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Determining NOEL/NOAEL in Repeated-dose Toxicity Studies, When the Low Dose Group Shows Significant Difference in Quantitative Data

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In repeated-dose 28-day oral toxicity study design, the low dose is fixed as the no observed effect level (NOEL). But, in practice the low dose usually shows significant difference in few measurable items in most of the studies. We investigated 109 of repeated-dose 28-day oral toxicity studies in rats conducted according to the Chemical Substance Control Law, Japan and examined the measurable items (functional observational battery, urinalysis, hematology, blood chemistry and absolute and relative organ weights) of the low dose group which showed a statistical significant difference ($P<0.05$) compared to the respective control groups. The investigation revealed that, 205/12,167 (1.6%) measurable items showed a significant difference in the low dose groups. The significant difference shown by urinalysis was high (3.3%), followed by clinical chemistry parameters, hematology, relative organ weights and absolute organ weights (1.8-1.1%). We conclude from the investigation that the low dose may be considered as NOEL, if the significant difference of measurable items of it is about 2% (maximum <5%), compared to the control. However, due consideration may be given to the clinical relevance of the items that showed a significant difference.

Key words: Repeated-dose toxicity study, statistical significant difference, rats, low dose, NOEL/NOAEL

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One of the main objectives of conducting repeated-dose toxicity studies is to arrive at no observed effect level/no observed adverse effect level (NOEL/NOAEL) (OECD, 2000). Most of the regulatory guidelines prescribe that the repeated-dose toxicity studies with rodents should be conducted with a minimum of three treatment doses (low, medium and high doses) and a control (OECD, 1995). The low dose is carefully chosen with the assumption that animals exposed to this dose level will not show any effect of the treatment compared to the control. But, most of the repeated-dose toxicity studies show some effect of the treatment in few parameters of the low dose group. In such cases considering the low dose as an NOEL/NOAEL may be questionable.

In the present study, data of functional observational battery (FOB), urinalysis, hematology, blood chemistry and absolute and relative organ weights obtained from 109 of 28-day repeated-dose toxicity studies in rats were examined to find the number of significant items of the above data of the low dose group showed significant difference compared to control. The authors suggested that the low dose can still be considered NOEL/NOAEL, if the number of items in this dose group that showed significant difference is minimum and clinically irrelevant.

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Material and Methods

The study reports of 109 of 28-day repeated-dose toxicity studies in rats conducted according to the Chemical Substance Control Law guideline of Japan (NITE, 2007) were obtained from the internet (Ministry of Health, Labour and Welfare, 2009). Group composition of the rats by this guideline is shown in Table 1. Of 109 studies examined, 37 and 72 studies had 5 and 4 groups of rats including one control group, respectively. Each group consisted of 5 rats/sex, except

Table 1. Group composition in Chemical Substance Control Law Guideline of Japan (NITE, 2007)

Group, dosage	Number of animal/sex	
	Dosing period for 28 days	Recovery period for 14 days
Control	10	5
Low	5	-
Medium	5	-
High	10	5

Table 3. Statistics analysis tools used for the repeated dose 28-day oral toxicity studies in rats

Analytical tools	Bartlett's homogenous test ANOVA Dunnett's multiple comparison test Scheffé's multiple comparison test Duncan's multiple range test Kruskal-Wallis's *H* test Nonparametric type Dunnett's test Nonparametric type Scheffé's test Steel test etc.

the control and high dose groups which consisted of 10 rats/sex. In most of the studies the doses were spaced by a factor of 3. In all studies, test article was administered by oral gavage.

Quantitative data (FOB, urinalysis, hematology, blood chemistry and absolute and relative organ weights) at terminal sacrifice of the above studies were used for the investigation in the present study (Table 2).

The statistical analyses carried out in most of the studies were similar to those explained by Kobayashi, *et al*. (2008), which is shown in Table 3. The studies used one-sided test, two-sided test or both one-sided and two-sided tests for examining the data. The data were examined at 5% significant level.

