pairs of group comparison, which are significantly different. For frequency type of data the Chi-square Test was conducted. If a significant difference was found in the Chi-Square Test, the Fisher's Exact Probability Test was conducted to determine the pairs of group comparison, which are significantly different. The level of significance was taken as $P<0.05$ or 0.01. Statistical analyses were performed by comparing the different dose groups with the vehicle control group using Path/Tox System (ver 4.2.2, Xybion Medical Systems Corporation, USA) and Statistical Analysis Systems (SAS/STAT Version 8.1, Cary, NC, USA).

RESULTS

Single Dose Toxicity Study

Mortality and LD_{50} values: The treatment-related death was not observed at any doses studied (Table 1). Consequently, the LD_{50} value of the test item was estimated to be higher than 37.5 g/kg for both sexes.

Table 1. Mortality and clinical signs of mice after single oral administration of DHP2

Dose (g/kg/day)	Male			Female		
	2.5	12.5	37.5	2.5	12.5	37.5
Mortality	0/3[a]	0/3	0/3	0/3	0/3	0/3
Clinical signs						
Decreased locomotive activity	-[b]	-	2/3	-	-	1/3

[a]Values are expressed as numbers of dead animals/total number of animals.
[b]No clinical signs were observed.

Table 2. Changes of body weights in mice after single oral administration of DHP2

Sex	Days after treatment	Dose (g/kg/day)		
		2.5	12.5	37.5
Male	0	25.7 ± 2.05[a]	25.9 ± 0.74	26.3 ± 0.75
	1	27.8 ± 2.80	28.0 ± 1.39	27.1 ± 1.06
	3	28.7 ± 3.42	28.2 ± 1.87	28.3 ± 2.15
	7	30.9 ± 3.55	30.3 ± 3.07	31.5 ± 2.55
	Gain	5.2 ± 1.50	4.4 ± 2.34	5.1 ± 1.80
Female	0	20.1 ± 0.98	22.4 ± 1.43	21.5 ± 1.10
	1	21.9 ± 1.19	23.4 ± 1.47	21.8 ± 0.40
	3	22.1 ± 0.92	23.8 ± 1.58	22.2 ± 1.22
	7	24.4 ± 1.15	25.0 ± 1.28	23.8 ± 1.44
	Gain	4.3 ± 0.32	2.6 ± 1.36	2.3 ± 2.54

[a]Values are presented as means ± S.D.

Table 3. Gross findings of necropsy in mice after single oral administration of DHP2

Sex	Dose (g/kg/day)		
	2.5	12.5	37.5
Male	0/3[a]	0/3	0/3
Female	0/3	0/3	0/3

[a]Values are expressed as numbers of abnormal animals/total number of animals.

Clinical signs: Decreased locomotive activity was found in two males and one female in the 37.5 g/kg groups, respectively. (Table 1).

Body weight changes: Normal body weight gains were observed in all dose groups during the study period (Table 2).

Gross findings: No treatment-related effects were found in all dose groups when the animals were macroscopically examined in the necropsy (Table 3).

2-Week Repeated Dose Toxicity Study

The treatment-related death was not observed at any doses studied. For general observation, one occur-

Table 4. Mean body weights of male and female mice after 2-week repeated oral administration of DHP2

	Dose (g/kg/day)			
	0	2.5	5	10
Male				
Day 0	29.4 ± 1.45[a]	29.9 ± 1.42	30.0 ± 1.56	29.9 ± 2.05
Day 3	31.0 ± 1.58	29.8 ± 2.51	30.8 ± 1.73	30.5 ± 2.04
Day 7	30.7 ± 1.04	30.4 ± 2.15	31.2 ± 2.54	31.6 ± 2.28
Day 10	30.8 ± 1.03	30.6 ± 2.43	30.6 ± 2.62	31.2 ± 2.10
Day 14	31.4 ± 1.43	31.4 ± 2.66	31.3 ± 1.97	31.6 ± 1.72
Female				
Day 0	24.4 ± 0.68	24.6 ± 1.07	24.5 ± 1.00	24.5 ± 0.94
Day 3	25.9 ± 0.78	25.1 ± 1.38	25.5 ± 0.57	25.2 ± 0.79
Day 7	26.2 ± 1.12	26.4 ± 1.24	25.1 ± 1.22	25.1 ± 1.20
Day 10	26.4 ± 1.02	25.4 ± 1.33	24.8 ± 1.16	24.7 ± 1.03
Day 14	27.9 ± 1.22	27.1 ± 1.38	25.6 ± 1.26*	25.1 ± 1.03**

