DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date APR - 6 1998

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	katsuobushi oligopeptide
Firm:	General Nutrition Corp.
Date Received by FDA:	March 13, 1998
90-day Date:	June 10, 1998

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after June 10, 1998.

Robert J. Moore, Ph.D.

3439 98 APR -9 P2:10

95S-0316

RPT27

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

MAR 13 1998

John P. Troup, Ph.D.
Vice President, Scientific Affairs
General Nutrition Corporation
300 Sixth Avenue
Pittsburgh, Pennsylvania 15222

Dear Dr. Troup:

This letter acknowledges receipt by the Food and Drug Administration (FDA) on March 13, 1998 of your notifications, dated March 2, 1998 and March 4, 1998, pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act), providing notice of your intent to introduce, or deliver for introduction into interstate commerce, the new dietary ingredients "ademetionine and katsuobushi oligopeptide.

The date that the agency received your notification submitted under 21 U.S.C. 350b(a), March 13, 1998, is the filing date for the notification. In accordance with the requirements of 21 U.S.C. 350b, for 75 days after the filing date, General Nutrition Corporation shall not introduce, or deliver for introduction, into interstate commerce any dietary supplement that contains either of these new dietary ingredients, ademetionine and katsuobushi oligopeptide.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Programs and Enforcement Policy
Office of Special Nutritionals

3440 '98 APR 9 P2:10

cc:
HFS-456 (file)
f/t:rjm:HFS-456:3/13/98:gnc.ack:disc26

GNC LiveWell™

John P. Troup, Ph.D.
Vice President,
Scientific Affairs

March 4, 1998

Linda S. Kahl, Ph.D.
Office of Special Nutritionals
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street (HFS-450)
Washington, DC 20204

Dear Dr. Kahl:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, General Nutrition Corporation ("GNC") located at 300 Sixth Avenue, Pittsburgh, PA 15222 wishes to notify the Food and Drug Administration that GNC will market a new dietary ingredient, Katsuobushi Oligopeptide, a peptide obtained from thermolysin digested Katsuobushi (dried bonito). Accordingly, enclosed please find two (2) copies of this notification.

The dietary supplement which contains Katsuobushi Oligopeptide will be sold in tablet and powder forms and will provide 1 gm of Katsuobushi Oligopeptide per serving. Suggested use will be three (3) times per day.

Attached please find a summary of the safety, stability and clinical reports along with other information which establish that this dietary ingredient, when used under the conditions suggested in the labeling of the dietary supplement, is reasonably expected to be safe. These supporting materials include:

(1) Product Specification & Process Manufacturing

(2) Summary of safety, stability and clinical reports

(3) Scientific study describing physiological effect and dosing

(4) Acute Toxicology

(5) References

Very truly yours,

John P. Troup, Ph.D.
Vice President, Scientific Affairs

JPT/jaj

Enclosures

SECTION 1-"Katsuobushi Oligopeptide"

Product Specification
Process Manufacturing

KATSUOBUSHI OLIGOPEPTIDE

The Nippon Synthetic Chemical Industry Co.,Ltd.

Functional Food Department

Name

"Katsuobushi Oligopeptide" is peptides obtained from thermolysin digested Katsuobushi (dried bonito). It inhibits Angiotensin(I) Converting Enzyme (ACE) activity.

Application field

"Katsuobushi Oligopeptide" inhibits angiotensin(I) converting enzyme (ACE) activity and helps people to keep blood pressure in good condition. It does not indicate any effect on healthy persons, but shows mild blood pressure lowering activity on borderline hypertensive or hypertensive persons.