Results and Discussion

The numbers of significant differences ($P<0.05$) detected in the items examined in different dose groups of 109 of 28-day repeated-dose toxicity studies in rats is given in Table 4. The total number of items examined in 109 studies is 12,167 and average items examined for a study is 111. As expected a dose-dependent increase in number of significant items was observed. In the case of low dose group, an average 1.6% of items showed significant difference and the maximum percent of significant items shown was 8.4%.

Among the data examined of all the treatment groups more items of urinalysis showed significant deference, compared to control, followed by the items of clinical chemistry and relative organ weight (Table 5). The percent items of FOB, hematology and absolute organ weight of the treatment groups showed a significant difference, compared to control, more or less in a similar magnitude.

Repeated-dose toxicity studies provide useful information for designing clinical trials of drugs. Though the doses are fixed and spaced in such a way that the low dose should constitute a NOEL/NOAEL, in most of the studies low dose group shows significant difference in few items, rendering difficulty in considering the low dose as NOEL/NOAEL. If the significant difference shown by the items of low dose group is 1 to 2% (maximum<5%), the low dose may still be considered as NOEL/NOAEL. In a recently conducted study it has been stated that the most appropriate techniques appear to be the trend test, comparison between treatment group and historical control by *t*-test, and confirmation that all individual values lie within the 95% confidence interval (2SD) of the historical control value, if a significant difference is admitted in the low dose (Kobayashi *et al*., 2010). However, due consideration should be given to the clinical relevance of these items.

Table 2. Items of quantitative data examined

Measurement data	Items examined
Functional observational battery	Grip strength, hindlimb foot splay, motor activity
Urinalysis	Urine volume, specific gravity, osmotic pressure, sodium, potassium, chloride
Hematology	White blood cell, differential leukocyte ratio, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet, reticulocyte, prothrombin time, activated partial thromboplastin time, etc.
Blood chemistry	Total protein, albumin/globulin ratio, total cholesterol, glucose, blood urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, triglyceride, inorganic phosphate, calcium, sodium, potassium, chloride, etc.
Absolute and relative organ weights	Brain, thyroid, thymus, heart, lungs, liver, spleen, adrenal glands, testes, ovaries, etc.

Table 4. Number of items showed a significant difference (P<0.05) from control group in each study

