

HKK No. 4123

August 8, 1994

Report on Acute Toxicity Study by Single  
Administration Using Mice

Testing Institution:

Address: 1-15-1 Kitasato, Sagamihara-shi, Kanagawa-ken

Name: KITASATO RESEARCH CENTER OF ENVIRONMENTAL  
SCIENCE

Administrator:

Haruo Ogura,  
Director



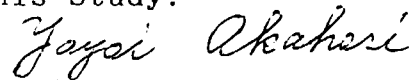
Study Director:

Masahito Aoki,  
Manager of Environmental Toxicology Section



Personnel Engaged in this Study:

Yayoi Akahori,  
Staff of Environmental Toxicology Section



## Summary

An undiluted KYUSEI EM-1 solution in a dose of 10 ml/kg was orally administered to male mice, and observation of clinical findings and measurement of body weight were made for one week. After the passage of one week from the administration, the mice were sacrificed and autopsied to make the macroscopic observation of each organ.

As a result, no abnormality such as death, diarrhea, piloerection, paralysis, spasm or a behavioral change was recognized.

The measurement of body weight showed a smooth tendency to increase, and no body weight decrease due to toxicity was observed.

As for the findings of the macroscopic observation on autopsy, no abnormality was recognized in each organ.

Based on the obtained results, it is presumed that no toxicity was observed within the range of the oral administration of the undiluted KYUSEI EM-1 solution in a dose of 10 ml/kg to male mice.

1. Purpose of test:

The toxicity was studied in forced oral administration of EM product to mice by assuming the case of erroneous drinking, etc.

2. Test substance:

(1) Trade name

KYUSEI EM-1

(2) Form

Aqueous solution

(3) Preserving conditions

Preserved at ambient temperature.

3. Supplier:

(1) Name

International Nature Farming Research Center  
EM Laboratory

(2) Address

678 Yoshizu, Shizuoka-shi, Shizuoka-ken

4. Test animals:

(1) Species and strain

Mice Slc:ddY male 4-week-old

(2) Name of supplier and address

Japan SLC, Inc.

3371-8 Koto-cho, Hamamatsu-shi, Shizuoka-ken

(3) Date of carrying-in

May 19, 1994

5. Feeding conditions:

(1) Kind and size of cages

Mouse cages made of aluminum

320 mm × 210 mm × 140 mm

(2) Number of animals per cage

10

- (3). Temperature and humidity of feeding room  
23 ± 2°C  
55 ± 15%
- (4) Lighting time  
Lighting for 12 hours from 8:00 a.m. to 8:00 p.m.
- (5) Name of feed and supplier  
Solid diet CL-2 for experimental animals  
CLEA JAPAN, INC.  
2-20-14 Aoba-dai, Meguro-ku, Tokyo
- (6) Method for feeding and amount  
The solid diet was fed ad libitum.
- (7) Kind of drinking water and method for feeding  
Tap water was placed in a water feed bottle and fed ad libitum.

6. Test methods:

(1) Method for administration

The administration was made for one sample group and one control group, and 10 animals were used for one group.

The sample was administered in a dose of 10 ml per kg body weight and mouse, and the sample was forcedly administered by using a metallic stomach tube for the mice.

As for the control, tap water was administered in the same dose according to the same method.

(2) Observation

Just after the administration of the sample, clinical findings were obtained. The body weight was measured on the day of administration, 4 days and one week after the administration and analyzed by Student's t-tests.

(3) Autopsy

The animals were sacrificed by bleeding one week after the administration and autopsied. Macroscopic

observation was made on the presence of a pathological change in each organ. The conditions in autopsy were photographed.

## 7. Results:

### (1) Results of observation on clinical findings

Death, diarrhea, piloerection, paralysis and abnormal behavior were not observed during the period just after the administration to the completion of the test.

### (2) Results of body weight measurement

Table-1 shows the results of body weight measurement.

No significant difference was noted during the period from the administration to one week by comparison with the control group.

### (3) Results of autopsy

As for the macroscopic observation in the autopsy, no pathological change was recognized in each organ in the control and sample dose groups (Photos 1 to 20).

## 8. Conclusion

As a result of the test, no abnormality was observed in the clinical findings, change in body weight and results of the macroscopic observation by autopsy. Therefore, it is presumed that no toxicity was recognized within the range of oral administration of the undiluted KYUSEI EM-1 solution in a dose of 10 ml/kg or below to male mice.

