From: Kathy Brailer on behalf of Claire Kruger
To: Morissette, Rachel
Cc: Dietrich Conze; Kathy Brailer; Jennifer Symonds; Claire Kruger
Subject: FW: questions for GRN 000800 gamma-oryzanol
Date: Monday, December 03, 2018 1:39:21 PM
Attachments: image007.png
image001.png
image003.png
Lee Lab Anim Res 2012.pdf
GRN 800 Response to FDA 12-3-18.pdf
Maruoka et al., 1972.pdf
Hasato et al. 1974.pdf

Dear Dr. Morissette,

In response to your e-mail below from November 19, 2018, attached are our responses to FDA's questions on GRN 000800. Please confirm receipt and let us know if you have any additional questions.

Best regards,

Claire Kruger, PhD, DABT, CFS
President
Senior Director Regulatory Affairs

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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, November 19, 2018 1:39 PM
To: Claire Kruger <ClaireK@chromadex.com>
Subject: questions for GRN 000800 gamma-oryzanol

Dear Dr. Kruger,

Please see attached our questions to be addressed for GRN 000800. Please let me know if you have questions.

Best regards,

Rachel Morissette, Ph.D.
Acting Supervisory Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
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ChromaDex
SPHERIX CONSULTING

December 3, 2018

Rachel Morissette, Ph.D.
Acting Supervisory Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
5001 Campus Drive, HFS-255
College Park, MD 20740

RE: Response to FDA Questions on GRN 800 - Gamma-oryzanol

Dear Dr. Morissette:

In response to your e-mail of November 19, 2018, following are our responses to FDA's questions on GRN 800. FDA's questions are in italics and our responses are in plain text.

1) *Oryza indicates that its commercial γ-oryzanol product derived from Oryza sativa Japonica rice bran consists of five different ferulates. The notice states "product specifications and other quality testing…in place to control the levels of the predominating sterol ferulates…"; however, it is not clear how Oryza will do this. The content of natural products tends to vary with growing conditions, such as season, weather, soil, etc. and across time. Please explain how Oryza will maintain or control the levels of chemical components in its product and what are the parameters associated with this.*

Although natural product composition can vary with growing conditions, the relative abundance of the predominating ferulates in ORYZA GAMMAX does not vary more than 10% from year to year (see Table 4 (page 11) of the GRN 800 and note that the batches were produced over the course of 2015 and 2016 (the dates of manufacture for the four batches presented in Table 4 are provided in Table 3 (page 10))). Importantly, Oryza controls the sum of the predominating sterol ferulates with the "γ-oryzanol" product specification (see Table 3) using a validated HPLC method that quantitates the amount of cycloartenol ferulate, 24-methylene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, cycloartanol ferulate, and cyclobranol ferulate. Thus, the product contains not less than 85% of the combined amount of cycloartenol ferulate, 24-methylene cycloartanol ferulate, campesterol ferulate, β-sitosteryl ferulate, cycloartanol ferulate, and cyclobranol ferulate. Additionally, by qualifying each batch for release against the γ-oryzanol specification, the levels of the predominating sterol ferulates are monitored.

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2) *On page 32 in the "Genotoxicity Studies" section (Section VI.B), Oryza states that the genotoxicity of γ-oryzanol is corroborated (i.e., substantiated) by several DNA-related tests. However, the subsequent discussion of the results of these various tests indicates that γ-oryzanol is not genotoxic. Please clarify these conflicting points.*

We agree that this statement is misleading and the studies in Section VI.B show that γ-Oryzanol is not genotoxic. We have therefore edited this paragraph to more accurately reflect the results of the genotoxicity testing. Please replace the first sentence of the discussion under "Section VI.B. Genotoxicity Studies" (page 32) with the following text:

"ORYZA GAMMAX is not expected to be genotoxic based on the results reported by Tsushimoto et al., 1991. Using a preparation of γ-oryzanol resuspended in acetone with an unknown sterol ferulate composition, Tsushimoto et al. conducted DNA repair (Rec assay), bacterial reverse mutation (Ames assay), and in vivo chromosomal aberration tests."

3) *In the summary of the toxicology studies, the NOAEL is stated as 3000 mg γ-oryzanol/kg body weight (bw)/day, the highest exposure level tested (e.g., page 33). However, in other sections of the notice, Oryza states that the NOAEL is 2000 mg/kg bw/day (e.g., page 3, point #7; page 44, paragraph 1; page 60, point #7). Please clarify the NOAEL selected by Oryza.*

This was a typographical error and the correct NOAEL is 2000 mg/kg bw/day. Please replace the second sentence in the discussion under "Section VI.C.1 Summary" (page 33) with the following sentence:

"The no observed adverse effect level (NOAEL) for ORYZA GAMMAX was determined to be 2000 mg/kg/day."

4) *In the notice, Oryza discusses a white substance observed in the feces of male and female rats at the 1000 and 2000 mg/kg bw/day exposure levels of γ-oryzanol (page 38), in addition to the 3000 mg/kg bw/day exposure, suggesting that the γ-oryzanol test substance is not readily absorbed. Oryza noted that the white substance was not observed consistently throughout the study. However, it was present for the last 33-34 days of the 1000 mg/kg/day treatment group and for the last 87-88 days of the 90-day study in the 2000 mg/kg bw/day treatment group. This suggests that the white substance was present consistently during exposure to γ-oryzanol. Please clarify this inconsistency.*

Thank you for identifying this inconsistency and we agree that the white substance was present in the feces during the study and likely the result of undigested test article (γ-oryzanol). Please replace the first paragraph on page 38 of the Notification with the following text:

"A white substance was observed in the feces of all males and females from day 57–58 to day 90 in the 1000 mg/kg body weight/day group and all males and females from day 2–3 to day 90 in the 2000 mg/kg body weight/day group. Previous work has found that γ-oryzanol is poorly absorbed (Mandak and Nystrom, 2012). Although not confirmed, the white substance was assumed to be ORYZA GAMMAX due to what is known about its poor absorption and presence in ORYZA GAMMAX-treated, but not control-treated rats. Importantly, it was not

toxicologically significant due to the fact that no macroscopic findings or lesions were noted in the gastrointestinal tract. There were also no abnormal ophthalmological findings in any rats. An open toe wound with nail damage, hemorrhage, and/or crust formation was observed on the left hind limb of one male on days 50–57 and on the right forelimb of one female on days 44–49 in the 2000 mg/kg body weight/day group. These findings were incidental changes unrelated to ORYZA GAMMAX, since they were not observed in any other rats throughout the study."

5) *Two corroborative animal studies with γ-oryzanol (i.e., chronic toxicity and developmental studies) are published in Japanese and no translated copy of the studies were provided. Please provide a certified English translation of these published studies.*

English translations of both studies were submitted with the original Notification. We have attached the English translations of these studies to this response to expedite review. The toxicology reports were translated by Oryza Oil & Fat Chemical Co., Ltd. based on the test results provided by the Institute of Medial Science, the University of Tokyo. Oryza hereby confirms that the translation and original test results are consistent.

6) *Oryza discusses the body weight results in the first paragraph on page 46. First, it is stated that there is no change in body weight at certain exposure levels. Later it is stated that there is a decrease in body weight at similar exposure levels in the same study. Possibly some relevant descriptive information is missing in this section. Please clarify this discrepancy.*

The two discrepant statements refer to the body weight changes in males and females and we agree that relevant descriptive information is missing. Please replace the paragraph that includes this discussion (beginning on the bottom of page 45 of the Notification) with the following text:

"In the 1000 mg/kg-treated group, one male rat died on the 17th day, one female rat died on the 73rd day, one female rat died on the 121st day, and on male rat died on the 128th day of the study. In the 300 mg/kg-treated group on male rat died on the 84th and one male rat died on 143rd day of the study. There were no deaths in the other groups. The causes of the deaths were not reported. Nasal inflammation was observed intermittently throughout the study in all groups including control animals and was most severe at approximately the 100th day. Although there was a transient decrease in the body weights of the γ-oryzanol-treated male rats during the 14th week of the study, there were no significant differences feed intakes or body weight gains during the study. Moreover, there were no significant differences in absolute body weights between the control- and γ-oryzanol-treated male rats at the end of the study. In female rats, significant reductions in absolute body weight were noted in the 30 and 300 mg/kg-treated groups at weeks 6, 8, 10, 12, 16, 18, 20, 24 and 25 and weeks 24 and 25 in the 100 mg/kg-treated group compared to the control group. No significant reductions were noted in the 1000 mg/kg group females compared to controls. Additionally, were no significant differences in feed intakes or body weight gains between the treated and control groups. Importantly, because the weight range reductions were not dose-dependent and there were no differences in body weight gain, the changes were considered to be the result of having fewer numbers of rats after the interim investigation rather than a toxic effect of the test article."

7) Throughout Part 6 of the notice, Oryza makes comparisons to "historical" control values for different studies, using that as justification as to why an effect is not treatment related. However, those historical control values (e.g., BUN, BWs, total cholesterol) or information about their source were not provided. Please provide the "historical" reference values and information cited throughout all relevant studies.

There are two references to "historical control values" in Part 6 of the Notification. One reference is in the third paragraph on page 38 and the other is in the third paragraph on page 44. The reference on page 38 is addressed in our response to question 8. The reference on page 44 is from a peer-reviewed study that was published in 1992. It will not be possible to obtain the supporting studies for the second reference.

8) *The treatment-related BUN increase in the 2000 mg γ-oryzanol/kg/day group and the treatment-related increases in total cholesterol in male rats (i.e., change at $p<0.01$ level) and in females (non-significant trend) at the 2000 mg γ-oryzanol/kg/day exposure level in the pivotal study are not adequately explained. Please address these findings.*

According to Table 16 (page 40) in GRN 800, the increases in BUN and total cholesterol were only seen in the γ-oryzanol-treated males and only the increases in the 2000 mg/kg bw/day-treated male were statistically significant compared to the control-treated males. No significant, non-significant trend, or dose-responsive effects were seen in the γ-oryzanol-treated females. Importantly, the increases in the BUN and total cholesterol noted in the γ-oryzanol-treated males were within the historical ranges reported by the laboratory that conducted the study (Lee et al., 2012) and not seen in the γ-oryzanol-treated females, indicating that the effects were not treatment-related. Moreover, this study was subjected to peer-review prior to publication and thus, the conclusions were deemed acceptable by the reviewers. We have revised the discussion of these results accordingly. Please replace the third paragraph on page 38 with the following text:

"There were no significant differences in hematology parameters in males and females between the γ-oryzanol- and control-treated groups (Table 15). Except for the blood urea nitrogen (BUN) and total cholesterol (T-Chol), which were significantly increased in males from the 2000 mg/kg/day group, there were no significant differences in the remaining blood chemistry parameters in males and females between the control and 1000, 2000 mg/kg/day-treated groups. Importantly, the significant increases in BUN and total cholesterol were considered to be not toxicologically significant because they were not observed in both sexes and were within the historical ranges reported by the laboratory that conducted the study (Lee et al., 2012)."

Also, please add the following reference to the reference list provided in section "VII. Supporting Data and Information":

"Lee, J.M., Lee, M.A., Do, H.N., Song, Y.I., Bae, R.J., Lee, H.Y., Park, S.H., Kang, J.S., and Kang, J.K. (2012). Historical control data from 13-week repeated toxicity studies in Crj:CD (SD) rats. Lab Anim Res *28*, 115–121."

9) Please provide the date (month, year) through which an updated literature search was conducted.

The literature search was completed on January 29, 2018.

Should you have additional questions, please let us know.

Sincerely,

(b) (6)

Claire L, Kruger, Ph.D., D.A.B.T.
President

Attachments

Hasato H, Yamamoto T, Tadokoro I, Kawamura A, Suzuki K, Nishioka H, Ougi M, Hamajima K, Goto A, Toyomasu T, Sakamoto M. γ-Oryzanol (Oliver table) chronic toxicity test. Kiso to Rinsho 1974; 8: 3417.

Lee, J.M., Lee, M.A., Do, H.N., Song, Y.I., Bae, R.J., Lee, H.Y., Park, S.H., Kang, J.S., and Kang, J.K. (2012). Historical control data from 13-week repeated toxicity studies in Crj:CD (SD) rats. Lab Anim Res *28*, 115–121.

Maruoka H and Kume M. Toxicity test on γ-oryzanol – Influence of γ-oryzanol on fetuses and offspring of mice and rats. Kiso to Rinsho. 1972, 6: 1717.

Kiso to Rinsho, 8, 3417 (1974)

γ-Oryzanol (Oliver Tablet) Chronic Toxicity Test

Toxicity Research Group, the Institute of Medical Science, the University of Tokyo
Hikozaemon Hasato, Tadashi Yamamoto, Ichiro Tadokoro
Akiyoshi Kawamura, Kiyoshi Suzuki, Hisatoshi Nishioka
Minoru Ougi, Kenji Hamajima, Atsushi Goto
Tsubasa Toyomasu, Motoko Sakamoto

Pathology Class, School of Medicine, Juntendo University
Takahiro Hashimoto, Sigeki Saiki

γ-oryzanol is a mixture of ferulic acid ester of several types of triterpene alcohol extractively separated from rice bran oil by Tsuchiya et al.[1)-3)] in 1953.
Regarding the pharmacological activities of γ-oryzanol, activities to accelerate rats' growth and stimulate their sexual glands[4)-6)] as well as activities on adenohypophysial cells[7)] have been reported.
Oliver Tablet contains 50 mg, 25 mg, or 5 mg of γ-oryzanol.
The author and group members carried out a test to study chronic toxicity of γ-oryzanol by orally administrating it to rats for 6 months (181 days). The results are described below.

I. Test samples and test method

1. Animals used and rearing conditions

140 rats, 70 male and 70 female 4 week old Wistar-Imamichi rats, were individually kept in gauge cages and used for the test after 6 days of preliminary observation. The room was controlled at a temperature of 24±2 °C and humidity of 50±10 % throughout the test period. Solid feed for mice and rats produced by Funabashi Nojo was freely accessible to the rats along with tap water from a water feed bottle.

2. Preparation of the drug solution and administration method

The drug was suspended in 0.5 % carboxymethylcellulose (CMC solution). It was forcibly administered orally in each group by the dose shown below while correcting the dose every week according to the rats' weight which was measured weekly. Only 0.5 % CMC solution was forcibly administered orally to the control group by the same amount.

3. Observation method and observation items

The weight of all rats was measured weekly throughout the administration period. The remaining feed amount was also measured to estimate the amount of feed that the rats in each group ingested.
On the 27th day after the administration of the drug started, 6 male and 6 female rats were

randomly sampled from each group except for the group that took 30 mg/kg. 5 male and 5 female rats were randomly sampled from the group that took 30 mg/kg. Blood was taken from them for hematological investigation, they were killed under ether anesthesia, partial inspection was performed, and the weight of each organ was measured in order to obtain interim study material to be used for histopathological investigation.