Study No.	Number of item	Number of items showed a significant difference[a]			
		Low dose	Medium dose	High dose	Top dose
1	120	0 (0.0)	2 (1.7)	4 (3.3)	10 (11)
2	104	6 (5.7)	5 (4.8)	4 (3.8)	-
3	108	1 (0.9)	2 (1.8)	3 (2.7)	-
4	128	2 (1.5)	10 (7.8)	23 (18)	-
5	98	4 (4.8)	10 (10)	17 (17)	-
6	120	0 (0.0)	4 (3.3)	10 (8.3)	24 (20)
7	146	2 (1.3)	6 (4.1)	42 (28)	-
8	118	1 (0.8)	1 (0.8)	0 (0.0)	1 (0.8)
9	118	1 (0.8)	2 (1.6)	27 (22)	-
10	116	1 (0.8)	10 (8.6)	26 (22)	-
11	116	2 (1.7)	10 (8.6)	26 (22)	-
12	156	3 (1.9)	11 (7.0)	36 (23)	-
13	106	2 (1.8)	5 (4.7)	30 (28)	-
14	100	7 (7.0)	11 (11)	23 (23)	-
15	100	2 (2.0)	2 (2.0)	2 (2.0)	-
16	128	3 (2.3)	10 (7.8)	44 (34)	-
17	96	5 (5.2)	0 (0.0)	4 (4.1)	-
18	118	2 (1.6)	0 (0.0)	1 (0.8)	4 (3.3)
19	114	0 (0.0)	1 (0.8)	1 (0.8)	-
20	102	4 (3.9)	1 (0.9)	6 (5.8)	-
21	94	3 (3.1)	6 (6.3)	2 (2.1)	-
22	128	0 (0.0)	2(1.5)	23 (17)	-
23	136	3 (2.2)	7 (5.1)	11 (8.0)	-
24	76	1 (1.3)	2 (2.6)	5 (6.5)	21 (27)
25	110	2 (1.8)	5 (4.5)	17 (15)	-
26	118	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.3)
27	116	0 (0.0)	2 (1.7)	9 (7.7)	23 (19)
28	100	2 (2.0)	4 (4.0)	37 (37)	-
29	158	5 (3.1)	12 (7.5)	31 (19)	-
30	120	0 (0.0)	2 (1.6)	10 (8.3)	-
31	134	0 (0.0)	0 (0.0)	4 (2.9)	16 (11)
32	126	1 (0.7)	6 (4.7)	12 (9.5)	30 (23)
33	106	3 (2.8)	1 (0.9)	23 (21)	-
34	128	0 (0.0)	3 (2.3)	3 (2.3)	38 (29)
35	118	0 (0.0)	9 (7.6)	24 (20)	40 (33)
36	54	0 (0.0)	2 (3.7)	9 (16)	-
37	106	0 (0.0)	2 (1.8)	6 (5.6)	-
38	122	1 (0.8)	5 (4.0)	44 (36)	-
39	100	6 (6.0)	17 (17)	27 (27)	-
40	104	1 (0.9)	3 (2.8)	7 (6.7)	-
41	104	2 (1.9)	4 (3.8	9 (8.6)	-
42	106	3 (2.8)	1 (0.9)	25 (23)	-
43	151	2 (1.3)	9 (2.9)	31 (20)	-
44	106	2 (1.8)	9 (8.4)	3 (2.8)	-
45	112	3 (2.6)	9 (8.0)	18 (16)	-
46	86	0 (0.0)	5 (5.8)	14 (16)	30 (34)
47	104	2 (1.9)	0 (0.0)	7 (6.7)	-
48	70	0 (0.0)	1 (1.4)	0 (0.0)	-
49	50	0 (0.0)	2 (4.0)	9 (18)	30 (60)
50	74	0 (0.0)	1 (1.3)	0 (0.0)	-
51	132	3 (2.2)	5 (3.7)	9 (6.8)	-
52	142	12 (8.4)	11 (7.7)	7 (4.9)	-
53	94	1 (1.0)	2 (2.1)	2 (2.1)	-