Specifications

"Katsuobushi Oligopeptide" powder

1.	Appearance	Light brown powder with no contamination of displeasure taste or odor
2.	Specified ingredient	Enzyme decomposed protein
3.	Content of (2)	more than 85%
4.	ACE inhibitory activity	Not more than 60 μg/ml
5.	Moisture content	Not more than 5%
6.	Arsenic	Not more than10 ppm as As
7.	Heavy metals	Not more than 20 ppm as Pb
8.	Standard plate count	Not more than 3000
9.	E.coli colony	Negative

Process manufacturing

Bonito

↓

Dried Bonito

↓

(Hot water extraction)

↓ → extract

Residue of extract

↓ ← Thermolysin, Water

(Enzyme reaction)

↓

(Heating)

↓

(Filtration)

↓

Filtrate

↓

(Sterilization)

↓

(Drying)

↓

"Katsuobushi Oligopeptide"

SECTION 2-"Katsuobushi Oligopeptide"

Summary of safety, stability and clinical reports

MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT & COMPANY IDENTIFICATION

CHEMICAL PRODUCT NAME	KATSUO-BUSHI OLIGOPEPTIDES
NAME OF MANUFACTURER	THE NIPPON SYNTHETIC CHEMICAL INDUSTRY CO.,LTD.
	1-88,1-CHOME OYODONAKA, KITA-KU,OSAKA, 531 JAPAN
NAME OF SECTION	FUNCTIONAL CHEMICALS DIVISION
	PLANNING AND COORDINATION DEPARTMENT
TELEPHONE NUMBER	(06) 440-5318
FAX NUMBER	(06) 440-5327

2. COMPOSITION/INFORMATION ON INGREDIENTS

SUBSTANCE/MIXTURE	MIXTURE
CHEMICAL NAME	PEPTIDES
UN CLASS	NOT APPLICABLE
UN NUMBER	-

3. HAZARDS IDENTIFICATION

CLASS NAME OF HAZARDOUS CHEMICALS FOR SDS IN JAPAN
NOT APPLICABLE

PHYSICAL AND CHEMICAL HAZARDS
MAY FORM EXPLOSIVE DUST-AIR MIXTURE

ADVERSE HUMAN HEALTH EFFECTS
CONSIDERED TO BE SAFE SINGLE ORAL INTAKE OF 30 GRAMS OF THE MATERIAL BY ADULTS
NEGATIVE IN THE AMES TEST

ENVIRONMENTAL EFFECTS
BIODEGRADABLE

4. FIRST AID MEASURES

EYE CONTACT

GENTLY RINSE THE AFFECTED EYES WITH CLEAN WATER FOR SEVERAL MINUTES.
REMOVE CONTACT LENSES IF EASILY POSSIBLE.
AND REFER FOR MEDICAL ATTENTION.

SKIN CONTACT

REMOVE ALL CHEMICALS BY FLUSHING WITH WATER

INHALATION

REMOVE THE VICTIM FROM CONTAMINATION TO FRESH AIR.
KEEP THE VICTIM QUIET.

INGESTION

CONSIDERED TO BE SAFE UP TO AROUND 2GRAMS/KG BODY WEIGHT BECAUSE THE MATERIAL IS FOOD.
RINSE MOUTH WITH WATER AND DILUTE WITH WATER.

---

5. FIRE-FIGHTING MEASURES

SPECIFIC HAZARDS WITH REGARD TO FIRE-FIGHTING MEASURES

SHUT OFF FUEL TO FIRE AND USE EXTINGUISHING MEDIA.

EXTINGUISHING MEDIA

DRY CHEMICAL POWDER, WATER SPRAY, CARBON DIOXIDE

---

6. ACCIDENTAL RELEASE MEASURES

SWEEP UP, PLACE IN AN BAG AND HOLD FOR WASTE DISPOSAL.
IF POSSIBLE, SPRAY WATER TO AVOID RAISING DUST.
FLUSH RESIDUAL SPILL WITH COPIOUS AMOUNTS OF WATER.

---

7. HANDLING AND STORAGE

HANDLING

HANDLE GENTLY TO AVOID RAISING DUST WHICH MAY FORM EXPLOSIVE DUST-AIR MIXTURE.