When the test ended (181st day after the test started), 6 male and 6 female rats were sampled from each group and 5 male and 5 female rats were sampled from the group that took 30 mg/kg. Blood was taken from them for hematological and biochemical investigation, they were killed under ether anesthesia, partial inspection was performed, and the weight of each organ was measured so that histopathological study materials were obtained.

Table 1

Group	No. of Rats		Drug Dose mg/kg/day	Administration Days
	Male	Female		
Control group	15	15	0	6 male and female rats were killed for interim investigation on the 27th day. The drug was forcibly administered orally to live rats for 181 days.
Group 1	15	15	1,000	
Group 2	15	15	300	
Group 3	15	15	100	
Group 4	10	10	30	

II. Test Results

1. Appearance

Nasal inflammation was observed on all rats throughout the test period. This symptom repeatedly came and went. Heavy nasal inflammation was observed on many rats especially around the 100th day after the test started. Some rats died between the 120th and 140th days.

2. Cases of Death

In the group that took 1,000 mg/kg of the sample, one male rat died on the 17th day, one female rat died on the 73rd day, one female rat died on the 121st day, and one male rat died on the 128th day.

In the group that took 300 mg/kg of the sample, one male rat died on the 84th day and one male rat died on the 143rd day. There were no deaths in other groups.

Partial inspection was performed on the dead rats for histopathological investigation.

3. Drug dose

Table 1 shows the drug dose. Since the dose was corrected every week according to the weight of each rat, the actual drug dose per rat increased as rats' weight increased.

4. Feed Intake

Tables 2-1 and 2-2 show the feed intake.

5. Weight change

Tables 3-1 and 3-2 show the mean weight of each group. Fig. 1 shows the weight curve. The weight of rats increased similarly in all groups. However, the rats experienced severe nasal inflammation and lost weight in the 14th week late August probably because of an unusually sudden drop in ambient temperature. They did not seem to quickly recover from this condition until the final investigation. When the control group and administration groups were compared, no significant difference was observed in the control group and 1,000 mg/kg, 300 mg/kg, and 100 mg/kg administration groups. However, a significant drop with a 5 % risk rate was found between the 30 mg/kg administration group and the control group in the 14th week. The significant drop observed only in the 30 mg/kg administration group is considered to have been caused because the number of rats decreased to 5 in group 1 after the interim investigation (27th day and later).

In female rats, no significant difference was observed between the control group and 1,000 mg/kg administration group. However, in the 300 mg/kg administration group, a significant drop with a 5 % risk rate was observed in all weeks except for the period between the 4th week and 14th week. In the 24th week, a significant drop with a 1 % risk rate was observed. In the 100 mg/kg administration group, a significant drop with a 5 % risk rate was observed from the 22nd week to the final investigation. In the 30 mg/kg administration group, a significant drop with a 5 % risk rate was observed in the 4th, 6th, 8th, 18th, 22nd, and 24th weeks, though the rats' weight was low overall. This is considered to have been caused because the number of rats was fewer than other groups after the interim investigation.

6. Hematological findings

To obtain hematological findings, the red blood cell count, white blood cell count, and the type of white blood cell were tested. Tables 4-1 and 4-2 show the results. No significant difference was observed in male rats at the interim and final investigations. In female rats at the interim and final investigations, a significant difference was observed in white blood cell count, red blood cell count, and percentage of lymphocytes and neutrophils as compared to the control group in some groups. However, no positive correlation with drug dose was observed. No changes considered to have been caused by the toxicity of the drug were observed either.

Table 2-1 Average (Feed) Intake

( ) ± S. E.

Test Group	Sex	Total intake (G)	Week 0	1	2	3	4	5	6	7	8	9	10
Control Group	♂	4270. 2		19. 4 (0. 31)	22. 9	25. 1	25. 3	24. 0 (0. 33)	24. 6	25. 2	24. 6	25. 6 (0. 45)	26. 3
1, 000 mg/kg/day	♂	4183. 9		19. 6 (0. 48)	23. 5	24. 5	25. 6	24. 1 (0. 58)	23. 9	25. 0	24. 3	24. 2 (0. 41)	23. 8
300 mg/kg/day	♂	4142. 3		19. 5 (0. 30)	23. 6	25. 0	26. 1	25. 0 (0. 48)	23. 9	25. 1	25. 1	23. 8 (0. 62)	24. 3
100 mg/kg/day	♂	4260. 3		19. 1 (0. 30)	23. 0	24. 9	26. 9	25. 9 (0. 71)	25. 5	25. 7	25. 2	25. 1 (0. 54)	25. 1
30 mg/kg/day	♂	4157, 6		19. 6 (0. 30)	23. 3	25. 2	25. 7	24. 6 (1. 01)	23. 1	23. 3	23. 9	24. 7 (1. 16)	22. 9
Control Group	♀	3193. 0		17. 2 (0. 41)	17. 4	18. 1	19. 5	19. 1 (0. 90)	18. 2	18. 4	17. 9	18. 4 (0. 84)	18. 3
1, 000 mg/kg/day	♀	3146. 5		18. 6 (0. 37)	18. 9	18. 8	18. 7	18. 7 (0. 47)	17. 6	17. 6	17. 3	17. 5 (0. 59)	17. 3
300 mg/kg/day	♀	2927. 6		17. 4 (0. 29)	17. 1	17. 6	16. 8	17. 1 (0. 52)	16. 3	16. 4	16. 8	17. 0 (0, 47)	16. 0
100 mg/kg/day	♀	3045. 5		17. 9 (0. 33)	17. 8	17. 7	17. 7	17. 9 (0. 55)	17. 6	17. 6	17. 4	17. 9 (0. 78)	17. 3
30 mg/kg/day	♀	2850. 8		17. 0 (0. 41)	17. 8	17. 1	14. 4	17. 9 (1. 59)	16. 8	16. 8	16. 4	16. 1 (0. 96)	16. 2

Table 2-2 Average (Feed) Intake

( ) ± S. E.

Week	Average Daily Intake														
11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
25. 2	25. 2	24. 6	21. 5 (0. 69)	17. 9	21. 3	23. 6	23. 1 (23. 1)	22. 3	25. 3	24. 7	24. 9 (0. 53)	24. 3	23. 7	23. 0	25. 0 (0. 53)
23. 5	23. 8	23. 9	19. 9 (1. 76)	19. 4	21. 3	22. 4	22. 8 (0. 61)	22. 3	23. 8	23. 7	24. 6 (1. 31)	24. 8	23. 8	23. 3	24. 0 (1. 15)
23. 4	24. 0	21. 7	18. 2 (0. 94)	19. 3	20. 7	21. 2	23. 0 (1. 21)	21. 8	24. 2	24. 3	23. 8 (0, 52)	24. 0	23. 2	21. 9	23. 7 (0. 44)
24. 4	24. 4	20. 3	16. 8 (2. 15)	18. 6	22. 9	23. 5	23. 3 (1. 36)	24. 4	25. 9	25. 2	24. 0 (0. 81)	24. 3	24. 2	23. 9	24. 7 (0. 47)
23. 9	23. 2	22. 4	13. 2 (3. 06)	18. 3	22. 3	23. 9	23. 9 (1. 04)	22. 4	24. 6	25. 0	25. 1 (1. 14)	24. 6	23. 6	25. 4	24. 5 (1. 32)
17. 3	17. 1	16. 9	12. 2 (1. 41)	15. 2	16. 7	18. 4	16. 2 (1. 43)	16. 7	19. 6	19. 1	19. 3 (0. 75)	19. 9	18. 5	17. 4	18. 1 (0. 65)
16. 9	16. 5	14. 9	12. 8 (1. 44)	13. 0	16. 9	16. 5	17. 2 (0. 82)	18. 2	18. 8	18. 7	18. 7 (1. 06)	18, 6	18. 5	19. 2	18. 6 (0. 59)
15. 7	15. 7	15. 3	14. 4 (0. 42)	14. 4	16. 1	15. 5	14. 2 (0. 99)	15. 5	17. 6	16. 9	17. 2 (0. 41)	17. 0	16. 3	15. 8	16. 1 (0. 65)
16. 5	16. 9	16. 0	13. 6 (0. 75)	14. 4	15. 5	17. 7	16. 0 (0. 67)	15. 3	17. 5	17. 0	17. 3 (0. 59)	17. 6	17. 1	16. 9	17. 7 (0. 86)
15. 4	15. 1	14. 0	12. 5 (1. 21)	16. 6	16. 0	13. 7	11. 8 (1. 90)	17. 1	17. 9	14. 9	15. 7 (0. 86)	16. 6	16. 1	15. 2	16. 1 (1. 25)

Table 3-1 Mean Weight

( ) ± S. E.

Test Group	Sex	Increase in Weight	Week 0	1	2	3	4	5	6	7	8	9	10
Control Group	♂	429.6	97.0 (1.13)	164.7	218.4	274.2 (4.26)	309.1	343.9	377.5 (4.17)	407.0	418.6	431.3 (5.54)	456.0
1,000 mg/kg/day	♂	412.1	96.3 (2.32)	164.4	220.2	268.3 (6.78)	313.0	342.9	372.4 (6.21)	401.0	423.3	434.7 (3.08)	457.8
300 mg/kg/day	♂	398.4	97.7 (1.28)	167.9	222.9	276.8 (4.33)	319.8	352.6	383.6 (5.53)	411.1	432.6	446.6 (3.08)	460.8
100 mg/kg/day	♂	422.0	98.3 (1.65)	166.3	220.5	273.4 (3.80)	321.6	353.9	389.3 (5.68)	415.3	434.7	448.4 (7.33)	465.3
30 mg/kg/day	♂	417.2	95.2 (1.56)	170.8	223.7	275.8 (6.54)	305.8	337.6	365.0 (8.63)	389.2	412.4	429.0 (12.30)	443.4
Control Group	♀	275.2	95.2 (1.49)	147.6	171.1	197.9 (3.61)	223.4	244.2	260.9 (8.90)	276.6	286.3	299.2 (10.89)	310.9
1,000 mg/kg/day	♀	247.7	98.7 (1.07)	151.6	177.4	205.8 (3.88)	228.3	247.8	266.2 (3.24)	277.8	287.8	296.7 (5.01)	304.8
300 mg/kg/day	♀	221.8	97.0 (1.55)	146.8	170.1	195.1 (3.05)	206.6	224.8	241.1 (2.90)	252.8	263.5	275.5 (4.44)	280.4
100 mg/kg/day	♀	232.0	97.0 (1.48)	150.2	176.5	203.1 (3.85)	223.9	239.3	260.0 (7.07)	271.6	281.6	289.1 (10.23)	300.1
30 mg/kg/day	♀	214.8	97.2 (2.55)	145.1	173.1	193.9 (5.98)	196.4	222.6	234.0 (7.92)	248.2	261.2	266.8 (13.91)	273.6

Table 3-2 Mean Weight

( ) ± S. E.

Week 11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
471.9	474.1 (9.68)	484.7	482.3	462.0 (14.74)	470.1	482.2	486.3 (13.43)	498.2	509.6	512.8 (10.70)	519.7	525.1	529.3	526.6 (14.02)	
466.7	460.3 (11.14)	487.8	463.6	472.4 (13.99)	467.1	472.9	481.3 (12.70)	490.9	495.3	500.3 (13.02)	505.9	512.6	509.1	508.4 (13.65)	
472.0	488.3 (2.84)	487.8	469.9	468.6 (8.56)	465.1	463.9	469.8 (11.13)	477.3	484.5	490.0 (9.04)	491.1	497.4	497.6	496.1 (9.10)	
475.3	485.6 (7.52)	474.3	457.1	458.0 (10.36)	478.3	472.7	474.6 (11.94)	493.8	500.3	508.0 (6.83)	502.3	516.9	521.4	520.3 (4.03)	
455.6	467.6 (14.44)	473.6	432.6	442.8 (15.39)	461.2	475.0	479.2 (13.42)	490.2	495.6	501.2 (19.42)	505.4	509.4	508.4	512.4 (19.96)	
316.3	322.8 (13.47)	328.9	317.6	327.8 (13.24)	333.1	343.8	337.4 (16.66)	345.2	351.6	358.4 (18.81)	362.4	367.8	373.0	370.4 (19.44)	
311.5	317.2 (5.40)	304.6	300.6	299.9 (15.87)	313.1	313.9	320.4 (14.00)	331.7	332.0	335.7 (13.77)	337.9	339.0	342.6	346.4 (18.78)	
284.5	292.7 (2.35)	297.4	299.1	300.4 (6.04)	304.1	305.0	293.9 (8.90)	300.3	305.5	310.8 (6.70)	314.6	318.4	316.1	318.8 (6.02)	
304.3	311.2 (11.70)	306.8	301.9	304.4 (6.67)	309.3	319.0	316.1 (8.35)	320.1	323.8	322.1 (8.59)	323.8	326.8	328.4	329.0 (7.15)	
278.0	286.2 (15.98)	281.6	283.6	286.4 (23.66)	299.4	287.2	285.4 (12.17)	299.6	302.6	302.8 (20.74)	304.6	307.4	314.2	312.0 (24.95)	

7. Biochemical findings

To obtain biochemical findings of plasma, S-GPT, S-GOT, blood sugar, S-alkaline phosphatase, cholesterol, urea nitrogen, LDH, Na, K, Cl levels, A/G ratio, and total protein were investigated. As shown in Tables 5-1 and 5-2, no significant changes were observed in any of the male and female groups. Although a slightly lower blood sugar level was observed in male rats of group 1 and female rats of group 3, the levels were within the normal range for rats.