[a]Values are presented as means ± SD (g).
* and ** indicate significant difference at $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Table 5. Food consumption of male and female mice after 2-week repeated oral administration of DHP2

	Dose (g/kg/day)			
	0	2.5	5	10
Male				
Day 0	4.6 ± 0.39[a]	5.2 ± 1.02	5.1 ± 0.18	5.6 ± 0.30
Day 1	4.4 ± 0.42	4.5 ± 0.74	4.9 ± 0.33	4.7 ± 0.18
Day 8	4.7 ± 0.05	6.0 ± 0.77	4.6 ± 0.28	4.5 ± 0.64
Day 14	4.8 ± 0.17	5.5 ± 0.18	4.5 ± 0.03	4.3 ± 0.69
Female				
Day 0	4.3 ± 0.45	4.3 ± 0.21	4.2 ± 0.73	4.4 ± 0.58
Day 1	4.7 ± 0.01	3.6 ± 0.08*	3.8 ± 0.42	4.2 ± 0.24
Day 8	5.3 ± 0.53	4.6 ± 0.14	4.0 ± 0.70	4.0 ± 0.94
Day 14	5.4 ± 0.04	4.9 ± 0.11	4.0 ± 0.26**	4.1 ± 0.19**

[a]Values are presented as means ± SD (g).
* and ** indicate significant difference at $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

rence of depilation in the 2.5 g/kg group, one occurrence of depilation in the 5 g/kg group and two occurrences of depilation in the 10 g/kg group were observed in males. For females, one occurrence of depilation in the vehicle control group was observed.

Body weight changes: Statistically significant decreases in body weight were noted in the 5 and 10 g/kg females at day 14 compared with the vehicle control (Table 4).

Food consumption: Statistically significant decreases in food consumption were noted in 2.5 g/kg females at day 1 and in the 5 and 10 g/kg females at day 14 compared with the vehicle control (Table 5).

Ophthalmoscopy: Ophthalmologic examinations did not reveal ocular lesions in any of the animals (data not shown).

Urinalysis: No significant difference between treatment groups and vehicle control group was seen for any urinary parameters (data not shown).

Hematology: No significant difference between treatment groups and vehicle control group was seen for hematology parameters (Table 6).

Table 6. Hematological findings in male and female mice after 2-week repeated oral administration of DHP2

	Dose (g/kg/day)			
	0	2.5	5	10
Male				
Leukocytes ($\times10^9$/l)	2.36 ± 1.27[a]	1.98 ± 0.27	1.62 ± 0.38	2.36 ± 0.87
Erythrocytes ($\times10^{12}$/l)	9.24 ± 0.69	9.29 ± 0.33	8.85 ± 0.29	9.08 ± 0.30
Hemoglobin (g/dl)	14.8 ± 0.83	15.5 ± 0.73	14.5 ± 0.47	14.6 ± 0.42
Hematocrit (%)	46.9 ± 2.48	48.2 ± 1.59	44.9 ± 1.24	45.5 ± 1.58
MCV (fl)	50.8 ± 1.25	51.8 ± 0.46	50.7 ± 1.06	50.1 ± 1.50
MCH (pg)	16.1 ± 0.34	16.7 ± 0.40	16.4 ± 0.46	16.2 ± 0.50
MCHC (g/dl)	31.6 ± 0.61	32.2 ± 0.65	32.4 ± 0.79	32.2 ± 0.27
Platelets ($\times10^9$/l)	1568 ± 220	1463 ± 285	1776 ± 73.4	1767 ± 203
Female				
Leukocytes ($\times10^9$/l)	4.98 ± 1.88	3.89 ± 1.70	5.90 ± 2.45	3.48 ± 1.67
Erythrocytes ($\times10^{12}$/l)	9.05 ± 0.24	9.09 ± 0.38	8.87 ± 0.35	8.85 ± 0.48
Hemoglobin (g/)	15.0 ± 0.30	14.7 ± 0.55	14.5 ± 0.53	14.6 ± 0.72
Hematocrit (%)	46.1 ± 1.14	46.2 ± 1.83	45.0 ± 1.56	45.2 ± 2.03
MCV (fl)	51.0 ± 0.54	50.9 ± 1.48	50.7 ± 1.40	51.1 ± 1.43
MCH (pg)	16.5 ± 0.37	16.2 ± 0.46	16.3 ± 0.44	16.5 ± 0.36
MCHC (g/dl)	32.4 ± 0.51	31.8 ± 0.16	32.2 ± 0.11	32.3 ± 0.86
Platelets ($\times10^9$/l)	1254 ± 168	1325 ± 106	1570 ± 208	1563 ± 90.9