STORAGE

STORE IN A COOL DRY DARK CLEAN LOCATION. KEEP AWAY FROM SUNLIGHT.

---

8. EXPOSURE CONTROL/PERSONAL PROTECTION

CONTROL PARAMETERS
NOT ESTABLISHED
ENGINEERING MEASURES
USE ADEQUATE VENTILATION AND IN CLOSED SYSTEMS.
PERSONAL PROTECTIVE EQUIPMENT
RESPIRATORY PROTECTION : DUST RESPIRATOR
EYE PROTECTION : SAFETY GLASSES, SAFETY GOGGLES
HAND,SKIN AND BODY PROTECTION : GLOVES, BOOTS, APRON

---

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE : LIGHT BROWN POWDER
BOILING POINT : –
VAPOUR PRESSURE : – (NON VOLATILE)
MELTING POINT : NA
DENSITY : NA
SOLUBILITY IN WATER: OVER 50%(25℃)

---

10. PHYSICAL HAZARD

FLASH POINT : NA
AUTOIGNITION TEMPERATURE : NA
EXPLOSION LIMIT : NA
FLAMMABILITY : FLAMMABLE
SPONTANEOUS COMBUSTIBILITY: NO
REACTIVITY WITH WATER : NO
OXIDIZIBILITY : NO
SELF–REACTIVITY : NO
EXPLOSIVITY : MAY FORM EXPLOSIVE DUST–AIR MIXTURES
STABILITY & REACTIVITY : THIS PRODUCT IS CONSIDERED A STABLE MATERIAL UNDER NORMAL AND ANTICIPATED STORAGE AND HANDLING CONDITIONS

---

11. TOXICOLOGICAL INFORMATION

ACUTE TOXICITY (1) : ORAL LD50 (MOUSE) OVER 2000MG
SUB-CHRONIC TOXICITY : NA
CHRONIC TOXICITY : NA
CORROSIVE AND IRRITANT PROPERTIES : NA
CARCINOGENIC EFFECTS : NA
MUTAGENIC EFFECTS (1) : NEGATIVE IN THE AMES TEST, UMU-ASSAY AND REC-ASSAY.
ALLEGENIC AND SENSITIZING EFFECTS : NA
EFFECTS ON THE REPRODUCTIVE SYSTEM : NA
TERATOGENIC EFFECTS : NA

12. ECOLOGICAL INFORMATION

BIODEGRADABILITY : THIS SUBSTANCE HAS GOOD BIODEGRADABILITY BECAUSE IT IS MADE FROM FISH MEAT.
BIOACCUMULATION : NA
FISH TOXICITY : NA

13. DISPOSAL CONSIDERATION

BURN IN AN INCINERATOR LITTLE BY LITTLE.

14. TRANSPORT INFORMATION

KEEP CARGO PROPERLY TO AVOID SPILL, WET, DAMAGE BY CARGO SHIFTING.

15. REGULATORY INFORMATION

FOOD SANITATION LAW

16. OTHER INFORMATION

(1) TOXICITY TEST RESULTS OBTAINED FROM AN ANALYTICAL LABORATORY

THE INFORMATION HEREIN MAY BE ALTERED BY UPDATED KNOWLEDGE OR TEST RESULTS.
THE INFORMATION HEREIN IS GIVEN BASED ON LATEST DATA, BUT THE NIPPON SYNTHETIC CHEMICAL INDUSTRY CO.,LTD. DOES NOT ASSUME ANY LIABILITY FOR THE ACCURACY OR COMPLETENESS OF THE INFORMATION.
SPECIAL CARE SHOULD BE TAKEN TO PREVENT POTENTIAL HAZARD IF A USER HANDLES THE MATERIAL FOR HIS OWN PARTICULAR USE.
ALL MATERIALS MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED IN CAUTION.
IT IS THE USER'S RESPONSIBILITY TO ESTABLISH SAFETY HANDLING PROCEDURE.