Table-1 Result of Measured Body Weight of Mice (unit : g)

Sample Name	Individual No.	Number of Days after Start of Administration (Days)		
		0	4	7
Control Group	1	26.56	28.43	30.68
	2	27.10	27.67	29.94
	3	28.00	29.51	33.60
	4	26.99	27.78	31.71
	5	24.91	26.50	28.27
	6	26.80	26.61	29.64
	7	26.80	28.26	30.85
	8	27.21	29.23	32.08
	9	26.60	27.46	30.49
	10	27.10	28.03	31.65
	Average	26.81	27.95	30.89
	Standard Deviation	0.78	0.98	1.47
EM-1	1	28.43	28.26	31.54
	2	25.70	26.73	28.68
	3	26.50	26.56	29.16
	4	26.58	28.23	31.05
	5	27.21	28.63	31.17
	6	26.47	28.44	31.09
	7	25.77	26.60	28.17
	8	27.34	29.06	32.65
	9	26.99	29.41	31.46
	10	27.13	28.87	31.10
	Average	26.81	28.08	30.62
	Standard Deviation	0.80	1.06	1.44



Photo 1 Control Group Individual No.1



Photo 2 Control Group Individual No.2



Photo 3 Control Group Individual No.3



Photo 4 Control Group Individual No.4



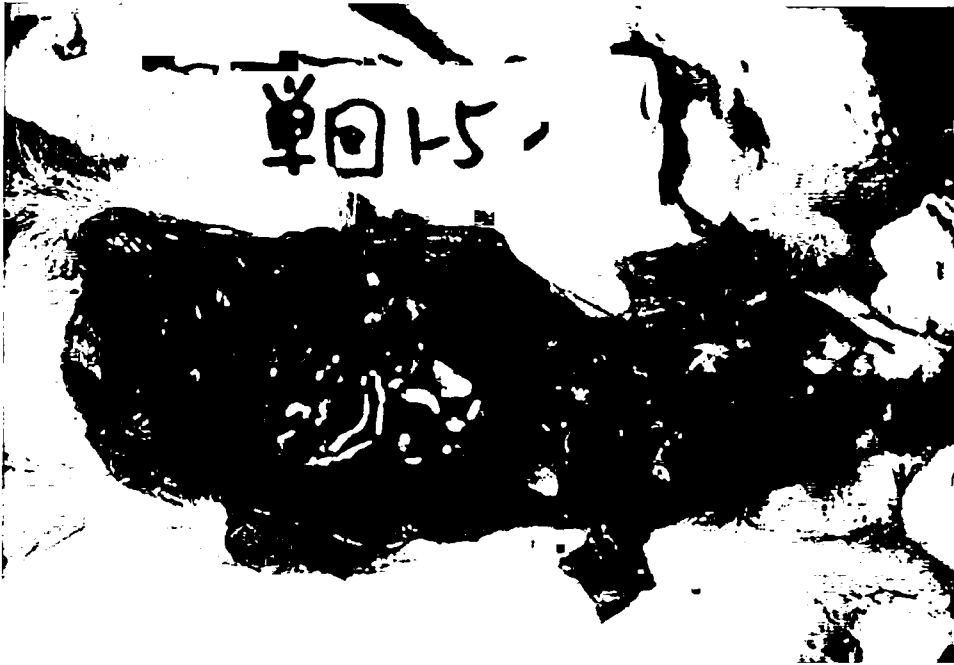


Photo 5 Control Group Individual No.5



Photo 6 Control Group Individual No.6



Photo 7 Control Group Individual No.7

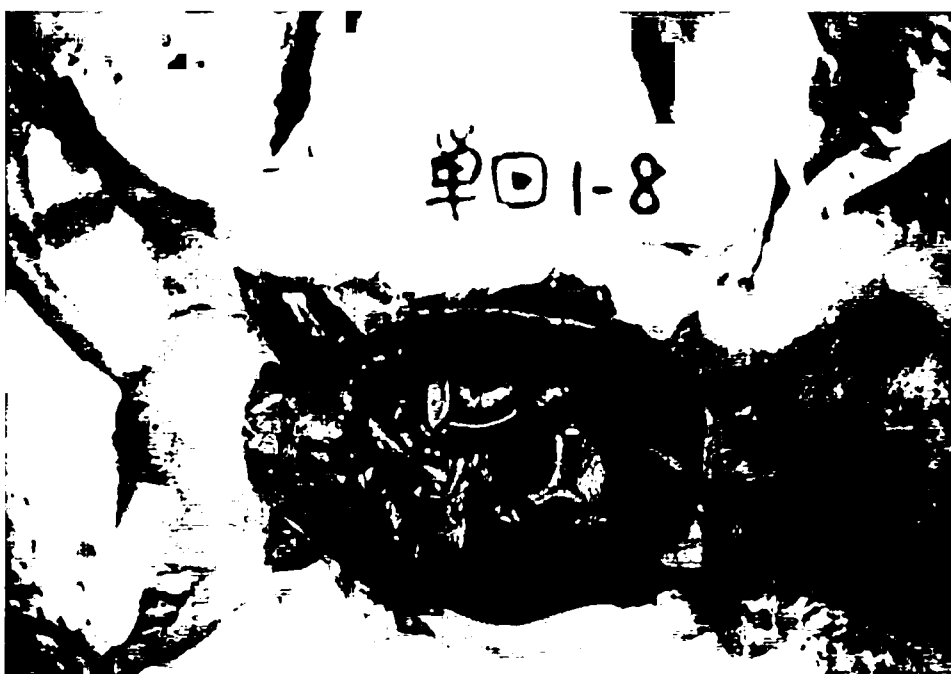


Photo 8 Control Group Individual No.8



Photo 9 Control Group Individual No.9



Photo 10 Control Group Individual No.10