[a]Values are presented as means ± SD.
MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 7. Serum biochemical findings in male mice after 2-week repeated oral administration of DHP2

	Dose (g/kg/day)				Normal range[b]
	0	2.5	5	10	
Aspartate aminotransferase (IU/l)	67.8 ± 9.94[a]	71.1 ± 22.1	65.0 ± 8.87	63.2 ± 8.88	-
Alanine aminotransferase (IU/l)	29.7 ± 7.86	28.0 ± 3.32	25.7 ± 2.23	22.7 ± 1.93	-
Alkaline phosphatase (IU/l)	363 ± 131	248 ± 49.6	279 ± 90.8	263 ± 90.2	-
Blood urea nitrogen (mg/dl)	33.9 ± 14.1	29.2 ± 3.54	22.9 ± 1.71*	25.5 ± 3.46	24.2 ± 5.73
Creatinine (mg/dl)	0.35 ± 0.25	0.24 ± 0.01	0.25 ± 0.04	0.20 ± 0.04	-
Glucose (mg/dl)	132 ± 18.2	138 ± 25.8	106 ± 22.1	124 ± 21.1	-
Total cholesterol (mg/dl)	116 ± 23.0	125 ± 22.3	121 ± 9.45	116 ± 9.85	-
Albumin/Globulin (ratio)	1.78 ± 0.18	1.67 ± 0.14	1.80 ± 0.03	1.79 ± 0.06	-
Total protein (g/dl)	5.27 ± 0.29	5.24 ± 0.20	5.28 ± 0.21	5.04 ± 0.24	-
Albumin (g/dl)	3.37 ± 0.12	3.27 ± 0.11	3.39 ± 0.14	3.24 ± 0.18	-
Creatine phosphokinase (IU/l)	145 ± 49.2	156 ± 82.1	113 ± 14.0	170 ± 64.3	-
Triglyceride (mg/dl)	60.7 ± 26.2	39.8 ± 23.0	63.3 ± 17.7	52.9 ± 7.46	-
Phospholipid (mg/dl)	190 ± 33.5	193 ± 29.8	200 ± 16.1	188 ± 15.3	-
Total bilirubin (mg/dl)	0.128 ± 0.021	0.113 ± 0.022	0.139 ± 0.015	0.118 ± 0.022	-

[a]Values are presented as means ± SD.
[b]Worford *et al.* (1986).
*indicates significant difference at $p<0.05$ level when compared with the control group.

Table 8. Organ weights in male mice after 2-week repeated oral administration of DHP2