Summary of safety information

Four weeks old Spontaneously Hypertensive Rats (SHR) were fed chow containing 0%(control) or 1% (40 times as much as the effective dose) of "Katsuobushi Oligopeptide" for six weeks.
In both groups no abnormality was found in average body weight, average body length or blood biochemical analysis result (lipometabolism, glicometabolism,inorganic substance metabolism, liver function, renal function).
Mutagenicity of "Katsuobushi Oligopeptide" was tested by Ames test, umu test, and Rec-assay.
In Ames test(reverse mutation of His and Trp auxotrophs), number of revertant colonies was higher than anticipated influence of free histidine in "Katsuobushi Oligopeptide". It suggested existence of something which may increase the revertant colonies. However, as the revertant colony increase was not so notable, the mutation potency of "Katsuobushi Oligopeptide" was not concluded to positive.
In both umu test and Rec-assay, mutagenicity was definitely negative.
All results mentioned above indicate that "Katsuobushi Oligopeptide" does not have mutagenicity.

An acute toxicity was examined with mice by OECD guidelines for toxicity tests of chemical substances (1987). In this test, neither death nor abnormality was observed among tested animals.
The LD50 of "Katsuobushi Oligopeptide" was over 2000 mg/kg by single oral administration.(section 4)

Thirteen healthy persons took "Katsuobushi Oligopeptide" 5g/day (2.5g each after breakfast and lunch) for two months. Before and after administration, no abnormality was observed in electrocardiogram, breast roentogenogram, blood pressure, body weight, blood test results, urinalysis, or subjective symptoms.

30 hypertensive volunteers were subjected to crossover control test of "Katsuobushi Oligopeptide" 3g/day for eight weeks. Any significant change of heart rate, body weight or abnormality of subjective or objective symptoms was not observed. (1)

No side effect or abnormality was found in other crossover control test by 37 borderline hypertensive subjects who took the peptide as Peptide soup. (2)

"Katsuobushi Oligopetide" is manufactured from Katsuobushi, one of traditional Japanese foods, with thermoase which is a brand name of thermolysin approved as food additives in Japan. It is expected to be decomposed to amino acids *in vivo*. That is a reason why "Katsuobushi Oligopeptide" has highly safety features.

Summary of stability information

Stability of "Katsuobushi Oligopeptide" was evaluated by storing spray dried powder of the peptide in screw capped bottles, at room temperature and 40°C in darkness for 18 months.
After the storage, any deterioration (LKPNM degradation or ACE inhibitory activity decrease) was not found.

SECTION 3-"Katsuobushi Oligopeptide"

Scientific Study Describing Physiological Effect and Dosing

Summary of scientific study

Katsuobushi Oligopeptide indicates anti-hypertensive activity by inhibiting Angiotensin Converting Enzyme (ACE). We summarized the results of investigations and studies concerning animal experiments and human clinical tests which support the above activity.

Angiotensin Converting Enzyme (ACE) catalyses a reaction to form Angiotensin II which has blood pressure elevating activity. It also stimulates the reaction to decompose Bradykinin which has anti-hypertensive activity. Therefore, ACE inhibitors are expected to show antihypertensive effect and actually several ACE inhibitory medicines have already been available.
So, we tried to find natural ACE inhibitors from enzyme digested food proteins.

The thermolysin digested Katsuobushi homogenate showed the strongest ACE inhibitory activity among all the substances tested by various digestive enzymes. Hot water extract of Katsuobushi itself or gastrointestinal protease (pepsin, trypsin or chymotrypsin) digest of Katsuobushi showed only weak ACE inhibitory activity. The residue of hot water extract of Katsuobushi has not been utilized as food or feed. So the residue with low fat content and low fish odor was suitable as a raw material for ACE inhibitor peptides.

