

HKK No. 4124

August 8, 1994

Report on Toxicity Study by Continuous Oral
Administration Using Mice

Testing Institution:

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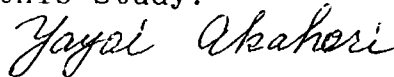
Manager of Environmental Toxicology Section



Personnel Engaged in this Study:

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Summary

A 1:100 diluted KYUSEI EM-1 solution was placed in a water feed bottle, fed and taken by male mice for 3 weeks ad libitum. Observation of clinical findings, intake of a diet, intake of drunk water and body weight measurement twice a week were made during the test period. After three weeks of administration, the mice were sacrificed and autopsied to carry out macroscopic observation of each organ.

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

No significant difference was noted in feed efficiency by comparison with the control group, and the sum total of the drinking water intake for 3 weeks was about 111 ml/animal amounting to 1.1 ml/animal converted into the sample undiluted solution.

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 continuous oral administration of the 1:100 diluted KYUSEI EM-1 solution to male mice for three weeks.

1. Purpose of Test:

The toxicity was studied in natural intake of the EM product suspended in feed water to mice by assuming the case of erroneous drinking, etc. for a long period (3 weeks).

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 preparing the sample
 - The test substance was diluted 1:100 with tap water drawn and allowed to stand for 24 hr.
- (2) 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 in a volume of 180 ml was placed in the feed water bottle at a time and fed to the mice ad libitum for 3 weeks.
 - As for the control, tap water was administered according to the same method.
- (3) Observation
 - Just after the administration of the sample, clinical findings were obtained. The body weight was measured on the day of administration and twice a week and analyzed by Student's t-tests.
 - The amount of taken drinking water (intake) and the intake of the diet were measured and analyzed by

Student's t-tests.

(4) Autopsy

The animals were sacrificed by bleeding three weeks 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 (Table-1).

(2) Results of body weight measurement

Table-2 shows the results of body weight measurement, and Table-3 shows the results of calculated specific body weight.

No significant difference was noted during the period from the start of administration to three weeks by comparison with the control group.

(3) Results of measurement of drinking water intake and feed intake

Table-4 shows the results of measured intake of drinking water per cage, and Table-5 shows the results of the calculated daily intake of drinking water per mouse. No significant difference in the intake of drinking water (the amount of drunk water) was noted by comparison with the control group.

The sum total of the drinking water intake of the test substance dose group for 3 weeks was about 111 ml/animal amounting to about 1.1 ml/animal converted into the sample undiluted solution.

Table-6 shows the results of the measured intake of the diet per cage, and Table-7 shows the results

of the calculated daily intake of the diet per mouse. Table-8 shows the feed efficiency. No significant difference was noted in the diet intake and feed efficiency by comparison with the control group.

(4) Results of autopsy

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

8. Conclusion

As a result of the test, no abnormality was observed in the clinical findings, change in body weight, intakes of drinking water and diet and results of the macroscopic observation by autopsy. Therefore, it is presumed that no toxicity was recognized within the range of continuous oral administration of the 1:100 diluted KYUSEI EM-1 solution to male mice for three weeks.

Table-1 Macroscopic Observation of Mice by Administration of Test Substance

Sample Name	Individual No.	Just after Administration	1st Week	2nd Week	3rd Week	In Autopsy
Control Group	1	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	2	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	3	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	4	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	5	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	6	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	7	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	8	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	9	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	10	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
EM-1	11	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	12	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	13	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	14	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	15	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	16	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	17	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	18	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	19	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality
	20	No Abnormality	No Abnormality	No Abnormality	No Abnormality	No Abnormality

Table-2 Results of Measured Body Weight of Mice (g)