Table 4. Continued

Study No.	Number of item	Number of items showed a significant difference[a]			
		Low dose	Medium dose	High dose	Top dose
54	116	5 (4.3)	7 (6.0)	3 (2.5)	-
55	98	7 (7.1)	3 (3.0)	23 (23)	-
56	104	6 (5.7)	12 (11)	21 (20)	-
57	88	2 (2.2)	1 (1.1)	6 (6.8)	-
58	98	0 (0.0)	0 (0.0)	8 (8.1)	-
59	100	0 (0.0)	0 (0.0)	1 (1.0)	8 (8.0)
60	104	2 (1.9)	8 (7.6)	37 (35)	58 (55)
61	96	1 (1.0)	7 (7.2)	16 (16)	-
62	128	1 (0.7)	3 (2.3)	25 (19)	-
63	138	2 (1.4)	6 (4.3)	49 (35)	-
64	128	1 (0.7)	1 (0.7)	12 (9.3)	-
65	130	0 (0.0)	3 (2.3)	9 (6.9)	33 (25)
66	104	0 (0.0)	6 (5.7)	42 (40)	-
67	94	6 (6.3)	3 (3.1)	4 (4.2)	-
68	106	3 (1.8)	3 (1.8)	3 (1.8)	-
69	110	0 (0.0)	0 (0.0)	2 (1.8)	21 (19)
70	122	0 (0.0)	2 (1.6)	4 (3.2)	0 (0.0)
71	162	1 (0.6)	2 (1.2)	9 (5.5)	-
72	136	1 (0.7)	3 (2.2)	18 (13)	-
73	104	1 (0.9)	0 (0.0)	1 (0.8)	4 (3.8)
74	106	0 (0.0)	0 (0.0)	7 (6.6)	19 (25)
75	112	7 (6.2)	1 (0.8)	1 (0.8)	-
76	118	1 (0.8)	3 (2.5)	6 (5.0)	13 (11)
77	90	0 (0.0)	0 (0.0)	2 (2.2)	14 (15)
78	116	0 (0.0)	4 (3.4)	2 (1.7)	5 (4.3)
79	106	1 (0.9)	0 (0.0)	4 (3.7)	8 (7.5)
80	62	2 (3.2)	2 (3.2)	2 (3.2)	3 (4.8)
81	96	1 (1.0)	1 (1.0)	0 (0.0)	-
82	124	3 (2.4)	3 (2.4)	30 (24)	53 (42)
83	110	1 (0.9)	3 (2.7)	13 (11)	-
84	100	1 (1.0)	1 (1.0)	2 (2.0)	-
85	102	1 (0.9)	2 (1.9)	2 (1.9)	14 (13)
86	120	4 (3.3)	12 (10)	15 (12)	29 (24)
87	162	1 (0.6)	4 (2.4)	16 (9.8)	-
88	134	3 (2.2)	1 (0.7)	3 (2.2)	17 (12)
89	90	1 (1.1)	2 (2.2)	2 (2.2)	-
90	108	1 (0.9)	2 (1.8)	1 (0.9)	-
91	100	2 (2.0)	2 (2.0)	4 (4.0)	-
92	92	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)
93	110	2 (1.8)	5 (4.5)	21 (19)	33 (30)
94	100	0 (0.0)	0 (0.0)	1 (1.0)	-
95	104	1 (0.9)	1 (0.9)	1 (0.9)	-
96	104	5 (4.8)	11 (10)	22 (21)	-
97	98	4 (4.0)	5 (5.1)	19 (19)	-
98	114	0 (0.0)	0 (0.0)	8 (7.0)	43 (37)
99	118	3 (2.5)	3 (2.5)	0 (0.0)	-
100	88	1 (1.1)	1 (1.1)	1 (1.1)	-
101	118	3 (2.5)	3 (2.5)	12 (10)	-
102	120	1 (0.8)	0 (0.0)	3 (2.5)	18 (15)
103	116	0 (0.0)	1 (0.8)	1 (0.8)	14 (12)
104	150	1 (0.6)	6 (4.0)	15 (10)	-
105	134	5 (3.7)	7 (5.2)	14 (10)	-
106	100	0 (0.0)	0 (0.0)	6 (6.0)	11 (11)

Table 4. Continued

Study No.	Number of item	Number of items showed a significant difference[a]			
		Low dose	Medium dose	High dose	Top dose
107	136	1 (0.7)	1 (0.7)	4 (2.9)	-
108	130	2 (1.5)	4 (3.0)	16 (12)	31 (23)
109	116	1 (0.8)	1 (0.8)	2 (1.7)	9 (7.7)
Number of study	109	109	109	109	37
Total	12,167	205	414	1318	731
Mean	112	1.9	3.8	12.1	20
Maximum	162	8.4%	17%	40%	60%
Minimum	50	0.0%	0.0%	0.0%	0.0%
Mode	-	0.0%	-	-	-

[a]Values in parentheses are percent of items.

Table 5. Number of quantitative data showed a significant difference (P<0.05) from control group

Quantitative data examined	Number of items examined	Number of items showed a significant difference[a]			
		Low dose	medium dose	High dose	Top dose
FOB	68	1 (1.4)	3 (4.4)	8 (11)	1/10 (10)
Urinalysis	392	13 (3.3)	30 (7.6)	81 (20)	40/96 (40)
Hematology	3,586	56 (1.5)	106 (2.9)	318 (8.8)	176/1198 (14)
Clinical chemistry	4,285	79 (1.8)	163 (3.8)	455 (10)	267/1426 (18)
Absolute organ weight	1,928	22 (1.1)	44 (2.2)	188 (9.7)	103/672 (15)
Relative organ weight	1,908	34 (1.7)	68 (3.5)	268 (14)	144/672 (21)
Total	12,167	205 (1.6)	414 (3.4)	1318 (10)	731/4074 (17)

[a]Values in parentheses are percent of items.

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