We studied both ACE inhibitory activity and antihypertensive effect of the thermolysin digest of Katsuobushi homogenate (hereinafter "Katsuobushi Oligopeptide") with rats. Systolic blood pressure of normotensive rats is temporarily elevated by intraveneous injection of 100 ng/kg of Angiotensin I (AI). However, this temporary elevation was completely inhibited by injecting "Katsuobushi Oligopeptide" intravenously at a dose of 10 mg/kg before the injection of AI. This fact shows that the blood pressure elevation by intravenous injection of AI was blocked by ACE inhibitory activity of "Katsuobushi Oligopeptide".

"Katsuobushi Oligopeptide" and gastrointestinal proteases (pepsin, trypsin, and chymotrypsin) digested Katsuobushi were given to Spontaneously Hypertensive Rats (SHR) by single oral administration to evaluate their antihypertensive activities. The single oral administration of "Katsuobushi Oligopeptide" at a dose of 500 mg/kg indicated significant and long antihypertensive effect of 20−25 mmHg 4−6 hours after the administration. On the other hand, 1.0 g/kg of gastrointestinal proteases digest of Katsuobushi hardly lowered blood pressure. This fact suggests that antihypertensive effect can not be obtained just by eating Katsuobushi, but when Katauobushi is taken as "Katsuobushi Olygopeptide", it shows antihypertensive activity. In addition, the ACE inhibitory activity of "Katsuobushi Oligopeptide" was not changed even after the further digestion by gastrointestinal proteases.
This result supports why "Katsuobushi Olygopeptide" can indicate antihypertensive activity without losing its power when taken by oral administration.

In order to examine antihypertensive activity by long term administration of "Katauobushi Oligopeptide", 3 weeks old SHR were fed chow diet containing 0.025−1.0% of the peptide for 7 weeks. Blood pressure, body weight, and chow intake were measured. Significant antihypertensive effect was recognized even in the group fed with 0.025% diet. The intake of "Katsuobushi Oligopeptide" in this group corresponded to 15mg/kg/day. It was confirmed that "Katauobushi Oligopeptide" has strong antihypertensive activity among ACE inhibitory peptides derived from food proteins. After the experiment, biochemical examination of blood (kidney function, liver function and lipometabolism) was also conducted, but no significant difference was found between the control group and all experimental groups. "Katsuobushi Oligopeptide" administration did not show any influence on the growth of SHR. This result supports the safety of the "Katsuobushi Oligopeptide" as it exhibits no side effects even when administered 40 times of the effective dosage.

Many ACE inhibitory peptides derived from food have been reported. However, most peptides do not have antihypertensive effect when they are taken by oral administration. We studied the reason for this phenomenon and found that the stability to ACE itself was the most important factor.
In order to evaluate the stability to ACE itself, apparent ACE inhibitory activity of various peptides were checked before and after the preincubation with ACE. The apparent ACE inhibitory peptides

can be classified into three types as follows.

a. Substrate type : This type of peptides is digested by ACE and its ACE inhibitory activity is lowered by preincubation. It is not effective by oral administration.

b. Inhibitor type : This type of peptides is not digested by ACE and so its activity will not be changed. It shows significant antihypertensive activity.

c. Pro-drug type : This type of peptides is digested by ACE or gastrointestinal proteases in vivo and converted into Inhibitor type peptides. It exhibits significant and long lasting activity by oral administration.

We confirmed that "Katsuobushi Oligopeptide" has stable ACE inhibitory activity even after the pre-incubation with ACE.