Sample Name	Individual No.	Number of Days after Start of Administration (Days)						
		0	4	7	11	14	18	21
Control Group	1	24.91	25.95	28.32	30.76	30.21	32.64	31.52
	2	25.88	27.12	29.54	32.51	32.25	35.00	35.29
	3	24.82	26.84	28.44	31.23	30.95	32.85	38.47
	4	28.74	30.90	32.32	35.02	34.50	37.55	32.32
	5	26.93	29.23	32.32	35.94	35.08	38.09	37.40
	6	26.58	28.68	31.15	33.21	34.13	36.96	35.65
	7	26.22	26.99	29.67	32.33	32.42	35.04	35.95
	8	26.61	28.68	29.45	33.68	34.06	34.74	36.00
	9	25.76	26.94	31.41	31.92	32.54	34.51	35.13
	10	25.57	26.86	29.81	32.15	33.17	35.26	34.95
	Average	26.20	27.82	30.24	32.88	32.93	35.26	35.27
	Standard Deviation	1.13	1.50	1.47	1.63	1.57	1.82	2.08
EM-1	1	24.20	25.76	27.62	30.09	30.56	32.22	32.39
	2	26.40	26.39	29.84	32.78	32.55	34.41	35.45
	3	27.03	28.29	30.37	32.33	32.26	33.91	34.02
	4	27.71	28.89	32.24	34.65	35.67	37.60	38.19
	5	25.94	27.98	31.39	34.99	36.03	37.64	38.02
	6	25.88	27.01	29.66	32.80	34.08	35.93	36.27
	7	26.06	27.88	30.47	32.95	34.06	36.34	36.40
	8	25.12	25.81	27.85	30.54	30.67	33.19	33.34
	9	25.51	26.61	29.52	32.56	33.68	35.43	35.54
	10	27.85	29.22	32.30	35.46	34.76	36.80	37.04
	Average	26.18	27.38	30.13	32.92	33.43	35.35	35.67
	Standard Deviation	1.12	1.24	1.60	1.76	1.90	1.86	1.93

Table-3 Results of Calculated Specific Body Weight

Sample Name	Individual No.	Number of Days after Start of Administration (Days)					
		4	7	11	14	18	21
Control Group	1	1.04	1.14	1.23	1.21	1.31	1.27
	2	1.05	1.14	1.26	1.25	1.35	1.36
	3	1.08	1.15	1.26	1.25	1.32	1.55
	4	1.08	1.12	1.22	1.20	1.31	1.12
	5	1.09	1.20	1.33	1.30	1.41	1.39
	6	1.08	1.17	1.25	1.28	1.39	1.34
	7	1.03	1.13	1.23	1.24	1.34	1.37
	8	1.08	1.11	1.27	1.28	1.31	1.35
	9	1.05	1.22	1.24	1.26	1.34	1.36
	10	1.05	1.17	1.26	1.30	1.38	1.37
	Average	1.06	1.16	1.26	1.26	1.35	1.35
	Standard Deviation	0.02	0.04	0.03	0.03	0.03	0.11
EM-1	11	1.06	1.14	1.24	1.26	1.33	1.34
	12	1.00	1.13	1.24	1.23	1.30	1.34
	13	1.07	1.12	1.20	1.19	1.25	1.26
	14	1.04	1.16	1.25	1.29	1.36	1.38
	15	1.08	1.21	1.35	1.39	1.45	1.47
	16	1.04	1.15	1.27	1.32	1.39	1.40
	17	1.07	1.17	1.26	1.31	1.39	1.40
	18	1.03	1.11	1.22	1.22	1.32	1.33
	19	1.04	1.16	1.28	1.32	1.39	1.39
	20	1.05	1.16	1.27	1.25	1.32	1.33
	Average	1.05	1.15	1.26	1.28	1.35	1.36
	Standard Deviation	0.02	0.03	0.04	0.06	0.06	0.06

Table-4 Intake of Drinking Water per Cage (ml)

Sample Name	Cage No.	Individual No.	1Week	2Weeks	3Weeks	Total	ml/Animal
Control Group	1	1-5	248	212	176	636	127.2
	2	6-10	132	256	225	713	142.6
EM-1	3	1-5	206	188	166	560	112.0
	4	6-10	204	192	162	558	111.6

Table-5 Daily Intake of Drinking Water per Mouse (ml)

Sample Name	Cage No.	Individual No.	1Week	2Weeks	3Weeks	Average
Control Group	1	1-5	7.09	6.06	5.03	6.06
	2	6-10	6.63	7.31	6.43	6.79
		Average	6.86	6.69	5.73	
		Standard Deviation	0.33	0.88	0.99	
EM-1	3	1-5	5.89	5.37	4.74	5.33
	4	6-10	5.83	5.49	4.63	5.31
		Average	5.86	5.43	4.69	
		Standard Deviation	0.04	0.08	0.08	

Table-6 Intake of Diet per Cage (g)

Sample Name	Cage No.	Individual No.	1Week	2Weeks	3Weeks
Control Group	1	1-5	163.7	183.1	158.5
	2	6-10	159.3	167.8	166.5
EM-1	3	1-5	185.4	185.5	183.7
	4	6-10	166.0	178.5	225.6