Organ	Dose (g/kg/day)			
	0	2.5	5	10
Body weight	31.4 ± 1.43[a]	31.4 ± 2.66	31.3 ± 1.97	31.6 ± 1.72
Brain (g)	0.450 ± 0.010	0.432 ± 0.043	0.440 ± 0.021	0.445 ± 0.013
per body weight (%)	1.68 ± 0.034	1.64 ± 0.118	1.62 ± 0.151	1.62 ± 0.034
Pituitary gland (g)	0.002 ± 0.0007	0.001 ± 0.0007	0.001 ± 0.0005	0.001 ± 0.0006
per body weight (%)	0.0075 ± 0.0026	0.0046 ± 0.0026	0.0049 ± 0.0018	0.0051 ± 0.0020
Liver (g)	1.23 ± 0.110	1.26 ± 0.107	1.16 ± 0.155	1.19 ± 0.098
per body weight (%)	4.58 ± 0.315	4.79 ± 0.139	4.23 ± 0.385	4.32 ± 0.231
Spleen (g)	0.070 ± 0.011	0.081 ± 0.029	0.068 ± 0.020	0.080 ± 0.008
per body weight (%)	0.262 ± 0.042	0.305 ± 0.088	0.246 ± 0.056	0.290 ± 0.028
Heart (g)	0.143 ± 0.013	0.140 ± 0.011	0.128 ± 0.009	0.134 ± 0.022
per body weight (%)	0.534 ± 0.053	0.533 ± 0.028	0.469 ± 0.037	0.485 ± 0.066
Lung (g)	0.177 ± 0.013	0.197 ± 0.012	0.180 ± 0.014	0.200 ± 0.015*
per body weight (%)	0.664 ± 0.054	0.747 ± 0.041	0.661 ± 0.075	0.729 ± 0.040
Thymus (g)	0.046 ± 0.011	0.046 ± 0.001	0.035 ± 0.012	0.040 ± 0.007
per body weight (%)	0.171 ± 0.040	0.173 ± 0.016	0.126 ± 0.041	0.144 ± 0.022
Kidneys (g)	0.411 ± 0.019	0.433 ± 0.055	0.372 ± 0.030	0.398 ± 0.047
per body weight (%)	1.54 ± 0.076	1.64 ± 0.094	1.36 ± 0.033*	1.43 ± 0.164
Prostates (g)	0.0098 ± 0.0035	0.0084 ± 0.0035	0.0080 ± 0.0016	0.0110 ± 0.0059
per body weight (%)	0.037 ± 0.013	0.032 ± 0.013	0.030 ± 0.007	0.040 ± 0.020
Testes (g)	0.209 ± 0.031	0.212 ± 0.025	0.226 ± 0.025	0.197 ± 0.020
per body weight (%)	0.782 ± 0.076	0.806 ± 0.087	0.827 ± 0.076	0.717 ± 0.062
Adrenal glands (g)	0.0054 ± 0.0012	0.0065 ± 0.0021	0.0056 ± 0.0017	0.0070 ± 0.0012
per body weight (%)	0.0203 ± 0.0045	0.0245 ± 0.0074	0.0205 ± 0.0071	0.0255 ± 0.0038
Epididymides (g)	0.0734 ± 0.0072	0.0724 ± 0.0061	0.0734 ± 0.0067	0.1280 ± 0.1177
per body weight (%)	0.275 ± 0.032	0.275 ± 0.016	0.268 ± 0.014	0.463 ± 0.417

[a]Values are presented as means ± SD.
*indicates significant difference at $p<0.05$ level when compared with the control group.

Serum biochemistry: Statistically significant decrease in blood urea nitrogen (BUN) was observed in *males of the 5 g/kg group* compared with the vehicle control group (Table 7). In females, no test item-related changes were observed in any treatment groups (data not shown).

Gross findings: At necropsy, no test item-related changes were observed in any treatment groups of both sexes (data not shown).

Organ weights: The statistically increased absolute

Table 9. Organ weights in female mice after 2-week repeated oral administration of DHP2