Fig. 1 Weight Curve (Forced oral administration for 6 months)

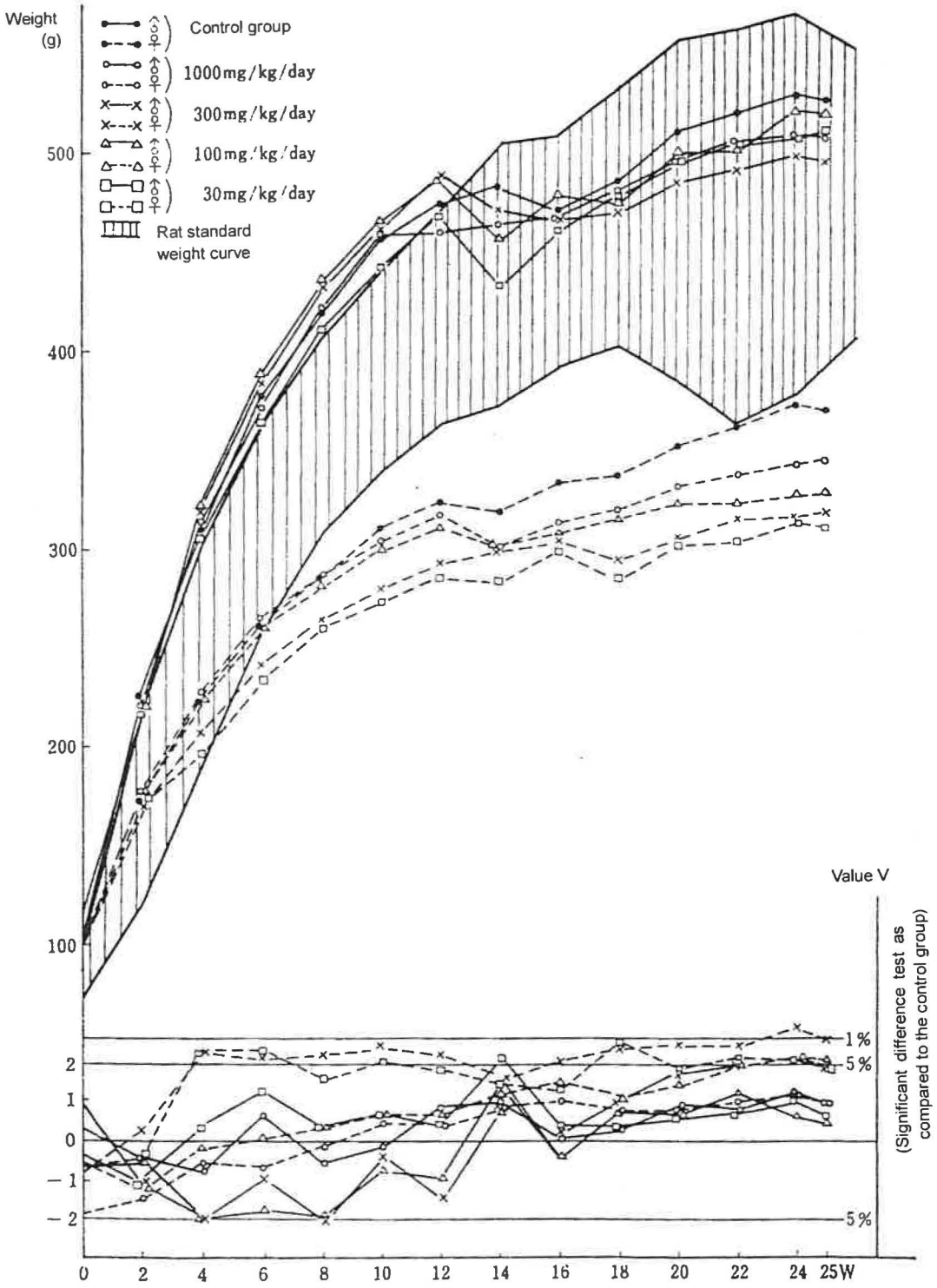


Table 4-1 Interim Investigation

Group	Sex		Red Blood Cell Count X10⁴	White Blood Cell Count	Differential Leukocyte Count: Lympho cytes	Neutro phils	Mono cyte	Eosino phil	Basophil
Control	♀	M	696. 2	5, 730	91. 8	6. 5	1. 08	0. 58	0. 08
		S E	6. 67	623	0. 67	0. 66	0. 28	0. 23	0. 08
Group 4	♀	M	617. 8**	5, 540	88. 0*	10. 5*	0. 50	1. 00	0
		S E	33. 51	570	1. 39	1. 43	0. 16	0. 27	
Group 3	♀	M	599. 4***	6, 020	84. 3**	13. 5*	1. 20	0. 50	0. 50
		S E	15. 23	365	7. 51	3. 05	0. 25	0. 27	0. 32
Group 2	♀	M	662. 3	5, 700	83. 5*	12. 8*	2. 33	1. 25	0. 17
		S E	20. 24	704	3. 36	2. 41	0. 70	0. 38	0. 11
Group 1	♀	M	680. 5	5, 630	83. 2**	14. 0**	1. 75	0. 50	0. 58
		S E	19. 61	415	1. 86	1. 88	1. 29	0. 13	0. 31
Control	♂	M	704. 0	6, 340	90. 1	8. 6	0. 90	0. 40	0
		S E	5. 96	1, 239	0. 82	1. 50	0. 18	0. 18	0
Group 4	♂	M	687. 6	8, 260	88. 3	10. 2	0. 80	0. 70	0
		S E	14. 99	1, 123	0. 75	0. 81	0. 34	0. 12	
Group 3	♂	M	703. 8	8, 930	87. 7	10. 9	0. 67	0. 58	0. 17
		S E	13. 13	10. 29	2. 72	3. 07	0. 25	0. 15	0. 11
Group 2	♂	M	710. 2	8, 170	85. 5	11. 2	2. 56	0. 75	0. 08
		S E	19. 94	8. 27	3. 98	2. 84	1. 15	0. 40	0. 08
Group 1	♂	M	692. 2	7, 670	89. 9	9. 2	0. 75	0. 33	0
		S E	8. 22	735	0. 97	1. 27	0. 36	0. 17	0

* 5 % significant difference, ** 1 % significant difference, *** 0.1 % significant difference

Table 4-2 Final Investigation

Group	Sex		Red Blood Cell Count X10⁴	White Blood Cell Count	Differential Leukocyte Count: Lympho cytes	Neutro phils	Mono cyte	Eosino phil	Basophil
Control	♀	M	795	11, 166	77. 25	19. 03	1. 83	1. 75	0. 08
		S E	11. 78	1491. 9	3. 38	3. 16	0. 21	0. 42	0. 08
Group 4	♀	M	734	6, 500*	82. 50	13. 90	2. 60	1. 00	0
		S E	11. 78	387. 3	3. 14	3. 10	0. 40	0. 44	0
Group 3	♀	M	706	9, 100	82. 83	13. 25	2. 33	1. 58	0
		S E	30. 10	364. 2	2. 13	2. 19	0. 40	0. 30	0
Group 2	♀	M	748	7, 883	82. 25	15. 75	1. 08	0. 83	0. 08
		S E	16. 87	566. 5	1. 82	1. 66	0. 45	0. 24	0. 08
Group 1	♀	M	738	8, 616	83. 50	12. 91	2. 33	1. 16	0. 08
		S E	17. 58	500. 2	2. 13	2. 04	0. 44	0. 40	0. 08
Control	♂	M	788	11, 050	81. 2	16. 4	1. 25	1. 0	0. 08
		S E	21. 38	719. 6	0. 88	0. 87	0. 30	0. 35	0. 08
Group 4	♂	M	793	9, 720	78. 2	19. 3	1. 60	0. 9	0
		S E	26. 57	835. 7	4. 54	4. 24	0. 29	0. 29	0
Group 3	♂	M	771	10, 733	73. 0	24. 8	0. 91	1. 16	0
		S E	17. 74	801. 9	3. 64	3. 52	0. 23	0. 30	0
Group 2	♂	M	718	10, 750	74. 0	22. 2	1. 50	2. 16	0
		S E	83. 33	1371. 6	2. 82	2. 93	0. 34	0. 55	0
Group 1	♂	M	848	10, 816	76. 5	21. 0	1. 08	1. 33	0. 08
		S E	31. 54	1196. 8	4. 35	4. 09	0. 23	0. 47	0. 08

* Significant difference with 5 % risk rate

Table 5-1 Biochemical Findings on Plasma (Interim)

($\bar{\chi} \pm SE$)

Group	Sex	Blood-Sugar(mg/dl) Oxidase method	S-GOT (Karmen-unit/ml) Colorimetry	S-GPT (Karmen-unit/ml) Colorimetry	S-Al-P (m-unit/ml) Colorimetry	Chole-sterol (mg/dl) Colorimetry	Urea-N (mg/dl) Urease method	LDH (m-unit/dl) Colorimetry	Na (mEg/l) Flame photometry	K (mEg/l) Flame photometry	Cl (mEg/l) Titrimetry (Schales法)	A/G Electrophoresis	T.P. (g/dl) Hitachi protein meter
Control Group	♂	149±21.0	20±2.2	8±0.9	57±5.3	87± 5.3	10±0.9	300±10.2 †9.8	154±0.8	4.0±0.20	103±0.3	1.3±0.26	6.3±0.20
4(1,000 mg/kg)	♂	143±14.6	18±1.6	9±0.7	71±1.5	83±10.4	12±0.7	347±7.1	154±0.9	3.8±0.09	104±0.2 †6.6	1.3±0.09	6.7±0.06
3(300 mg/kg)	♂	155±7.7	26±4.4	8±0.7	71±3.6	92±5.7	11±0.7	311±11.1	154±0.6	4.0±0.13	105±0.3	1.3±0.20	6.6±0.18
2(100 mg/kg)	♂	148±4.9	25±5.1 ††19.1	9±1.0	57±5.2	86±3.1	12±0.8	295±3.5	153±0.7	4.0±0.16	105±0.4	1.2±0.09	6.4±0.09
1(30 mg/kg)	♂	123±19.8	38±9.3	8±0.8	55±5.6	80±7.8	11±0.8	337±35.3	153±0.9	4.0±0.13	104±0.5	1.3±0.10	6.5±0.26
5(Ferulic acid)	♂	133±7.8	14±0.9	8±0.7	15±7.3	99±11.5	13±0.5	314±9.5	152±1.1	4.0±0.09	104±0.6	1.4±0.14	6.4±0.11
Control Group	♀	86±15.9	26±5.2	6±0.9	44±3.6	92±5.5	16±1.0	334±18.4	155±0.8	3.3±0.29	103±0.7	1.1±0.11	6.6±0.07
4(1,000 mg/kg)	♀	141±7.3	29±5.4	6±1.5	74±5.2	90±5.4	13±0.9	296±6.1	156±1.6	3.1±0.28	103±0.7	1.2±0.08	6.3±0.07
3(300 mg/kg)	♀	160±17.6	23±3.7	9±0.8	46±3.6	90±7.8	14±1.3 †5.4	348±34.9 †6.8	153±0.9	3.3±0.27	103±0.7	1.2±0.09	6.3±0.15
2(100 mg/kg)	♀	114±22.7	27±4.1	10±2.3	48±4.7	95±16.4	11±1.3 †5.8	306±7.4 †††12.1	154±1.0	3.4±0.07	104±0.5	1.2±0.20	6.4±0.09
1(30 mg/kg)	♀	118±14.5	17±1.6	5±0.5	48±2.4	85±3.5	13±1.2	297±7.7 †5.7	153±0.8	3.3±0.08	104±0.4	1.2±0.10	6.6±0.09
5(Ferulic acid)	♀	115±6.9	24±0.6	5±0.6	41±4.5	92±4.9	14±0.8	305±9.8	153±0.9	3.5±0.10	104±0.5	1.2±0.10	6.5±0.15

有意差 † 5% †† 2.5% ††† 1%

Table 5-2 Biochemical Findings on Plasma (Final)

($\bar{\chi} \pm SE$)

Group	Sex	Blood-Sugar(mg/dl) Oxidase method	S-GOT (Karmen-unit/ml) Colorimetry	S-GPT (Karmen-unit/ml) Colorimetry	S-Al-P (m-unit/ml) Colorimetry	Chole-sterol (mg/dl) Colorimetry	Urea-N (mg/dl) Urease method	LDH (m-unit/dl) Colorimetry	Na (mEg/l) Flame photometry	K (mEg/l) Flame photometry	Cl (mEg/l) Titrimetry (Schales法)	A/G Electrophoresis	T.P. (g/dl) Hitachi protein meter
Control Group	♂	193±13.7	76±10.2	14±2.1	130±9.5	181±58.4	16±0.7	227±42.7	152±0.4	3.6±0.06	105±0.4	1.2±0.08	6.9±0.04
4	♂	185±11.6	80±6.4	15±1.8	125±13.05	300±18.3	15±7.0	209±26.2	154±0.7	3.5±0.07	104±1.0	1.3±0.14	6.9±0.13
3	♂	215±12.9	75±7.9	17±2.1	124±21.6	266±24.8	15±0.1	320±81.1	153±0.9	3.6±0.00	104±0.6	1.4±0.11	6.7±0.05
2	♂	190±6.2	65±4.8	16±1.6	114±15.3	185±10.3	15±0.7	270±38.9	154±1.2	3.7±0.04	104±0.5	1.4±0.04	6.9±0.29
1	♂	146±8.5	83±8.4	13±1.1	177±30.0	236±7.9	16±1.7	231±32.9	155±0.7	3.6±0.07	105±0.6	1.3±0.09	7.2±0.11
Control Group	♀	212±26.7	69±12.3	23±3.8	89±4.3	207±14.6	18±1.0	353±39.6	152±0.5	3.5±0.12	105±0.2	1.3±0.13	6.7±0.13
4	♀	198±12.2	45±7.4	15±1.1	83±5.5	246±22.1	17±1.1	266±15.4	154±0.9	3.6±0.13	105±0.7	1.3±0.09	6.8±0.12
3	♀	132±21.4	62±14.4	19±2.7	75±5.0	215±11.5	20±1.4	423±81.9	156±1.2	3.5±0.16	106±0.5	1.5±0.13	7.1±0.09
2	♀	199±13.7	46±4.9	14±1.6	88±8.5	226±7.6	20±2.4	264±35.7	154±1.4	3.6±0.11	106±0.4	1.2±0.11	6.7±0.10
1	♀	192±12.4	49±15.2	16±3.2	91±24.5	197±11.5	21±3.3	226±25.2	154±1.1	3.5±0.07	105±0.5	1.4±0.06	6.8±0.09

Table 6-1 Organ Weight (Interim Investigation) (Mean value g; *mg)

Test Group	Sex	Weight	Brain	Heart	lung	Liver	Kidney	Spleer	Adrenal Gland*	Thymus Gland	Pituitary Gland*	Testis/ Ovary	Pancreas
Control	♂	280.8	1.86	1.20	1.66	12.36	2.74	0.74	57.2	0.88	9.4	2.56	0.80
30 mg group	♂	285.0	1.84	1.16	1.68	12.40	2.50	0.68	56.0	0.84	8.4	2.64	0.90
100 mg group	♂	271.8	1.82	1.20	1.62	12.28	2.56	0.64	54.8	0.76	7.4	2.48	0.90
300 mg group	♂	285.2	1.74	1.24	1.52	12.52	2.76	0.66	52.6	0.78	10.6	2.64	0.80
1,000 mg group	♂	262.6	1.68	1.06	1.64	11.78	2.58	0.58	59.4	0.82	8.8	2.50	0.82
												Ovary	
Control	♀	196.2	1.63	0.70	1.08	7.98	1.70	0.45	66.5	0.58	9.0	98.0	0.43
30 mg group	♀	202.8	1.72	0.90	1.30	8.96	1.94	0.60	72.2	0.58	8.6	122.0	0.88
100 mg group	♀	201.8	1.68	0.88	1.32	9.24	1.86	0.60	59.6	0.66	9.4	115.8	0.84
300 mg group	♀	202.0	1.74	1.00	1.32	9.80	1.94	0.58	69.8	0.58	10.8	118.2	0.78
1,000 mg group	♀	195.8	1.76	1.00	1.18	9.08	1.80	0.54	65.2	0.62	8.4	110.8	0.74

Table 6-2 Organ Weight (Final Investigation) (Mean value g; *mg)

Test Group	Sex	Weight	Brain	Heart	lung	Liver	Kidney	Spleer	Adrenal Gland*	Thymus Gland	Pituitary Gland*	Testis/ Ovary	Pancreas
Control	♂	521.6	2.10	2.02	2.70	17.58	3.74	1.08	66.2	0.50	16.0	3.23	1.41
30 mg group	♂	512.4	2.12	1.42	3.02	17.26	3.66	2.06	58.0	0.42	11.6	3.00	1.46
100 mg group	♂	530.4	2.08	2.08	2.52	17.65	3.66	1.08	71.8	0.58	11.8	2.92	1.18
300 mg group	♂	498.0	2.10	1.94	2.74	18.14	3.68	1.04	62.2	0.42	15.8	2.80	1.28
1,000 mg group	♂	523.2	2.14	1.96	3.74	18.50	3.72	1.12	74.4	0.60	13.8	3.14	1.34
												Ovary	
Control	♀	368.4	1.84	1.44	2.06	11.36	2.40	0.84	44.4	0.74	10.2	114.0	1.08
30 mg group	♀	312.0	1.82	1.28	2.10	8.74	2.10	0.66	61.8	0.44	10.2	113.2	0.86
100 mg group	♀	326.0	1.86	1.18	2.14	10.40	2.42	0.72	57.4	0.56	11.0	114.2	1.04
300 mg group	♀	328.4	1.90	1.18	1.60	9.42	2.28	0.76	68.2	0.48	12.6	107.2	0.90
1,000 mg group	♀	355.0	1.80	1.20	1.94	10.06	2.44	0.72	76.8	0.62	10.6	113.6	1.06

III. Anatomicopathological Findings

Tables 6-1 and 6-2 show the weight of each organ. When the rats' lungs were examined, prominent single or multiple lung abscesses were observed in one rat in the 1,000 mg group, two rats in the 300 mg group, one rat in the 100 mg group, and one rat in the control group. Other than that, there were no significant changes.