Table-7 Daily Intake of Diet per Mouse (g)

Sample Name	Cage No.	Individual No.	1Week	2Weeks	3Weeks
Control Group	1	1-5	4.68	5.23	4.53
	2	6-10	4.55	4.79	4.76
EM-1	3	1-5	5.30	5.30	5.25
	4	6-10	4.74	5.10	6.45

Table-8 Diet Intake and Feed Efficiency of Mice (g)

Sample Name	Individual No.	Body Weight Gain (g)	Diet Intake (g)		Feed Efficiency (%)
			One Animal/ 3Weeks	One Animal/ Day	
Control Group	1	6.61	101.06	4.81	6.51
	2	9.41	101.06	4.81	9.31
	3	13.65	101.06	4.81	13.51
	4	3.58	101.06	4.81	3.54
	5	10.47	101.06	4.81	10.36
	6	9.07	98.72	4.70	9.19
	7	9.73	98.72	4.70	9.86
	8	9.39	98.72	4.70	9.51
	9	9.37	98.72	4.70	9.49
	10	9.38	98.72	4.70	9.50
	Average	9.07	99.89	4.76	9.08
	Standard Deviation	2.58	1.23	0.06	2.58
EM-1	11	8.19	110.92	5.28	7.38
	12	9.05	110.92	5.28	8.16
	13	6.99	110.92	5.28	6.30
	14	10.48	110.92	5.28	9.45
	15	12.08	110.92	5.28	10.89
	16	10.39	114.02	5.43	9.11
	17	10.34	114.02	5.43	9.07
	18	8.22	114.02	5.43	7.21
	19	10.00	114.02	5.43	8.77
	20	9.19	114.02	5.43	8.06
	Average	9.49	112.47	5.36	8.44
	Standard Deviation	1.47	1.63	0.08	1.31