Eight ACE inhibitory peptides which have strong activity(Ile-Tyr, Phe-Gln-Pro, Ala-Leu-Pro-His-Ala, Ile-Trp-His-His-Thr, Leu-Lys-Pro-Asn-Met, Ile-Lys-Pro-Leu-Asn-Tyr, Asp-Tyr-Gly-Leu-Tyr-Pro, and Ile-Val-Gly-Arg-Pro-Arg-His-Gln-Gly) were isolated from " Katsuobushi Oligopeptide" and their primary structures were identified. [3] Polypeptides having IC50 values below 10 μM, i.e. IY (IC50=2.1μM, One letter abbreviation is used to represent amino acids), IWHHT (IC50=5.8μM), LKPNM(IC50=2.4μM), IVGRPRHQG(IC50=2.4μM) were synthesized and fed to SHR at a dose of 60mg/kg by single oral administration. All synthesized peptides showed significant anti-hypertensive activity. IY, which is ACE inhibitor type peptides exhibited the maximum antihypertensive effect of 19 mmHg at 2 hr after administration. The pro-drug type peptides (IWHHT, LKPNM, and IVGRPRHQG) required two hours or more time to reach their maximum effect than their corresponding activated peptides (IWH, LKP, and IVGRPR) and exhibited long lasting activities. They need to be absorbed and converted into active forms *in vivo*.

LKPNM which is a typical pro-drug type peptide and contained about 1.7% in "Katsuobushi Oligo-peptide" is converted into LKP with 3.2 times stronger activity (IC50=0.32μM) than the pro form. As LKPNM peptide indicated as strong activity as Captopril with SHR experiment, this peptide was thought as representative one in "Katsuobushi Oligopeptide".

On the basis of the results mentioned above, the antihypertensive effect to humans was studied. 30g of "Katsuobushi Oligopeptide" (contains LKPNM 64mg) was given by single oral administration to three healthy persons and six hypertensive patients. Among three healthy persons, any clear variation of systolic blood pressure was not observed, but systolic blood pressure decreased in all patients. [4] A long term oral administration effect of "Katuobushi Oligopeptide" was evaluated by giving 2g (3.3mg as LKPNM) of the peptide three times a day (after each meal) to four hypertensive patients. Significant antihypertensive activity was recognized three of them. Remaining one patient showed no antihypertensive effect by ACE inhibitor drug treatment after this "Katsuobushi Oligopeptide" experiment. [5]

Cross over design examination was conducted to 30 hypertensive volunteers. The daily dosage of "Katsuobushi Oligopeptide" was set at 3g (5mg as LKPNM) by the result of SHR experiment.
In this test, significant antihypertensive activity was observed in both the first term and the second term group of subjects. Effective ratio (% of subjects who indicated blood pressure lowering effect) were 60% and 67% in the first and second group respectively. [1]

All results of these clinical tests indicate that "Katsuobushi Oligopeptide" taken by humans is stable against gastrointestinal proteases, absorbed without losing the ACE inhibitory activity and worked to help over 60% of essential hypertensive subjects to control their blood pressure with no side effect.

SECTION 4-"Katsuobushi Oligopeptide"

Acute Toxicology

TEST REPORT

No. OS55110581-3

Client The Nippon Synthetic Chemical Industry Co., Ltd.

Test Substance Enzyme Digest of Fish

Test Item Acute Toxicity Test

The result of the test substance delivered to Japan Food Research Laboratories on November 16th, 1992 is as reported below.

December, 21st 1992

(Foundation) Japan Food Research Laboratories

Acute Toxicity Test

Summary

An acute oral toxicity test of the enzyme digest of fish was conducted using mouse according to the OECD toxicity test guidelines for chemical substances(1987).

Three levels of dosage of 2,000, 1,670, and 1,390 mg/kg of the test substance were administered by single oral administration to male and female mice. Neither abnormality in, nor death of, test animals were observed.

Client

The Nippon Synthetic Chemical Industry Co.,Ltd.

Test Substance

Enzyme Digest of Fish

Period of Testing

From 25th, November of the 4th year of Heisei to 21st, December of the 4th year of Heisei

Test Laboratory

Food Research Laboratories, Osaka Branch

Chemists and Associates who Conducted the Test

Shinichi Katsuda, Mayumi Morita, and Shigeko Kamioka

1. Purpose of Test

In order to confirm the acute oral toxicity of the test substance to mouse, the oral toxicity test was conducted according to OECD guidelines for the toxicity tests of chemical substances(1987).