One liver abscess each (about 3 to 4 mm size) was observed in two rats in the 1,000 mg group and one rat in the control group. An abscess of 6.8×4.5×4.2 cm size was found in the retroperitoneal space of one rat in the 1,000 mg group. The left kidney was buried in this abscess, causing a kidney abscess associated with it. Small abscesses with the same properties were observed under the skin, prostatic gland, and the back side of the liver.

Six rats died before the end of the test period all from pneumonia. Two male rats and two female rats in the 1,000 mg group and one male and female rat in the 300 mg group died.

Histogenetically, there were a few cases of sporadic appearance of small fat droplets in liver cells. However, positive correlation with the drug dose was not confirmed.

No significant change was observed in the heart, kidney, brain, pancreas, thyroid gland, submaxillary gland, pituitary gland, testis, ovary, gastrointestinal tract, bone marrow, or bone. No findings indicating chronic toxicity was observed either.

No epithelial necrosis was observed in the duct of submaxillary gland, especially in the striated part. Other than minor small intercellular vacuoles observed sporadically in high-concentration groups, there was no significant change. No significant changes were observed in the acinar epithelium either.

The lower extremities of the rats including knee joints were examined using ultrasoft x-ray radiography films. No deformations caused by atrophy or degeneration of bone or cartilage were observed.

After the aortic wall was examined by the elastin fiber staining method, no abnormalities such as elastolysis were observed.

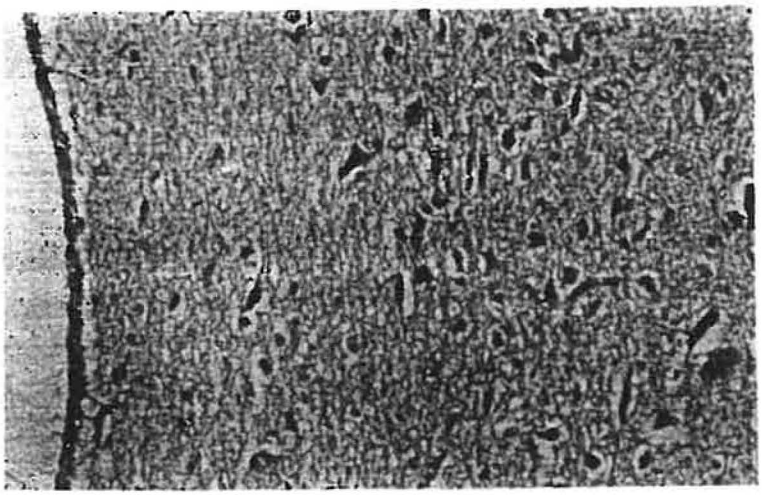
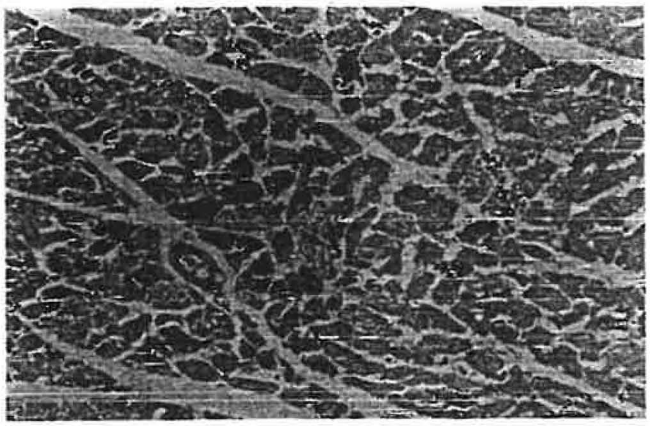


Photo 1 1,000 mg/kg
(1-♂-11)
Brain 25×10

Photo 2 1,000 mg/kg group
(1-♂-2)
Heart 25×10



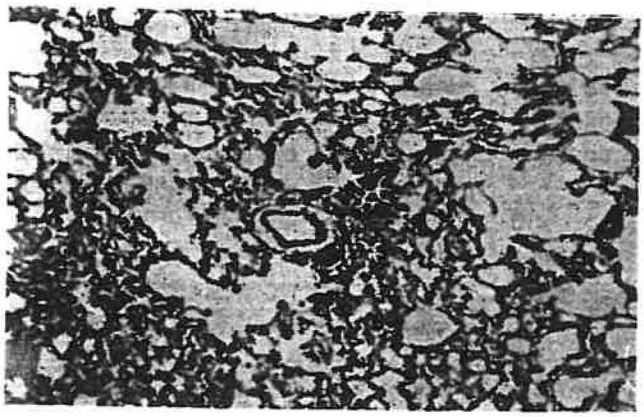


Photo 3 1,000 mg/kg group
(1-♂-11)
Lung 25×10

Photo 4 1,000 mg/kg group
(1-♂-11)
Liver 25×10

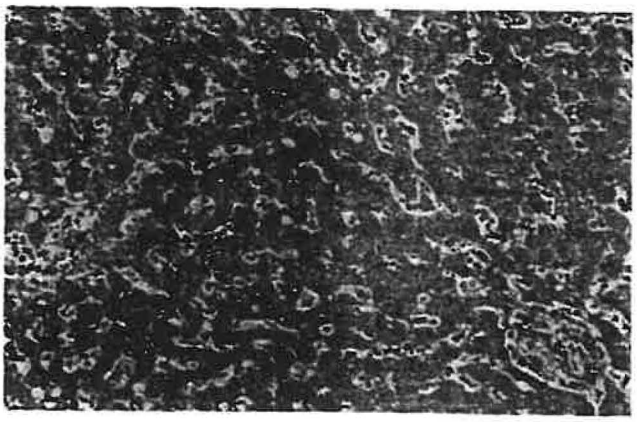


Photo 5 1,000 mg/kg group
(1-♀-11)
Kidney 25×10

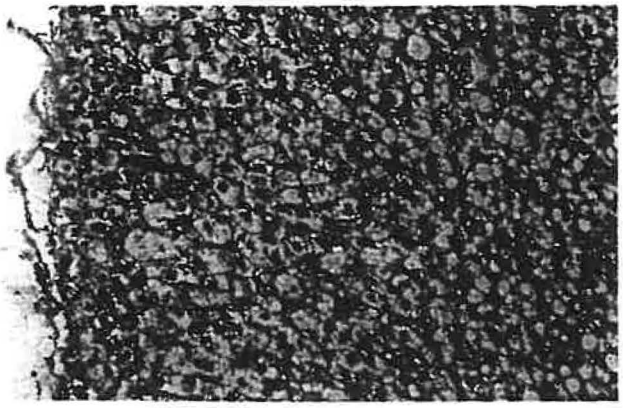
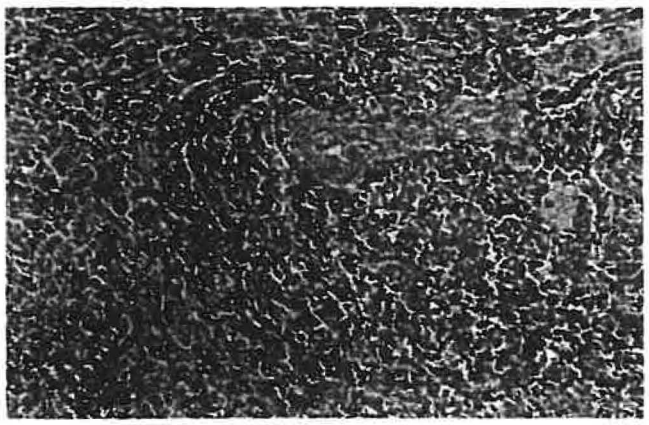


Photo 6 1,000 mg/kg group
(1-♀-3)
Adrenal gland 25×10

Photo 7 1,000 mg/kg group
(1-♀-11)
Spleen 25×10



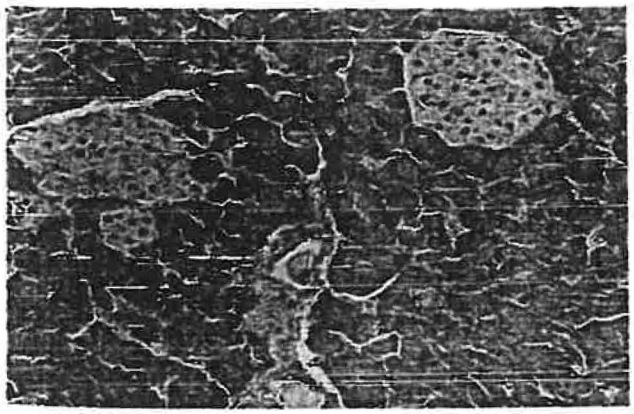


Photo 8 1,000 mg/kg group
(1-♂-11)
Spleen 25×10

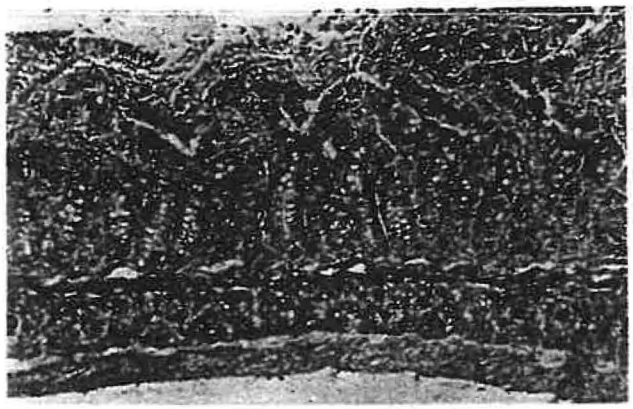
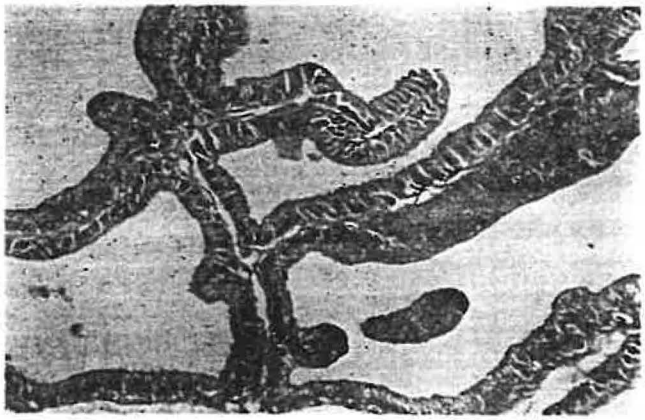


Photo 9 1,000 mg/kg group
(1-♂-11)
Duodenum
10×10

Photo 10 1,000 mg/kg group
(1-♂-11)
Prostate gland 25×10



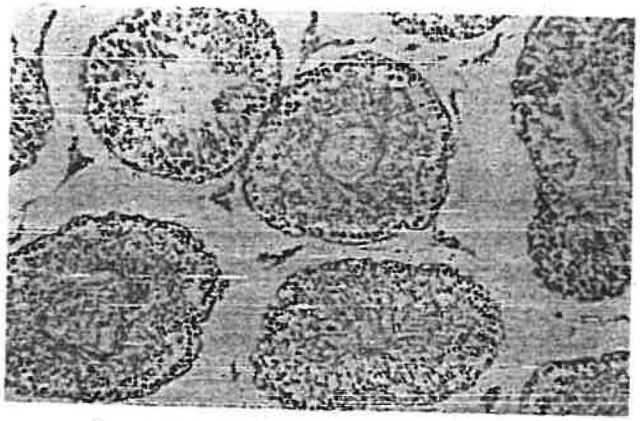


Photo 11 1,000 mg/kg group
(1-♂-11)
Testis 10×10

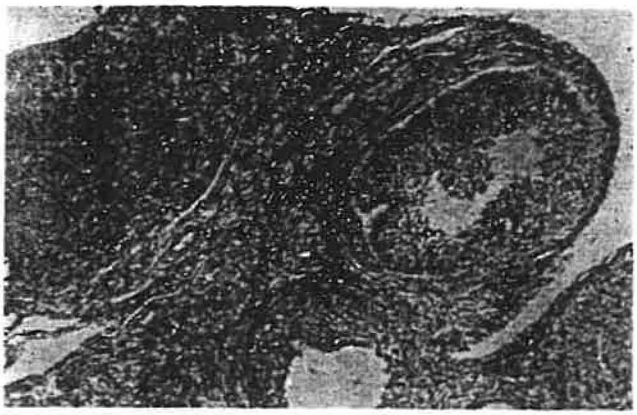
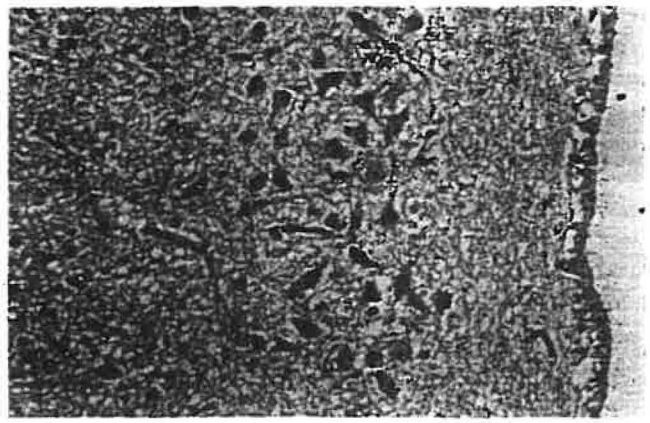