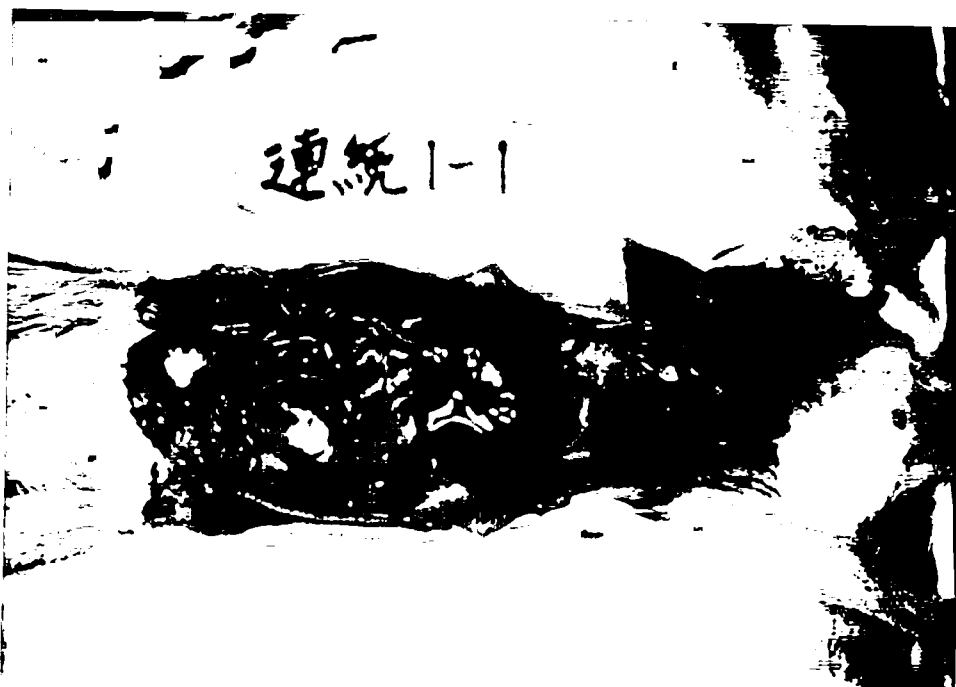


Photo 1 Control Group Individual No.1



Photo 2 Control Group Individual No.2



Photo 3 Control Group Individual No.3

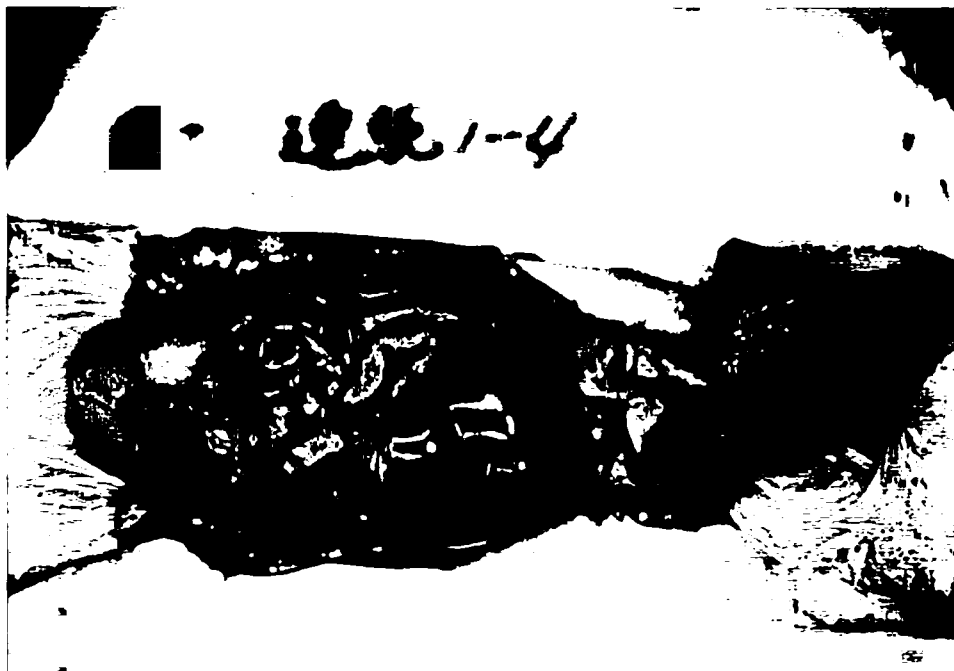


Photo 4 Control Group Individual No.4

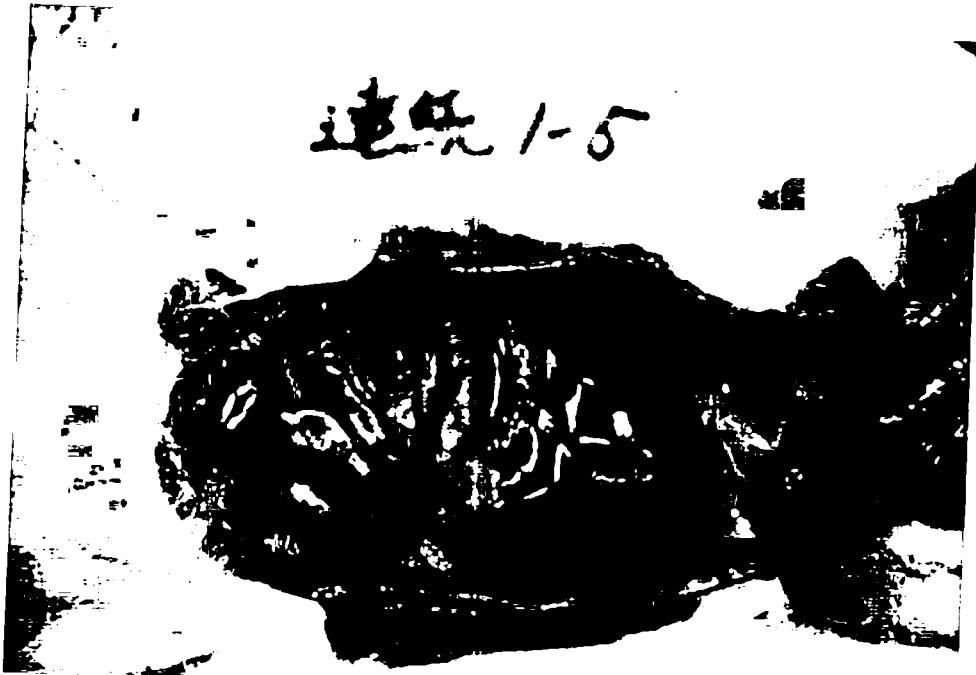


Photo 5 Control Group Individual No.5