2. Test Substance

Enzyme Digest of Fish

Description: Light Brown Powder

3. Preparation of Test Solution

A solution containing 200 mg of the test substance in 1 ml was prepared using purified water as solvent and this solution was subjected to testing.

4. Test Animals

Four weeks old ICR series male and female mice that were bought from Japan SLC, Inc. were preliminarily bred for 1 week to confirm that they are not abnormal in health. Afterwards, they were subjected to the test. Each 10 test animals were accommodated in a polycarbonate cage and bred in a breeding room, in which the temp. and lighting timewere set at 23 ± 2 ℃ and 12 hours per day, respectively. The feed (CE-2, Nippon Clea Corporation) and drinking water(tap water) were allowed to freely ingest.

5. Test Method

Test animals were subjected to fasting for 4 hours before administration of the test substance. 10 mice were used for each level of dosage.

After having weighed body weights of each mouse, a single dosage of the test substance corresponding to 3 levels(the coefficient = 1.2) of 1,390, 1,670, and 2,000 mg/kg were forcibly administered orally by means of a mouse stomach sonde.

Observation continued for 14 days. Observation was conducted often on the day of administration and once per day after the second day including the second day. and body weight was measured after a lapse of each one week. All animals were sacrificed and necropsied after expiration of the test period. Additionally. solvent control groups consisting of each 5 female and5 male animals were administered solvent of 10 ml/kg and other handlingand operation were conducted by the same procedure as those for test groups.

The obtained data of body weight were subjected to a statistical analysis at a level of significance of 5 % by means of an analysis method of variance.

6. Test Results

1) Death examples and mortality (Tabel -1 and Table-2)

No example of death in both male and female groups. was observed during observation period.

2) General Situation

In all animals including both female and male animals, any abnormality was not observed during the whole observation period from just after administration to the end of observation period.

3) Change of body weight(Table-3 abs Table-4)

In body weights measured at 1 and 2 weeks after administration, any significant difference among average body weights of all groups was not found.

4) Results of anatomical examinations

In anatomical examinations conducted after the test, any abnormality in representative organs was not observed in both male and female groups.

7. Consideration

The acute oral toxicity test of the test substance was conducted to mice.

OECD guidelines for toxicity tests of chemical substances (1987) indicate to conduct a detailed test for evaluation of LD_{50} value in the case in which the dead animal, even if it were one, was recognized at the administration level of 2,000 mg/kg. In this test conducted according to these guidelines, neither any dead animal nor any abnormality in anatomical examinations was observed.

Therefore, the lethal dosage by a single oral administration of the test substance to mouse was concluded to be above 2,000 mg/kg to both male and female mouse.

Table-1 Dead animals and Mortality(Male)

Dosage (mg/kg)	After Administration (hr)			(day)								Mortality
	<1	3	5	1	2	3	4	5	6	7~13	14	
1,390	0	0	0	0	0	0	0	0	0	0	0	0/10
1,670	0	0	0	0	0	0	0	0	0	0	0	0/10
2,000	0	0	0	0	0	0	0	0	0	0	0	0/10
Control	0	0	0	0	0	0	0	0	0	0	0	0/5

Table-2 Dead animals and Mortality(Female)

Dosage (mg/kg)	After Administration (hr)			(day)								Mortality
	<1	3	5	1	2	3	4	5	6	7~13	14	
1,390	0	0	0	0	0	0	0	0	0	0	0	0/10
1,670	0	0	0	0	0	0	0	0	0	0	0	0/10
2,000	0	0	0	0	0	0	0	0	0	0	0	0/10
Control	0	0	0	0	0	0	0	0	0	0	0	0/5