Photo 12 1,000 mg/kg group
(1-♀-4)
Ovary 10×10

Photo 13 Control group
(C-♂-7)
Brain 25×10



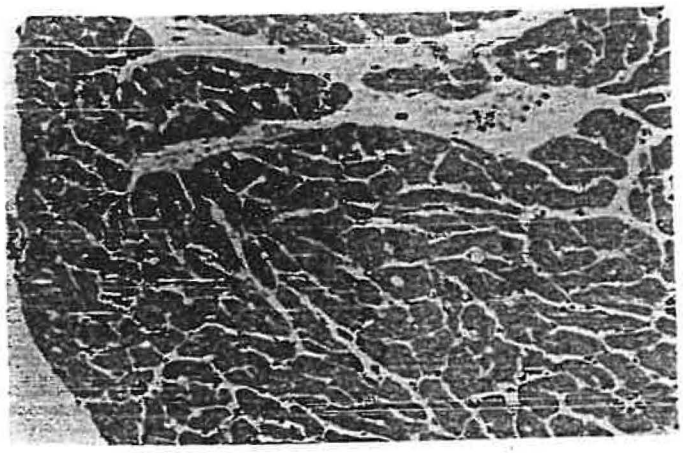


Photo 14 Control group
(C-さ-9)
Heart 25×10

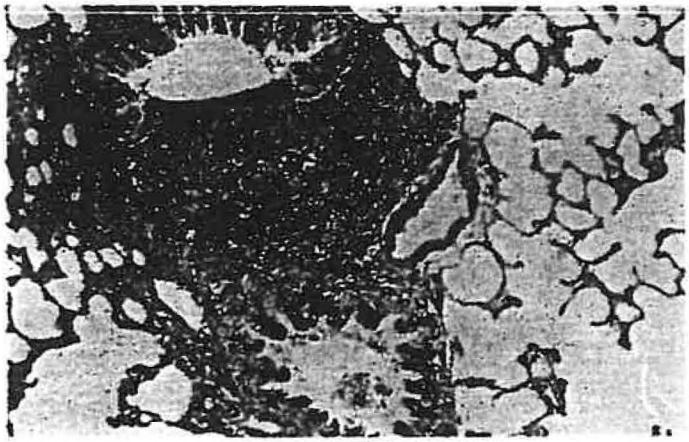
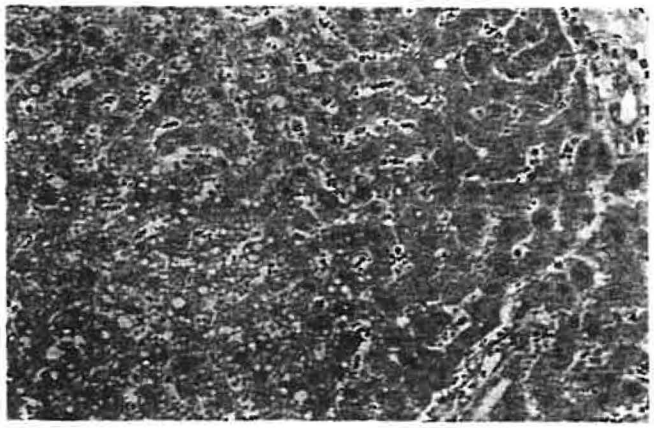


Photo 15 Control group
(C-さ-7)
Lung 10×10

Photo 16 Control group
(C-さ-9)
Liver 25×10



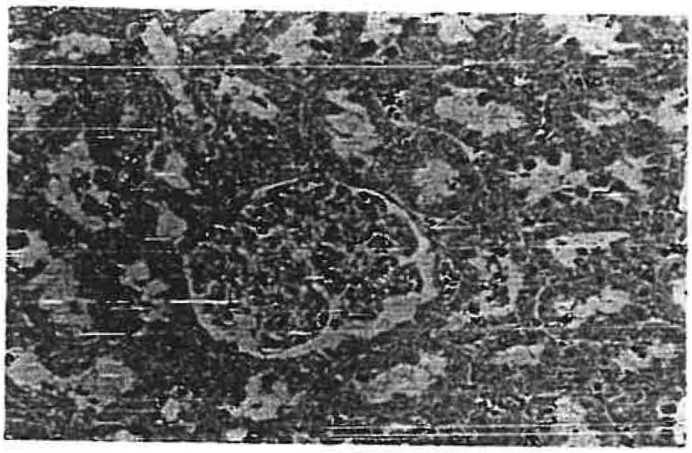


Photo 17 Control group
(C-さ-13)
Kidney 25×10

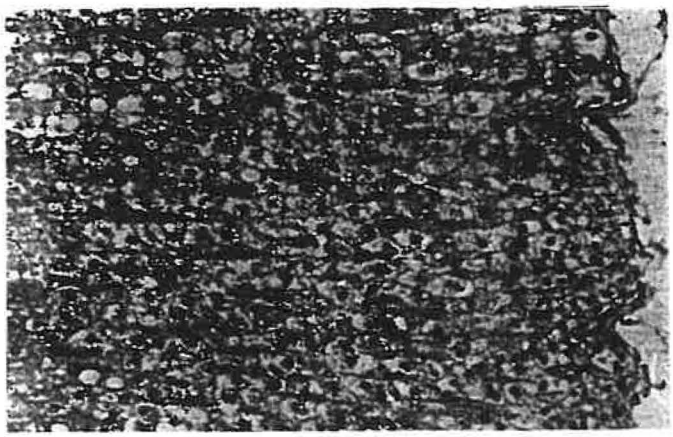
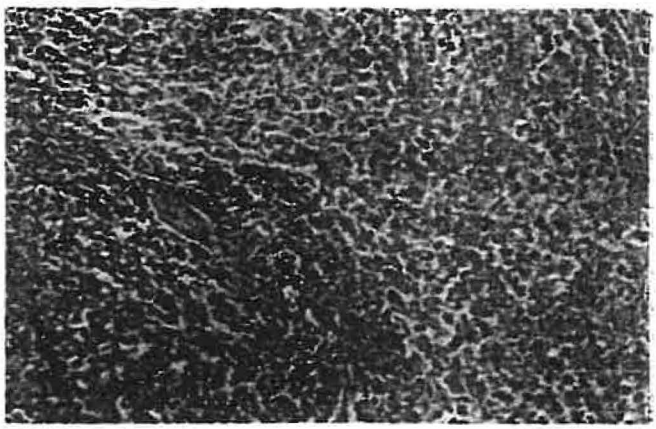


Photo 18 Control group
(C-さ-13)
Adrenal gland 25×10

Photo 19 Control group
(C-さ-13)
Spleen 25×10



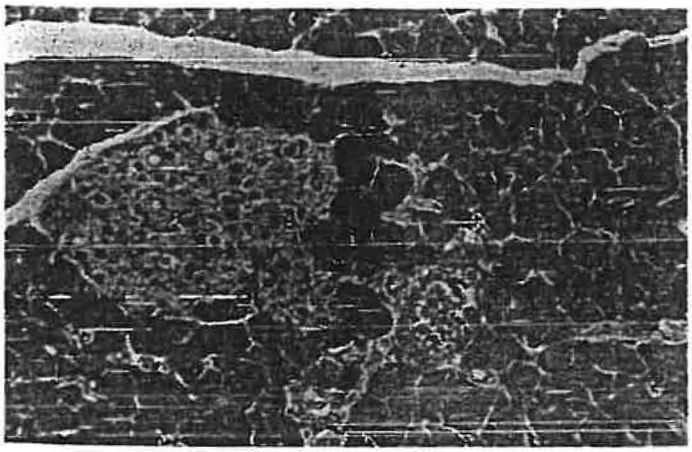


Photo 20 Control group
(C-♂-13)
Spleen 25×10

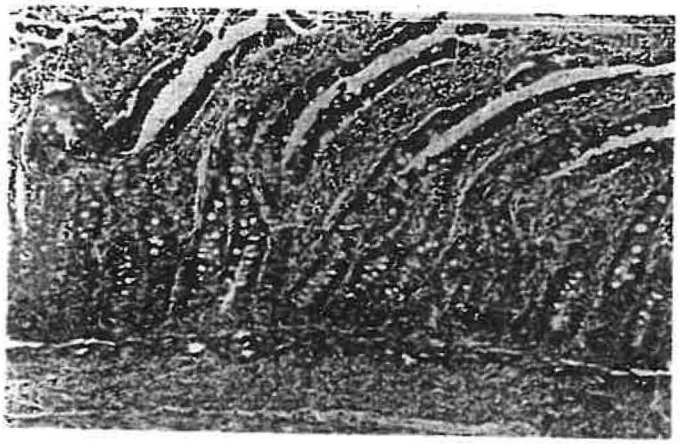
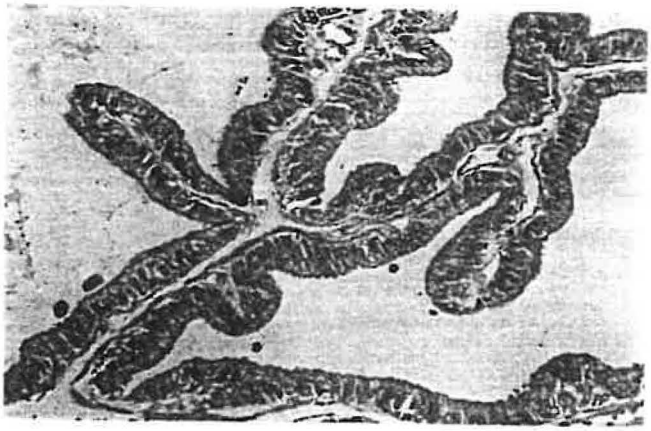


Photo 21 Control group
(C-♂-7)
Duodenum 10×10

Photo 22 Control group
(C-♂-9)
Prostate gland 25×10



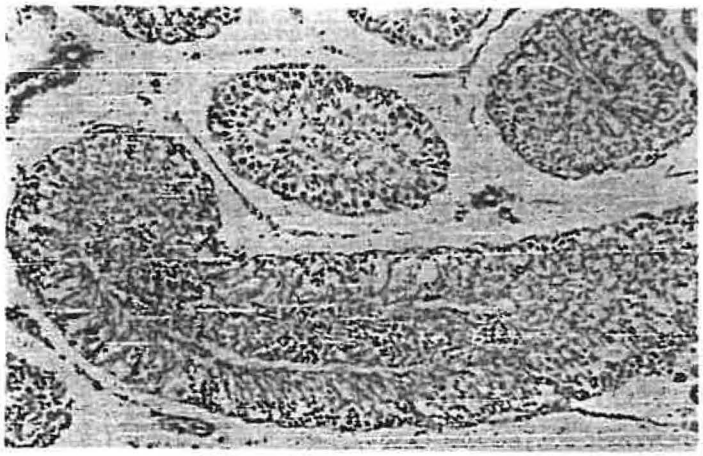


Photo 23 Control group
(C-♂-9)
Testis 10×10

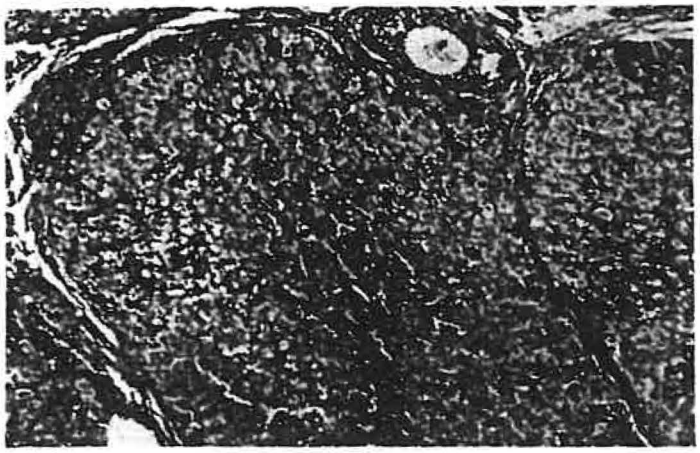


Photo 24 1,000 mg/kg
(1-♀-4)
Ovary 10×10

Photo 25 1,000 mg/kg
(1-♂-4)
Submaxillary gland 40×10

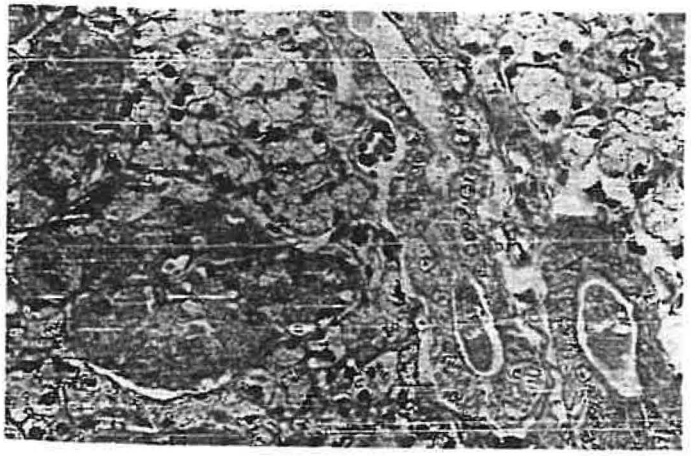


Photo 26 1,000mg/kg Group
(1-♀-4)
Submaxillary gland 40×10

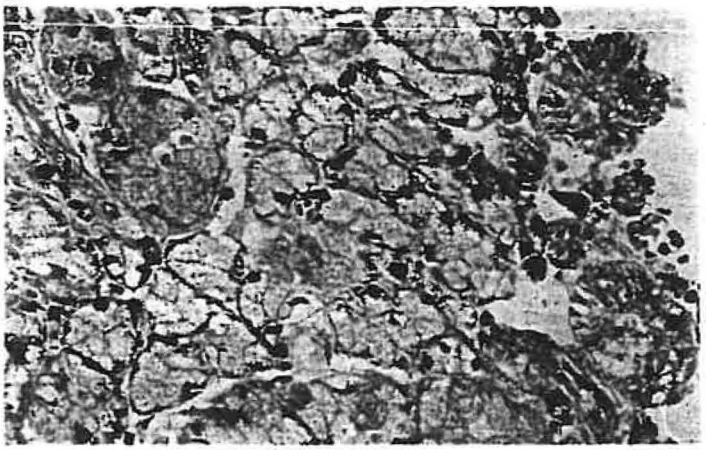
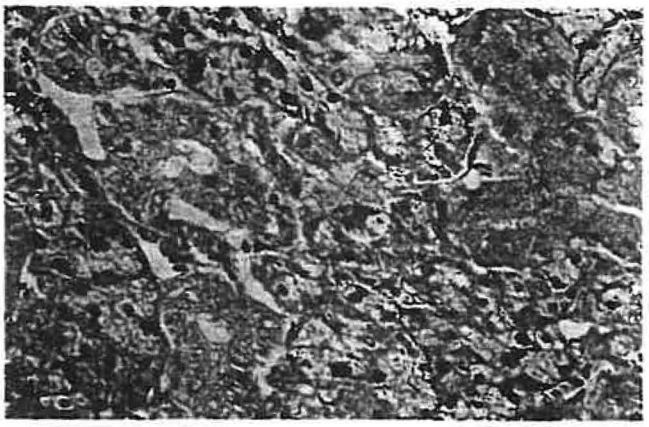


Photo 27 Control group
(C-♂-8)
Submaxillary gland
40×10

Photo 28 Control group
(C-♀-6)
Submaxillary gland
40×10



Summary

A chronic toxicity test was carried out on Oliver Tablet for 181 days between May 17 and November 14 in 1971 using 140 Wistar-Imamichi rats, 70 each of male and female. The drug was administered by 1,000 mg/kg, 300 mg/kg, and 100 mg/kg daily. It was suspended in 0.5 % CMC and forcibly administered to the rats orally. During the administration period, four rats died in the 1,000 mg/kg administration group and two rats in the 300 mg/kg administration group on the 17^{th} to 143^{rd} days. The number of dead rats was the same for male and female. It was indicated that all of these rats died from pneumonia, where significant correlation with the drug can hardly be observed.