Organ	Dose (g/kg/day)			
	0	2.5	5	10
Body weight	27.9 ± 1.22[a]	27.1 ± 1.38	25.6 ± 1.26	25.1 ± 1.03
Brain (g)	0.459 ± 0.034	0.448 ± 0.015	0.444 ± 0.022	0.447 ± 0.038
per body weight (%)	1.96 ± 0.088	1.98 ± 0.098	2.01 ± 0.086	2.07 ± 0.147
Pituitary gland (g)	0.002 ± 0.0009	0.002 ± 0.0006	0.002 ± 0.0004	0.002 ± 0.0003
per body weight (%)	0.0074 ± 0.0038	0.0084 ± 0.0026	0.0073 ± 0.0017	0.0084 ± 0.0015
Liver (g)	1.00 ± 0.060	1.00 ± 0.059	0.92 ± 0.043	0.891 ± 0.046*
per body weight (%)	4.29 ± 0.156	4.41 ± 0.204	4.17 ± 0.119	4.12 ± 0.174
Spleen (g)	0.082 ± 0.013	0.080 ± 0.006	0.080 ± 0.010	0.070 ± 0.018
per body weight (%)	0.353 ± 0.059	0.353 ± 0.018	0.362 ± 0.047	0.323 ± 0.077
Heart (g)	0.121 ± 0.015	0.109 ± 0.007	0.104 ± 0.006	0.107 ± 0.009
per body weight (%)	0.517 ± 0.053	0.481 ± 0.030	0.471 ± 0.034	0.493 ± 0.042
Lung (g)	0.181 ± 0.009	0.173 ± 0.008	0.174 ± 0.011	0.170 ± 0.015
per body weight (%)	0.775 ± 0.042	0.763 ± 0.054	0.789 ± 0.068	0.785 ± 0.066
Thymus (g)	0.063 ± 0.009	0.055 ± 0.014	0.049 ± 0.017	0.041 ± 0.009
per body weight (%)	0.269 ± 0.038	0.241 ± 0.058	0.223 ± 0.075	0.190 ± 0.040
Kidneys (g)	0.292 ± 0.015	0.303 ± 0.031	0.259 ± 0.019	0.275 ± 0.018
per body weight (%)	1.25 ± 0.046	1.34 ± 0.128	1.17 ± 0.090	1.27 ± 0.067
Uterus (g)	0.101 ± 0.036	0.077 ± 0.018	0.089 ± 0.041	0.094 ± 0.050
per body weight (%)	0.432 ± 0.156	0.339 ± 0.070	0.400 ± 0.187	0.431 ± 0.220
Ovaries (g)	0.0097 ± 0.0026	0.0099 ± 0.0021	0.0091 ± 0.0013	0.0084 ± 0.0019
per body weight (%)	0.041 ± 0.009	0.043 ± 0.009	0.041 ± 0.005	0.039 ± 0.008
Adrenal glands (g)	0.0082 ± 0.0020	0.0080 ± 0.0008	0.0080 ± 0.0010	0.0079 ± 0.0019
per body weight (%)	0.0351 ± 0.0074	0.0352 ± 0.0022	0.0361 ± 0.0045	0.0365 ± 0.0096

[a]Values are presented as means ± SD.
*indicates significant difference at $p<0.05$ level when compared with the control group.

weight of lung in the 10 g/kg males, decreased relative weight of kidney in the 5 g/kg males (Table 8) and decreased absolute weight of liver in the 10 g/kg females were observed compared with the vehicle control group (Table 9).

DISCUSSION

In the present study we reported the results of the single and 2-week repeated oral administration to ICR mice performed as a part of the preclinical safety evaluation program for DHP2. For a single dose toxicity study, DHP2 was administered by gavage to ICR mice at dose levels of 2.5, 12.5 and 37.5 g/kg and evaluations during the 7-day observation period including mortality, clinical signs, body weight changes, and gross findings were performed. For 2-week repeated dose toxicity study, DHP2 was administered by gavage to mice at dose levels of 0, 2.5, 5 and 10 g/kg for 2 weeks.

In the single dose toxicity study, decreased locomotive activity was found in the 37.5 g/kg group and treatment-related effects on mortality, body weight changes and gross findings were not observed. In the 2-week repeated dose toxicity study, depilation was not thought to attribute to the test item administration because it occurred at a low frequency and did not show a dose-response relationship. Statistically significant decreases in body weight noted in the 5 g/kg and 10 g/kg females at day 14 was due to the decreased food intake temporarily, therefore it was not considered to be treatment related. Mortality, ophthalmoscopic examination, urinalysis and hematology revealed no abnormalities in all treatment groups of both sexes. In serem biochemistry, the statistically significant decrease of BUN in the 5 g/kg males was considered not toxicologically significant since this change was within the limit of normal biological variation and not exhibit a dose-response relationship. The statistically increased absolute weight of lung in the 10 g/kg males, decreased relative weight of kidney in the 5 g/kg males and decreased absolute weight of liver in the 10 g/kg females were negligible, not associated with the gross findings and within the limits of normal biological variation, so these changes were of doubtful toxicological significance (Wolford *et al.*, 1986; Kang *et al.*, 1995).

Based on these results, it was concluded that the single oral dose of DHP2 produced no toxic effects in ICR mice at the dose level of 37.5 g/kg, and that the LD_{50} value was estimated to be over 37.5 g/kg for both sexes. Also, 2-week repeated oral dose of DHP2 did not cause any toxic effect to ICR mice at the dose level

of 10 g/kg. In the condition of this study, the NOAEL of DHP2 is considered to be over 10 g/kg for both sexes.

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