(TRANSLATION)

Toxicity (LD 50)

Report of Experiment Result No. 41040689-2

Materials submitted for experiment:	PICO
Description:	Manufactured on March 20, Showa Era 63 (A.D. 1988).
Experimental items:	Experiment of acute and severe toxicity.

Experimental result of the above-mentioned materials, submitted to this Food Research Center on April 11, Showa Era 63 (A.D. 1988) is as follows.

May 11, Hese Era 63 (A.D. 1988)

Foundation

Food Research Center in Japan

Tokyo Head Office:	Code 151, No. 52 – 1, Motoyoyoki Shibuyaku, Tokyo.
Osaka Branch Office:	Code 564, No. 3 – 1, Tatesawacho Fukitashi, Osaka County.
Nagoya Branch Office:	Code 460, No. 5 – 13, Chome 4, Otsu, Nakaku, Nagoya City.

Test of Acute and Severe Toxicity

1. Name of company applying for testing:

PICO Technology Co., Ltd.

2. Name of tested materials:

PICO (manufactured on March 20, Showa Era 63 (A.D. 1988)).

3. Method of consuming:

Through mouth.

4. Animals under experiment:

Male or female rats of DDY – N breed.
Age on weekly basis and initial weight: about 5 weeks.
Male rats: 22- 24 grams. Female rats: 20 –22 grams.

5. Temperature of laboratory:

22 °C ± 2 °C

6. Term of experiment:

From April 21, Showa Era 63 (A.D. 1988) to April 28 of the same year.

7. How to prepare experimental solution:

Mincing the tested material, and mixing 35 grams thereof with olive oil (subject to Standard of Medical Materials of Japan) to make the volume of 100 cubic centimetres (liquid bearing tested materials suspending at 35% of volume). This solution shall be used in the experiment.

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8. How to feed the experimental solution:

1) Feeding
Through Sonde medicine plastic tube into stomach once, and through mouth.

2) Computation for LD 50
By Propit method.

3) Ratio of feeding concentration and the experimental animals.
1 : 1.2

4) Number of animals used in one experiment.
10 male and female rats.

9. Result of Testing:

Sex	No. of Experiment	Quantity of Feeding (mg/kg)	Ratio of Death Later			Ratio of Death (%)	LD 50 Value (mg/kg)
			Hours		Days		
			5	15	1 2 3 4 5 6 7		
Male	1	12,153	0/10	0/10	1/10……...0/10	0	>21,000
	2	14,583	0/10	0/10	1/10……...0/10	0	
	3	17,500	0/10	0/10	1/10……...0/10	0	
	4	21,000	0/10	0/10	1/10……...0/10	0	
Female	1	12,153	0/10	0/10	1/10……...0/10	0	>21,000
	2	14,583	0/10	0/10	1/10……...0/10	0	
	3	17,500	0/10	0/10	1/10……...0/10	0	
	4	21,000	0/10	0/10	1/10……...0/10	0	

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10. Symptoms of Toxicity:

After feeding of solution for about 10 minutes, both male and female animals were moving by themselves a little slower. After about 30 minutes, they released light brown excrement, which should have come from the fed solution. Meanwhile, the area around their anus was moist, and the hair around the anus was foul. However, after feeding for about 3 –15 hours, every animal returned to normal state, and there has not been any abnormal symptom at all since then.

11. Opinion on separate experiments:

There was no abnormal symptom to any essential organ of any experimental group after feeding of solution at all.

12. Opinions:

Regarding this experiment, the solution of 21,000 milligrams per kilogram of the animal's weight has been fed, but neither male nor female animals became dead at all. The volume of 21,000 milligrams per kilogram is equal to liquid of 60 cc. per kilogram, which is the maximum volume to feed the rats. Besides, the feeding liquid is pasty, which represents the maximum concentration to be fed. Therefore, the LD 50 value acquired from the experiment of both male and female animals is higher than the level of 21,000 milligrams per kilogram.

The weight of each rat is subject to the attached table.

Venue of Experiment: 821 Machikura, Naritashi, Chiba County.

Name of Experiment Organization:
Feedstuff Scientific Research Center Foundation,
Scientific Feedstuff Association of Japan.

Name of Experimenting Party:
Noro Shigetsugu, Shimitsu Toshimasa, Yonemochi Senri,
Kasahaya Hiroyi, Yamsaki Hiroaki.

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Attached Table 1

Weight of Each Rat under Experiment (Male)

Feeding Quantity / Weight / No. of Rat		Initial Stage (g)	Final Stage (g)	Feeding Quantity / Weight / No. of Rat		Initial Stage (g)	Final Stage (g)
12,153 (mg/kg)	1	22	23	17,500 (mg/kg)	21	22	24
	2	22	24		22	22	24
	3	22	26		23	22	24
	4	22	27		24	22	26
	5	22	28		25	22	26
	6	24	24		26	24	24
	7	24	25		27	24	25
	8	24	25		28	24	25
	9	24	26		29	24	25
	10	24	26		30	24	26
	Average	23.0	25.4		Average	23.0	24.9
14,583 (mg/kg)	11	22	22	21,000 (mg/kg)	31	22	24
	12	22	23		32	22	26
	13	22	24		33	22	26
	14	22	25		34	22	26
	15	22	28		35	22	27
	16	24	24		36	24	24
	17	24	25		37	24	25
	18	24	25		38	24	26
	19	24	27		39	24	27
	20	24	28		40	24	27
	Average	23.0	25.1		Average	23.0	25.8

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Attached Table 2
Weight of Each Rat under Experiment (Female)

Feeding Quantity / Weight No. Of Rat		Initial Stage (g)	Final Stage (g)	Feeding Quantity / Weight No.of Rat		Initial Stage (g)	Final Stage (g)
12,153 (mg/kg)	101	20	23	17,500 (mg/kg)	121	20	20
	102	20	24		122	20	22
	103	20	24		123	20	23
	104	20	24		124	20	24
	105	20	25		125	20	24
	106	22	24		126	22	24
	107	22	25		127	22	24
	108	22	26		128	22	25
	109	22	26		129	22	25
	110	22	26		130	22	26
	Average	21.0	24.7		Average	21.0	23.7
14,583 (mg/kg)	111	20	20	21,000 (mg/kg)	131	24	24
	112	20	23		132	20	24
	113	20	23		133	20	25
	114	20	24		134	20	25
	115	20	24		135	20	26
	116	22	24		136	22	23
	117	22	24		137	22	23
	118	22	25		138	22	24
	119	22	25		139	22	24
	120	22	26		140	22	26
	Average	21.0	23.8		Average	21.0	24.4

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