On the weight curve, the weight of rats in all groups demonstrated a similar level of increase. Positive correlation between the weight and drug dosage was not observed based on the experiments we previously carried out. No significant difference was caused by the drug. In blood pictures or biochemical properties of plasma, no change related to the administration of the drug was observed.

No remarkable chronic toxicity was observed in general organ examination from a histopathological viewpoint.

No abnormalities in the salivary gland or ossification disorder in cartilage of the epiphyseal area described for Kashin-Beck disease were observed.

References

1) Tomotaro Tsuchiya, Ryohei Kaneko: Koka Zasshi, 56, 295, 1953

2) Tomotaro Tsuchiya, Ryohei Kaneko: Toko Shiho, 49, 141, 1954

3) Ryohei Kaneko, Tomotaro Tsuchiya: Koka Zasshi, 57, 526, 1954

4) Tomotaro Tsuchiya et al.: Toko Shiho, 53, 235, 1958

5) Tomotaro Tsuchiya et al.: ibid, 54, 143, 1959

6) Tatsuo Katsuki et al.: Bulletin of the Nippon Veterinary and Zootechnical College (currently Nippon Veterinary and Animal Science University), 7, 56, 1958

7) Atsumi Ishihama et al.: Sanfujinka no Jussai, 13, 625, 1964

Kiso to Rinsho, 6, 1717 (1972)

Toxicity Test on γ–Oryzanol
-Influence of γ–Oryzanol on Fetuses and Offspring of Mice and Rats

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Summary

6 mg/kg and 600 mg/kg of γ–oryzanol was orally administered to ICR-JCL mice daily from the 7th to 12th day of pregnancy and to Wistar rats from the 9th to 14th day of pregnancy and the influence on dams, fetuses, and offspring was examined.

Although the weight of pregnant dams slightly increased after the administration of γ–oryzanol, there was no significant influence from the administration of γ–oryzanol.

There was no significant change found as a result of visual observation of fetuses, observation of stained bone samples, or visual observation by fixation in Bouin's solution, indicating no influence on teratogenicity or the skeletal development.

When offspring was observed, there was almost no change considered to be caused by the administration of γ–oryzanol. There was no significant change in external features from visual observations such as differentiation status. No deformation of organs or bones was observed either. The results supported that γ–oryzanol has no teratogenicity once again.

Introduction

γ–oryzanol was first extracted and separated from rice bran oil by Tsuchiya et al.[1-3] in 1953. Studies of Tsuchiya[4, 5] and Shimizu et al.[6-12] clarified its chemical structure, ferulic acid ester mixture of several types of triterpene alcohols.

For pharmacological activities of γ–oryzanol, activities to accelerate the growth of rats and stimulate their sexual glands[13-15] and activity on adenohypophysial cells[16] have been reported. According to Wakao et al.[17], γ–oryzanol has an effect similar to vitamin E.

Clinically, γ–oryzanol has long attracted a great amount of attention for its activity to control the autonomic nervous system. It has been confirmed to be effective for indefinite complaints of autonomic nerve imbalance and menopause. γ–oryzanol is also used to treat so-called whiplash[18] and its therapeutic effects have been examined in various fields[19][20].

The author and group members administered γ–oryzanol to pregnant mice and rats and evaluated its influence on their fetuses and offspring.

Test method

1. Animals used and environmental conditions

Female ICR-JCL nulliparous mice (approx. 25 g) and female Wistar nulliparous rats (approx.

220 g) were used.

Even though the mice showed visually apparent estrus symptoms, rats in proestrus were selected by the vaginal smear method. Both mice and rats were left with mature male animals of the same kind overnight. On the next morning, mice with a copulatory plug and rats with sperm in their vagina were determined to be pregnant. The day was defined as day zero of pregnancy. Both mice and rats were stored in an individual cage after the pregnancy test.

Solid feed made by Oriental Yeast Co., Ltd. (MF for mice, NMF for rats) was given to the test animals. Tap water was available whenever they wanted.

Room conditions were maintained at certain levels; temperature of 22±2 °C and humidity of 55±5 %.

2. Administration method, dose, and timing

Since γ–oryzanol was determined to be administered orally in clinical tests, γ–oryzanol was orally administered forcibly using a tube for oral administration in the test.

In the group for small dose administration, 6 mg/kg (maximum regular dose for humans) of γ–oryzanol was administered which was six times more than the regular dose for humans, 1 mg/kg. In the group for large dose administration, 600 mg/kg, which was 100 times more than the group for small dose, was administered.

γ–oryzanol was suspended in a 0.5% CMCNa solution so that the administration dose per animal was 10 ml/kg in all groups. The same amount of normal saline solution was administered to the animals of the control group.

γ–oryzanol was administered to mice on the 7^{th} to 12th days of their pregnancy and to rats on the 9^{th} to 14^{th} days of their pregnancy for six days respectively. It was administered once a day in the morning in principle.

For the test on fetuses, 20 dams were used in both mouse and rat groups. For the test on offspring, 5 dams were used.

3. Observation items

[Observation of dams]

Pregnant mice were weighed for 18 days and pregnant rats for 21 days starting on the day of the pregnancy test. Their general conditions were monitored and principle organs in their abdominothoracic areas were visually observed during Caesarean section.

[Observation of fetuses]

Pregnant mice and rats were anesthetized with chloroform and put to death from exsanguination on the 18^{th} day and 21^{st} day of their pregnancy respectively. After Caesarean section, the total number of implants, the number of live fetuses, and the number of resorbed or dead fetuses were counted.

Live fetuses were weighed, their sex was determined, and the external appearance and

abdominal viscera were checked for any abnormalities. Intra-abdominal viscera of approximately two-thirds of the total live fetuses was observed mainly to check for missing parts. Then, they were fixed using alcohol, made into alizarin red S-stained samples by the Dawson method[21] to be used for observation of their skeletons.

Skeletal development was observed referring to the ossification of breast bone, tail caudal vertebra, and toe bone. For mice, ossification of their ankle bone and calcaneal bone was also observed.

After fixation using Bouin's solution, the internal parts of the remaining live fetuses were observed by the razor blade sectioning method. Their head was observed referring to the method considered by Barrow et al.[22] Furthermore, partial inspection of the thoracoabdominal areas was performed in order to check for any abnormalities in principle organs.

Stained bone samples and fetuses fixed in Bouin's solution were observed under a low-power stereoscopic microscope.

[Observation of offspring]

Dams were brought to give natural birth and γ–oryzanol's influence on their offspring was examined. The number of offspring was counted, their sex was determined, and the external appearance was checked for any abnormalities within 24 hours after birth.

The date of the delivery was counted as day zero. Dams raised the offspring until the 21st day after birth. Then, differentiation conditions such as detachment of auricles, eruption of incisors, opening of eyelids, and fur appearance were observed.

When differentiation conditions were observed, external appearance of the offspring and behavioral abnormalities behavior were monitored.

Offspring was weighed until the 21st day after the birth. External appearance of live offspring was examined, their behavior was observed, and auditory, pain, and righting reflex tests were performed at the time of weaning on the 21st day.

Partial inspection was performed on most weaning offspring in order to check for visceral abnormalities. Tainted bone samples from one male and one female offspring born from each dam were created just like fetuses' samples and they were checked for any bone abnormalities.

Test results

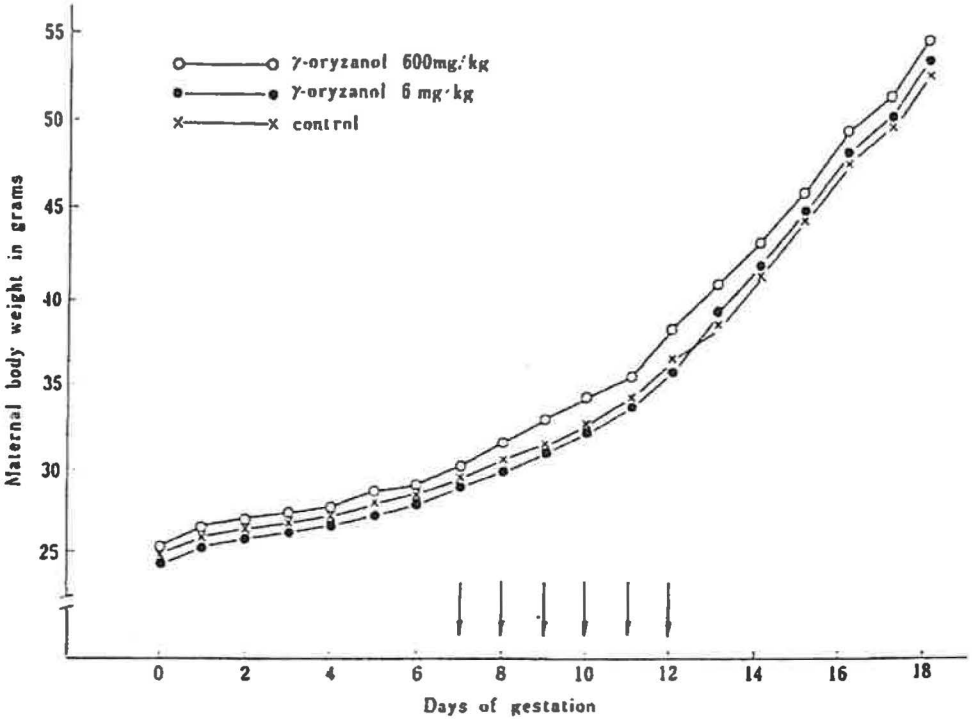
I. Mice

1) Influence on dams' body

Fig. 1 shows the change of dams' weight.

The weight of dams in the group that took 600 mg/kg of γ–oryzanol increased slightly more in the later administration period as compared to the other administration group. The weight of dams in the group that took 6 mg/kg of γ–oryzanol increased by a similar amount to the control group.

Fig. 1 Mean body weight of pregnant mice throughout the period of pregnancy Arrows indicate oral administration of the drug.



No dam died or miscarried during the test period. There was no specific change in dams in partial inspection of principle organs during Cesarean section. As a result of observation of general conditions, there was no specific change in dams in the groups that took γ–oryzanol as compared to the control group.

2) Influence on fetuses

Table 1 shows the results of observation of fetuses.

Table 1 Influence of γ-oryzanol on mouse fetuses

Drug and Dose	Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of dams	20	20	20
Total implants (lit. size±S.D.)	233(11.8±2.13)	231(11.5±1.32)	198(9.9±1.20)
No. of fetuses	11 (4.7)	18 (7.8)	5 (2.5)
Dead or resorbed (%)[a]	D) 6(2.5), R) 5(2.1)	D) 2(0.9), R) 16(6.9)*	D) 0, R) 5(2.5)
Live (lit. size±S.D.)	222(11.1±2.48)	213(10.7±1.40)	193(9.7±1.27)
Sex ratio (♂/♀)	1.13(118/104)	1.20(116/97)	1.07(100/93)
Fetal weight (g) ♂	1.39±0.09	1.46±0.13	1.45±0.12
(Mean±S.D.) ♀	1.34±0.08	1.41±0.13	1.46±0.13
External abnormalities (%)[b]	Club foot, 4 (1.8) Bent tail, 1 (0.5)	Club foot, 4 (1.9) Hematoma, 1 (0.5)	Club foot, 1 (0.5)

* $P<0.05$

a) % of total implants b) % of live fetuses D) Dead fetuses R) Resorbed fetuses

Total implants and total number of live fetuses of the group that took 600 mg/kg of γ–oryzanol were slightly less as compared to the control group. However, the difference was not significant. The percentage of dead fetuses for this group was 2.5 % which was lower than the control group's 4.7 %.

The percentage of dead fetuses in the group that took 6 mg/kg of γ–oryzanol was 7.8 % which was slightly higher as compared to the percentage of the control group which was 4.7 %, though the difference was not significant. The number of resorbed fetuses was significantly higher in the group that took 6 mg/kg of γ–oryzanol as compared to the control group. However, there was no significant difference in the total implants or total number of live fetuses.

Mean body weight of live fetuses of γ–oryzanol administration groups was almost the same as the one of the control group both for males and females.

From external observation, one case of club foot was found in the group that took 600 mg/kg of γ–oryzanol, four cases of club foot (photo 1) and one case of hematoma were found in the group that took 6 mg/kg of γ–oryzanol, and four cases of club foot and one case of bent tail were found in the control group. The occurrence rate of all abnormalities was as low as 0.5 to 1.9 %.

Photo 1 Mouse γ-oryzanol 6 mg/kg
Club foot

In observation of intra-abdominal viscera mainly to check for missing parts, there were no fetuses with any visceral abnormalities.

Tables 2 to 4 show the results of observation of stained bone samples and Table 5 shows the summary.