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

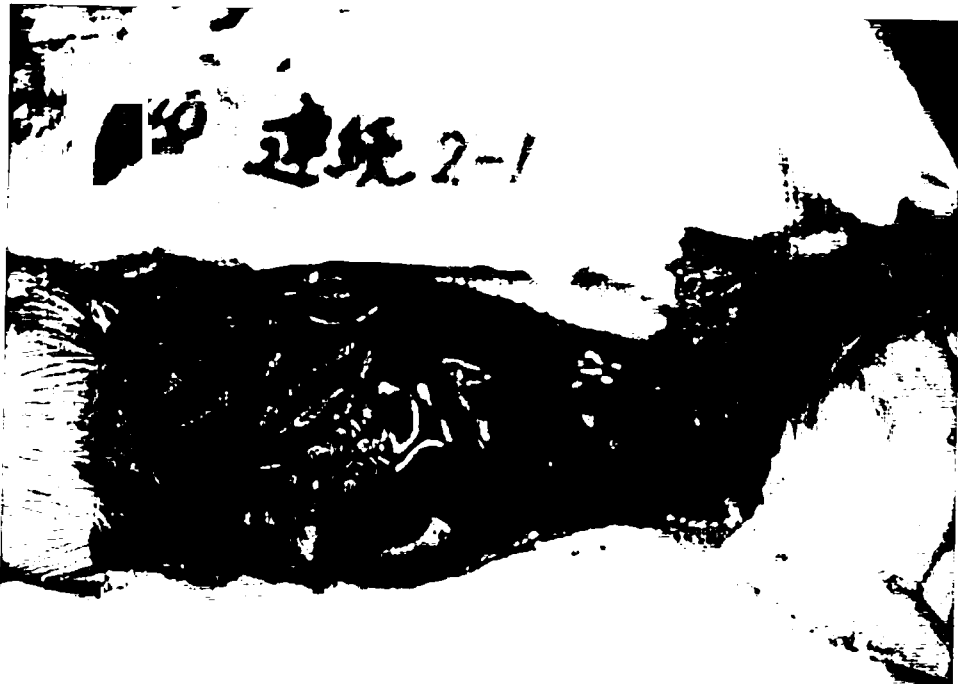


Photo 11 Sample Dose Group Individual No.1



Photo 12 Sample Dose Group Individual No.2



Photo 13 Sample Dose Group Individual No.3



Photo 14 Sample Dose Group Individual No.4

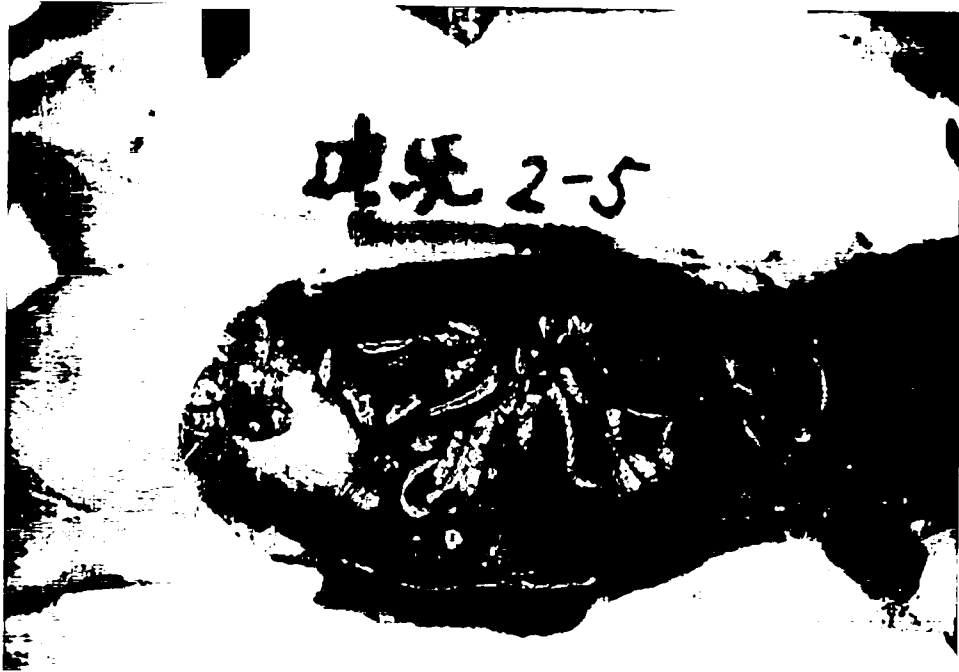