Photo 11 Sample Dose Group Individual No.1

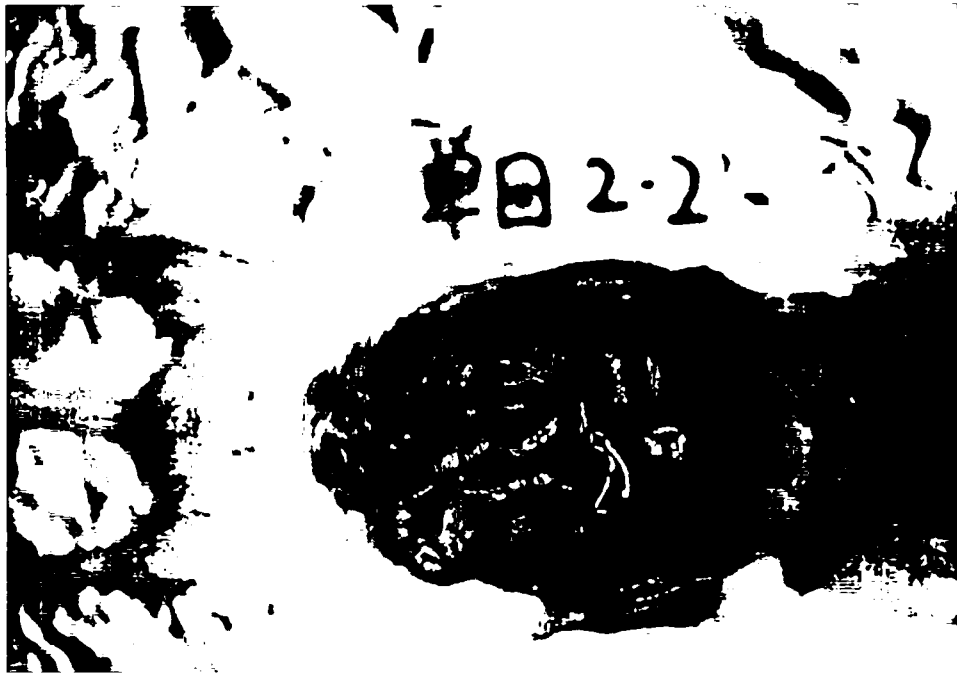


Photo 12 Sample Dose Group Individual No.2



Photo 13 Sample Dose Group Individual No.3

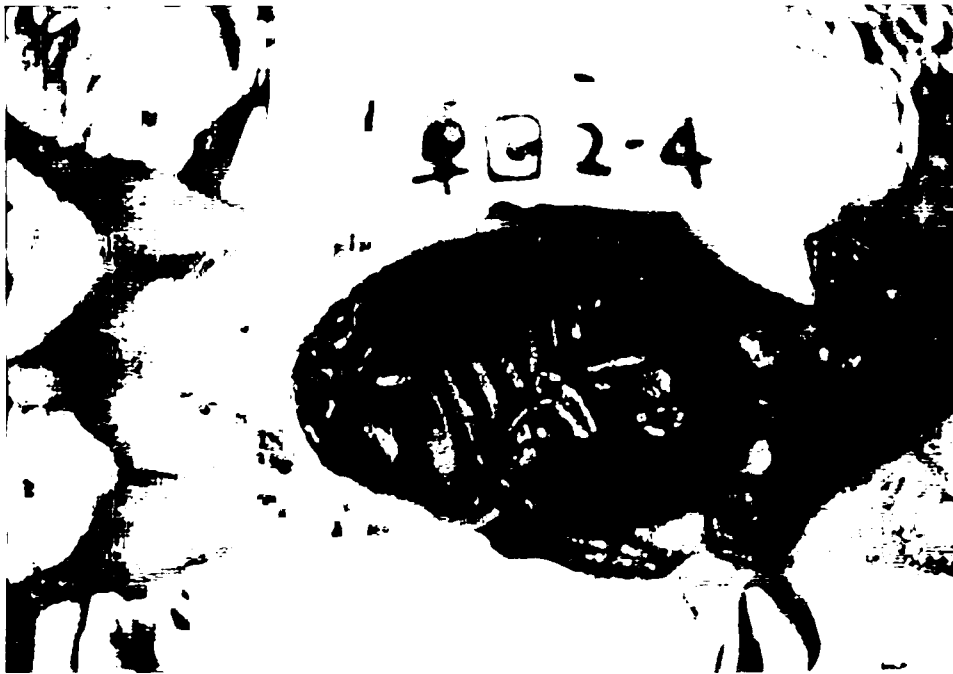


Photo 14 Sample Dose Group Individual No.4



Photo 15 Sample Dose Group Individual No.5



Photo 16 Sample Dose Group Individual No.6



Photo 17 Sample Dose Group Individual No.7



Photo 18 Sample Dose Group Individual No.8

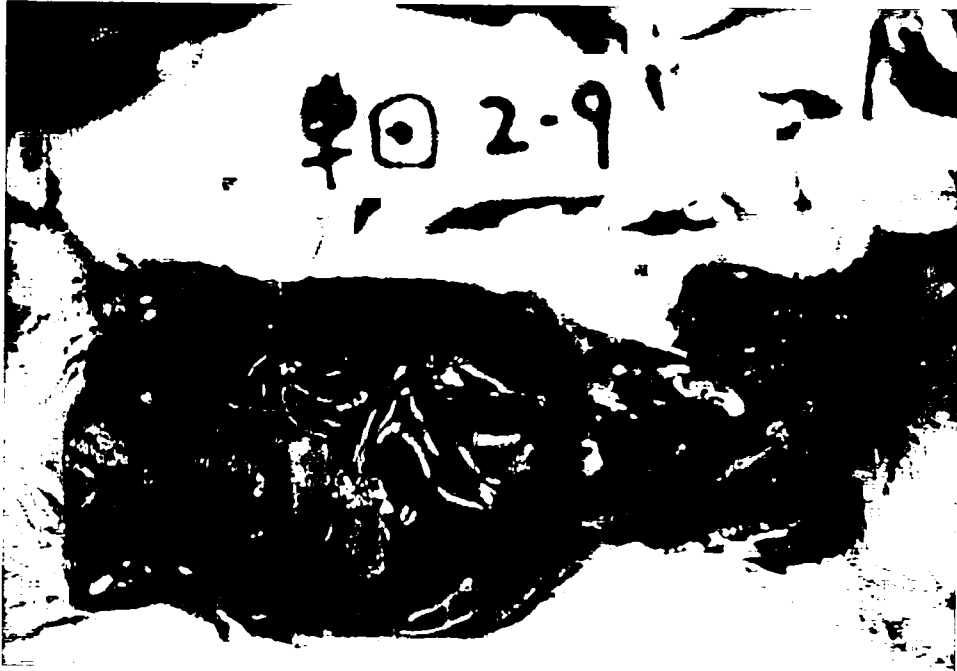


Photo 19 Sample Dose Group Individual No.9



Photo 20 Sample Dose Group Individual No.10