Table 2 Influence of γ-oryzanol on skeletal development of mouse fetuses

Control ICR-JCL strain

No. of experimental animals / Observation	1	2	3	4a)	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Malformations																					0
Variations																					
Split or bifurcation of atlas																					0
Split or bifurcation of axis						1											1		1		3
Cervical rib															1						1
14th rib	2	1	1								2	3	2	1	1	1					13
Extra sternebrae																					0
Asymmetry of sternebrae			2	1	1										1						5
Types of ossification																					
Delayossification of sternebrae	1		2	1	1										1						6
Total fetuses examined	160																				
Total variational fetuses	21																				
Total variational observation	22																				
Total variational fetuses / Total fetuses examined ratio	13.1%																				

a) fetus with 2 variations

Table 3 Influence of γ-oryzanol on skeletal development of mouse fetuses (2)

γ-oryzanol (daily dose 6 mg/kg) ICR-JCL strain

No. of experimental animals / Observation	1	2	3	4	5a)	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Malformations																					0
Variations																					
Split or bifurcation of atlas	1			1											1						3
Split or bifurcation of axis																			1		1
Cervical rib																					0
14th rib		2		1		3			1	5	3	2	1	2				2	1		23
Extra sternebrae																					0
Asymmetry of sternebrae			1		1										1				1		4
Types of ossification																					
Delayossification of sternebrae				1												1					2

Total fetuses examined	132
Total variational fetuses	30
Total variational observation	31
Total variational fetuses / Total fetuses examined ratio	22.7%

a) fetus with 2 variations

Table 4 Effect skeletal development of mouse fetuses (3)

γ-oryzanol (daily dose 600 mg/kg) ICR-JCL strain

No. of experimental animals / Observation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
Malformations																					0
Variations																					
Split or bifurcation of atlas				1					1												2
Split or bifurcation of axis																	1				1
Cervical rib																					0
14th rib	2			1		5					1		1					2			12
Extra sternebrae											2										2
Asymmetry of sternebrae									1	2		1					1				5
Types of ossification																					
Delayossification of sternebrae												1							1		2

Total fetuses examined	128
Total variational fetuses	20
Total variational observation	22
Total variational fetuses / Total fetuses examined ratio	15.6%

a) fetus with 2 variations

Table 5 Influence of γ-oryzanol on skeletal development of mouse fetuses

(Summary) ICR-JCL strain

Drug and Dose	Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of fetuses examined	160	132	128
Skeletal malformations (%)			
Skull	0	0	0
Axial skeleton	0	0	0
Appendicular skeleton	0	0	0
Skeletal variations (%)			
Split or bifurcation of atlas	0	3(2.3)	2(1.6)
Split or bifurcation of axis	3(1.9)	1(0.8)	1(0.8)
Cervical rib	1(0.6)	0	0
14th rib	13(8.1)	23(17.4)*	12(9.4)
Extra Sternebrae[a]	0	0	2(1.6)
Asymmetry of sternebrae	5(3.1)	4(3.0)	5(3.9)
Total variational fetuses / Total fetuses examined ratio	13.1%	22.7%*	15.6%
Development of ossification			
Delayossification of sternebrae	6(3.7)	2(1.5)	2(1.6)
No. of caudal vertebrae	8.0±1.51	8.6±1.99	8.7±1.99
No. of proximal phalanges in forepaw[b]	4.0±0.13	4.0±0.19	4.0±0
No. of proximal phalanges in hindpaw[b]	4.6±0.44	4.9±0.25	4.9±0.15
No. of middle phalanges in forepaw[b]	2.4±0.72	2.7±0.59	2.9±0.06
No. of middle phalanges in hindpaw[b]	0.9±1.08	1.3±1.26	1.8±1.22
Calcaneous	132(82.3)	108(81.9)	110(85.9)
Talus	13(8.9)	17(12.9)	12(9.4)

a) Appeared between the 5th and 6th sternebrae

b) Mean±S.D.

* $P<0.05$

No malformation was found in any group. Variations such as split or bifurcation of atlas; split or bifurcation of axis; cervical rib, 14th rib, and extra sternebrae (photo 2); and asymmetry of sternebrae (photo 3) were observed. A 14th rib was found in 17.4 % in the group that took 6 % γ–oryzanol, which was significantly higher as compared to the control group. However, the occurrence rate of bone variation except for a 14th rib was as low as 0.8 to 3.9 % and there was no significant difference between the γ–oryzanol administration groups and the control group.

Photo 2 Mouse : γ-oryzanol 600 mg/kg
Extra sternebrae

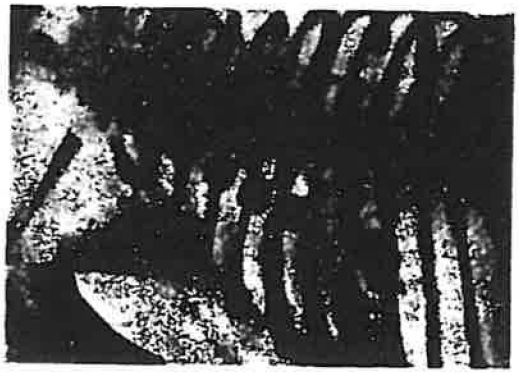
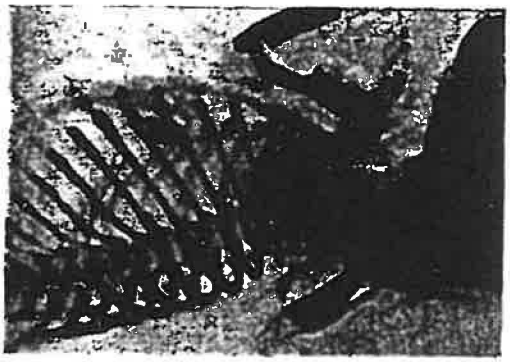


Photo 3 Mouse γ-oryzanol 600 mg/kg
Asymmetry of (5 th) sternebrae



Skeletal development of breast bone; the number of tail caudal vertebra, the number of proximal phalanges of toe bone, the number of middle phalanx, and the number of fetuses with ossified ankle bone and calcaneal bone were used as indexes to examine skeletal development. These values were slightly higher in the γ–oryzanol administration groups as compared to the control group. However, the difference was not significant and it was confirmed that there was no influence of γ–oryzanol in skeletal development.

Table 6 shows the results of observation of fetuses fixed in Bouin's solution.
There was no noticeable difference in the γ–oryzanol administration groups or the control group.

Table 6 Observation of some organs by razor blade sectioning method[a]

Drug and Dose	Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of observed fetuses	55	67	62
No. of abnormal fetuses	0	0	0

a) Organs observed were : brain, eye, mouth, heart, lung, kidney, gonad etc.

3) Influence on offspring

Table 7 shows the results of raising offspring until the 21st day after birth. Table 8 shows the results of observation of external differentiation. Fig. 2 shows the increase in their weight.
The total number of offspring was almost the same in the γ–oryzanol administration groups and the control group. There were dead offspring sporadically in the group that took 6 mg/kg of γ–oryzanol. 25 % of the total offspring died by the 21st day and the rearing rate in this group was 75 % which was significantly lower as compared to the control group.

Table 7 Influence of γ-oryzanol on mouse offsprings

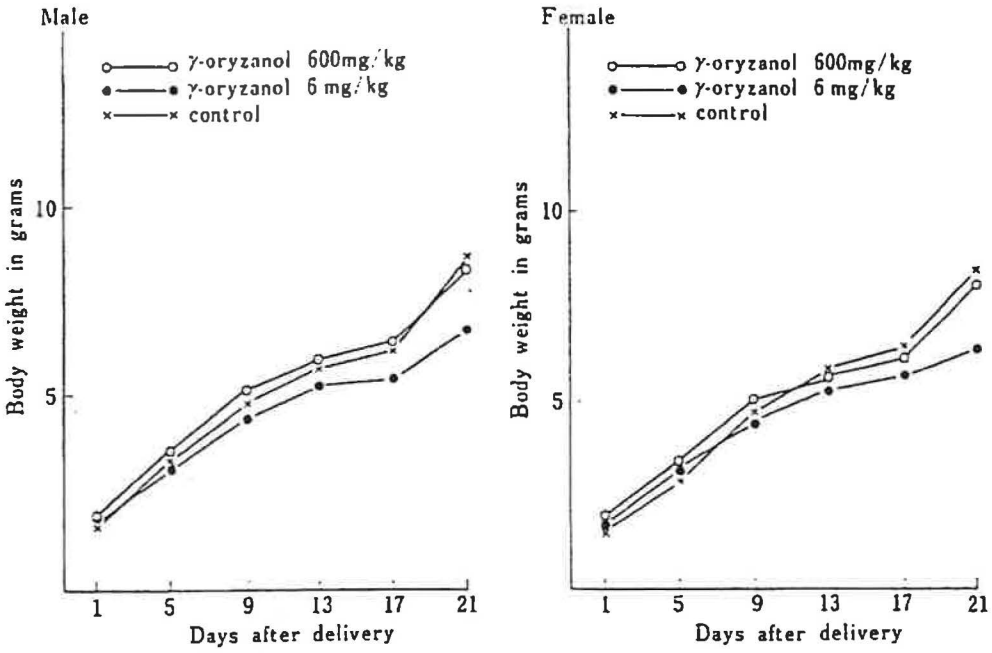
Drug and Dose		Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of dams		5	5	5
Total offsprings (lit. size±S. D)		56(11.2±1.30)	56(11.2±1.92)	54(10.8±1.64)
No. of live offsprings				
(Days after delivery)	1	56	54	54
	5	56	52	54
	9	56	51	54
	13	56	49	54
	17	56	42	54
	21	56	42	54
Total weanlings (lit. size±S. D)		56(11.2±1.30)	42(8.4±2.25)	54(10.8±1.64)
Sex ratio (♂/♀)		1.57(33/21)	1.41(24/18)	1.43(33/23)
Rate of weanling (%)		100	75	100

Table 8 Influence of γ-oryzanol on external differentiation of mouse offsprings

Drug and Dose		Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
Time scale of external features[a]				
(Days after delivery)				
Detachment of auricles	♂	3.8±0.46	4.2±0.31	4.0±0.00
	♀	3.8±0.42	4.5±0.50	4.0±0.07
Eruption of incisor	♂	10.5±0.51	10.5±0.46	10.7±0.25
	♀	10.4±0.50	10.5±0.50	10.7±0.41
Opening of eyelids	♂	14.0±1.04	14.2±0.36	14.2±0.44
	♀	14.0±1.36	14.3±0.47	14.2±0.45
Behavioral abnormalities		—	—	—
Abnormalities of auditory, pain and righting reflex		—	—	—

a) Mean±S. D.

Fig. 2 Mean body weight of young mice after delivery



Increase of weight was slightly less in the group that took 6 mg/kg of γ–oryzanol. However, the difference was not significant as compared to the control group.

When differentiation was observed, detachment of auricles was slightly delayed in the group that took 6 mg/kg of γ–oryzanol, though the delay was within a day. Eruption of incisor and opening of eyelids were confirmed on almost the same day in both γ–oryzanol administration groups and the control group. Fur appeared around the 10th day, both in the γ–oryzanol administration groups and control group.

No abnormalities were found in the external appearance; general behavior; or auditory, pain, and righting reflex in three week old live weanlings.

There was no noticeable variation in observation of principle organs and stained bone samples.

II. Rats

1) Influence on dams' body

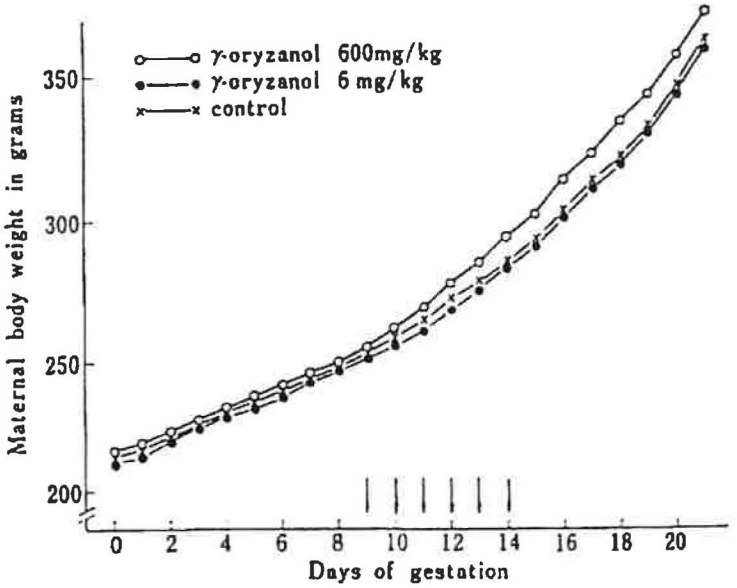
Fig. 3 shows the change of dams' weight.

The weight of dams in the group that took 6 mg/kg of γ–oryzanol increased by a similar amount to the control group. The weight of dams in the group that took 600 mg/kg of γ–oryzanol increased between the end of administration and the end of the test period slightly more as compared to the control group, though the difference was not significant.

No influence was found in general conditions after administration just like the results for mice. There was no specific change in dams in partial inspection of principle organs during

Cesarean section.

Fig. 3 Mean body weight of pregnant rats throughout the period of pregnancy



Arrows indicate oral administration of the drug.

2) Influence on fetuses

Table 9 shows the results of observation of fetuses.

Total implants and total number of live fetuses of the group that took 600 mg/kg of γ–oryzanol were similar to the control group. The values of the group that took 6 mg/kg were slightly less as compared to the control group. However, the difference was not significant. The percentage of dead fetuses for this group was 2.5 % which was lower than the control group's 4.7 %.

Table 9 Influence of γ-oryzanol on rat fetuses

Drug and Dose	Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of dams	20	20	20
Total implants (lit. size±S. D.)	232(11.6±1.94)	190(9.5±2.79)	230(11.5±2.47)
No. of fetuses	6(2.6)	5(2.6)	3(1.3)
Dead of resorbed (%)[a]	D) 0, R) 6(2.6)	D) 2(1.0), R) 3(1.6)	D) 2(0.9), R) 1(0.4)
Live (lit. size±S. D.)	226(11.3±2.17)	185(9.3±2.95)	227(11.4±2.65)
Sex ratio (♂/♀)	1.21(124/102)	1.13(98/87)	1.10(119/108)
Fetal weight ♂	5.43±0.46	6.00±0.42	5.87±0.45
(Mean±S. D.) ♀	5.35±0.46	5.73±0.39	5.63±0.49
External abnormalities (%)[b]	Hematoma 3(1.3)	—	Hematoma 2 (0.9) Bent tail 1 (0.4)

a) % of total implants b) % of live fetuses D) Dead fetuses R) Resorbed fetuses

The rate of dead fetuses for the group that took 600 mg/kg of γ–oryzanol was 1.3 % and the

rate for the group that took 6 mg/kg was 2.6 % which were not significantly different. Mean body weight of live fetuses of γ–oryzanol administration groups was slightly higher as compared to the control group both for males and females.

From external observation, two cases of hematoma and one case of bent tail were found in the group that took 600 mg/kg of γ–oryzanol and three cases of hematoma were found in the control group.

The occurrence rate of all abnormalities was as low as 0.4 to 1.3 %. There were no fetuses with any visceral abnormalities in the γ–oryzanol administration groups or control group.

Tables 10 to 12 show the results of observation of stained bone samples and Table 13 shows the summary. No malformations were found in any group.