Photo 15 Sample Dose Group Individual No.5



Photo 16 Sample Dose Group Individual No.6



Photo 17 Sample Dose Group Individual No.7



Photo 18 Sample Dose Group Individual No.8

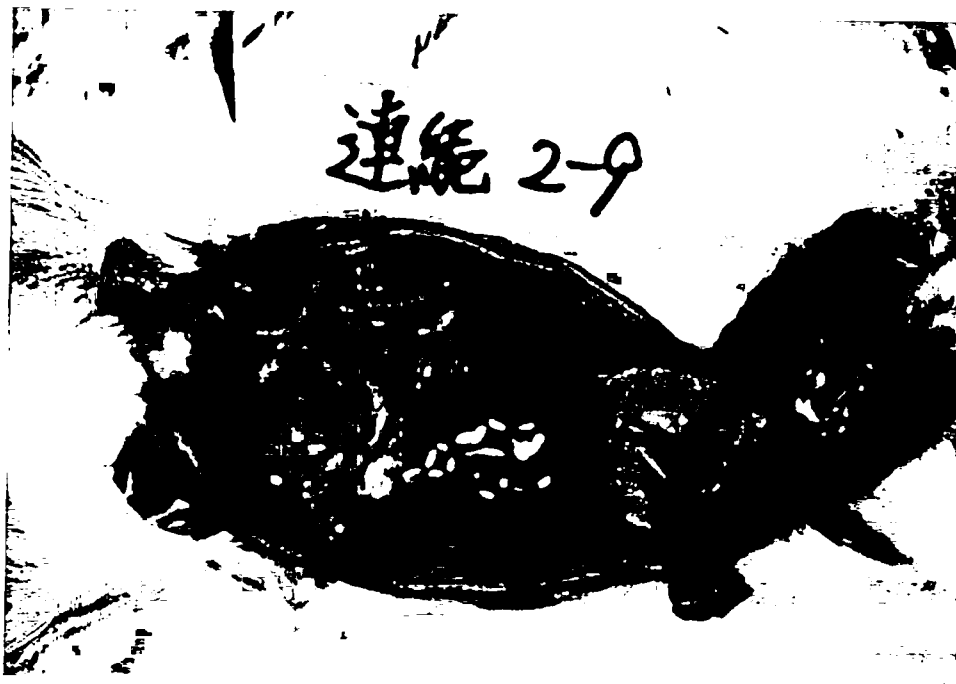


Photo 19 Sample Dose Group Individual No.9

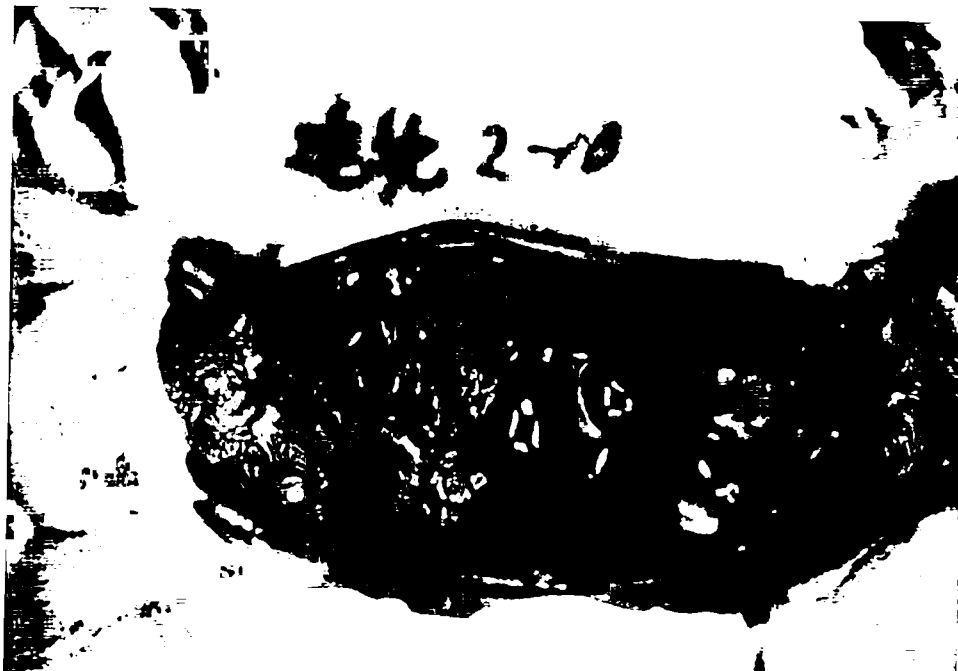


Photo 20 Sample Dose Group Individual No.10