Table 10 Influence of γ-oryzanol on skeletal development of rat fetuses

Control — Wistar strain

Test animal No. / Observation	1	2	3	4	5	6	7	8 a)	9 a)	10	11	12 a)	13	14	15	16	17	18	19	20	Total
Malformations																					
Variations																					0
Non-ossified of 5th sternebrae									1			1			1						3
Asymmetry of sternebrae	1			1				1	2		1		1								7
Transformation or split of thoracic body	1																				1
Split of thoracic arch								1													1
14th rib		1	1	2						3	2	6	2	4				4	2	1	28
Split of lumbar arch								1					1		1						3
Types of ossification																					
Delayossification of sternebrae							2		1	2							2				7
Total fetuses examined												145									
Total variational fetuses												40									
Total variational observations												43									
Total variational fetuses / Total fetuses examined ratio												27.6%									

a) fetus with 2 variations

Table 11 Influence of γ-oryzanol on skeletal development of rat fetuses

γ-oryzanol (daily dose 6 mg/kg) Wistar strain

Test animal No. / Observation	1	2	3	4	5	6	7	8	9	10 a)	11	12	13	14	15	16	17 a)	18	19	20	Total
Malformations																					0
Variations																					
Non-ossified of 5 th sternebrae																					0
Asymmetry of sternebrae						1		1		1							1				4
Transformation or sprit of thoracic body			1																		1
Sprit of thoracic arch																					0
14 th rib	1			2	2			4		2		1		4	1		4	3		2	26
Sprit of lumbar arch	1																				1
Types of ossification																					
Delayossification of sternebrae	1			1	1					1	3	3					2	1			13

Totel fetuses examined	115
Total variational fetuses	30
Total variational observations	32
Total variational fetuses / Total fetuses examined ratio	26.1

a) fetuse : with 2 variations

Table 12 Influence of γ-oryzanol on skeletal development of rat fetuses

γ-oryzanol (daily dose 600 mg/kg) Wistar strain

Test animal No. / Observation	1	2	3	4	5	6	7	8	9	10 a)	11	12	13	14 b)	15	16	17 a)	18	19	20	Total
Malformations																					0
Variations																					
Non-ossified of 5 th sternebrae														1							1
Asymmetry of sternebrae	2						2	1		1		1		2				2			11
Transformation or split of thoracic body														2							2
Split of thoracic arch																					0
14 th rib	3	3			4	5			1	6	1	1	3			3	8				38
Split of lumbar arch																	1				1
Types of ossification																					
Delayossification of sternebrae			1									1						1	1		4

Total fetuses examined	142
Total variational fetuses	49
Total variational observations	53
Total variational fetuses / Total fetuses examined ratio	34.5%

a) fetuse with 2 variations
b) fetuse with 3 variations

Table 13 Influence of γ-oryzanol on skeletal development of rat fetuses (Summary)

Drug and Dose	Control	γ-oryzanol 6 mg/kg	
No. of fetuses examined	145	115	142
Skeletal maformations (%)			
Skull	0	0	0
Axial skeleton	0	0	0
Appendicular skeleton	0	0	0
Skeletal variations (%)			
Non-ossified of 5 th sternebrae	3(2.1)	0	1(0.7)
Asymmetry of sternebrae	7(4.8)	4(3.5)	11(7.7)
Transformation or split of thoracic body	1(0.7)	1(0.9)	2(1.4)
Split of thoracic arch	1(0.7)	0	0
14 th rib	28(19.3)	26(22.6)	38(26.8)
Split of lumbar arch	3(2.1)	1(0.9)	1(0.7)
Total variational fetuses / Total fetuses examined ratio	27.6%	26.1%	34.5%
Development of ossification			
Delayossification of sternebrae	7(4.8)	13(11.3)	4(2.8)
No. of caudal vertebrae[a]	6.9±1.23	7.3±0.92	7.0±1.25
No. of proximal phalanges in forepaw[a]	3.8±0.55	3.9±0.46	3.7±0.71
No. of proximal phalanges in hindpaw[a]	1.8±1.59	2.1±1.57	2.1±1.58
No. of distal phalanges in forepaw[a]	5.0±0.00	5.0±0.00	5.0±0.00
No. of distal phalanges in hindpaw[a]	5.0±0.26	5.0±0.13	5.0±0.17

a) Mean ±S. D.

In the γ–oryzanol administration groups, variations such as non-ossified of the fifth sternebrae, asymmetry of sternebrae (photos 4 and 5), transformation or split of thoracic body (photo 6), split of lumbar arch, 14th rib, and split of lumber arch (photo 7) were observed. These variations were found in the control group as well and there were no significant differences between the administration groups and the control group.

Photo 4 Rat: γ-oryzanol 600 mg/kg
Asymmetry of 2 nd-5 th sternebrae

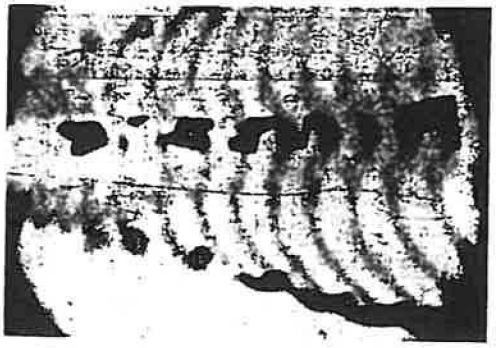


Photo 5 Rat: γ-oryzanol 6 mg/kg
Asymmetry of 5 th sternebrae

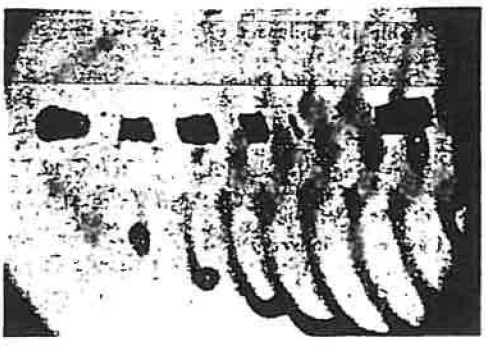


Photo 6 Rat : γ-oryzanol 6 mg/kg
Transformation of thoracic body

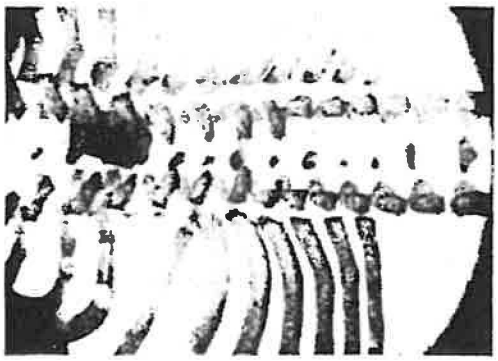


Photo 7 Rat Spit of 3rd lumbar arch

Skeletal development of breast bone; the number of tail caudal vertebra, the number of proximal phalanges of toe bone, and the number of distal phalanges were used as indexes to examine skeletal development. These values were similar to the values of the control group.

Table 14 shows the results of observation of fetuses fixed in Bouin's solution. No variation was observed except for two cases of ureterohydronephrosis (photo 8) in the group that took 600 mg/kg of γ–oryzanol.

Table 14 Observation of some organs by razor blade sectioning method

Drug and Dose	Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of observed fetuses	55	62	62
No. of abnormal fetuses	0	0	2[b]

a) Organs observal were : brain, eye, mouth, heart, lung, kidney, gonad etc.
b) Ureterohydronephrosis.

Photo 8 Rat : γ-oryzanol 600 mg/kg
Ureterohydronephrosis

3) Influence on offspring

Table 15 shows the results of raising offspring until the 21st day after the birth. Table 16 shows the results of observation of external differentiation. Fig. 4 shows the increase in their weight.

Table 15 Influence of γ-oryzanol on rat offsprings

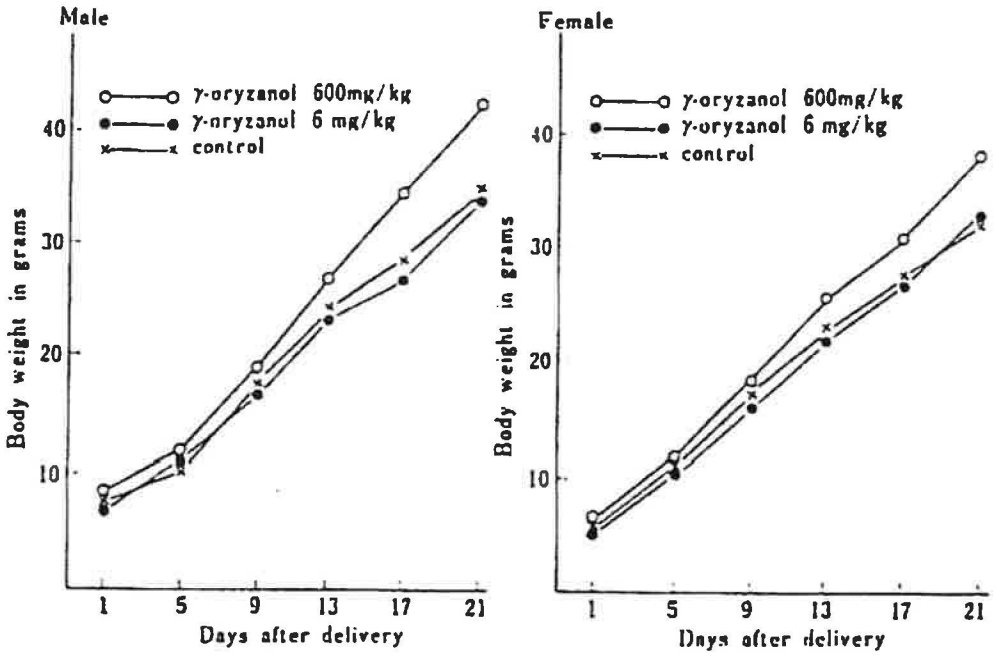
Drug and Dose		Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
No. of dams		5	5	5
Total offsprings (lit. size ±S.D.)		61(12.2±1.59)	60(12.0±1.87)	45(9.0±2.92)
No. of live offsprings (Days after delivery)	1	61	60	45
	5	61	57	42
	9	61	57	42
	13	61	57	42
	17	61	57	42
	21	61	57	42
Total weanlings (lit size ± S.D.)		61(12.2±2.59)	57(11.4±2.41)	42(8.4±2.19)
Sex ratio (♂/♀)		1.03(31/30)	0.96(28/29)	0.91(20/22)
Rate of weanling (%)		100	95	93

Table 16 Influence of γ-oryzanol on external differentiation of rat offsprings

Drug and Dose		Control	γ-oryzanol 6 mg/kg	γ-oryzanol 600 mg/kg
Time scale of external features[a] (Days after delivery)				
Detachment of auricles	♂	3.2±0.42	3.3±0.48	3.4±0.42
	♀	3.6±0.40	3.3±0.48	3.2±0.38
Eruption of incisor	♂	10.4±0.54	10.4±0.61	10.1±0.96
	♀	10.2±0.54	10.4±0.62	10.0±0.68
Opening of eyelids	♂	16.3±0.90	16.2±0.69	16.4±0.92
	♀	16.3±0.82	16.2±0.62	16.3±0.53
Behavioral abnormalities		—	—	—
Abnormalities of auditory pain and righting reflex		—	—	—

a) Mean±S.D.

Fig. 4 Mean body weight of young rats after delivery



Although the total number of offspring was slightly lower in the group that took 600 mg/kg of γ–oryzanol as compared to the control group, the difference was not significant. Some offspring died in the initial stage of rearing in all groups. Three offspring died in each γ–oryzanol administration group. The rearing rate was 93 % in the group that took 600 mg/kg of γ–oryzanol and 95 % in the group that took 6 mg/kg, which were good results.

When differentiation was observed, detachment of auricles, eruption of incisor, and opening of eyelids were confirmed on almost the same day in both γ–oryzanol administration groups and the control group. Fur appeared on around the 9th day both in the γ–oryzanol administration groups and control group.

Although weight of offspring in the group that took 600 mg of γ–oryzanol increased more as compared to the control group, the difference was not significant.

Weight increase of offspring in the group that took 6 mg of γ–oryzanol was similar to the control group.

No abnormalities were found in the external appearance; general behavior; or auditory, pain, and righting reflex in live weanlings, just as in mice.

No abnormalities were found in partial inspection of principle organs of live weanlings or in observation of stained bone samples.

Discussion and conclusion

Influence of γ–oryzanol on fetuses and offspring was investigated using pregnant mice and rats.

In the test, 6 mg/kg (maximum regular dose for human) of γ–oryzanol, which was six times more than regular dose for humans, was administered in the group for small dose administration. In the group for large dose administration, 600 mg/kg, which was 100 times more than the group for small dose, was administered. Normal saline solution was administered to the animals of the control group.

No dam died or miscarried during the test period. There was no specific change in their general conditions.

Yamamoto[23] and Wakao[17] et al. reported that γ-oryzanol has an activity to accelerate growth. When the weight of dams was compared, the increase of weight of pregnant mice and rats in the group that took 600 mg/kg of γ–oryzanol was slightly higher as compared to the control group. There was no significant difference in total implants and total number of live fetuses between the administration groups and control group. The number of resorbed fetuses of mice in the group that took 6 mg/kg of γ–oryzanol was significantly different from the control group. However, the death rate of the group that took 6 mg/kg of γ–oryzanol was 7.8 % including resorbed fetuses, which was lower than the natural death rate of ICR-JCL mice 9.4 %[24], 10.2 %[25], indicating that the death of the fetuses was not caused by the administration of γ–oryzanol. This can be confirmed by the fact that the death rate in the group that took 600 mg/kg of γ–oryzanol was 2.5 %, which was lower than the rate of the control group.

In the observation of fetuses, a few cases of club foot and hematoma were found in mice and a few cases of hematoma and bent tail were found in rats. No other abnormalities were observed.

In observation mainly to check for missing principle organs, there was no fetus with abnormalities. Only two cases of ureterohydronephrosis were found in rat fetuses fixed in Bouin's solution in the group that took 600 mg/kg of γ–oryzanol.

No bone malformation was found in stained bone samples.

In some mice, variations such as split or bifurcation of atlas; split or bifurcation of axis; cervical rib, 14th rib; extra sternebrae; and asymmetry of sternebrae were observed. In some rats, asymmetry of sternebrae, 14th rib, non-ossified fifth sternebrae, transformation or split of thoracic body, split of lumbar arch, and split of lumber arch were observed. These variations were found in the control group as well and there was no significant difference in their occurrence rate between the administration groups and the control group except for 14th rib (mice, γ-oryzanol 6 mg/kg).

Skeletal development of breast bone; the number of tail caudal vertebra, the number of proximal phalanges of toe bone, the number of middle phalanx, the number of distal phalanges and the number of fetuses with ossified ankle bone and calcaneal bone were used as indexes to examine skeletal development. These values were almost the same in the γ–oryzanol administration groups and control group, indicating that there is no influence of γ–oryzanol in skeletal development.

When the change in the weight of offspring was examined, increase of weight was slightly less in the mice in the group that took 6 mg/kg of γ–oryzanol. However, the difference was not significant. There was no indication of prohibited weight increase in the group that took 600 mg/kg of γ–oryzanol. The weight of rats in the group that took 600 mg/kg of γ–oryzanol increased adequately.

Rearing rate of mice three weeks after the birth was as low as 75 % in the group that took 6 mg/kg of γ–oryzanol. However, the rate was 100% in the γ–oryzanol administration group. Differentiation conditions in the γ–oryzanol administration groups were good as well. No abnormalities were found in the external appearance; general behavior; or auditory, pain, and righting reflex in mice or rats.

In general, no deformities were found in fetuses born from dams that γ–oryzanol was administered to. Most of the variations observed in administration groups were also found in the control group and the degree was within the range of natural occurrence. Thus, γ–oryzanol is not considered to be teratogenic. γ–oryzanol does not show any influence on skeletal development either.

The fact that no appearance abnormalities, visceral malformations, or bone malformations were found from observation of three week old weanlings confirms that γ–oryzanol is not teratogenic.

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